Study Title: **Pharmacokinetics of Intramuscular Versus Subcutaneous Administration of Benzathine Penicillin G (Bicillin® L-A)**

Abbreviated Title: The IM versus SC Bicillin® L-A PK Study
Protocol No: U1111-1216-5903
Clinical Trial Registration #: XXXXXXXX
HREC approval: approved by: XXXXXXXX
Study Product/s: Benzathine Penicillin G (Bicillin® L-A)
Principal Investigator: A/Prof Laurens Manning

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1. INVESTIGATOR AGREEMENT

Study Title: Pharmacokinetics of Intramuscular Versus Subcutaneous Administration of Benzathine Penicillin G (Bicillin® L-A)

I agree:

- To assume responsibility for the proper conduct of the study;
- To conduct the study in compliance with this protocol, with any future protocol amendments, and with any study conduct procedures;
- To ensure that all persons involved with this study are adequately informed about the study-related duties and functions as described in the protocol;
- Not to implement any changes to the protocol without prior review and approval from the Human Research Ethics Committee/s approving the protocol, except where necessary to eliminate an immediate hazard to the subjects, or where permitted by all applicable regulatory requirements (for example, administrative aspects of the study);
- That I am aware of, and will comply with "Good Clinical Practice" (GCP), Declaration of Helsinki, and all applicable TGA/NHMRC requirements;
- That I, and any persons employed on this project, will provide up-to-date curriculum vitaes and any declaration of financial and ownership interests in this project.

Investigator name: ____ Associate Professor Laurens Manning _____

Date: ____ 31/07/2018 _____

31/07/2018

X 

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Signed by: sslvpn.telethonkids.org.au

2. STUDY SYNOPSIS

TITLE	Pharmacokinetics of Intramuscular Versus Subcutaneous Administration of Benzathine Penicillin G (Bicillin® L-A)
OBJECTIVES	Primary Aim: 1. To compare the pharmacokinetics, in particular, the rate of absorption of Bicillin® L-A administered by the subcutaneous route and the intramuscular route in healthy adults Secondary aims: 1. To measure the pain experienced by participants administered Bicillin® L-A by the subcutaneous route compared to the intramuscular route 2. To measure the frequency, types, and severity of adverse events related to Bicillin® L-A administered by the subcutaneous route compared to the intramuscular route
DESIGN	A single-blinded study in 2x2 crossover using two doses of Bicillin® L-A administered by the subcutaneous and intramuscular route with a 10-week wash-out period between doses. Serum penicillin levels and pain scores just prior to injection and at pre-determined intervals for six weeks following each injection will be collected. An ultrasound scan will be used to guide and confirm injection site and document location of medication bolus on injection day and local changes at injection sites at four weeks after injection.
OUTCOMES	Primary outcome measure: 1. The rate of absorption of Bicillin® L-A administered subcutaneously and intramuscularly Secondary measures: 1. Time above the minimum inhibitory concentration (0.02mg/mL) for *Streptococcus pyogenes* 2. Pain scores associated with receiving Bicillin® L-A administered subcutaneously and intramuscularly 3. Type, number, and severity of adverse events following subcutaneous compared to intramuscular administration of Bicillin® L-A 4. Type and number of ultrasound-detected local changes following subcutaneous compared to intramuscular administration of Bicillin® L-A
STUDY DURATION	Six months (each participant will be in the study for 18 weeks)
INTERVENTIONS	Healthy male participants meeting all the inclusion criteria and none of the exclusion criteria will be divided into two groups. Each participant will receive an injection of Bicillin® L-A, followed by a ten-week wash-out period during which sampling will occur in the 1st six weeks after injection. After the wash-out period, participants will receive the second injection using the alternate route with sampling over the next six weeks. Sampling will include serum penicillin levels, pain scores, and ultrasound examinations of injection sites at pre-determined periods to inform outcomes.
NUMBER OF PARTICIPANTS	15 participants

Abbreviations:

AE	Adverse event
ANZCTR	Australian and New Zealand Clinical Trials Registry
AR	Adverse reaction
ARF	Acute rheumatic fever
AUC	Area under curve
BPG	Benzathine penicillin G
Cmax	Maximum serum drug concentration
CRF	Case report form
CT	Computerised tomography
DBS	Dried blood spot
DMSB	Data and safety monitoring board
DoH	Declaration of Helsinki
GAS	Group A streptococcus
GCP	Good clinical practice
HREC	Human research ethics committee
IM	Intramuscular
IMP	Investigational medical product
ISM	Independent Safety Monitor
IV	Intravenous
Ka	Rate of absorption
NRS	Numeric rating scale
MIC	Minimum inhibitory concentration
NHMRC	National Health and Medical Research Council
NSAID	Non-steroidal anti-inflammatory drug
PK	Pharmacokinetic
RHD	Rheumatic heart disease
RSI	Reference safety information
SAE	Serious adverse event
SAP	Statistical analysis plan
SAR	Serious adverse reaction
SC	Subcutaneous
SP	Secondary prophylaxis
SUSAR	Suspected unexpected serious adverse reaction
TGA	(Australian) Therapeutic Goods Administration
US	Ultrasound

TABLE OF CONTENTS

3. GENERAL INFORMATION

3.1. Protocol full title:

Pharmacokinetics of intramuscular versus subcutaneous administration of Benzathine penicillin G (Bicillin® L-A)

3.2. Principal investigator:

Associate Professor Laurens Manning

3.3. Person(s) authorised to sign the protocol amendments:

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3.4. Investigator(s) responsible for conducting study:

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Prof Jonathan Carapetis
Dr Robert Hand
Dr Robert Henderson
Dr Sam Salman
Dr Rosemary Wyber
Dr Joseph Kado

3.5. Confidentiality statement

All information found within is the property of Telethon Kids Institute, and Harry Perkins Institute of Medical Research, and is therefore provided to you in confidence. Only authorised personnel may access this information and it is understood that its contents shall not be disclosed without written authorisation from the Principal Investigator, Associate Professor Laurens Manning.

4. TRIAL SUMMARY

Benzathine penicillin G (BPG) is marketed in Australia as Bicillin® L-A, produced by Pfizer. BPG has been in use since the 1950s for treatment and prevention of recurrent episodes of acute rheumatic fever (ARF). Current guidelines recommend a 900mg dose of BPG every 28 days for a minimum of 10 years to prevent the progression to rheumatic heart disease (RHD). ARF and RHD predominantly affect children and young adults in resource-limited settings. Australia and New Zealand have a high burden of disease despite their economic status, with Indigenous populations in Northern Australia having ARF rates of 150-380 per 100,000.[1] RHD affects an estimated 33.4 million people worldwide, resulting in the death of approximately 319,400 people each year.[2] BPG is also recommended for the first line treatment of most types of syphilis (including in pregnant women to prevent maternal-foetal transmission of syphilis), which affects approximately 18 million people worldwide.[3]

The pain associated with BPG injection is often cited as a reason for contributing to the low rates of adherence below the recommended 80% for ARF/RHD patients (11 of 13 BPG injections/year).[4,5]

Several studies have suggested that a significant percentage of people intended to receive intramuscular injections may in fact be having subcutaneous injections due to thickness of gluteal fat, especially in women due to fat distribution.[6,7] The use of the subcutaneous route to administer medications is widely accepted, although the paucity of data around pharmacokinetics and safety with antibiotics is still an issue.[8]

This investigator-initiated trial aims to demonstrate that Bicillin® L-A injected via the subcutaneous route has an improved pharmacokinetic profile and tolerability when compared with intramuscular administration (current standard) in healthy adults.

5. OBJECTIVES / AIMS / SIGNIFICANCE

5.1. Primary Objective

1. To compare the rate of absorption of Bicillin® L-A administered by the subcutaneous route and the intramuscular route in healthy adults.

5.2. Secondary Objective/s

1. To measure the pain experienced by participants administered Bicillin® L-A by the subcutaneous route compared to the intramuscular route.
2. To measure the frequency and types of adverse events related to Bicillin® L-A administered by the subcutaneous route compared to the intramuscular route.

5.3. Significance

Numerous studies have demonstrated that intended intramuscular (IM) injections are commonly not administered intramuscularly but instead are administered to the subcutaneous (SC) tissues – especially intra-adipose - particularly in females and/or those who are classified as obese.[6,7] Despite the significant variability between individuals and between different studies of BPG pharmacokinetics, there have been no comparative studies to assess route as an explanation for these variances in pharmacokinetics.[9,10] The results of this clinical trial have several important clinical applications, namely:

1. Determining if the route of administration of BPG has significant impact on pharmacokinetic properties of the medication.
2. Ability to guide clinicians as to whether BPG should be delivered to an IM or SC site, or either.
3. Better understanding whether there is a difference in injection-related pain between IM and SC injections.
4. Determining if a future reformulated long-acting penicillin should be delivered preferentially at an IM or SC site.

6. RATIONALE / BACKGROUND

6.1. Indications for BPG treatment

Since the 1950s, BPG has been used extensively for the treatment of many infectious diseases. The unique characteristics of BPG, namely the prolonged serum concentration for up to four weeks, have resulted in BPG's continual use in ARF/RHD secondary prophylaxis programs.[9,11] The development of bacterial resistance of some organisms against penicillin G, has resulted in a reduction of primary indications for BPG, however, it remains the antibiotic of choice for several infections, particularly those caused by group A streptococcus (GAS), which has never developed penicillin resistance.

6.1.1. Streptococcal pharyngitis

Group A streptococcal pharyngitis is a common cause of a bacterial sore throat.[12] Bicillin® L-A is recommended by the Therapeutic Guidelines of Australia for the treatment of acute pharyngitis in individuals unable to take oral antibiotics or non-compliant with a course of oral antibiotics. It is only recommended for those at high risk of ARF.

6.1.2. Impetigo

Impetigo is a common skin condition, especially in resource-limited settings, and is caused by *Streptococcus pyogenes* or *Staphylococcus aureus*.[13] The Therapeutic Guidelines recommend a single dose of Bicillin® L-A 1.2M IU given IM to treat impetigo in remote Australia. Alternatives do exist, although these require a minimum five-day course of oral antibiotics (co-trimoxazole).

Bowen et al. in a systemic review in 2015 estimated the global point prevalence of impetigo to be approximately 162 million cases. In Australia, they used the data from 10 population prevalence studies of Indigenous remote communities to determine a median prevalence of 44.5%. Using the 2011 census data, they estimated the number of remote Indigenous children affected by impetigo aged 0-15 years was 15,696.[14]

6.1.3. Syphilis and other treponemal diseases

There are an estimated 18 million cases of syphilis (*Treponema pallidum*) worldwide, with an additional 5.6 million new cases in the 18-49 year old age group expected in 2012.[15] The World Health Organization recommends BPG as the first line treatment of *syphilis* in all cases, including pregnant women, except in the case of late syphilis (>2years). Courses of treatment range from a single 2.4MIU BPG dose, to a weekly injection for three weeks. Alternatives exist for treatment of syphilis, although they have disadvantages. Ceftriaxone has been shown to be equally effective in non-pregnant individuals, but requires a 10 day intravenous/intramuscular regimen.[15,16] Oral therapy with azithromycin treats the mother but not the foetus, and doxycycline has teratogenic effects.[15] Different subspecies of *Treponema palladium* also cause the skin diseases yaws, bejel, and pinta, and are susceptible to treatment with BPG.[17]

6.1.4. Acute rheumatic fever – secondary prophylaxis

It is estimated that 33.4 million people worldwide live with RHD, resulting in approximately 319,400 deaths each year. The majority of these cases are in resource-limited settings.[18,19] Over 471,000 cases of ARF occur each year.[19] Australia, New Zealand, and Pacific nations carry a disproportionately large burden of disease in their Indigenous populations. Australia continues to have endemic areas of high rates of ARF and RHD.[20,21] In the Northern Territory, rates are amongst the highest in the world. In the 5-14-year age group, ARF rates are between 162/100,000 and 228/100,000 for males and females respectively. Sixty-one percent will go on to develop RHD within 10 years. Once diagnosed, 27% developed heart failure within five years.[21]

Secondary prophylaxis (SP) is the administration of four-weekly BPG injections to prevent the recurrence of ARF. Current Australian guidelines recommend patients are administered SP for a minimum of 10 years (sometimes lifelong in severe cases).[4] BPG is the only medication which has been shown to affect outcome in ARF.[11,22,23]

Although some clinicians suggest alternatives antibiotics, such as oral azithromycin, these options have not been shown to be superior, and have the potential for rapidly promoting increased antimicrobial resistance. Oral regimens have more complicated dosing schedules with suboptimal protection (e.g. oral penicillin V).[24-26]

Whilst ARF and RHD are completely preventable by treating recurrent GAS infections, adherence to SP is generally poor. Australian guidelines recommend 80% of SP injections (11 of 13) are achieved;

the reality is much lower than this.[27] Many factors contribute to this, including education, health awareness, and access to health care. Fear of injection and injection pain are often cited as reasons for poor adherence.[5] Some clinicians advocate for the addition of lignocaine to BPG to help reduce pain and/or the use of a Buzzy® device.[28,29] The use of lignocaine and Bicillin® L-A has not been studied in vivo, however, addition of lignocaine to powdered BPG has shown no significant difference in pharmacokinetics (PK).[30]

6.2. Pharmacokinetics of BPG

BPG has favourable characteristics which make it ideal for slow-growing organisms and for SP. Its long serum half-life compared to other forms of penicillin, allows for the serum levels to remain above minimum inhibitory concentration (MIC) for several weeks. This unique characteristic of BPG is due the slowed absorption from the IM depot formed on injection. Initial studies in the 1950s showed the superiority of BPG when compared to sulfadiazine and oral penicillin. Subsequent studies went on to establish the PK profile and show superiority of BPG for SP.[11]

Stollerman et al. in 1955 compared BPG and oral penicillin efficacy in 329 patients with ARF or RHD; 145 patients received BPG 1.2MIU via 4-weekly intramuscular injection, 111 patients received oral penicillin V 100,000 units twice daily, and 73 received oral sulfadiazine 1gm daily. There were 0, 2 and 5 recurrences respectively, of ARF through the equivalent 242, 170, 130 patient years, respectively. They did note that on some occurrences when inadvertently given subcutaneously, BPG had a longer duration of discomfort, but they did not quantify the severity. Compared to healthy controls who had carriage rates of GAS of 19.3%, the intervention groups had lower rates of GAS: 1.4%, 13.1% and 10.7% respectively for BPG, oral penicillin, and sulfadiazine respectively.[11]

The ability to detect serum penicillin levels four weeks after administration above the in-vitro MIC, and demonstrated clinical superiority have resulted in BPG being the preferred agent for SP. Whilst the PK profile of BPG has been studied extensively, there has been increasing evidence to suggest that current dosing regimens are insufficient.[9,31,32] During the 1990s, evidence emerged of lower than expected plasma drug levels after IM injection. A case was made for revising the dosing of BPG by Currie et al. 1994, who compared the protective plasma concentration of 1.2MIU, 1.8MIU, and 2.4MIU in adults. At 3 weeks, 50%, 73%, and 100% respectively had adequate penicillin levels. Only 24% (1.2MIU), 39% (1.8MIU), and 56% (2.4MIU) had protective levels at four weeks.[10]

It was noted that there were some individuals who had significantly different PK profiles and the authors postulated that this could be due to inadvertent adipose administration, but were unable to demonstrate this in their cohort.[10]

In 2011, Broderick et al. conducted a PK study whereby BPG was administered to healthy military recruits, and noted that levels were lower than expected. In total, 329 adult males were

administered 1.2MIU of BPG, and blood samples were taken for PK analysis. Nine days after BPG delivery, 50% of the cohort had a concentration of penicillin G less than 0.02µg/mL, the accepted standard for protection against GAS.[9] The researchers did not draw any conclusions as to the possible causes of this variation.

