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Original Article

Percutaneous re-circulating isolated limb perfusion of gentamicin in a large animal model: targeted delivery of gentamicin to limb

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Abstract: Objective: We have developed a percutaneous recirculation system (V-Vascular, V-V) to enable delivery of high levels of antibiotic to the limb in an isolated and targeted manner for the treatment of limb infection. Background: Chronic and acute limb infections are relatively commonplace in a variety of wound types. Infection can become refractory to existing treatment strategies and can cause complications associated with wound healing, lead to amputation and even death. Methods: Gentamicin was delivered to the ovine hind limb (4 mg/kg) using the V-V system, a 'closed' recirculatory catheter system that draws blood from the venous system and returns it to the artery via an oxygenator, or via intra-venous (IV) infusion. Samples of muscle, bone and synovial fluid of the limb were collected at 30 and 60 min post administration of gentamicin. Results: There was a significantly greater concentration of gentamicin observed in the bone and skeletal muscle of limbs receiving the antibiotic via V-V at 30 min post administration compared to IV delivery, (bone V-V 0.05 ± 0.04, I.V 0.004 ± 0.001 mg/L $p<0.05$; muscle V-V 0.005 ± 0.001, I.V 0.002 ± 0.0005 mg/L $p<0.05$) and bone and synovial fluid at 60 min post administration (bone V-V 0.06 ± 0.02, I.V 0.005 ± 0.001 mg/L $p<0.05$; synovial fluid V-V 34.58 ± 14.9, I.V 3.03 ± 0.59 mg/L $p<0.05$). Conclusions: These results suggest that the use of percutaneous recirculation is a safe and effective method for delivering a greater concentration of antibiotic to the limb without systemic implications.

Keywords: Wound infection, percutaneous delivery, gentamicin, limb

Introduction

It was estimated in 2010 that 285 million people worldwide are living with diabetes and that this number will increase to more than 400 million by 2030 [1]. Whilst the cardiovascular complications of diabetes contribute the major proportion of diabetes related mortality a range of other complications contribute to the wide range of morbidities experienced by patients with diabetes. Of these, diabetes related foot complications are estimated to affect 25% of all individuals with diabetes [2], 50% of which will become clinically infected [3] requiring hospitalization, wound debridement and, in 11.2% of cases, amputation during the first 3 years from initial diagnosis [4]. The 'diabetic foot' is characterized by a complex interplay of contributory factors including peripheral neuropathy, peripheral arterial disease, impaired wound healing and excessive plantar pressure [5-7].

Whilst existing antibiotics are effective at treating infection in the majority of cases, the diabetic patient often presents with factors that further complicate patient and infection management including peripheral vascular disease [8, 9], renal impairment [9, 10] and cardiac disease [10]. The existence of these co-morbidities may contribute to the inability to control infection, due to an incapability to achieve adequate and sustained concentrations of antibiotic at the site of infection without systemic consequence, including organ damage. For example, whilst gentamicin is an effective antimicrobial agent, the dose adjustment required in the patient with renal impairment, may lead to in the delivery of an inadequate antibiotic dose leading to suboptimal tissue levels [11].

Accordingly we developed an antibiotic delivery system for the lower limb which allows the administration of elevated levels of antibiotic

