ISSN: Enhanced Systemic Absorption of Hyaluronidase Augmented Subcutaneous Administered Ampicillin in a Mouse Model

Albert Nakanishi1*, Thomas Burris2 and Kristine Griffit2

1Department of Pediatrics, Saint Louis University School of Medicine, USA
2Department of Pharmacology and Physiological Sciences, Saint Louis University School of Medicine, USA

Abstract

Background: Intravenous access may be difficult in young children and drugs or fluids administered subcutaneously may pose advantages in health resource limited environments such as rural clinics, refugee camps or disaster situations. Hypodermoclysis is the subcutaneous (SQ) infusion of fluids; it was the major way of hydrating patients prior to intravenous catheters. The technique is simple, safe and effective in the treatment of mild to moderate dehydration in adults and children. Hyaluronidase is an enzyme and tissue permeability modifier and when administered with fluids subcutaneously aids in the transfer of fluids into the intravascular space. Drugs administered subcutaneously and augmented with hyaluronidase may reveal enhanced systemic absorption.

Objective: We investigated the pharmacokinetics of ampicillin given subcutaneously to mice with and without SQ hyaluronidase.

Methods: Ten-week-old C57BL/6 mice in 3 study groups: 1) Ampicillin (50 mg/kg, 75 mg/kg and 100 mg/kg) w/SQ hyaluronidase (0.1cc), 2) Ampicillin (50 mg/kg, 75 mg/kg and 100 mg/kg) w/SQ saline, 3) Ampicillin (50 mg/kg, 75 mg/kg and 100 mg/kg) administered IM. Plasma levels were assessed at 30, 60, 120 and 240 min by ELISA.

Results: Ampicillin administered SQ and augmented with hyaluronidase had enhanced absorption. Maximum concentration (Cmax) and Area Under the Curve (AUC) data also revealed improved absorption of ampicillin augmented with hyaluronidase vs IM ampicillin. Metabolism of ampicillin appears to follow first-order kinetics.

Conclusion: Ampicillin administered with hyaluronidase had enhanced absorption and appears to be equivalent to IM administered ampicillin pharmacokinetically.

Introduction

Hypodermoclysis is the Subcutaneous (SQ) infusion of fluids; it was the major way of artificially hydrating patients from the early 1900’s until the invention of the intravenous catheter in the 1950’s [1]. The technique was simple, safe and effective in the treatment of mild to moderate dehydration in adults and children [2,3]. Hyaluronidase is an enzyme and tissue permeability modifier used as an adjuvant to increase the dispersion and absorption of injected fluids subcutaneously. Intravenous access may be difficult in young children and drugs or fluids administered via the SQ route may pose advantages in heath resource limited environments such as rural clinics, refugee camps or disaster situations around the world [4]. Drugs administered subcutaneously and augmented with hyaluronidase may reveal comparable systemic absorption when compared to Intravascular (IV) or Intramuscular (IM) administration. This may be a viable alternative to parenteral administration in patients with difficult IV access or when the oral route of administration is not a reasonable option. The purpose of this study is to describe the pharmacokinetics of hyaluronidase augmented ampicillin in a mouse model, comparing SQ administration versus IM administration with serum levels of ampicillin measured over time.

Methods

Six-week-old C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME) and housed in standard cages and maintained on standard mouse chow diet (Research Diets). Care and feeding will be conducted by the Comparative Medicine Department at Saint Louis University.
School of Medicine. There were 3 study groups: 1. Ampicillin w/ hyaluronidase administered SQ. 2. Ampicillin w/saline administered SQ and 3. Ampicillin administered IM. Ampicillin was mixed and injected subcutaneously over the scapula with hyaluronidase (0.1 cc) over a range of doses, 50 mg/kg, 75 mg/kg and 100 mg/kg in a total volume not to exceed 2 ml. A second group of mice was injected subcutaneously with just ampicillin at 50 mg/kg, 75 mg/kg and 100 mg/kg in a total volume not to exceed 2 ml. A third group of mice received IM ampicillin at 50 mg/kg, 75 mg/kg and 100 mg/kg, in a volume not to exceed 2 ml. Four mice were used per dose and groups of mice were sacrificed at 30 min, 60 min, 120 min, and 240 min after administration of the drug and blood was collected. Levels of ampicillin in the blood will be assessed by a beta-lactam ELISA kit (Randox Laboratories, Crumlin, CO). Hyaluronidase (rHUPH20) an FDA approved drug is produced by genetically engineered Chinese Hamster Ovary (CHO) cells containing a DNA plasmid encoding for a soluble fragment of human hyaluronidase (PH20). The purified hyaluronidase glycoprotein contains 447 amino acids with an approximate molecular weight of 61,000 Daltons. Hyaluronidase recombinant is supplied as a sterile, clear, colorless, non-preserved, and ready for use solution. Each mL contains 150 USP units of recombinant human hyaluronidase with 8.5 mg sodium chloride, 1.4 mg dibasic sodium phosphate, 1 mg albumin human, 0.9 mg edetate disodium, 0.3 mg calcium chloride, and sodium hydroxide added for pH adjustment. Hyaluronidase recombinant has an approximate pH of 7.0 and an osmolality of 290 to 350 mOsm. Ampicillin is a penicillin beta-lactam antibiotic with a molecular weight of 349,404 Daltons and is manufactured as a sterile injectable powder easily reconstituted with sterile water. Animal studies were performed in accordance with Saint Louis University’s IACUC standards.