6.3. Reformulation of BPG

BPG is a key component of SP programs for treatment and prevention of ARF/RHD. It is widely acknowledged that BPG is not an 'ideal medication' for this purpose owing to the pain and other adverse reactions. There is growing support for the reformulation of penicillin to provide better injectable SP, and to produce a product that is longer acting, less painful, and/or more reliable in its pharmacokinetics.[33,34]

The need for a reformulation product is well established. Wyber et al. 2016, assessed the ideal characteristics of a penicillin reformulation for SP, using a panel of RHD experts, including clinicians. Their conclusion was that an acceptable reformulation would need to: be administered subcutaneously; have a dosing schedule greater than six weeks; be less or no more painful than existing BPG; be cold-chain independent; and of comparable cost. [34]

6.4. Adverse events and safety of BPG in humans

6.4.1. Local adverse events

Adverse events related to BPG administration include local and systemic events. Of the local adverse events, pain is probably the most problematic and is further expanded below. Other local adverse events/reactions range in severity from minor injection site reaction, erythema, and sterile abscesses (see Table 1 below) to more severe but rare events like myositis (s6.4.3), fibrosis and Nicolau syndrome are described later.

6.4.2. Pain of BPG IM injections

The pain associated with IM BPG injection is frequently cited as a reason for lack of compliance to SP.[28,34] Whilst the exact mechanism behind the pain is poorly understood, studies have shown that patients who receive BPG injections do not 'get used to' injections, as some believe.[5,28,29] There have been several interventions that have shown some efficacy in reducing the pain in children and adolescents receiving BPG injections. Studies have demonstrated a reduction in pain scores with the co-administration of lignocaine. Powdered BPG can be reconstituted with lignocaine in place of sterile water. The resultant injection has significantly reduced pain with no significant alteration in pharmacokinetics.[30] New Zealand Ministry of Health recommends the addition of lignocaine to Bicillin® L-A to reduce injection pain.[35] This practice is not endorsed by the manufacturer due to potential loss of sterility and is not routinely used in Australia. Use of the Buzzy®, and with addition of lignocaine to Bicillin® L-A, have been shown to reduce pain for up to 12 hours in populations of

children, however, the efficacy decreases with age. Additionally, application of pressure and temperature packs are two methods that have shown significant reduction in pain scores with BPG injections.[28,30,36] These interventions do not make the procedure painless, however, they do significantly reduce the pain experienced by children.

6.4.3. Other local adverse events

A case report of myositis in a seven-year-old child receiving Bicillin® L-A reported by Francis et al. in 2016[37], demonstrated MRI-confirmed myositis. The individual was receiving SP for ARF, observed symptoms of left hip mono-arthritis, and noted to have elevated inflammatory markers. Noting that he had not received his usual four-weekly Bicillin® L-A injection, he was given a dose during admission; two days after injection, he underwent MRI for the presenting left hip pain, which incidentally revealed significant inflammatory changes on the ipsilateral side of injection, consistent with myositis. The child's left hip pain responded to aspirin, however, the right-sided myositis remained an issue for a further five days. MRI also revealed granulomata likely resulting from previous IM injections.[37]

6.4.4. **Systemic adverse events**

Whilst generally regarded as a safe medication, there have been several unexplained deaths in children with RHD-related heart failure receiving BPG. This has resulted in other medication regimens being substituted despite studies showing reduced efficacy.[23,38] A survey by the World Heart Federation indicated that 21% of the 39 respondents had a patient die from anaphylaxis post–injection.[39] The reporting mechanisms are often poor or non-existent, not allowing for discrimination as to whether these cases are due to true anaphylaxis or another mechanism that is not well understood.[17]

Berkovitch et al. 2017 describe near-fatal and fatal non-allergic reactions to BPG and describe them as occurring within a few minutes after administration of BPG, but the cohort described included only two patients aged 10 and 12 years with fatal outcomes, for whom SP BPG was prescribed. Both children had severe mitral insufficiency. The other seven cases ranged in age from 66-93 years old and were prescribed BPG for recurrent erysipelas (6) and sinusitis(1).[40] Wyber et al., (personal correspondence, unpublished data, April 2018) also describe a retrospective survey of a cohort of patients aged 12-35 years old from low and middle-income countries who were prescribed BPG for secondary prevention of ARF. Of note, all cases were documented to have valvular heart disease (RHD), with four of five that reported severity documenting severe RHD. In this cohort, only three of the 10 fulfilled the level 1 Brighton Criteria for anaphylaxis.

In 1991, the International Rheumatic Fever Study Group undertook a study to assess BPG administration in 1,790 patients from 11 countries. Over 32,430 injections were administered over an equivalent of 2,736 patient years. Four episodes of anaphylaxis occurred (an injection frequency of 0.012%) and one patient died (an injection frequency of 0.0031%). All of the individuals who had

an anaphylactic reaction had RHD and the patient that died had severe mitral valve disease and was undergoing treatment for chronic congestive heart failue.[41]

Galvao et al. 2013, primarily assessed the safety of BPG in the treatment of syphilis in pregnant women. In their systematic review, they included the evaluation of 10 prospective studies and three retrospective studies, covering discrete periods between 1946 and 2008. They identified 13 studies representing 3,466,780 patient episodes where penicillin was prescribed. There were four deaths identified as relating to BPG: two in 1954, one in 1958 and one in 1991 (mentioned above). There were seven episodes of anaphylaxis identified in the studies relating to BPG. No serious adverse events were reported in the 1,244 pregnant women receiving BPG. The researchers reported the pooled absolute risk of death to be 0% (95% CI 0-0), and the pooled absolute risk of anaphylaxis to be 0.002% (95% CI 0-0.003). Of all pooled adverse reactions recorded, 6,377 adverse reactions occurred amongst 3,465,322 patient episodes, equating to an absolute risk of 0.169% (95% CI 0.073- 0.265).

Whilst there have been reports of adverse events (AE) in Australia thought to be related to Bicillin® L-A, a search of the Database of Adverse Event Notifications for the period 1/1/1971 until 31/12/2016 revealed only 22 reported adverse reactions. Of these, only 13 had Bicillin® L-A as the only administered medication suspected for the AE. The TGA has records for 10 of the 13 cases, which are summarised in the below table. At the time of search, there had been no recorded death due to Bicillin® L-A injection, either as a single agent or when co-administered with other medications.[42]

Table 1: Summary list of the 10 cases where Bicillin® L-A was the only administered medication

Date of entry	MedDRA reaction terms
18/1/1973	Rash erythematous
06/05/1987	Pyrexia, injection site pain
11/7/1994	Cyanosis, dyspnoea, face oedema, peripheral ischaemia
8/12/1999	Injection site reaction
8/12/1999	Injection site abscess
26/2/2001	Cardiac arrest, hypotonia, seizure
2/11/2010	Gait disturbance, injection site reaction
2/11/2010	Erythema, pain, rash, swelling
2/11/2010	Injection site reaction, otitis media, pyrexia
2/11/2010	Injection site reaction, pyrexia

6.5. Intramuscular versus subcutaneous administration of antibiotics in animals

Animal studies have demonstrated different PK properties of antibiotics when given via alternate routes.[43,44] Despite the widespread use of BPG in humans, there is a lack of clinical studies investigating possible PK differences with different administration route.

Lavy et al. 1999, compared pharmacokinetics of clindamycin hydrochloride when delivered intravenously, intramuscularly, and subcutaneously. Using a partial crossover trial with 12 dogs, they compared the pharmacokinetics as well as monitoring the dogs for signs of pain/irritation at site of injection. Four of the dogs were noted to have clinical signs indicative of slight pain after IM injection; this was not observed in the SC group. The results showed IM and SC routes of administration produced a significantly larger area under the curve (AUC) than intravenous (IV) delivery. There was no significant difference between the IM vs SC routes, however, they did note a significant rise in serum creatinine phosphokinase (CPK) for 48 hours after administration of IM clindamycin. There was no significant increase in CPK in the subcutaneous group, or with the administration of IM saline. It was suggested this rise was due to muscle damage, although no imaging or biopsy studies were undertaken.[43]

6.6. Intramuscular versus subcutaneous administration in humans

All routes of parenteral medication administration carry risks, which vary with the method chosen. SC injections are often selected for low volume medications with a desire for slow and prolonged mechanism of action. IM injections are chosen due to being highly vascular, and causing tolerable pain depending on the volume and characteristics of the injection. The increased vascularity shortens time of onset (in comparison to the SC route) whilst maintaining a depot for increased half-life (in comparison to intravenous). IV injections are the most rapid of the three routes, although this often results in the most rapid clearance of the administered medication. Additionally, some medications are contraindicated for IV injection, but approved for other routes, BPG being one such example.[45,46]

IM injections are an invasive procedure and not without risk. Babhulkar 1985, reported 11 case reports of unilateral triceps fibrosis, due to recurrent IM injections. Seven of these cases received oxytetracycline; because of the fibrosis, patients were unable to fully flex their elbow, and in some cases, were unable to feed themselves. Initially, all cases were prescribed physiotherapy with varying results. Eight cases required surgery throughout the study. Intraoperative findings were of fibrous bands in the triceps. The authors noted that the contractures were similar to those reported due to injections in the quadriceps and deltoid muscles.[47]

Haramati et al. 1994, studied 338 sequential pelvic computerised tomography (CT) scans for the location and depth of buttock granulomas, and the thickness of SC fat. Their results identified 164 granulomas in 67 patients: 152 of the granulomas in the SC fat, and 12 in the muscle tissue. They

also noted the thickness of SC fat in the upper, outer quadrant to be 5.0cm +/-1.9cm. They did not assess the reason or the intended target of injection.[48]

Serious adverse reactions, e.g. 'Nicolau Syndrome' (Livedoid dermatitis) associated with IM injection are thought to result from inadvertent intravascular injection of medication intended for muscle, although the exact underlying pathology is not well understood. They are well described in the literature.[49,50] It manifests in extreme pain immediately post-injection, a painful livedoid rash, and evidence of skin ischaemia. Microscopic evaluation shows evidence of thrombosis of small vessels. Involvement of the deeper tissue, including muscle, subsequently resulting in compartment syndrome has been documented, and severe cases have resulted in permanent ischaemia to affected limbs.[50-52]

There are significant adverse reactions noted in the literature from all routes, and the route chosen is often a combination of drug characteristics, desired onset/clearance, tolerability, and known AE profiles.

6.7. Intramuscular versus subcutaneous administration in animal studies to assess procaine benzylpenicillin/benzathine penicillin G

While no animal studies directly compare BPG administration routes, there are numerous studies in different mammals which have demonstrated altered pharmacokinetics of combination penicillin formulations depending on route and location of injection.[44,53] Ranheim et al. 2002, compared the effects of IM versus SC administration of procaine/benzathine penicillin G in piglets, using a stratified parallel design with six litters comprising 57 piglets in total. The three arms of the study compared the administration of: 33,000 IU/kg; 100,000 IU benzathine procaine penicillin (Peni-KEL L.A. 15 +15, 150,000IU benzathine penicillin G/ 150,000IU procaine penicillin); and procaine penicillin G (Penivet) 100,000IU/kg. The first three litters received the above doses via IM injection, with blood samples taken at: 0,2,6,10,14,24,32 hours, 2,2.5,3,4,5,7,9,13 days following injection. The experiment was replicated with the subsequent three litters with the medications given by SC injection. Results showed a statistically significant difference between maximum plasma concentration (Cmax) and area under the curve (AUC), and mean residence time in all groups in favour of the SC concentration with prolonged duration of effect above MIC. The results showed no adverse effects with comparison between IM and SC administration.[44]

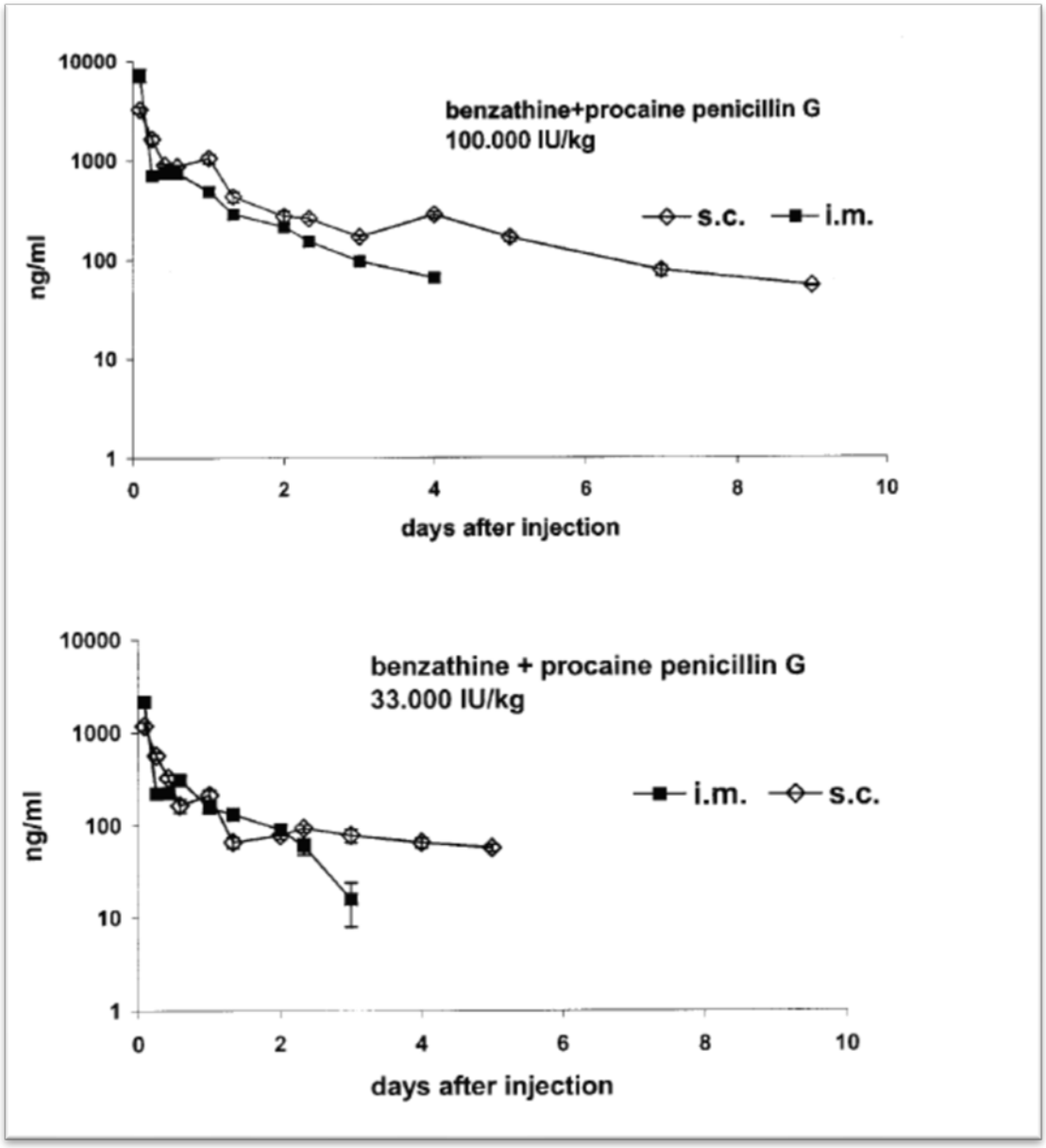

Figure 1: Displaying the difference in plasma penicillin concentration between intramuscular (i.m.) or subcutaneous(s.c.). Top: benzathine + procaine penicillin G (100,000IU/Kg); bottom: benzathine + procaine penicillin G (33,000 IU/kg) [*Ranheim et al. (2002)*]