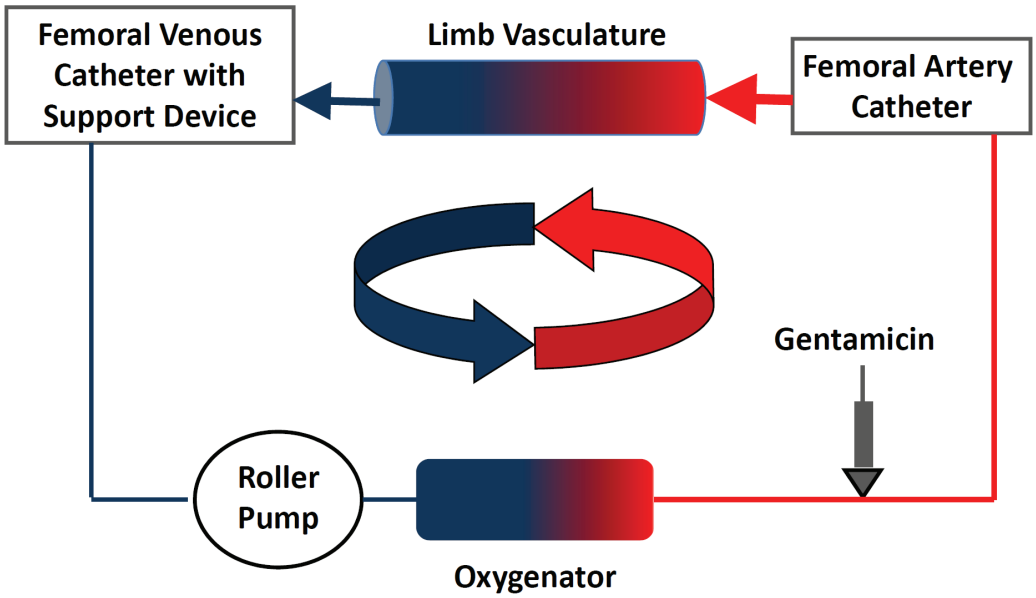

Figure 1. Schematic of the V-Vascular system.

locally to the site of infection in an isolated manner for extended periods whilst also limiting the systemic exposure of potentially toxic agents.

Materials and methods

All animal care and procedures were in accordance with the guidelines laid down by the National Health and Medical Research Council and approved by the Institutional Animal Ethics Committee (Department of Primary Industries, Victoria).

Limb isolation

A total number of 15 crossbred sheep were used for this study with a mean weight of 55kg. Under general anesthesia (induction, 2 mg/kg propofol; maintenance, isoflourane 2% in oxygen), a 9 Fr vascular sheath was placed in a carotid artery and an 11 Fr sheath placed in a jugular vein. Limb isolation was performed using a percutaneous recirculation circuit ('V-Vascular', Osprey Medical, Minneapolis, USA), shown in **Figure 1**. Under fluoroscopic guidance balloon catheters were positioned in the femoral vein and artery of the sheep via the carotid artery and jugular vein. Once in position a nitinol basket was placed within the femoral vein at the tip of the balloon catheter to provide support and prevent collapse of the vessel during recirculation. The system acts by capturing the femoral venous return, via the customized balloon catheter, which is oxygenated with a membrane oxygenator, the reoxygenated blood is then returned to the femoral artery via a roller pump.

Safety study

An initial pilot study with a 10 minute recirculation time was conducted in n=4 sheep to assess the safety of the system which is required to maintain adequate oxygen saturation without altering blood pH or increasing lactate within the limb. Blood was sampled from the arterial and venous arms of the circuit at baseline and at 2 minute intervals and analysed using the i-STAT system (Abbott Point of Care Inc.). Parameters assessed were O_2 and CO_2 saturation, lactate and blood pH. Arterial pressure was also monitored during recirculation. Histopathology of the artery/vein was also assessed following recirculation, samples were formalin fixed and embedded in paraffin, slides were cut and stained using haematoxylin and eosin (H&E) stain.

Gentamicin delivery

Gentamicin was administered using one of two methods, in both cases samples of the hind limb (bone, skeletal muscle and synovial fluid) were taken at 30 and 60 minutes post initiation of administration to determine Gentamicin lev-

