Development and Feasibility of a Porcine Model of Amlodipine Toxicity

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Abstract

Introduction Toxicity related to calcium-channel blockers remains a significant cause of morbidity and mortality. Amlodipine-induced shock is unique in that its mechanism of action is thought to occur in part via the release of nitric oxide (NO) in the peripheral vasculature. Specific therapeutic interventions, including methylene blue (an NO scavenger), have been suggested, but efficacy studies are severely limited. To facilitate a larger porcine study into the effect of various interventions on amlodipine toxicity, we undertook this model development and feasibility study.

Methods Intravenous amlodipine was prepared by dissolving commercially obtained amlodipine tablets in dimethylsulfoxide. The concentration of the drug was verified using ultraviolet spectroscopy. We administered this solution to three animals in order to determine a toxic dose, capable of facilitating a two-arm study of amlodipine toxicity.

Results The first pig died rapidly after the bolus infusion. The second pig developed mild toxicity, but the dissolution of the plastic tubing by the solvent and subsequent leakage limited the interpretability of the result. The third animal developed expected toxicity with an infusion rate between 2.0 and 5.5 mg/kg/h.

Conclusion This study demonstrates a potentially repeatable model of amlodipine-induced toxic shock using intravenous administration of amlodipine and several methodological considerations for researchers undertaking similar work.

Keywords Pilot · Methylene blue · Poison-induced cardiogenic shock · Pig · DMSO

Introduction

Although calcium channel blockers represent a small proportion of exposures reported to US poison centers, they account for a disproportionate 5.2% of the fatalities [1]. Despite interventions such as normal saline (NS), calcium, high-dose insulin (HDI), and vasopressors, many patients with amlodipine toxicity succumb to refractory shock [2, 3]. Amlodipine induces shock not only through L-type calcium channel blockade [4], but also via release of nitric oxide (NO) in the

peripheral vasculature [4–6]. This makes it unique in the class of dihydropyridine (DHP) calcium channel blockers. Methylene blue, an NO scavenger, has been used clinically in amlodipine-induced refractory shock [7–12] with its efficacy studied in a single rat model [13]. A recent systematic review has argued there is insufficient evidence for routine use of methylene blue in the treatment of poison-induced cardiogenic shock (PICS) [6]. Vasopressors have been used widely for DHP toxicity, and norepinephrine (NE) is frequently used in this context, although no single inotrope has been shown to be consistently effective [4].

We ultimately intended to embark on a randomized, two-armed study comparing methylene blue to norepinephrine therapy in the context of amlodipine-induced hemodynamic shock. Authors have used swine models in multiple previous studies investigating various cardiovascular parameters [14, 15] and hemodynamic shock induced by various drugs [16–20]. To our knowledge, no swine models have been developed to specifically investigate amlodipine-induced cardiogenic shock; in order to move forward with the aforementioned two-armed comparison study, this toxicity model would have to be developed. Therefore, the purpose of this

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developmental pilot was to achieve a feasible porcine amlodipine toxicity model. We describe the novel preparation of the study drug and a three-pig pilot study, including learning points from several pitfalls and limitations.

Methods

Amlodipine Preparation

Clinically, amlodipine is administered as an oral formulation. In order to directly control the amount of circulating amlodipine in our study, we made the decision to administer it intravenously (IV); this has been successful in previous toxicity models using other drugs [16–19, 21]. The goal of this approach was to mimic the oral absorption of the drug, while avoiding the unpredictable nature of gastrointestinal absorption and the need to measure serum drug concentrations.

To our knowledge, amlodipine for IV administration is not available commercially. Therefore, we planned to dissolve the drug in solution. We needed a solvent both able to dissolve amlodipine and simultaneously unlikely to have a major impact on the cardiovascular system to avoid compromising the ultimate study goal. Amlodipine is a relatively difficult substance to dissolve; it is nearly insoluble in water and minimally soluble in ethanol [22]. Amlodipine has been reported to be freely soluble in methanol [23]. Several manufacturers report dimethyl sulfoxide (DMSO) to be an effective solvent for amlodipine (Enzo Life Sciences, Farmingdale, NY: 25 mg/mL; Selleck Chemical, Houston, TX: 82 mg/mL; Tocris Bioscience, Bristol, UK: 56.7 mg/mL; Sigma Aldrich, St Louis, MO: > 20 mg/mL). Amlodipine was found to be quite soluble in DMSO in a similar model development study [24]. Trauma researchers injected swine with a bolus dose of DMSO (as a control) at approximately 2 mg/kg with mild decrease in mean arterial pressure in a hemorrhagic shock model [25]. Researchers utilizing a canine model noted minimal hemodynamic effects in doses less than 1 g/kg DMSO [26]. Injection of DMSO directly into the pulmonary vasculature of pharmaceutical grade DMSO (> 99%) found no hemodynamic effects at a low dose [27]. Despite the risk of minimal hemodynamic effects utilizing DMSO, we determined that the only other possible solvent that amlodipine is significantly soluble in, methanol, poses a higher risk from a metabolic perspective.