6.8. Evidence to support the failure of intramuscular injection in humans

In 1982, Cockshott et al. investigated the administration of IM injections into the upper outer quadrant of the buttock with CT. 213 patients were studied; the weight of participants was measured in 123 individuals - 60 women and 63 men. They compared skin to muscle measurements for a given weight. Women were found to have 2.5cm additional gluteal fat thickness for a given weight. It was estimated that more than 85% of males and 95% of females would have received their IM injections outside the gluteal muscle when using 3.5cm needles.[6] The researchers also noted the presence of adipose calcification, suggesting previous 'IM injections' were possibly intra-lipomatous.[6]

Chan et al. 2006, observed a similar rate of failure of IM injections. Using CT imaging, they assessed upper outer quadrant gluteal IM injections in adults; only 32% were confirmed IM when using the standard 3cm 23G needle. There was significant difference between genders: 56% were correctly administered for males, compared with only 8% for females. Of the patients with a BMI of 25-29.9kg/m^2, 33% received IM injections. Above a BMI of 30kg/m^2, no patients received correct administration of IM injections.[7]

In 1975, Vukovich et al. noted significant PK variation between sexes in the absorption of cephadrine, specifically when administered in the gluteus maximus. In this study, 12 volunteers (six male, six female) received intramuscular injections in the deltoid, vastus lateralis, and gluteus maximus on different occasions. As demonstrated below in Figure 2, cephadrine serum concentrations were similar between sexes for the deltoid and vastus lateralis. However, when administered via the gluteus maximus, there was an unexplained significant difference in peak serum concentration. They did not comment on the possible reasons behind the difference.[54] Burbridge et al. 2005, reported similar significant differences in gluteal SC fat thickness between men and women, and suggested that using standard IM needles would result in 34% failure rate (14% males, 54.7% females).[55]

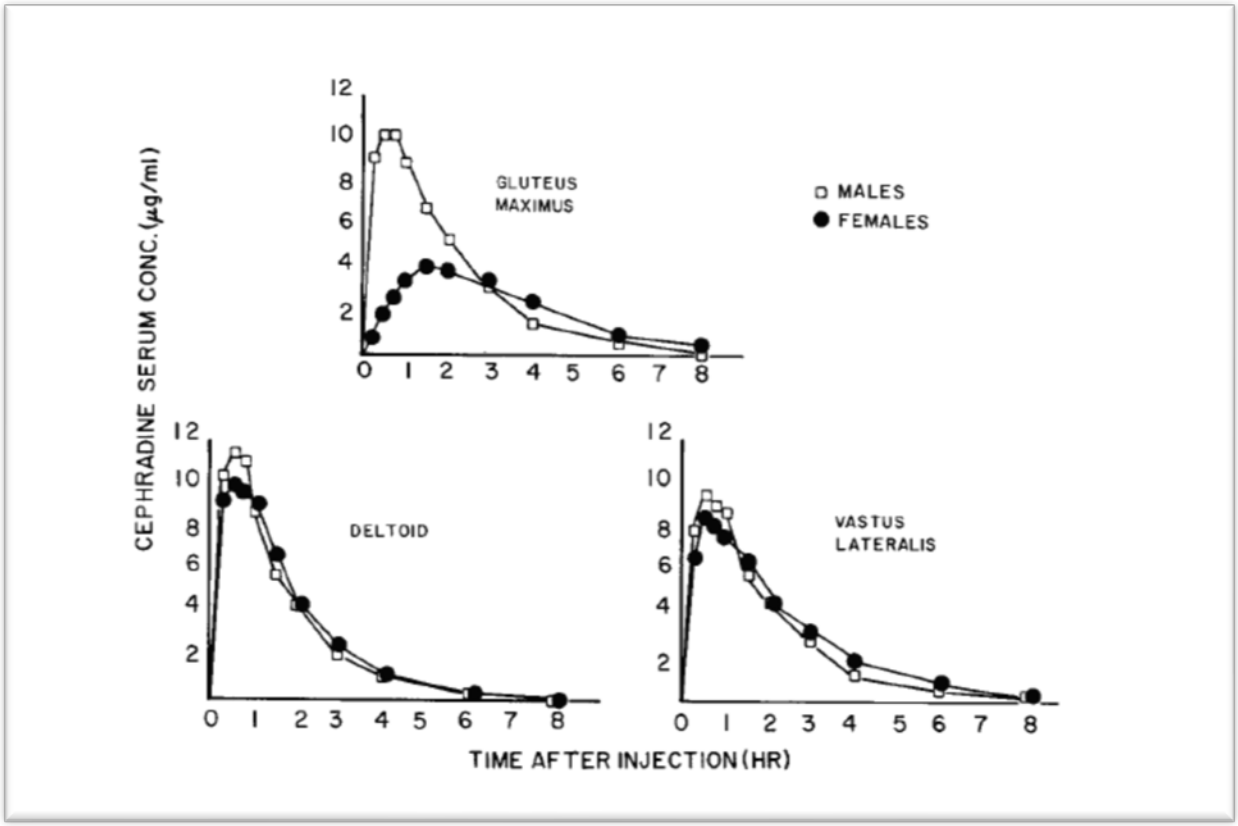

Figure 2: Time to cMAX was 2.05 hrs (females) vs 0.63 hrs (males). Peak concentration was 4.30+/-0.92µg/ml (females) vs 11.08 +/- 1.11 µg/ml (males), both statistically significant (p<0.05). [*Vukovich et al. (1975)*]

6.9. Evidence that subcutaneous administration of medication has preferable characteristics

The above studies give support to the hypothesis that IM injections can be inadvertently administered to the SC/adipose tissue, possibly affecting the PK in certain individuals. Cephadrine, aspirin, and dapsone have all displayed PK differences which have been attributed to intra-adipose injections.[56,57] The ideal characteristics of secondary prophylaxis is a long-acting depot of medication which is released in a predictable fashion to ensure that plasma levels remain above the MIC for *Streptococcus pyogenes*, the causative organism of ARF. The ability to extend the duration of therapy by altering route of administration has benefits for the patient (fewer injections), health care providers (human resources), and governments (financial savings).

Whilst there have been no human comparative studies with BPG investigating alternate routes, human studies involving other medications have revealed significant PK differences.[54,58] In 2016, El Samad et al., compared the safety and tolerability of SC teicoplanin versus IV administration for the treatment of bone and joint infections. Thirty patients received a total of 1460 subcutaneous injections; doses of less than 800mg per dose were used and mixed into 50ml saline, then subsequently administered by gravity feed. It was noted that doses below 600mg were associated with fewer adverse reactions by SC injection, and no patient had a Grade 3 or above local reaction at any dose. The local reactions included: pain; swelling; erythema; warmth; itching; haematoma; and telangiectasia, with patients reporting greater pain at the end of the infusion compared to the start. Between 36-50% of participants experienced pain throughout the study, although it was not significant enough to withdraw from the study. The pharmacokinetics of teicoplanin were more favourable with the SC route of administration. A significantly higher C_{min} +6.51mg/L was noted, with SC vs IV (p<0.001).[58]

6.10. Subcutaneous administration of antibiotics

Forestier et al. 2015, performed a national survey of The French Infectious Diseases Society (SPILF) and French Society of Geriatrics and Gerontology (SFGG) society networks. Of the 1800 surveys emailed to members, 382 responded from 160 hospitals across France. Three hundred and sixty-seven (96.1%) of respondents admitted to administering SC antibiotics. The most frequently prescribed subcutaneous antibiotic was ceftriaxone, being prescribed by all but one physician (n=366), with ertapenem (n=122, 33.2%), teicoplanin (n=144, 39.2%), aminoglycosides (n=129, 35.1%), amoxicillin (n=56, 15.3%), cefepime (n=9), imipenem (n=6), piperacillin-tazobactam (n=2), amoxicillin-clavulanic acid (n=2), ceftazidime (n=1), doripenem (n=1) also reported. The number of respondents who treated 6-10 or more patients per month via the SC route was 117 (31.8%). Two hundred and eighty-five respondents recorded their duration of SC therapy to be between 4-14 days; 69 respondents administered SC antibiotics for more than 14 days. Reasons for choosing SC therapy included IV/IM contraindicated, oral route contraindicated, avoiding oral polypharmacy, palliative care, and facilitating hospital discharge.[8]

Of the reasons for not prescribing SC antibiotics, the most frequent was the lack of PK data: 59.7% (n=219); 29.2% (n=107) cited the lack of marketing authorisation. Survey respondents did not comment on the adverse reaction rate or tolerance by patients, although given such a high number of clinicians admitting to using therapy for more than four days, it is suggestive that patient tolerance is acceptable.[8]

6.11. Pain of subcutaneous injections in humans

Berteau et al. 2015, investigated the effect of viscosity of injection in healthy adult volunteers. Using a comparative crossover design, they enrolled 24 adults (12 male, 12 female). They compared either low (0.02mL/s) or high (0.3mL/s) flow rates as well as viscosity of suspension of sodium chloride +/- hyaluronic acid at different viscosities: 1, 8-10, 15-20cP. They showed no difference in pain scoring due to injection rate (p=0.79), however, they showed a significant difference in pain scores dependent on the viscosity of the injection. Higher viscosity was associated with a lower pain score: visual analogue scale (VAS)=12.6mm vs 22.1mm. (p=0.0002). Volume of injection 2 or 3mL did not reveal a significant difference in pain (p=0.89).[59]

6.12. Clinically significant reduction in pain

The VAS has been well validated as a method for determining differences in pain in clinical practice. Todd et al. 1996, demonstrated that a difference of 13mm (95% CI, 10-17mm) on a 100mm scale was a clinically significant change in pain severity.[60] This was validated in a larger study by Gallagher et al. 2001, in which they were able to replicate Todd et al. in 96 patients.[61] Bijur et al., 2003, compared the VAS (0-100) to numerical reported scale (NRS) (0-10) and found no significant difference in the recorded pain levels.[62] Cepeda et al. 2003, undertook a prospective study investigating what determined a clinically significant reduction in post-operative pain scores. They determined that a reduction in pain of 1.3 units in the NRS was clinically significant for those with moderate pain (NRS=6). With severe pain (NRS=8) the minimum clinically significant reduction in pain was 1.8 units.[63]

6.13. Using ultrasound to determine the depth of injection and complications

The use of ultrasound (US) to determine and document the placement of both IM and SC injections is well described in the literature.[64,65] The US findings of complications of IM and SC injections are described for other drugs but only case reports of rare complications after BPG are described.[51,66,67] Confirmation of US-guided IM injection with MRI has demonstrated the reliability of US as a means of determining the depth of injection and avoidance of critical structures like blood vessels and nerves.[64] While the use of CT scan and MRI are more definitive in defining structures, their cost, associated risks, and utility at the bedside make them less applicable in the acute clinical setting.[68-70]

6.14. Potential risks and benefits of BPG to humans

6.14.1. Potential risks

AEs to Bicillin® L-A, are uncommon. Despite being the only current form of BPG licenced for use in Australia, there have been episodes of 'stockouts.[33,71] During these periods, a powdered form of BPG was used. A search of the Database of Adverse Event Notifications – from 1st January 1971 through to 18th February 2017 for all types of benzathine penicillin reveals only 22 case reports, 13 in which Bicillin® L-A was the only medication administered. There were no adverse reactions noted for the powdered BPG used during stockouts.[42] Prior to 1995, Bicillin® L-A was only available in Australia in the 2.4 MIU unit prefilled syringe. It required clinicians to either use half of the dose or decant the Bicillin into a secondary syringe prior to administration.[33] Since 1995, there have been 11 adverse reactions reported to the TGA, seven in which Bicillin®L-A was the only administered medication. Of the seven, one reported a cardiac arrest, hypotonia, and seizure. One reported injection site reaction with abscess, four reported injection site reaction, one reported gait disturbance, three reported pyrexia, one reported otitis media, and one reported erythema, pain, rash, and swelling. From the period September 1997 through to May 2014, a total of 20,641 prescriptions were filled for Bicillin® L-A, representing a total of 206,410 doses prescribed.[71] This corresponds to an almost zero absolute risk of reaction. BPG is a commonly used medication; the reported side effects/adverse drug reactions are mentioned in the product information sheet in Appendix 1.[72]

Intramuscular injection is a frequently-used route of parenteral drug administration. In IM injections, the skin is broken, SC tissue is traversed, and medication injected into the muscle. IM injections have been shown to have increased absorption into systemic circulation compared to SC injections.[73] The most common side effects are pain, redness, and localised swelling. There is a small risk of: infection, damage to muscle, nerve, skin necrosis, inadvertent vascular damage/injection, anaphylactic reaction, and death. Most of the serious complications involving tissue necrosis will resolve with conservative management; a small percentage of severe cases may require medical procedures e.g. fasciotomy, if complicated by compartment syndrome.[45,51] As with any drug, anaphylactic reactions can cause death in its most severe cases.

The risk of IV injection is present in SC injections, although the rates of inadvertent vascular damage are significantly lower than XX? owing to the reduced blood supply of SC tissue. SC granulomas are a common incidental finding on imaging; uncommonly, SC injections are thought to be responsible for the development of calcific granulomas and fat necrosis.[48] Kawai et al., 2014, reported on the rate of injection site reaction to depot SC injection of leuprorelin acetate. Over a 5-year period at Tokai University Hospital, they identified 13 out of 335 (3.88%) of patients had a reaction to the depot injection. Eleven cases of SC induration were reported, as well as one ulceration and one SC ulceration and mass. Additionally, they examined case reports from 1999 onwards and identified 37 cases of SC granuloma formation. They noted that almost half of the granulomas spontaneously resolved (15/37).[74]

6.14.2. Known / potential benefits

The participants will not benefit directly from this study and most risks are transient in nature (pain, redness). The outcomes of the study will benefit those required to have secondary prophylaxis for ARF and RHD. Good adherence to secondary prophylaxis with BPG is effective in reducing recurrence and progression of ARF to RHD.[23] Children often have significant pain associated with their injection, and the pain and fear of pain is frequently cited as a reason for poor adherence to the 4-weekly injections.[5,17,30] This cohort of individuals are the ones who will directly benefit from this study. Bicillin® L-A has a narrow spectrum of activity, which is beneficial for antimicrobial resistance. GAS has remained exquisitely sensitive to penicillin, with no documented isolates of Strep pyogenes resistant to penicillin identified.[75] It does, however, display resistance to other classes of antibiotics e.g. macrolides and lincomycin.[26] Rates of resistance to macrolide antibiotics vary according to local factors and can rapidly change.[25]

The people to benefit from this trial are those who also suffer from the other diseases treated by BPG: pharyngitis, cellulitis, syphilis yaws, bejel, and pinta. Of those to benefit the most are individuals who require repeated BPG injections, with an estimated 33.4 million people living with RHD worldwide and an annual 319,400 deaths due to RHD.[2] This alone is sufficient reason to undertake this study. Additionally, there are approximately 5.6 million new cases each year of syphilis and an estimate prevalence of 0.5% amongst the 15-49 year age group, giving a worldwide prevalence of more than 18 million.[15] Approximately 162 million individuals are estimated to be suffering from impetigo at any one time.[14] There are an estimated 616 million cases of GAS pharyngitis per year.[19] BPG is currently listed as an essential medication by the WHO; given its widespread use, any individual who needs treatment may benefit from an improved delivery method. Whilst the Bicillin® L-A is used in high resource countries owing to the cost and cold chain dependence, the results of this study will provide valuable information for future planned BPG reformulation and RHD control programs in other countries.