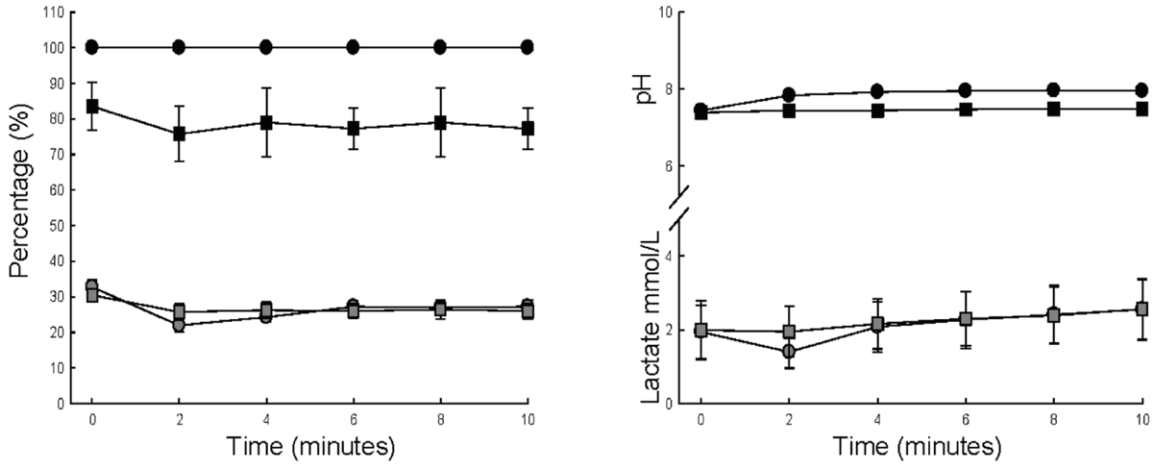

Figure 2. Blood chemistry during recirculation procedure. A. oxygen (black) and CO_2 (grey) saturation for arterial (circles) and venous (squares); B. lactate and pH for arterial (circles) and venous (squares). values mean ± sem, * $p<0.05$.

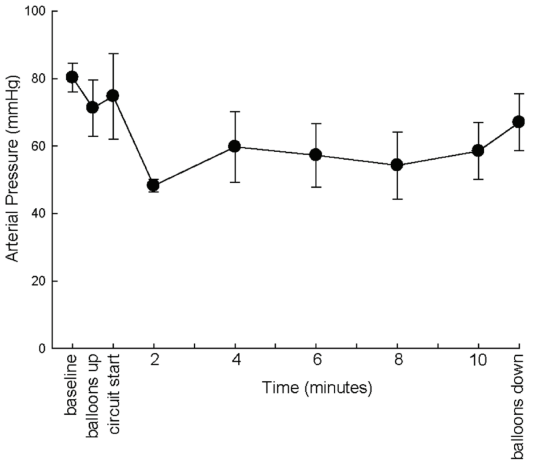

Figure 3. Limb arterial pressures during recirculation procedure, values mean ± sem.

els. The control group received 4 mg/kg via the standard intra-venous route (n=6) while the second group received the antibiotic using percuateneous recirculation as described above for 30 minutes (n=5). In the recirculation group, once stable blood flow through the circuit was established, Gentamicin 4 mg/kg was delivered into the antegrade limb of the circuit over two minutes and recirculation continued for 30 minutes. To limit the systemic appearance of Gentamicin, femoral venous blood was collected for 2 minutes without recirculation at the conclusion of antibiotic administration, the catheters removed and recirculation allowed to return to normal for 30 min prior to the final samples of bone, skeletal muscle and synovial fluid (60 min post initiation of gentamicin delivery).

Following sampling at the 60 minute time point, animals were euthansed (potassium chloride 30 mk/kg, i.v) and samples from the liver, kidney and lung of the sheep obtained to examine Gentamicin levels.

Gentamicin levels

Bone, skeletal muscle and synovial fluid samples from the limb, as well as kidney, liver and lung, were weighed and placed in saline. The tissue was ground, centrifuged and the supernatant removed for analysis of gentamicin levels using Flourescence Polarisation Immunoassay (Abbott AxSYM Gentamicin).

Statistical analysis

The study was conducted in a blinded fashion, analyses of the samples was conducted by researchers unaware from which group the data was obtained. Data are expressed as mean ± sem. Between or within group comparisons were performed using unpaired or paired t-tests where data was normally distributed, whilst non-normal data were analysed using rank sum test. A p value less than 0.05 was considered statistically significant.