**Pharmacokinetics**

The absorption and metabolism of ampicillin subcutaneously with and without hyaluronidase was assessed. A third group of mice who received ampicillin IM was also assessed. The plasma level of ampicillin was measured at 30 min, 60 min, 120 min and 240 min after administration of ampicillin. Pharmacokinetic parameters such as Maximum Concentration (Cmax), Area Under the Curve (AUC), and half-life (t1/2) was determined and potential differences between ampicillin and ampicillin plus hyaluronidase and ampicillin administered IM was evaluated by Student’s t test to determine if they are statistically significant (p <0.05). Power analysis for this pilot study is based on ampicillin AUC data in children with the assumption of a 50% increase of serum ampicillin when administered and augmented with hyaluronidase versus ampicillin administered without hyaluronidase. Ampicillin therapeutic levels are about 175 ughr/ml with a SD of 60 ughr/ml. Based on a power calculation of 0.8 at an alpha of 0.05 a sample size of 4 mice per antibiotic group was determined for a total of 36 mice [5].

### Results

Ampicillin administered SQ with hyaluronidase or saline had enhanced absorption at all drug doses at 30 min when compared to IM ampicillin but not statistically significant vs IM administration (Figure 1A-1C). IM ampicillin at 75 mg/kg and 100 mg/kg had higher serum levels at the 60 min mark when compared to either methods of administering ampicillin sub-cutaneously (Table 1). AUC data also trended upward with higher serum levels of ampicillin augmented with or without hyaluronidase when compared to IM (Figure 1D-1F). Overall ampicillin administration revealed a first order degradation kinetics with a half-life of approximately 0.32 hrs.

### Discussion

In heath resource limited environments around the world. Drugs administered subcutaneously may provide a viable option when the IV, IM or oral route is not feasible. The technical skill to establishing
a SQ access is lower than required for IV and less painful especially in children where vascular access can be an problematic with less than a 70% success rate even in skilled hands [3,4]. Ampicillin was chosen for its continued utility as a first line antibiotic in the treatment of Community Acquired Pneumonias (CAP) worldwide. In children less than 5 years of age, bacteria pneumonias is a major cause of morbidity and mortality especially in developing countries [6,7]. Ampicillin is effective against a range of both Gram positive and Gram negative organisms and is usually administered parenterally with the oral formulation of ampicillin used when hospitalization is not required [6,7].

Hyaluronidase is an enzyme which aids as a spreading factor under the skin by reversibly breaking down hyaluronan part of the collagen Extracellular Matrix (ECM) of the subcutaneous space, by reducing the viscosity and bulk of this barrier to drug or fluid flow aids in their systemic absorption. In 2005, the FDA approved a human recombinant form of hyaluronidase (rHuPH20) for subcutaneous use [8]. Drugs administered subcutaneously must travels through the Extra Cellular Matrix (ECM) then absorbed into the vascular system including the lymphatics to interact with the target tissue. The interstitial fluid space is tightly regulated by hydrostatic and oncotic pressure gradients from the lymphatic and capillary networks [9]. The rate of drug administration will be a function of both these forces in addition to the collagen matrix representing a significant barrier to drug or fluid flow through this potential space. The interstitial fluid volume of the skin accounts for approximately 25% of total body water in man and is in constant equilibrium with the intracellular fluid and plasma compartments [10]. Water-soluble, low molecular weight compounds (<1 kDa) are drawn into the vascular compartment due to plasma oncotic pressure gradients, other low molecular weight compounds such as electrolytes, morphine sulfate, and insulin are absorbed into the vascular space within minutes, as long as adequate capillary flow and oncotic pressure gradients exist [11]. Larger proteins (>150 kDa) access the vascular space mainly by way of bulk flow through the cascading lymphatics. Lymphatic capillaries have a larger diameter than blood capillaries and have a discontinuous basement membrane favoring the ability to absorb large molecules [11,12]. Large molecules and proteins may take several days to achieve adequate serum levels or may be metabolized and rendered inactive. Factor VIII is inactivated subcutaneously and thus must be administered intravenously to be bio available [13]. In a rat model looking at peg interferon alpha-2b and infliximab augmented with rHuPH20 both reveal enhanced absorption when administered subcutaneously when compared to IV administration and AUC data were similar for both groups [8]. The study also revealed that injection of rHuPH20 by itself was non inflammatory with no effect on vascular structures or permeability with rapid regeneration of the ECM due to the short half-life of rHuPH20 after injection [8]. In another animal study, ceftazidime administered SQ in hypo perfused guinea pigs with just warm compress or mentholated warm compresses over the injection site had higher plasma levels of ceftazidime than those guinea pigs without the warm compresses when compared to IV administration [14]. Lymphatic flow is enhanced when external factors such as heat, massage, local inflammation, or even movement of the body part which received the SQ injection, all improving the systemic absorption of the SQ administered drug [12].