Table 2: Estimated number of potential beneficiaries of this BPG study

Disease	Estimated Burden
ARF/RHD	33.4 million
Pharyngitis	616 million/yr
Impetigo	162 million cases
Syphilis	18 million

This study will determine if Bicillin® L-A, when given at an equivalent dose, is tolerated via SC injection. This information will also provide further background knowledge regarding potential future reformulation of BPG.

6.15. Name and description of the intervention or product(s) used in this trial

The investigational medical product (IMP) for this trial is Bicillin® L-A 1016.6mg (1.2 MIU) in 2.3ml. This product is registered with the Australian Therapeutics Goods Administration (TGA AUST R 147169).

7. TRIAL DESIGN

7.1. Description

This is a phase 1, investigator-initiated singled-blinded, longitudinal two by two crossover clinical trial to assess the pharmacokinetics of Bicillin® L-A administered to healthy adult volunteers. Participants will be recruited using the Linear Clinical Research volunteer database. They will undergo screening to ensure that they fit the inclusion criteria detailed in section 8. Baseline physical measurements and blood sampling will occur as outlined in section 9. Randomisation and blinding will occur once the participant has satisfied the inclusion criteria as outlined in section 10.

Participants will receive two injections in total. It will be a randomized two by two longitudinal crossover trial with a washout period of 10 weeks between administration periods. The number of participants in the study will be 15.

Participants will be randomised to either receive:

(a) Group A: IM injection → washout → SC injection, OR

(b) Group B: SC injection → washout → IM injection

Table 3: Representation of the study periods

	Duration (weeks)	Group A	Group B
Administration Period 1	6	IM	SC
Washout period	4	Washout	Washout
Administration Period 2	6	SC	IM
Follow up (telephone)	2	Follow up	Follow up

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7.2. Hypotheses

Primary hypothesis:

1. Administering Bicillin® L-A via the SC route will result in a clinically significant reduction in the rate of absorption (Ka) compared with IM absorption. This will result in clinically relevant prolongation of the duration that penicillin concentrations remain above the minimum inhibitory concentration (0.02mg/ml) for *Streptococcus pyogenes*.

Secondary hypothesis:

1. Administering Bicillin® L-A via the SC route will result in improved or comparable tolerability compared to the current recommended IM route.

7.3. Primary endpoint

(a) Determination of the Ka for Bicillin® L-A administered via the SC route.

7.4. Secondary endpoints

(a) Pain scores related to administration of Bicillin® L-A for the SC and IM routes.
(b) Defined AEs related to SC and IM routes of administration of Bicillin® L-A.
(c) Time above the MIC for Bicillin® L-A administered via the SC and IM routes.

7.5. Study stopping rules

Individual subjects will be stopped from continuing in the trial if they:

(a) display signs or symptoms of a penicillin allergy.
(b) have a serious adverse reaction.
(c) request discontinuation.

An individual subject who wishes to stop the trial after first dosing will be withdrawn from the study and followed up as per the withdrawal section 8.5.

The trial can be stopped at any stage at the discretion of:

(a) the Principal Investigator (PI)
(b) the study sponsor
(c) suspected unexpected serious adverse reaction (SUSAR), after discussion with the ethics committee

8. SELECTION AND WITHDRAWAL OF PARTICIPANTS

8.1. Study population

Participants will meet **all** the inclusion criteria and **none** of the exclusion criteria.

8.2. Study numbers

15 participants are required to perform this study.

8.3. Study inclusion criteria

Participants who meet **all** the inclusion criteria are eligible to be a participant in the trial:

(a) Male aged 18 - 65 years at the time of screening.

(b) BMI between 18.5kg/m^2 and 25kg/m^2.
(c) No history of chronic renal impairment or significant liver dysfunction.
(d) No prior documented allergy to penicillin, cephalosporin antibiotics.
(e) Participants who are considered likely to adhere to the trial guidelines for the duration of the trial.
(f) Sign and dated informed consent in accordance with Good Clinical Practice (GCP) / Declaration of Helsinki (DoH) (Appendix 5).

8.4. Study exclusion criteria

Patients who meet **any** of these criteria are not eligible for participation in the trial:

(a) Currently taking penicillins or **Use of any penicillin-based antibiotics from screening through to the final study visit. The use of probenecid, NSAIDs, or other medications which may significantly alter the Bicillin® L-A PK will also not be permitted within 14 days prior to study drug administration until completion of the final follow-up visit, with the exception of occasional paracetamol and ibuprofen use.**).
(b) Participation in another clinical trial in the three months preceding the trial.
(c) Planned participation in another clinical trial concurrently.
(d) Planned operation/absence from the study site during the duration of the study.
(e) Known penicillin allergy or soy allergy.
(f) History of any clinically significant hip/gluteal surgery/radiotherapy.
(g) Use of any **prescription medication or over-the-counter medication, herbal products, vitamins or minerals, within 7 days prior to study drug administration until completion of the final follow-up visit, unless in the opinion of the Principal Investigator or delegate the medication will not compromise participant safety or interfere with study procedures or data validity**
(h) **Participants must be non-smokers and must not have used any tobacco products within 1 month prior to screening**

8.5. Participant withdrawal

8.5.1. Process of withdrawal

Participants will be able to withdraw from the study at any stage without consequence. In the event of a participant withdrawal, they will be treated without prejudice; reasons for withdrawal will be recorded if volunteered. They will not need to have any specific treatment in the event of withdrawal. i.e., no antidote medication.

8.5.2. Data to be collected for withdrawn participant(s)

Participants who withdraw from the trial will have all data up to point of withdrawal included in the trial results.

8.5.3. Replacement of participants

Withdrawal of a participant will not result in replacement recruitment unless the withdrawal occurs before the first injection because of the cross-over methodology of the trial.

8.5.4. The follow-up of withdrawn participants

The withdrawn participant will still be able to receive medical care if related to the investigational product as per the GCP guidelines. If they have received a dose of Bicillin® L-A, they will be monitored for a minimum of 30 days.

9. STUDY PROCEDURES

9.1. Description of assessments and procedures

9.1.1. Informed consent

Signed written consent must be obtained from the participant in accordance with GCP guidelines; informed consent is an ongoing process throughout the trial and may be withdrawn freely at any time by the participant without reason.

9.1.2. Eligibility criteria

Participants must meet all inclusion criteria and no exclusion criteria to be eligible to participate.

9.1.3. Medical history / examination

During screening, participants will have history examination performed which will include, but is not limited to:

(a) History of renal disease.
(b) History of liver disease.
(c) History within the last 12 months of gluteal intramuscular or subcutaneous injection or operation.
(d) Adverse reaction to injections / phlebotomy.
(e) Known allergies.
(f) Screening physical exam, including vital signs, height, weight, and auscultation.

9.1.4. Laboratory tests

Baseline blood testing will occur during screening and on injection days via means of phlebotomy. The following standard testing will be performed:

(a) Haematology: complete blood count.
(b) Clinical chemistry: urea, glucose, creatinine, sodium, potassium, chloride and bicarbonate, lactate dehydrogenase, calcium, total protein, magnesium, phosphate, albumin, cholesterol,

and uric acid [eGFR >90ml/min/m2 will be considered normal using the CKD-EPI and the absence of albuminuria on dipstick].

(c) Liver function tests (only at screening): aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, gamma-glutamyl transferase [<1.5 x ULN (ALT, GGT) will be considered normal].

(d) Serology (only at screening): HIV, Hepatitis B and C.

Urine dipstick for blood, protein, leukocytes, nitrite, and drug screening along with breath alcohol testing will be conducted at screening and on injection days.

Participants who undergo screening, who have a significant abnormality detected, will be referred to their general practitioner for further investigation and management as deemed appropriate by the PI or delegate.

9.1.5. Dried blood spot assays to measure penicillin

Dried blood spots (DBS) have been used for decades in clinical application, the most common being the Guthrie test. Over the last decade, refinement of the DBS technology has allowed the expansion of the gamut of testing possible with the DBS. Hepatitis B, HIV, and malaria parasites are all able to be detected with DBS in addition to therapeutic drug monitoring. The DBS has several advantages. The blood required is minimal: each standard sample is only 10-20µL instead of the usual 3000-10000µL (3-10ml) required for traditional venesection in adults. The complications from venepuncture include inadvertent arterial puncture, haematoma, thrombophlebitis, and bruising. DBS, sampled from the fingers, are not associated with these complications. Development of the DBS system has allowed for more ethical PK studies in children. This method has recently been validated for penicillin, ceftriaxone, and ertapenem. DBS samples can be taken serially and impregnated onto filter paper which is stored with desiccant without the need for processing of plasma and cell pellets. A small diameter disc can be subsequently punched out from the filter paper and the drug eluted into a liquid matrix prior to liquid chromatography-mass spectroscopy (LC/MS).[76-78]

9.1.6. Pain scoring

Pain scoring will be measured using the numeric rating scale (NRS), which is a valid method for recording pain in this setting.[79] . The NRS has been validated in adults for recording pain; the scoring system has 11 (0-10) different values anchored by terms describing pain severity extremes: 0=none, 1-3=mild, 4-6=moderate, and 7-10=severe. A score of 10 indicates the worst pain imaginable. The NRS is a widely accepted tool for the quantitative assessment of pain, and has the ability to detect significant changes in pain and is comparative to other methods of pain reporting such as the visual analogue scale, whilst having the advantage of being easier to record, with less chance of error.[79] As mentioned in section 6.13, there is variability in the definition of 'significant reduction' in pain. With a moderate pain score of NRS=4, a change of 1.3 units are required to be

'minimally clinically significant'.[63] Additionally, participants will describe the character of the pain using the phrases "throbbing", "dull", or "sharp". They will be asked if the pain prevents them from undertaking any desired activities. E.g. walking. Pain will be assessed at pre-determined intervals (as described earlier) and if present, continue to be assessed at each follow-up visit until pain levels return to zero. It will be noted if additional medication is taken e.g. paracetamol or ibuprofen.[80]

9.1.7. Monitoring of skin irritation

The participant will be questioned regarding symptoms of pain, character, itchiness, pallor or erythema bleeding, and heat. Participants will be inspected by a study team member for evidence of skin erythema at day 1 and 2, with continued visual inspection if there was presence of pain or irritation as reported by the participant (days 4, 7, 14, 21, 28) until resolution. Area of irritation or erythema if present will be measured in length and width.

9.1.8. Contraception

It is recommended that male participants with a female partner of child-bearing potential, use a condom for all sexual intercourse for the entire duration of the study until completion of all follow-up visit

9.1.9. Sampling schedule

It is expected the study will occur over an 18-week period. There will be a maximum of 26 DBS samples taken from each participant through the study period. Additionally, baseline blood tests will be performed in the screening period. A serum sample will be taken at 12 hours and at 14 days post-injection to compare penicillin levels to DBS. Participants will be recruited in month 1; after consent and screening tests, they will then be randomised to receive IM or SC Bicillin® L-A in month 1 as per the schedule (a-e):

(a) Enrolment and screening (2 weeks).

Potential participants will be screened according to the clinical criteria as described in section 6.5. They will then undergo the consent process, the required screening including history, and examination and screening blood tests as outlined in section 9.1.4-9.1.5. If any significant abnormalities are found on screening, they will be referred as clinically appropriate for further testing via their general practitioner and exit the study. Those who fulfil all inclusion and no exclusion criteria will then be blinded and randomised to Group A or Group B (see Table 3).

(b) Administration period 1 (6 weeks).

Participants will be randomised to either the SC or IM administration of Bicillin® L-A in the upper outer quadrant of buttock at day zero. The IMP (Bicillin® L-A) will be prepared as per the manufacturer's instruction in the product information sheet. Injection will occur with a 20G needle of appropriate length. The route of administration will be confirmed via real-time ultrasound imaging; the procedure is outlined in section 10.6. DBS samples will be taken at: baseline, then t=2,

6, 12, and 24 hours, followed by day 2, 3, 5, 7, 14, 21, 28, and 42. There is some flexibility in sampling times as demonstrated in Table 4 below. Pain scoring will be taken routinely at: baseline, t=2, 6, 12, 24, 48, and 72 hours, then at day 5, 7, 14, 21, 28, and 42 if injection-associated pain is recorded at the prior visit. Venesection will be conducted at baseline, 12 hours after the dose of BPG and at day 14 for baseline biochemical and haematological profile and to demonstrate concordance between plasma and DBS penicillin concentrations.

(c) Washout period (4 weeks).

Participants will undergo a washout period of at least 28 days after administration period 1.

(d) Administration period 2 (6 weeks).

After the washout period, participants will receive alternate administration of IM or SC Bicillin® L-A, dependent on the route of administration in period 1. The IMP (Bicillin® L-A) will be prepared as per the manufacturer's instruction in the product information sheet. Injection will occur with the 20G needle supplied as part of the Bicillin® L-A product. The route of administration will be confirmed via real-time ultrasound imaging. The procedure is outlined in section 10.6. DBS samples will be taken at: baseline, t=2, 6, 12, and 24 hours, followed by day 2, 3, 5, 7, 14, 21, 28, and 42. There is some flexibility in sampling times as demonstrated in Table 4 below. Pain scoring will be taken routinely at: baseline, t=2, 6, 12, 24, 48, and 72 hours, then at day 5, 7, 14, 21, 28, and 42 if injection-associated pain is recorded at the prior visit. Venesection will be conducted on days 0 and 14 to verify the DBS levels.

(e) Post-sampling telephone follow-up (2 weeks).

Participants will be followed up after a 14-day period by telephone to confirm no persisting AEs; if present, they will be formally reviewed by a study team member.

Table 4: Sampling schedule for both administration periods 1 & 2

T: hrs (days)	0	2	6	12	24 (1)	48 (2)	72 (3)	120 (5)	168 (7)	336 (14)	504 (21)	672 (28)	1008 (42)
DBS	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Sampling window‡	-	±5 min	± 15 mins	± 30 mins	± 4 hours	± 8 hours	± 12 hours	± 1 day	± 1 day	± 2 days	± 2 days	± 2 days	± 2 days
Pain score	Y	Y	Y	Y	Y	Y	Y	Y*	Y*	Y*	Y*	Y*	Y*
Venesection	-	-	-	Y	-	-	-	-	-	Y	-	-	-
USS	Y	-	-	-	-	-	-	-	-	-	-	Y	-

DBS = dried blood spot; Y = yes; * = possible measurement; USS = ultrasound scan of injection site (see appendix 8) ; ‡ = time in minutes/hours/days following Bicillin® L-A injection.