Results

In the initial pilot safety study oxygen saturation and carbon dioxide levels did not change significantly ($p>0.05$) throughout the recirculation

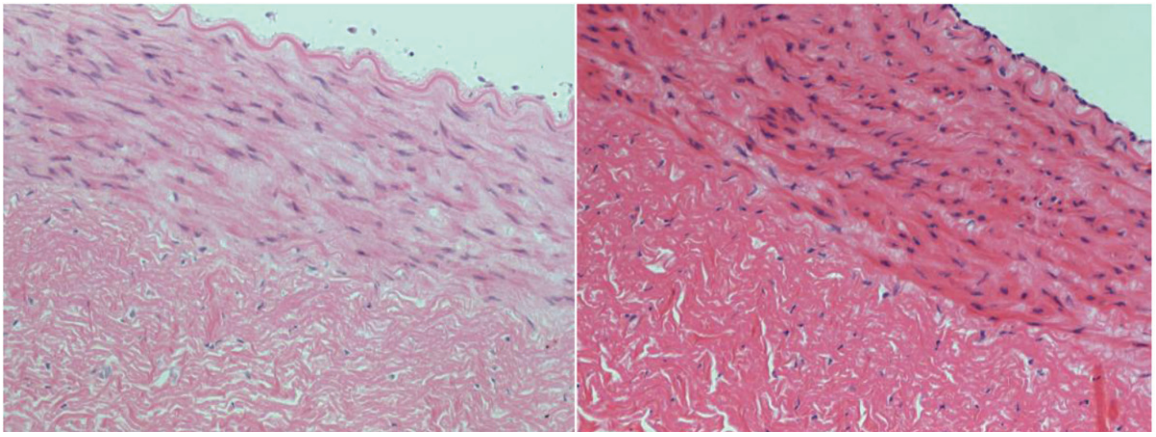

Figure 4. Representative images of arterial wall following the recirculation procedure. Haematoxylin and Eosin staining, x100.

procedure (**Figure 2A**) while lactate and pH was also not significantly altered (**Figure 2B**, $p>0.05$). Pressure within the femoral artery was maintained without increase during all aspects of the recirculation protocol (**Figure 3**, $p>0.05$). Following removal of the catheters, histological tissue samples collected from the artery show no sign of inflammation or damage occurring as a consequence of isolated recirculation (**Figure 4**).

A significantly greater concentration of Gentamicin was achieved in the bone and muscle of the sheep hind limb following 30 min of recirculation delivery compared to intra-venous administration ($p<0.05$, **Figure 5A** and **5B**). Although the catheters were removed from the limb and circulation allowed to return to normal in the V-V delivery group, there was still a significant difference in the concentration of Gentamicin found in the bone and synovial fluid of the limb in compared to I.V. group ($p<0.05$, **Figure 5A** and **5C**).

Levels of Gentamicin were also determined in the liver, lung and kidney for both groups following the 60 min time point. There was no significant difference between the two groups for the organs examined although a trend was observed for reduced uptake of the antibiotic in the V-V recirculation group (**Figure 6**).

Discussion

Infective foot complications are a significant cause of morbidity in patients living with diabetes, and in conjunction diabetes with attendant infection or ischemia is the leading cause of lower limb amputation. Using the principles of local chemotherapy delivery established for patients with refractory malignancy (eg. melanoma) in the lower limb, we developed a similar concept for the delivery of antibiotics to the lower limb. In particular, in a significant proportion of diabetic patients, infection remains refractory to conventional treatment due to the inability to achieve sufficient antibiotic tissue levels when delivered systemically [11]. In the current study we examined the safety and effectiveness of a non-surgical, catheter-based delivery system ('V-Vascular') for the targeted delivery of Gentamicin. The system provides arteriovenous recirculation of the delivered antibiotic, while maintaining normal physiology within the limb as demonstrated by the maintenance of blood gases and perfusion pressure.

The use of the V-Vascular system resulted in a significantly higher concentration of the antibiotic in the treated limb even after a 30 min washout period once the recirculation procedure was completed. In addition, the resultant 10-fold increase in synovial uptake of Gentamicin in the limb, once delivery is complete, may act as a reservoir of the antibiotic, producing a subsequent sustained release and maintained antibiotic level within the area of infection, prolonging the effectiveness of the agent.