Clinical studies in humans revealed similar or enhanced systemic absorption of fluids and electrolytes when administered subcutaneously and facilitated with rHuPH20 and often compared to intravenously administered fluids [15-17]. In a phase IV trial using rHuPH20 facilitated SQ rehydration in children with dehydration, 94% of the patients were successfully rehydrated with no adverse events, clinicians found the procedure easy to perform for 96% of the patients with 90% of parents reporting being satisfied or very satisfied overall with the SQ process [16]. An ED randomized clinical trial of rHuPH20 facilitated SQ vs IV rehydration in mild to moderated dehydrated children found 100% success in placement of the SQ line versus 78% for the IV group with similar volumes of bolus isotonic fluid received and in a quicker fashion, 3.1hrs vs 6.6 hrs when taking consideration for time it took to establish an IV in a child [17].

In healthy adult volunteers, ceftriaxone augmented subcutaneously with rHuPH20 revealed comparable AUC absorption when compared to IV administered ceftriaxone, other than local swelling or erythema of the injection site no adverse events were seen.
In an adult study with advanced disease the use of rHuPH20 improved the absorption of subcutaneously administered morphine with reduced time for maximum concentration (T_{max}) from 13.8 minutes to 9.2 minutes when compared to SQ morphine with saline [19]. In a pre-clinical trial of subcutaneous administered ondansetron with rHuPH20 in healthy adults, the Cmax was 35% higher than IM administered ondansetron, 43% lower than IV administered and 126% higher than oral administered ondansetron [20]. The pharmacokinetics of ampicillin and tobramycin administered by Hypodermoclysis was compared to IV administration in healthy young adults (<50 yo) and older (>65 yo) volunteers and found in general that tobramycin by Hypodermoclysis had lower T_{max} when compared to IV and smaller AUC concentration after a single dose of the drug. Ampicillin also had lower T_{max} for both set of volunteers but had higher AUC concentrations for ampicillin when compared to IV administration [21]. This was similar to our data where the addition of rHuPH20 was not statistically significant versus saline in the level of ampicillin yet was better than IM administered ampicillin over time.

In dehydrated children bolus fluid administration is often faster when delivered SQ subcutaneously as well as being less painful and simpler to perform [16,17]. Controversy exists in aggressive fluid resuscitation as to whether routine bolus administration of fluids in sick children is actually safe. In a randomized double blinded study in septic African children receiving bolus resuscitation versus slower administered IV fluids the 28 day morbidity and mortality was less in the non-bolus resuscitation group [22]. Freeman also demonstrated rapid rehydration was not superior to standard IV hydration in children with gastroenteritis [23]. Hypodermoclysis may offer a gentler and less painful method of resuscitating children with sepsis or dehydration with better clinical outcomes.

There were limitations to our study, serum levels of ampicillin augmented with hyaluronidase revealed enhanced absorption at the 50 mg/kg and 75 mg/kg but less so with the 100 mg/kg dose. This latter finding was thought to be due to some of the drug precipitating out of solution at the higher concentration of ampicillin when administered in the same 2 cc volume that all the mice received subcutaneously. We used a mouse model for its simplicity and cost effectiveness, but the SQ space of a mouse is different from a humans. The main difference between the subcutis of a human versus other animal species is the prominent striated panniculus carnosus muscle in lower species, reduced in non-human primates and absent in humans [24,25]. There also exists greater mobility of the skin in scruff species versus the skin of humans which may account for the similar absorption of ampicillin in our study whether administered SQ with saline or augmented with rHuPH20.

Oral route of therapy remains the mainstay of treatment in the emergency department setting especially in children but when oral therapy fails a number of methods are available for systemic access with Intravenous (IV), Nasogastric (NG), Intraosseous (IO) and Subcutaneous (SQ) routes the most studied. All these methods of access in patients have their limitations and risks [26].

In sum, subcutaneous administered fluids and medications is a promising tool for the treatment of patients when the oral or intravenous routes are sub optimal. Ampicillin augmented with or without hyaluronidase and administered subcutaneously may represent a viable route in the treatment of infections when health resources are limited. Future studies in patients hospitalized with pneumonia and treated subcutaneously would be novel. The technique is easy to perform and may benefit patient care in the pre-hospital arena, rural clinics, refugee camps, mass casualty situations or when epidemics arise. Currently, rHuPH20 needs refrigeration for enzymatic stability with increased clinical interest a more portable form would be desirable.

References