A study sampling schedule from screening through to follow up phone call is included as Appendix 7 for ease of reference.

9.2. Not / permitted medications during the study

Administration of penicillin-based antibiotics will not be permitted during the study period as this may interfere with the results of assays. The use of probenecid, NSAIDs, or other medications which may significantly alter the Bicillin® L-A PK will also not be permitted during the study period. Participants will be asked to avoid all prescription and over-the-counter medications and supplements for at least seven days prior to commencement and during the study. Probenecid and NSAIDs other than paracetamol and ibuprofen should be avoided for 14 days prior and during the study. The following rescue medication will be permitted:

(a) Loratadine, 10mg, non-sedating antihistamine, for the treatment of itch.
(b) Prednisolone, 1mg/kg, steroid, for treatment of rash.
(c) Adrenaline 0.3mg/0.3ml auto-injector IM will be available at time of administration in the event of anaphylactic reaction.

10. Study Treatment

10.1. Randomisation and blinding

(a) Randomisation of participants to first injection of either SC or IM injection will occur post-enrolment. Randomisation will be by means of a random number generator.
(b) Blinding will occur in a single blinded fashion as it is not possible to blind the clinician who will administer the injection. Both injections will utilise the outer upper quadrant of the gluteal area and participants will be blinded to whether injection is SC or IM.

10.2. Methods to reduce bias

Bias - results will not be processed until after all data collection is complete. The potential sources include the participant perception of pain associated with injection, and previous experience. The same clinician will perform all injections and will be instructed to follow the same procedure for each injection to minimise bias.

10.3. Maintenance of any blinding records or randomisation codes and procedures for breaking codes

Maintenance of randomisation codes will be in accordance with GCP; if a subject is admitted to hospital for management from a complication of the administration of trial product, the study coordinator will break blinding to allow for medical care to proceed unhindered.

10.4. IMP dosing, registration, formulation, packaging, labelling, and storage

10.4.1. Registration

Benzathine Penicillin G is marketed in Australia under the market name of Bicillin® L-A, and is registered by the TGA. Other forms of the drug exist as a combination medication but are not marketed in Australia. Bicillin® L-A comprises a single active compound: BPG. Attached is the product information sheet from Pfizer Australia in Appendix 1, which contains further information regarding the susceptible bacteria, storage, administration dosage, and over-dosage information.

There is no comparator product in this trial.

10.4.2. Formulation

BPG is comprised of a suspension of two benzylpenicillin molecules and a benzathine molecule forming a white crystalline powder that is very slightly soluble in water and sparingly soluble in alcohol. It is approved for deep IM injection. Bicillin® L-A refers to the resultant aqueous suspension which also contains a citrate buffer and, as weight/volume, approximately 0.5% lecithin, 0.6% carboxymethylcellulose, 0.6% povidone, 0.1% methylparaben, and 0.01% propylparaben. Bicillin® L-A is an opaque, viscous suspension. In Australia, it is available in 2.3ml corresponding to 1,200,000 units of penicillin G. Administration of Bicillin® L-A 1,200,000 units via deep IM injection results in prolonged serum concentrations of the active metabolite; penicillin G levels of 2.25ng/mL may still be detectable for four weeks.

See PIS for additional information.

10.4.3. Packaging, labelling and storage

The IMP (Bicillin® L-A) will be labelled in accordance with annex 13 of the "guide to good manufacturing practice for medicinal products annexes".[81] The IMP is registered by the therapeutic good administration of Australia. The IMP will be refrigerated at 2-8°C, and will not be frozen. The product information sheet from Pfizer Australia is attached (see Appendix 1), and contains further information regarding the susceptible bacteria, storage, administration dosage, and over-dosage information.

10.5. Accountability of IMP

The investigator is responsible for maintaining adequate records of the disposition of the received IMP, including date, quantity and serial/batch numbers, records of who received the IMP, and records of any IMP destroyed/discarded (intentionally/unintentionally). All records will be available for inspection by the monitor or sponsor if required.

10.6. Preparation, administration and dose

Bicillin® L-A will be prepared in accordance with the manufacturer's guidelines.

Intramuscular injection

1. On morning of injection, the Bicillin® L-A 1016.6mg or 1.2MIU (2.3ml) and supplied needle will be removed from the fridge at least 3 hours prior to injection time to allow suspension to reach room temperature. The syringe will later be further warmed by rolling the syringe between palms prior to injection.
2. The syringe will be placed in a clean field.
3. The participant will have consent checked and will be placed in prone position. Participants will nominate a buttock for the first administration period and asked to expose the buttock area. In second administration period, the opposite buttock will be used.
4. The injection will be undertaken in a clinical suite screened for privacy, with access to medical resuscitation equipment; adrenaline will be available. The injection will be performed by a suitably qualified investigator who has undertaken the online training module on BPG injection developed by Pfizer with RHDAustralia (https://www.rhdaustralia.org.au/administering-bicllin).
5. A screen will be set up to prevent participant observation of the US monitor and injection site. Landmarks will be palpated to estimate the dorso-gluteal injection site.
6. US will be used to confirm the injection site and determine the thickness of SC tissue. Distance from skin to at least 5mm into gluteal muscle will be measured.
7. Skin will be cleaned and prepped using alcohol swab.
8. Using sterile technique, the needle will be attached to the syringe.
9. Manual pressure will be applied over the site for 30 seconds prior to injection.
10. Formation of 'Z' tract. The needle will be inserted rapidly at an angle of 90° to skin surface with no pressure on the plunger. Confirmation of needle tip location in IM tissue with US and a safe distance from vasculature and nerves. Aspiration to prevent complication of IV injection: if aspiration of blood is noted, the needle will be withdrawn and removed, and the blood expelled from the chamber. The needle will be discarded, and a new needle attached. Step 6 will be repeated.
11. The Bicillin® L-A will be injected slowly in 8-10 small aliquots over 3 minutes, monitored with a timer.
12. The needle will be removed, and light pressure applied directly over the injection site with gauze.
13. The time of completion of the injection will be documented, and sampling using DBS and NRS post-injection carried out as scheduled using the end of injection time as the reference point.
14. The participant will be monitored for AEs in the clinical trial facility for 12 hours post-injection.
15. Participant pain scores will be recorded at the time intervals specified above.

16. The participant will be discharged and instructed to follow up at the appropriate times.

Subcutaneous injection

1. On morning of injection, the Bicillin® L-A 1016.6mg or 1.2MIU (2.3ml) and supplied needle will be removed from fridge at least 3 hours prior to injection time to allow suspension to reach room temperature. The syringe will later be further warmed by rolling the syringe between palms prior to injection.
2. The syringe will be placed in a clean field.
3. The participant will have consent checked and placed in prone position. Participants will nominate a buttock for the first administration period and asked to expose the buttock area. In second administration period, the opposite buttock will be used.
4. The injection will be undertaken in the clinical suite screened for privacy, with access to medical resuscitation equipment; adrenaline will be available. The injection will be performed by a suitably qualified investigator who has undertaken the online training module on BPG injection developed by Pfizer with RHDAustralia (https://www.rhdaustralia.org.au/administering-bicllin).
5. A screen will be set up to prevent participant observation of the US monitor and injection site. Landmarks will be palpated to estimate the dorso-gluteal injection site.
6. US will be used to confirm the injection site and determine the thickness of SC tissue. Distance from skin to the gluteal muscle will be measured.
7. Skin will be cleaned and prepped using alcohol swab.
8. Using sterile technique, the needle will be attached to the syringe.
9. Manual pressure will be applied over the site for 30 seconds prior to injection.
10. The needle will be inserted rapidly at an angle of 45° to skin surface with no pressure on the plunger. Confirmation of needle tip location in SC tissues with US and safe distance from vasculature and nerves. Aspiration to prevent complication of inadvertent IV injection: if aspiration of blood is noted, the needle will be withdrawn and removed, and the blood expelled from the chamber. The needle will be discarded, and a new needle attached. Step 6 will be repeated.
11. The Bicillin® L-A will be injected slowly 3 minutes monitored with timer.
12. The needle will be removed, and light pressure applied directly over the injection site with gauze.
13. The time of completion of the injection will be documented, and sampling using DBS and NRS post-injection carried out as scheduled using the end of injection time as the reference point.
14. The participant will be monitored for AEs in the clinical trial facility for 12 hours post-injection.
15. Participant pain scores will be recorded at the time intervals specified above.
16. The participant will be discharged and instructed to follow up at the appropriate times.

10.7. Treatment adherence

The IMP will be administered by a study investigator; no IMP will be required to be taken outside of the direct supervision of a study investigator.

11. Assessment of Safety

11.1. Definitions

Table 5: List of definitions for assessment of safety

Adverse Event (AE)	Any untoward medical occurrence in a patient or clinical trial participant administered a medicinal product and that does not necessarily have a causal relationship with this treatment. An AE can be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the study medication, whether or not considered related to the study medication.
Adverse Reaction (AR)	Any untoward and unintended response in a participant to an IMP related to any dose administered to that participant. Comment: All adverse events judged by either the reporting investigator or the sponsor as having a reasonable possibility of a causal relationship to an IMP would qualify as adverse reactions. The expression 'reasonable causal relationship' means to convey, in general, that there is evidence or argument to suggest a causal relationship.
Safety Critical Adverse Events	Adverse events and/or laboratory abnormalities identified in the protocol as critical to safety evaluations that should be reported to the sponsor according to the reporting requirements specified in the protocol.
Serious Adverse Event (SAE)/Serious Adverse Reaction (SAR)	Any adverse event/adverse reaction that: (a) results in death (b) is life-threatening (c) requires inpatient hospitalisation or prolongation of existing hospitalisation (d) results in persistent or significant disability/incapacity (e) is a congenital anomaly or birth defect Note: Life-threatening in the definition of a serious adverse event or serious adverse reaction refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe. Note: Medical and scientific judgement should be exercised in deciding whether an adverse event/reaction should be classified as serious in other situations. Important medical events that are not immediately life-threatening or do not result in death or hospitalisation, but may jeopardise the participant or may require intervention to prevent one of

	the other outcomes listed in the definition above should also be considered serious.
Significant Safety Issue (SSI)	A safety issue that could adversely affect the safety of the participants or materially impact on the continued ethical acceptability or conduct of the trial. e.g.: A SAE that could be associated with the trial procedures and that requires modification of the conduct of the trial. A hazard to the patient population, such as lack of efficacy of an IMP used for the treatment of a life-threatening disease. A major safety finding from a newly completed animal study (such as carcinogenicity). A temporary halt/termination of a trial for safety reasons. Recommendations of the safety monitor, where relevant for the safety of the participants, such as an increase in frequency or severity of an expected adverse reaction. Single case events (e.g. toxic epidermal necrolysis, agranulocytosis, hepatic failure) that lead to an urgent safety measure.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	An adverse reaction that is both serious and unexpected. A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out in reference safety information - summary of product characteristics relating to the trial in question.
Unexpected Adverse Reaction (UAR)	An adverse reaction, the nature or severity of which is not consistent with the Reference Safety Information (RSI). Note: The RSI should be contained in the Investigator's Brochure for an unapproved medicinal product or Product Information (or another country's equivalent of the Product Information) for an approved medicinal product.
Urgent Safety Measure (USM)	A measure required to be taken in order to eliminate an immediate hazard to a participant's health or safety. Note: This type of significant safety issue can be instigated by either the investigator or sponsor and can be implemented before seeking approval from Human Research Ethics Committees (HRECs) or institutions.

NB: To avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Seriousness" is the regulatory definition supplied above, and is based on patient/event outcome or action criteria usually associated with events that pose a threat to a participant's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

11.2. Procedures for recording adverse events

AEs will be reported using the following guidance:

(a) All AEs occurring after enrolment until study completion or withdrawal, that are observed by an investigator/member of the health care team, or that are reported by the participant, will be detailed in the Case Report Form (CRF), whether or not attributed to the study.

(b) Pre-existing medical conditions (present before start of the AE collection period) are considered "concurrent medical conditions" and should not be recorded as AEs. However, if the participant experiences a worsening or complication of such a condition, the worsening or complication should be recorded as an AE. Investigators should ensure that the AE term recorded captures the change in the condition (e.g., "worsening of")

(c) Each AE should be recorded to represent a single diagnosis. Accompanying signs or symptoms (including abnormal laboratory values) should NOT be recorded as additional AEs.

(d) Changes in laboratory values and vital signs are only considered to be AEs if they are judged to be clinically significant, e.g., if some action or intervention is required. If abnormal laboratory values or vital signs are the result of pathology for which there is an overall diagnosis, the diagnosis only should be reported as one AE.

(e) AEs considered related to the study as judged by a medically qualified investigator or the sponsor, will be followed either until resolution, or the event is considered stable.

11.3. Reporting procedures for Serious Adverse Events

All SAEs will be recorded from the point of enrolment until the 14-day follow-up period, outlined in trial timeline. Follow-up information will be provided as necessary. A member of the site research team will obtain information from the hospital or clinic to enable reporting to the sponsor and the HRECs. All SAEs will require a report to be prepared by a suitably qualified medical practitioner for the principal investigator. All SAEs must be reported on the SAE reporting form and emailed to the sponsor within one working day of the site study team becoming aware of the event. In addition, all SAEs must be reported to the approving HREC within 72 hours of the study team's awareness of the event. Additional and further requested information (follow-up or corrections to the original case) will be detailed on a new SAE Report Form and emailed to the sponsor. Once the SAE Notification Form is received by the sponsor, the PI (or delegate) will investigate the expectedness of the event and forward the report to the Independent Safety Monitor (ISM). All serious, related, and unexpected (SUSARs) SAEs will be reported to the TGA by the sponsor.

As per the 2016 updated NHMRC position statement on safety monitoring of clinical trials involving therapeutic goods, AEs, SAEs, external SUSARs, and SUSARs six-monthly line listings to the HREC will no longer be reported. Instead, the sponsor will provide an annual safety report to the HREC in lay language. SUSARs which occur on site and significant safety issues will still be reported to the institution (within 72 hours). A figure outlining the safety reporting flowchart is attached as Appendix 2.

11.4. Independent Safety Monitor

The ISM should receive full details of all SAEs. SAEs should be reported to the ISM within three days of the sponsor receiving notification of the event. A list of all safety events will be provided to the ISM at the end of each month of the study period; the ISM can request further investigation or interim analysis of any/all events, at his/her discretion. The ISM will discuss any safety concerns with the PI at any point throughout the study period.

11.5. Principal Investigator's responsibilities

The PI or delegate should:

(a) Capture and assess all AEs that occur at the site as required and in accordance with the protocol.

(b) Report to the sponsor within 24 hours of becoming aware of the event:
   a. all SAEs, except those that are identified in the protocol as not needing immediate reporting.
   b. any occurrences of congenital anomaly/birth defect arising from any pregnancy of a participant (or partner).
   c. all urgent safety measures instigated by the site.

(c) Report to the sponsor as specified in the protocol:
   a. all safety-critical events.
   b. any additional requested information relating to reported deaths.

(d) Report to the institution within 72 hours of becoming aware of the event:
   a. all significant safety issues.
   b. SUSARs arising from the local site (reported when, in the investigator's judgement, a SUSAR has occurred. The investigator should not unblind the SUSAR for the purposes of reporting to their institution).