The ability to provide targeted and isolated delivery enables the use of the standard prescription of, in this case, gentamicin to produce higher concentrations in the limb as compared

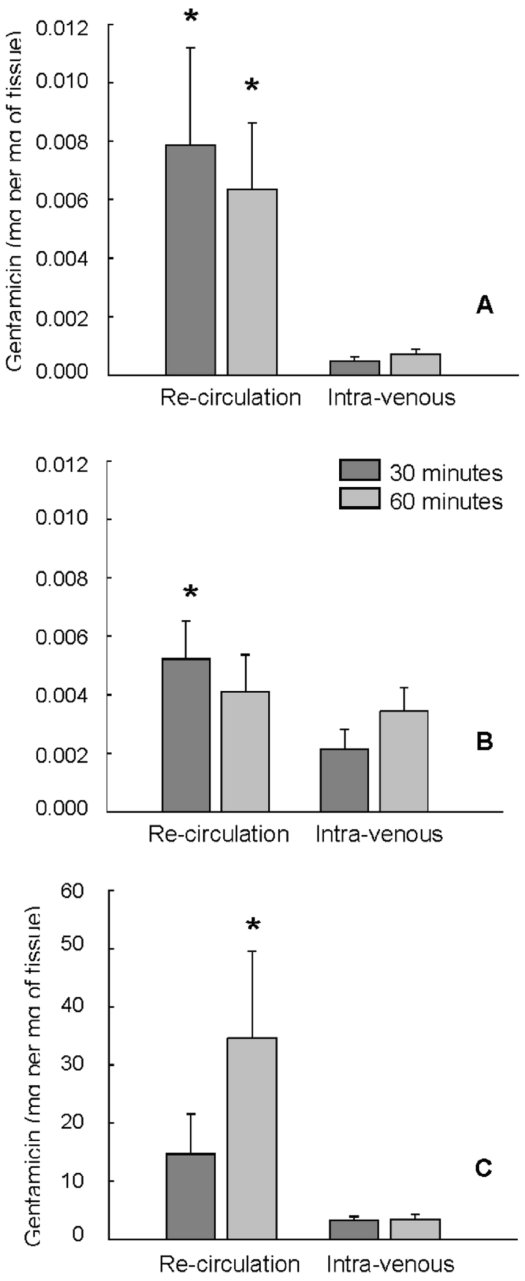

Figure 5. Gentamicin levels in limb tissue following recirculation or intra-venous administration at 30 min and 60 min post delivery. A. bone; B. muscle; C. synovial, values mean ± sem, * p<0.05 compared to intravenous delivery.

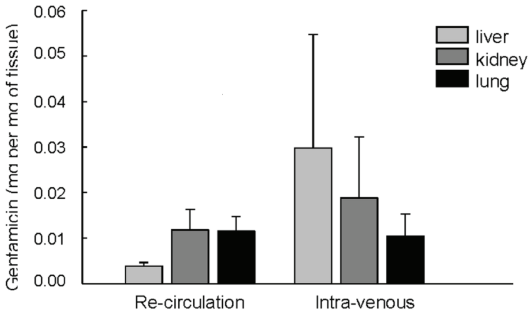

Figure 6. Gentamicin levels in organs following re-circulation or intra-venous administration at 60 min post delivery. values mean ± sem, * p<0.05.

to that normally achieved. In the present study we found evidence of some Gentamicin within the liver, lung and kidney albeit non-significant. Leakage from the lower limb may have resulted from lymphatic drainage, which could be abrogated by the concomitant use of a tourniquet in man however this is not practical in the sheep hind limb.

While there are other methods of isolated limb delivery [12, 13], the recirculatory system employed in this study maintained homeostasis of the limb. It is known that correction of tissue oxygenation aids in the treatment of wounds, driving redox sensitive gene expression and signal transduction beneficial to the healing response [14, 15]. Therefore, the increased/maximal oxygenation of the perfusate circulated within the system during antibiotic administration may actually enhance the ability to combat infection [16].

Conclusions

Taken together the present study demonstrates for the first time the application of a non-surgical technique for augmenting the delivery of antibiotics to the lower limb. This approach may have direct utility for the management of diabetic patients at high risk of tissue or limb loss due to refractory infection.

Disclosure

John Power, David Kaye and Melissa Byrne are stockholders of Osprey Medical.

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