11.6. Causality

The relationship of each adverse event to the trial medication must be determined by a medically qualified investigator according to the following definitions:

Related: The adverse event follows a reasonable temporal sequence from trial medication administration. It cannot reasonably be attributed to any other cause.

Probably related: The adverse event follows a reasonable temporal sequence from trial medication administration. Although one or more other causes can be reasonably attributed, it is most reasonably attributed to the study medication.

Possibly related: The adverse event follows a reasonable temporal sequence from trial medication administration, but it can be more reasonably attributed to another cause.

Not Related: The adverse event is probably produced by the participant's clinical state or by other modes of therapy administered to the participant.

11.7. Expectedness

Expectedness will be determined according to the product information sheet. Any SAE considered to be unexpected and related to the study drug will be reported to the TGA.

11.8. SUSAR reporting

All SUSARs will be reported to the TGA. For fatal and life-threatening SUSARS, this will be done no later than seven calendar days after the Sponsor or delegate is first aware of the reaction. Any additional relevant information will be reported within eight calendar days of the initial report. All other SUSARs will be reported within 15 calendar days. The PI or delegate will also inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants immediately.

11.9. Procedures for the treatment of immediate adverse reaction

The study investigator who is performing the injection of the participant must we acquainted with the safety procedures at the Linear clinical suites. They will hold the relevant qualification of senior first aid or hospital life support. The site will have appropriately qualified and trained personnel on site to respond to any AE during the 12-hour period of observation post-injection.

Any acute onset illness with typical skin features (urticarial rash or erythema/flushing, and/or angioedema), *plus* involvement of respiratory and/or cardiovascular and/or persistent severe gastrointestinal symptoms.
OR
Any acute onset of hypotension or bronchospasm or upper airway obstruction where anaphylaxis is considered possible, even if typical skin features are not present. For example:

- Difficult/noisy breathing
- Swelling of tongue
- Swelling/tightness in throat
- Difficulty talking and/or hoarse voice
- Wheeze or persistent cough
- Persistent dizziness or collapse

The Australasian Society of Clinical Immunology and Allergy (ASCIA) guidelines for acute management of anaphylaxis is attached as Appendix 6.

In the event of anaphylaxis, the following should occur:

1. Cease injecting Bicillin® L-A and removal of the needle.
2. Activation of the ‘medical emergency system’.

3. Administration of adrenaline 1:1000 - 0.5mg IM into lateral thigh.
4. Commencement of resuscitation as appropriate.
5. Transfer of participant to hospital as required by local facility policy.
6. Cessation of further injections until PI is contacted and situation is discussed.

12. Data management / statistical analysis / record keeping

12.1. Interim analysis

No interim analysis will be performed given the time and cost associated with penicillin DBS assay. Performing the assay for part of the samples will also increase the chance of systematic error.

12.2. Sample size and power calculation

Sample size was calculated based on a clinically significant and likely reduction in the primary outcome measure, namely the rate of absorption (Ka). This was set at around 40% for the following reasons: i) based on simulation data for children this would increase the median time above 0.02mg/L by around one week from 15 to 22 days and ii) animal studies comparing IM to SC dosing observed this difference (Ranheim et al[44]).

Power calculations were performed within NONMEM using a combination of local data in children and a published population PK model of IM benzylpenicillin in adults (Neely et al[82]) to perform Monte-Carlo mapped power implemented via Perl-Speaks-NONMEM. The simulations assumed a slower absorption with SC dosing using the crossover design and the planned sampling schedule. There was over 90% power with an alpha of 0.05 (significance level of 5%) to detect a difference of 40% with 11 participants. There was also around 80% power to detect a 30% difference with 14 participants.

To account for possible loss to follow-up and unpaired data, a target of 15 participants was set.

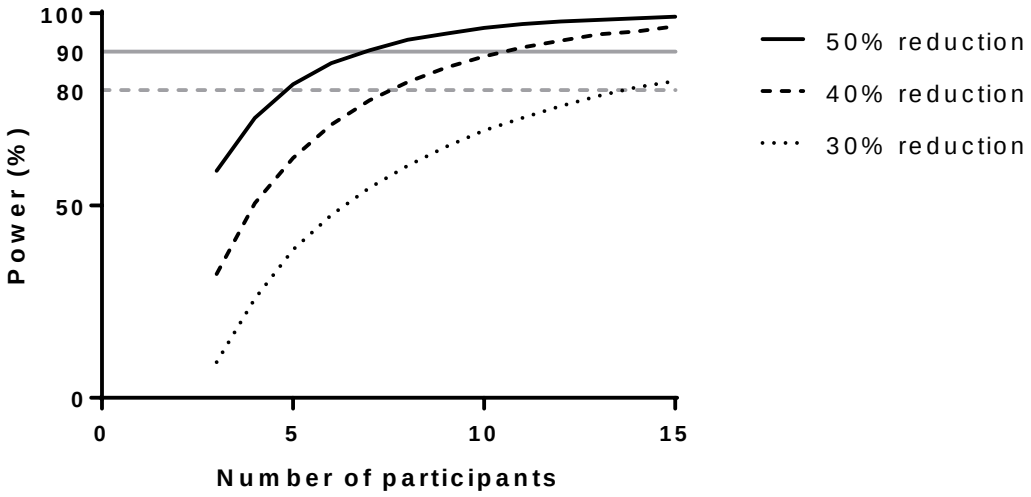

Figure 3: Power calculations for study recruitment

Rationale of sample size for pain assessment:
Using a t-test with matched pairs, discrimination of 2 points with a SD of 1.7 should be attainable. Studies have shown that a reduction of pain by 30% is considered clinically significant. Therefore, to determine a difference in pain between the two routes, we considered a 30% difference to also be significant. We assumed a correlation between groups of 0.3; hence, n=15 results in power to discriminate of 94.6%; n=10 will result in power of 79.8% and n=12, 87.9%.

12.3. Level of significance to be used

The significance level is 5% (0.05).

12.4. Procedures for reporting any deviation(s) from the original statistical plan

Any significant deviation from the PI-approved original Statistical Analysis Plan (SAP) will be discussed with the study statistician and pharmacokineticist, documented as appropriate, and reflected in any publications arising out of this study.

12.5. Selection of participants to be included in the analyses

All dosed participants who contribute at least one penicillin concentration value will be included in the summary statistics and PK analysis.

12.6. Information on how data will be managed, including coding for computer analysis and data handling

Details will be included regarding these processes if the data is sent off-site (e.g. encryption). Clinical trial records should be retained for a minimum of 15 years from the completion of the trial.

12.6.1. Access to data / data recording

All study data will be entered via paper and electronic CRFs. All data will be entered onto the password-protected electronic database. Data queries will be raised, and data cleaned by the data manager/study statistician. Direct access of data will be granted to authorised representatives from the sponsor, HREC, and funding bodies to permit trial-related monitoring, audits, and inspections.

12.6.2. Encryption

All data will be stored electronically with password protection. If data needs to be transported, an encrypted storage media will be used.

12.6.3. Archiving

All paper records will be scanned. Long term storage of the clinical trial data will be in accordance with GCP, for a minimum of 15 years in a password-protected folder. Data will be stored on the sponsor server and any physical collection sheets will be transferred and stored at the sponsor in a locked cupboard for the duration required to comply with GCP.

12.7. Procedure for accounting for missing, unused, and spurious (*false*) data

Reasons for missing data pertaining to the primary endpoint and safety endpoints (including withdrawal of consent, loss to follow-up, removal from study due to serious side effects, death, or inability to obtain any laboratory results) will be indicated. Statistical methods to account for sparse or missing data are available within the NONMEM software and will be documented in the SAP.

13. QUALITY CONTROL AND QUALITY ASSURANCE

13.1. Statement

The study will be conducted according to GCP, the DoH, the NHMRC criteria for the ethical conduct of research in humans, and the NHMRC criteria for research in Indigenous Australians. The study will be submitted to the Bellberry ethics committee for relevant approval. The study investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996 (REF).

This study will be notified on the Clinical Trials Notification scheme by the sponsor and registered on the Australian New Zealand Clinical Trials Registry (ANZCTR).

14. MONITORING/AUDIT

14.1. Quality assurance audit / inspection statement

The study may be subject to an audit at the request of the HREC. In the event of an audit, all relevant documentation must be made available to the auditor(s).

14.2. Procedures for monitoring and auditing

All investigators will permit study-related monitoring and audits, providing direct access to source data/documents. We also acknowledge this may include review by external sponsors, HRECs, and institutional governance review bodies.

15. ETHICS

15.1. Statement of compliance

The clinical trial will be conduction according to TGA, DOH, GCP, and the NHMRC criteria for the ethical conduct of research in humans. This trial will be submitted to Bellberry HREC for approvals. The study protocol, information statements, consent forms, advertising materials and any other documents required for ethics approval will be submitted to the relevant HRECs for approval before the study commences. Participant information sheets and explanatory material are attached in Appendix 4. Informed consent will be obtained from all individuals in their recruitment stage according the principles outlined in the DOH, GCP, and the TGA. Written and verbal versions of the Participant Information Sheet and Informed Consent will be presented to the participants, detailing no less than: the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol; the known side effects; and any risks involved in taking part. It will be clearly stated that the participant does not have to participate in the study and is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

15.2. Informed consent form

The informed consent discussion and written materials (including pictorial information) must address explanations of the following:

1. That the study involves research;
2. The purpose of the study;
3. The study procedures to be followed, including all invasive procedures;
4. The participant's responsibilities;
5. Those aspects of the study that are experimental;
6. The reasonably foreseeable risks and inconveniences to the subjects;
7. The reasonably foreseeable benefits to the participant;
8. The compensation and/or treatment available in the event of study-related injury;
9. The anticipated prorated payment, if any, for participation in the study;
10. The anticipated expenses, if any, for participating in the study;
11. That participation in the study is voluntary and that participants may refuse participation and withdraw from the study at any time, without penalty or loss;
12. That various authorities and personnel, such as study monitors and ethics committees, will be granted access to the subject's study records and original medical records for the purpose of verification of study procedures or data;
13. That study records will be kept confidential and not made publicly available (unless required by law to do so);
14. That the participant will be informed in a timely manner if any new information becomes available that may affect their willingness to continue in the study;

15. The person(s) to contact for further information regarding the study, the rights of study subjects, and who to contact in the event of study-related injury;
16. The foreseeable circumstances and/or reasons under which a subject's participation in the study may be terminated;
17. The expected duration of a subject's participation in the study; and
18. The approximate number of subjects involved in the study.

15.3. Participant confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials, date of birth, and participant ID number on the CRF. All records will use these three identifiers in the electronic database. A password-protected document will contain the information relating to name, emergency contact, and study number in case of adverse reaction to the IMP. All documents will be stored securely and only accessible by study staff and authorised personnel.

16. ADMINISTRATION

16.1. Participant reimbursement

Participants will be compensated for their participation to cover the costs of travel, parking, and other inconveniences. The value of the reimbursement will be dependent on the participant involvement.

17. FINANCE AND INSURANCE

17.1. Budget

Financing for the trial will be provided by the End RHD Centre of Research Excellence (END RHD CRE) and Telethon Kids Institute. Additional funds are currently being sought from the Wesfarmers Centre of Vaccines and Infectious Diseases. The study is fully funded.

17.2. Insurance

Insurance for the clinical trial will be provided by the sponsor, Telethon Kids Institute. Telethon Kids Institute holds adequate insurance.

17.3. Indemnity

Negligent Harm: Indemnity and/or compensation for negligent harm arising specifically from an accidental injury for which the Telethon Kids Institute is legally liable as the sponsor will be covered by the Telethon Kids Institute.

Non-Negligent Harm: Indemnity and/or compensation for harm arising specifically from an accidental injury, and occurring because of the research subjects' participation in the study for which the Telethon Kids Institute is the Research Sponsor will be covered by the Telethon Kids Institute.

18. PUBLICATION POLICY

This study will be published in peer-reviewed journals. The PI will co-ordinate dissemination of data from this study. All publications (e.g., manuscripts, abstracts, oral/slide presentations, book chapters) based on this study will be provided by the PI for review by each study investigator prior to submission. Authorship will be determined in line with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals published by the International Committee of Medical Journal Editors.

In summary, authorship will be limited to those who have:

1. Contributed substantially to the conception and design of the study; or the acquisition, analysis or interpretation of data for the work; AND
2. Drafted the work or revised it critically for important intellectual content; AND
3. Provided final approval of the version to be published; AND
4. Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
5. Acquisition of funding or general supervision of the research group alone does not constitute authorship. Although authorship will be inclusive and offered to all people who significantly participate in the study, the final decision on authorship of any publication will be the responsibility of the Principal Investigator.

In accordance with the DoH, the clinical trial will be registered with the ANZCTR prior to commencement.

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20. Appendices

20.1. APPENDIX 1: Product information: Bicillin® L-A

PRODUCT INFORMATION

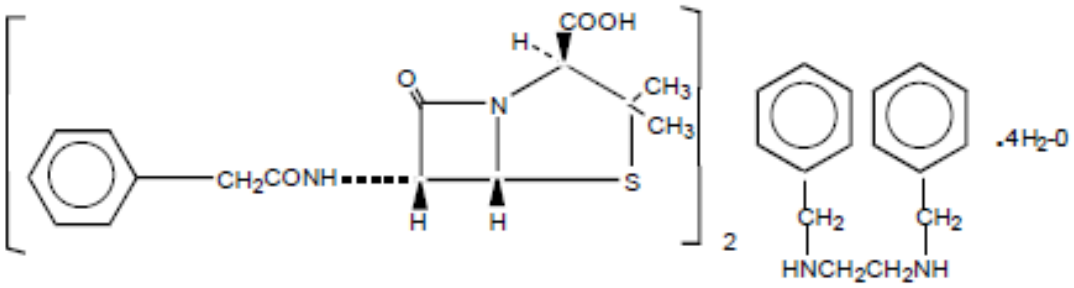
BICILLIN® L-A Suspension for Injection
(Benzathine Benzylpenicillin tetrahydrate)
for deep IM injection only

NAME OF THE MEDICINE

BICILLIN L-A 1,200,000 Units/ 2.3 mL pre-filled syringe with needle, containing Benzathine Benzylpenicillin tetrahydrate 1016.6 mg / 2.3 mL.

BICILLIN L-A (sterile benzathine benzylpenicillin suspension) is chemically designated as (2S,5R,6R)-3,3-dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid compound with *N,N'*-dibenzylethylenediamine (2:1), tetrahydrate.

Its chemical structure is as follows:



Chemical Formula: $(C_{16}H_{18}N_2O_4S)_2 \cdot C_{16}H_{20}N_2 \cdot 4H_2O$

Molecular Weight: 981.18

CAS Number: 41372-02-5

DESCRIPTION

Benzathine benzylpenicillin occurs as a white or almost white powder. It is very slightly soluble in water, freely soluble in dimethylformamide and in formamide, slightly soluble in ethanol (96 per cent).

BICILLIN L-A contains benzathine benzylpenicillin (the benzathine salt of benzylpenicillin) in aqueous suspension with sodium citrate buffer and water for injection; and as w/v, approximately 0.5% lecithin, 0.5% carmellose sodium, 0.6% povidone, 0.1% methyl hydroxybenzoate and 0.01% propyl hydroxybenzoate.

BICILLIN L-A suspension in the disposable pre-filled syringe formulation is viscous and opaque.

PHARMACOLOGY

Microbiology

Benzylpenicillin exerts a bactericidal action against penicillin-sensitive micro-organisms during the stage of active multiplication. It acts through the inhibition of biosynthesis of cell-wall peptidoglycan, rendering the cell wall osmotically unstable. It is not active against the penicillinase-producing bacteria or against organisms resistant to beta-lactams because of alterations in the penicillin-binding proteins.

The following *in-vitro* data are available but the clinical significance is unknown. Benzylpenicillin exerts high *in vitro* activity against Staphylococci (except penicillinase-producing strains), Streptococci (Groups A, C, G, H, L and M) and Pneumococci. Other organisms sensitive to benzylpenicillin are: *Neisseria gonorrhoea*, *Corynebacterium diphtheria*, *Bacillus anthracis*, Clostridia spp, *Actinomyces bovis*, *Streptobacillus moniliformis*, *Listeria monocytogenes* and Leptospira spp. *Treponema pallidum* is extremely sensitive to the bactericidal action of benzylpenicillin.

Pharmacokinetics

Absorption

Intramuscular benzathine benzylpenicillin is absorbed very slowly into the bloodstream from the intramuscular site and converted by hydrolysis to benzylpenicillin. This combination of hydrolysis and slow absorption results in blood serum levels much lower but much more prolonged than other parenteral penicillins.

Intramuscular administration of 225 mg of benzathine benzylpenicillin in adults results in blood levels of 22.5 to 37.5 nanogram per mL, which are maintained for 4 to 5 days. Similar blood levels may persist for 10 days following administration of 450 mg and for 14 days following administration of 900 mg. Blood concentrations of 2.25 nanogram per mL may still be detectable 4 weeks following administration of 900 mg.

Distribution

Approximately 60% of benzylpenicillin is bound to serum protein. The drug is distributed throughout the body tissues in widely varying amounts. Highest levels are found in the kidneys with lesser amounts in the liver, skin and intestines. Benzylpenicillin penetrates into all other tissues and the spinal fluid to a lesser degree.

Excretion

With normal kidney function, the drug is excreted rapidly by tubular excretion.

In neonates and young infants and in individuals with impaired kidney function, excretion is considerably delayed.

INDICATIONS

Intramuscular benzathine benzylpenicillin is indicated in the treatment of infections due to penicillin-sensitive micro-organisms that are susceptible to the low and very prolonged serum levels common to this particular dosage form. Therapy should be guided by bacteriological studies (including sensitivity tests) and by clinical response.

The following infections will usually respond to adequate dosage of intramuscular benzathine benzylpenicillin:

Streptococcal infections (Group A - without bacteraemia). Mild-to-moderate infections of the upper respiratory tract (eg., pharyngitis).

Venereal infections - Syphilis, yaws, bejel and pinta.

Medical conditions in which benzathine benzylpenicillin therapy is indicated as prophylaxis:

Rheumatic fever and/or chorea - Prophylaxis with benzathine benzylpenicillin has proven effective in preventing recurrence of these conditions. It has also been used as follow-up prophylactic therapy for rheumatic heart disease and acute glomerulonephritis.

CONTRAINDICATIONS

Previous hypersensitivity reaction to any of the penicillins.

PRECAUTIONS

Allergic reactions

Serious and occasionally fatal hypersensitivity (anaphylactoid) reactions have been reported in patients on penicillin therapy. Although anaphylaxis is more frequent following parenteral therapy, it has occurred in patients on oral penicillins. These reactions are more apt to occur in individuals with a history of sensitivity to multiple allergens. There have been well-documented reports of individuals with a history of penicillin hypersensitivity reactions who have experienced severe hypersensitivity reactions when treated with a cephalosporin. Before therapy with a penicillin, careful inquiry should be made concerning previous hypersensitivity reactions to penicillins, cephalosporins and other allergens. If an allergic reaction occurs, the drug should be discontinued and the patient treated with the usual agents, e.g., pressor amines, antihistamines and corticosteroids. Severe anaphylactoid reactions require emergency treatment with adrenaline. Oxygen and intravenous corticosteroids and airway management, including intubation, should also be administered as indicated.

Penicillin should be used with caution in individuals with histories of significant allergies and/or asthma.

Whenever allergic reactions occur, penicillin should be withdrawn unless, in the opinion of the physician, the condition being treated is life-threatening and amenable only to penicillin therapy.

Administration precautions

Do not inject intravenously or admix with other intravenous solutions. There have been reports of inadvertent intravenous administration of benzathine which has been associated with cardiorespiratory arrest and death.

Inadvertent intravascular administration, including inadvertent direct intra-arterial injection or injection immediately adjacent to arteries, of BICILLIN L-A and other penicillin preparations has resulted in severe neurovascular damage, including transverse myelitis with permanent paralysis, gangrene requiring amputation of digits and more proximal portions of extremities, and necrosis and sloughing at and surrounding the injection site. Such severe effects have been reported following injections into the buttock, thigh and deltoid areas. Other serious complications of suspected intravascular administration which have been reported include immediate pallor, mottling or cyanosis of the extremity, both distal and proximal to the injection site, followed by bleb formation; severe oedema requiring anterior and/or posterior compartment fasciotomy in the lower extremity.

Severe effects and complications following accidental intravascular administration have most often occurred in infants and small children. Prompt consultation with an appropriate specialist is indicated if any evidence of compromise of the blood supply occurs at, proximal to, or distal to the site of injection.

Do not inject into or near a nerve. Injection into or near a nerve may result in permanent neurological damage.

Quadriceps femoris fibrosis and atrophy have been reported following repeated intramuscular injections of penicillin preparations into the anterolateral thigh.

Antibiotic-associated pseudomembranous colitis

Antibiotic-associated pseudomembranous colitis has been reported with many antibiotics including penicillin. Treatment with antibacterial agents alters the normal flora of the colon leading to overgrowth of *C. difficile*.

C. difficile produces toxins A and B which contribute to the development of *Clostridium difficile* associated diarrhoea (CDAD). Hypertoxin producing strains of *C. difficile* cause increased morbidity and mortality, as these infections can be refractory to antimicrobial therapy and may require colectomy. CDAD must be considered in all patients who present with diarrhoea following antibacterial use. Careful medical history is necessary since CDAD has been reported to occur over two months after the administration of antibacterial agents.

The severity of the colitis may range from mild to life-threatening. It is important to consider this diagnosis in patients who develop diarrhoea or colitis in association with antibiotic use (this may occur up to several weeks after cessation of antibiotic therapy). Mild cases usually respond to drug discontinuation alone. However, in moderate to severe cases, appropriate therapy with a suitable oral antibacterial agent effective against *C. difficile* should be

considered. Fluids, electrolytes and protein replacement should be provided when indicated. Drugs which delay peristalsis, e.g. opiates and diphenoxylate with atropine (Lomotil) may prolong and/or worsen the condition and should not be used.

Non-susceptible organisms and superinfections

Prolonged use of antibiotics may promote the overgrowth of non-susceptible organisms, including fungi. Should superinfection occur, appropriate measures should be taken.

Streptococcal infections

In streptococcal infections, therapy must be sufficient to eliminate the organism otherwise the sequelae of streptococcal disease may occur. Cultures should be taken following completion of treatment to determine whether streptococci have been eradicated.

Blood and kidney function tests

In prolonged therapy with penicillin and particularly with high-dosage schedules, periodic evaluation of the renal and haematopoietic systems is recommended.

Fluids, electrolytes and protein replacement therapy should be provided when indicated.

Use in pregnancy

Category A - Drugs which have been taken by a large number of pregnant women and women of child-bearing age without any proven increase in the frequency of malformations or other direct or indirect harmful effects on the fetus having been observed.

Although generally considered to be safe, BICILLIN L-A should be used during pregnancy only if clearly needed.

Use in lactation

Soluble penicillin is excreted in breast milk. The effect on the infant, if any, is not known. Caution should be used when BICILLIN L-A is administered to a nursing woman.

Pediatric use

(See INDICATIONS and DOSAGE AND ADMINISTRATION sections.)

Use in the elderly

BICILLIN L-A is known to be mainly excreted by the kidney, the risk of toxic reactions to this drug may be greater in patients with impaired renal function (see PHARMACOLOGY section). It may be useful to monitor renal function in elderly patients.

Effects on laboratory tests

Penicillins can interfere with the copper sulphate reagent method of testing for glycosuria, resulting in falsely elevated or falsely decreased readings. Such interference does not occur with the glucose oxidase method.

INTERACTION WITH OTHER MEDICINES

Tetracyclines may antagonise the bactericidal effect of penicillin and concurrent use of these drugs should be avoided.

The rate of excretion of the penicillins is decreased by concomitant administration of probenecid which prolongs, as well as increases, blood levels of the penicillins.

ADVERSE EFFECTS

As with other penicillins, untoward reactions of the sensitivity phenomena are likely to occur, particularly in individuals who have previously demonstrated hypersensitivity to penicillins or in those with a history of allergy, asthma, hay fever, or urticaria.

The following adverse reactions have been reported:

General: Hypersensitivity reactions including the following: skin eruptions (maculopapular to exfoliative dermatitis), urticaria, laryngeal oedema, fever, eosinophilia; other serum sickness-like reactions (including chills, fever, oedema, arthralgia and prostration), and anaphylactic/anaphylactoid reaction (including shock and death).

Fever and eosinophilia may frequently be the only reaction observed.

Gastrointestinal: Pseudomembranous colitis. Onset of pseudomembranous colitis symptoms may occur during or after antibacterial treatment (see PRECAUTIONS section).

Haematologic: Haemolytic anaemia, leucopenia, thrombocytopenia

Neurologic: Neuropathy

Urogenital: Nephropathy, acute interstitial nephritis

As with other treatments for syphilis, the Jarisch-Herxheimer reaction has been reported.

The following adverse events have been temporally associated with parenteral administration of benzylpenicillin:

Body as a Whole: Hypersensitivity reactions (including allergic vasculitis, pruritus, fatigue, asthenia, and pain); aggravation of existing disorder; headache.

Cardiovascular: Cardiac arrest; hypotension; tachycardia; palpitations; pulmonary hypertension; pulmonary embolism; vasodilation; vasovagal reaction; cerebrovascular accident; syncope.

Gastrointestinal: Nausea, vomiting; blood in stool; intestinal necrosis.

Haematological: Lymphadenopathy.

Injection Site: Injection site reactions (including pain, inflammation, lump, abscess, necrosis, oedema, haemorrhage, cellulitis, hypersensitivity, atrophy, ecchymosis, and skin ulcer); neurovascular reactions (including warmth, vasospasm, pallor, mottling, gangrene, numbness of the extremities, cyanosis of the extremities, and neurovascular damage).

Metabolic: Elevated BUN, creatinine, and SGOT.

Musculoskeletal: Joint disorder; periostitis; exacerbation of arthritis; myoglobinuria; rhabdomyolysis.

Nervous System: Nervousness; tremors; dizziness; somnolence; confusion; anxiety; euphoria; transverse myelitis; seizures; coma.

A syndrome manifested by a variety of CNS symptoms such as severe agitation with confusion, visual and auditory hallucinations, and a fear of impending death (Hoigne's syndrome), has been reported after administration of benzylpenicillin procaine and, less commonly, after injection of the combination of benzylpenicillin benzathine and benzylpenicillin procaine. Other symptoms associated with this syndrome, such as psychosis, seizures, dizziness, tinnitus, cyanosis, palpitations, tachycardia, and/or abnormal perception in taste, also may occur.

Respiratory: Hypoxia; apnoea; dyspnoea.

Skin: Diaphoresis.

Special Senses: Blurred vision; blindness.

Urogenital: Neurogenic bladder; haematuria; proteinuria; renal failure; impotence; priapism.

DOSAGE AND ADMINISTRATION

Use a concentration of 442 mg/mL when measuring part doses. The quantity of benzathine benzylpenicillin is based on 1,200 Units/mg potency.

Streptococcal (Group A) upper respiratory infections (for example, pharyngitis)

A single injection of 1,200,000 Units for adults.

A single injection of 900,000 Units for older children.

A single injection of 300,000 to 600,000 Units for infants and for children under 27 kg.

Venereal infections

Syphilis - Primary, secondary and latent - 2,400,000 Units (1-dose). Late (tertiary including neurosyphilis) - 2,400,000 Units at 7-day intervals for three doses.

Congenital (with normal CSF) - under 2 years of age: 50,000 Units/kg body weight; ages 2-12 years; adjust dosage based on adult dosage schedule.

Yaws, bejel and pinta - 1,200,000 Units (single injection).

Prophylaxis - for rheumatic fever and glomerulonephritis

Following an acute attack, benzathine benzylpenicillin (parenteral) may be given in doses of 1,200,000 Units once a month or 600,000 Units every 2 weeks.

TO ADMINISTER

Because of the high concentration of suspended material in this product, the needle may be blocked if the injection is not made at a slow, steady rate.

Administer by DEEP, INTRAMUSCULAR INJECTION in the upper, outer quadrant of the buttock. In infants and small children, the midlateral aspect of the thigh may be preferable. When doses are repeated, vary the injection site.

Method of administration is the same as with conventional syringe. Remove needle cover by grasping it securely; twist and pull. Introduce needle into patient, aspirate by pulling back slightly on the plunger, and inject.

Discard any unused portion.

OVERDOSAGE

There have been no reported overdoses with BICILLIN L-A. Penicillin in overdosage has the potential to cause neuromuscular hyperirritability and convulsive seizures. This is particularly so if the penicillin is given intravenously or to patients with renal failure.

PRESENTATION AND STORAGE CONDITIONS

BICILLIN L-A benzathine benzylpenicillin tetrahydrate injection is a white fluid suspension and supplied as follows:

2.3 mL pre-filled glass syringe containing 1,200,000 Units benzathine benzylpenicillin tetrahydrate, equivalent to 1016.6 mg; *packs of 5 x 2.3 mL and 10 x 2.3 mL syringes.

*Not all pack sizes available.

Storage Conditions

Store at 2 to 8°C. Refrigerate, do not freeze.

BICILLIN L-A may be stored below 30°C, for a single period of up to 2 months, prior to expiry. The date the product is placed outside of refrigerated storage and stored below 30°C

should be written in the space provided on the carton. After storage outside of refrigeration, the product should be discarded and cannot be returned to refrigerated storage.

NAME AND ADDRESS OF THE SPONSOR

Pfizer Australia Pty Ltd
38-42 Wharf Road
West Ryde NSW 2114

POSION SCHEDULE OF THE MEDICINE

Schedule 4 - Prescription Only Medicine

DATE OF FIRST INCLUSION IN THE AUSTRALIAN REGISTER OF THERAPEUTIC GOODS (THE ARTG)

19 July 1996

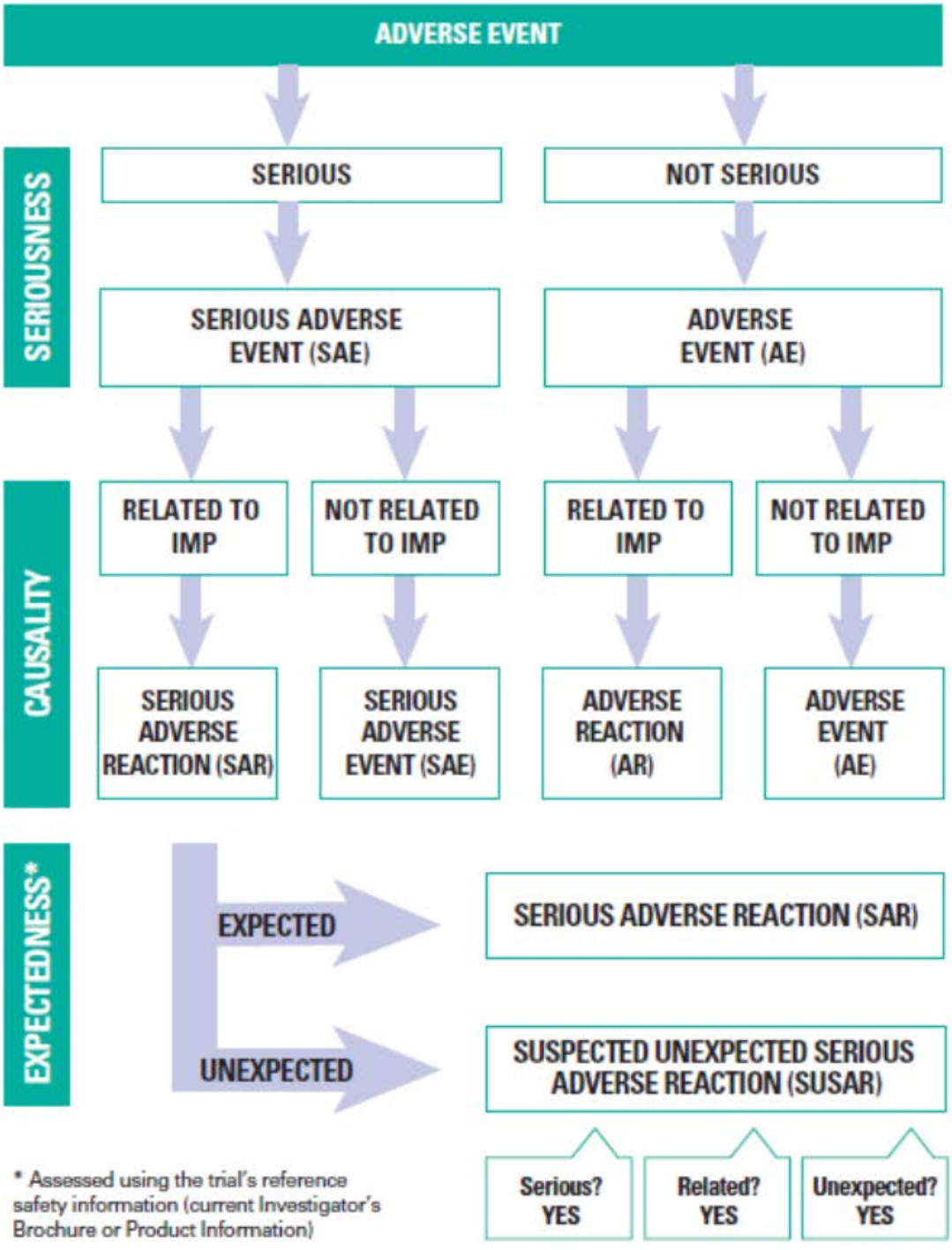
DATE OF MOST RECENT AMENDMENT

04 September 2017

® Registered trademark

20.2. APPENDIX 2: Adverse event flow sheet

B. Safety Reporting Assessment Flowchart: IMP Trial



Adapted from the NIHR Clinical Trials Toolkit

20.3. APPENDIX 3: Case Report Form

The CRF :

Telethon Kids Institute will develop a Medrio database and e-forms to cover both the intensive 12-hour observation and the follow up periods.

20.4. APPENDIX 4: Participant information and Consent Form

The participant information sheet and consent forms will be developed in conjunction with the team at Linear to align with the protocol, Telethon Kids Institute's, and Linear's processes.

20.5. APPENDIX 5: Declaration of Helsinki

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the: 29th WMA General Assembly, Tokyo, Japan, October 1975 35th WMA General Assembly, Venice, Italy, October 1983 41st WMA General Assembly, Hong Kong, September 1989 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996 52nd WMA General Assembly, Edinburgh, Scotland, October 2000 53th WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added) 55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added) 59th WMA General Assembly, Seoul, October 2008.

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data. The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and

quality.

8. In medical practice and in medical research, most interventions involve risks and burdens.

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.

10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.

14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.

15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards, but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.

16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.

18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimise the impact of the study on their physical, mental and social integrity.
24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.
25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.
26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.
27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.
28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents

giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.

30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:

The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo, or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.

35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

20.6. Guideline for acute management of anaphylaxis

https://www.allergy.org.au/images/stories/pospapers/ASCIA_Acute_Management_of_Anaphylaxis_Guidelines_2015.pdf

ascia
australasian society of clinical immunology and allergy
www.allergy.org.au

Guidelines

Acute management of anaphylaxis

These guidelines are intended for primary care physicians and nurses providing first responder emergency care.

Anaphylaxis definition

Any **acute onset illness** with **typical skin features** (urticarial rash or erythema/flushing, and/or angioedema), **PLUS** involvement of **respiratory** and/or **cardiovascular** and/or persistent severe **gastrointestinal** symptoms.
OR
Any **acute onset of hypotension or bronchospasm or upper airway obstruction** where anaphylaxis is considered possible, **even if typical skin features are not present**.

Signs and symptoms of allergic reactions

Mild or moderate reactions
- Swelling of lips, face, eyes
- Hives or welts
- Tingling mouth
- Abdominal pain, vomiting (these are signs of anaphylaxis for insect allergy)

Anaphylaxis
Watch for any one of the following signs of anaphylaxis:
- Difficult/noisy breathing
- Swelling of tongue
- Swelling/tightness in throat
- Difficulty talking and/or hoarse voice
- Wheeze or persistent cough
- Persistent dizziness or collapse
- Pale and floppy (young children)
- Vomiting and/or abdominal pain for insect stings/bites

Immediate action

1. Remove allergen (if still present).
2. Call for assistance.
3. Lay patient flat. Do not allow them to stand or walk. If breathing is difficult, allow them to sit.
4. **Give INTRAMUSCULAR INJECTION (IMI) OF ADRENALINE (epinephrine) without delay using an adrenaline autoinjector if available OR adrenaline ampoules and syringe.**
   - **1:1000 IMI** into outer mid-thigh
   - 0.01mg per kg up to 0.5mg per dose
   - Repeat every 5 minutes as needed.
   - If multiple doses required or a severe reaction, consider adrenaline infusion if skills and equipment available.

Adrenaline (epinephrine) dosages chart			
Age (years)	Weight (kg)	Vol. adrenaline 1:1000	Adrenaline autoinjector
<1	5-10	0.05-0.1 mL	
1-2	10	0.1 mL	10-20 kg (~1-5yrs) 0.15mg (green labelled device)
2-3	15	0.15 mL	
4-6	20	0.2 mL	
7-10	30	0.3 mL	>20kg (~>5yrs) 0.3mg (yellow labelled device)
10-12	40	0.4 mL	
>12 and adults*	>50	0.5 mL	

*For pregnant women, a dose of 0.3mg should be used.

5. Call ambulance to transport patient if not already in a hospital setting.

If required at any time, commence cardiopulmonary resuscitation.

ALWAYS give adrenaline autoinjector FIRST, then asthma reliever if someone with known asthma and allergy to food, insects or medication has SUDDEN BREATHING DIFFICULTY (including wheeze, persistent cough or hoarse voice) even if there are no skin symptoms.

Positioning of patient

- Laying the patient flat will improve venous blood return to the heart.
- By contrast, placing the patient in an upright position can impair blood returning to the heart, resulting in insufficient blood for the heart to circulate and low blood pressure.
- The left lateral position is recommended for patients who are pregnant to reduce the risk of compression of the inferior vena cava by the pregnant uterus and thus impairing venous return to the heart.
- Fatality can occur within seconds if a patient stands or sits suddenly.
- For mainly respiratory reactions, the patient may prefer to sit and this may help support breathing and improve ventilation. BEWARE this may trigger hypotension. Monitor closely. Immediately lay the patient flat again, if there is any alteration in conscious state or drop in blood pressure.
- If vomiting, lay the patient on their side (recovery position).
- Patients must not be walked to/from the ambulance, even if they appear to have recovered.
- Infographics are included in ASCIA Action Plans to reinforce correct positioning.

Supportive management (when skills and equipment available)

- Check pulse, blood pressure, ECG, pulse oximetry, conscious state.
- Give high flow oxygen if available and airway support if needed.
- Obtain IV access in adults and hypotensive children.
- If hypotensive, give IV normal saline 20mL/kg rapidly and consider additional wide bore IV access.

Additional measures - IV adrenaline infusion in clinical setting

If inadequate response or deterioration start IV adrenaline infusion, given by staff who are trained in its use or in liaison with an emergency/critical care specialist.

- Mix 1 mL of 1:1000 adrenaline in 1000 mL of normal saline.
- Start infusion at 5 mL/kg/hour (~0.1 µg/kg/minute).
- Titrate rate up or down according to response.
- Monitor continuously.

IV adrenaline infusions should be used with a dedicated line, infusion pump and anti-reflux valves wherever possible.

CAUTION: IV boluses of adrenaline are NOT recommended without specialised training as they may increase the risk of cardiac arrhythmia.

Additional measures to consider if IV adrenaline infusion is ineffective

For Upper airway obstruction	• Nebulised adrenaline (5mL i.e. 5 ampoules of 1:1000). • Consider intubation if skills and equipment are available
For persistent hypotension/ shock	• Give normal saline (maximum of 50mL/kg in first 30 minutes). • Glucagon (1-2mg IMI or IV as starting dose) especially for patients on beta blockers or has heart failure. • In adults, selective vasoconstrictors metaraminol (2-10mg) or vasopressin (10-40 units) only after advice from an emergency medicine/critical care specialist.
For persistent wheeze	Bronchodilators: • Salbutamol 8 - 12 puffs of 100µg using a spacer OR 5mg salbutamol by nebuliser. • Note: Bronchodilators do not prevent or relieve upper airway obstruction, hypotension or shock Corticosteroids: • Oral prednisolone 1 mg/kg (maximum of 50 mg) or intravenous hydrocortisone 5 mg/kg (maximum of 200 mg). • Note: Steroids must not be used as a first line medication in place of adrenaline.

Antihistamines and corticosteroids

Antihistamines:
- Antihistamines have no role in treating or preventing respiratory or cardiovascular symptoms of anaphylaxis.
- Do not use oral sedating antihistamines as side effects (drowsiness or lethargy) may mimic some signs of anaphylaxis.
- **Injectable promethazine should not be used** in anaphylaxis as it can worsen hypotension and cause muscle necrosis.

Corticosteroids:
- The benefit of corticosteroids in anaphylaxis is unproven.
- It is common practice to prescribe a 2-day course of oral steroids (e.g. oral prednisolone 1 mg/kg, maximum 50 mg daily) to hopefully reduce the risk of symptom recurrence after a severe reaction or a reaction with marked or persistent wheeze.

Observe patient for at least 4 hours after last dose of adrenaline

Relapse, protracted and/or biphasic reactions may occur. Patients require overnight observation if they:
- Had a severe or protracted anaphylaxis (e.g. required repeated doses of adrenaline or IV fluid resuscitation), OR
- Have a history of asthma or severe/protracted anaphylaxis, OR
- Have other concomitant illness (e.g. asthma, history or arrhythmia), OR
- Live alone or are remote from medical care, OR
- Present for medical care late in the evening.

The true incidence of biphasic reactions is estimated to occur following 3-20% of anaphylactic reactions.

Follow up treatment

Adrenaline autoinjector
- If there is a risk of re-exposure (e.g. stings, foods, unknown cause) then prescribe an adrenaline autoinjector before discharge, pending specialist review.
- Teach the patient how to use the adrenaline autoinjector using a trainer device and give them an ASCIA Action Plan for Anaphylaxis (see ASCIA website www.allergy.org.au).

Allergy specialist referral
- Refer ALL patients who present with anaphylaxis for specialist review
- The allergy specialist will:
  - Identify/confirm cause.
  - Educate regarding avoidance/prevention strategies, management of comorbidities.
  - Provide ASCIA Action Plan for Anaphylaxis - preparation for future reactions.
  - Initiate immunotherapy where available (some insect venoms).

Documentation of episodes
Patients should be advised to document the circumstances of episodes of anaphylaxis to facilitate identification of avoidable causes (e.g. food, medication, herbal remedies, bites and stings, co-factors like exercise) in the 6-8 hours preceding the onset of symptoms.

The ASCIA allergic reactions event record form can be used to collect this information
www.allergy.org.au/health-professionals/anaphylaxis-resources/anaphylaxis-event-record

Preparation: Equipment required for acute management of anaphylaxis

The equipment on your emergency trolley should include:
- Adrenaline 1:1000 (consider adrenaline autoinjector availability in rural locations for initial administration by nursing staff)
- 1ml syringes; 21 gauge needles
- Oxygen
- Airway equipment, including nebuliser and suction
- Defibrillator
- Manual blood pressure cuff
- IV access equipment (including large bore cannulae)
- Pressure sleeve (aids rapid infusion of fluid under pressure)
- At least 3 litres of normal saline

Acknowledgements

The information in these guidelines is consistent with the Australian Prescriber Anaphylaxis Management wall chart www.australianprescriber.com

These guidelines are based on the following international guidelines:
- International Liaison Committee on Resuscitation (ILCOR) and Australian and New Zealand Committee on Resuscitation (ANZCOR) guidelines
- American Academy of Allergy, Asthma and Immunology (AAAAI) anaphylaxis parameter
- World Allergy Organisation (WAO) anaphylaxis guidelines

20.7. Appendix 7- Study Schedule of Assessments

	Screen	Period 1 and Period 2														Washout Period	Follow-up Period
	2 weeks	Day 0					Day 1	Day 2	Day 3	Day 5	Day 7	Day 14	Day 21	Day 28	Day 42	4 weeks	14 Days after Completing Period 2
Event ▼ Study Hour ▶		Pre-dose	0 h	2	6	12	24	48	72								
Informed Consent	X																
Inclusion/Exclusion Criteria	X	X															
Demographics																	
Medical History	X																
Height	X																
Physical Exam (incl. weight)	X																
Vital Signs	X	X		X	X	X											
Clinical Laboratory Tests	X	X															
HIV, Hepatitis B/C Testing	X																
Urine Drug / Alcohol Breath Test	X	X															
Dried Blood Sampling PK Sample		X		X	X	X	X	X	X	X	X	X	X	X	X		
PK Blood Sample (from a vein)						X						X					
Pain Score			X	X	X	X	X	X	X	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]		
Monitoring of Skin Irritation							X	X	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]		
Ultrasound Scan of Injection Site			X											X			
Study Medication Administration			X														
Adverse Events		◄	———	———	———	———	———	———	———	———	———	———	———	———	———	———	►
Record Current Medications	◄	———	———	———	———	———	———	———	———	———	———	———	———	———	———	———	►
In Clinic Confinement		◄	———	———	———	►											
Outpatient Visit	X						X	X	X	X	X	X	X	X	X		
Telephone Call Follow-up																	X

[1] Participants should be resting in a supine position for at least 5 minutes prior to and during the measurement of vital signs. When vital signs are scheduled at the same time as blood draws, the vital signs will take priority. Additional vital signs may be performed as clinically indicated.

[2] Assessments performed only if pain or irritation was recorded at the previous visit.