Commercially available amlodipine tablets (Mylan Pharmaceuticals, Morgantown, WV) were used to separate the active drug from the insoluble congeners. Two hundred fifty commercially obtained 10 mg amlodipine tablets were ground and thoroughly mixed with 300 mL of dimethylsulfoxide (DMSO). This mixture was then sonicated for 15 minutes, with the resulting product separated using vacuum filtration. This solution was then centrifuged and the

supernatant collected. This was then diluted and placed in a Jasco-720 ultraviolet-visible (UV/vis) spectrophotometer. The concentration was measured by determining absorption at 360 nm and then compared to a previously derived absorption versus concentration plot. This comparison plot was obtained using pure amlodipine powder (HPLC-grade; Sigma Aldrich, St Louis, MO); 360 nm is the wavelength of maximum absorption for the pure amlodipine standard.

Instrumentation

The Institutional Care and Use Committee of the HealthPartners Institute approved all protocols. We performed the experimental protocol in the HealthPartners Animal Care Facility, which is a secured animal care facility that is United States Department of Agriculture licensed and accredited with the American Association for Accreditation of Laboratory Animal Care.

Three Yorkshire-Duroc cross swine (median weight 41.6 kg) were anesthetized with an intramuscular injection of tiletamine and xylazine. Response to a brief pinch of the toe was used to assess for adequate sedation. Once sedated, animals were instrumented in a fashion similar to several previous studies undertaken by this group [16–18, 21]. A tracheostomy was performed and the animal was placed on a ventilator. Mechanical ventilation was maintained throughout the study (initial settings $V_t = 10$ mL/kg, PEEP = 5, $FiO_2 = 50\%$) and FiO_2 was adjusted to maintain an oxygen saturation greater than 90%. Once on the ventilator, sedation was maintained using a combination of isoflurane and nitrous oxide. While prioritizing adequate sedation by monitoring pain response, sedation was minimized so as to mitigate the hemodynamic effects of the anesthetic agents; this balance has been well established [16–18, 21]. Femoral access was obtained to facilitate venous blood sampling as well as for fluid and medication administration. ECG electrodes were attached to continuously monitor heart rate. We also obtained arterial access via the femoral approach to obtain arterial blood gases and continuously monitor blood pressure. A Swan-Ganz catheter was placed into the right external jugular after a venous cut-down procedure and floated into the pulmonary artery for cardiac output measurements as determined by thermodilution.

Toxicity Modeling

Once anesthetized and instrumented, we initiated an infusion of normal saline (NS) at 1.5 mL/kg/h and monitored the animals for 20 min. Following this period, baseline parameters were recorded. Based on previous experience with porcine models of poison-induced shock, as well as the known pharmacokinetics of amlodipine, along with some related previous work [24], our initial planned approach was to administer a 2 mg/kg bolus, followed by an infusion of 0.3 mg/kg/h. This

dose would be adjusted in the subsequent pigs if a predefined point of toxicity was reached too quickly or not at all during the 5-hour study period. This predefined point of toxicity (P_1) was defined as a 25% reduction in baseline cardiac output (CO) multiplied by mean arterial pressure (MAP); this P_1 has been used multiple times previously [16–18, 20, 21, 28]. After achieving P_1 , all animals would receive a 20 mL/kg bolus of NS to simulate resuscitation. Animals were then monitored during ongoing amlodipine infusion (to mimic ongoing absorption) until the end of the 5-h monitoring period or death. Any animal that survived to the 5 hours would be euthanized using standard laboratory protocols.

Results

Amlodipine Preparation

Using pure amlodipine powder dissolved in DMSO, a UV absorption versus concentration plot was obtained at 360 nm (Fig. 1). The data were fit with a trendline of the form $A = \epsilon bc$, where A , ϵ , b , and c are the absorption, molar absorptivity coefficient, path length, and concentration, respectively. The line had a slope of $10.9 \pm 0.24 \text{ mL mg}^{-1}$ ($4.5 \times 10^3 \pm 98 \text{ L mol}^{-1}$), a y-intercept of -0.044 , and an R^2 value of 0.998. With a 1 cm path length, the molar absorptivity coefficient at 360 nm was therefore $4.5 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$. Two hundred fifty 10-mg amlodipine tablets were used in the extraction procedure. A sample of the supernatant, diluted 100 times, had an absorbance of 0.76. This represents an undiluted concentration of 6.9 mg mL^{-1} ($1.7 \times 10^{-4} \text{ mol L}^{-1}$). Thus, 2070 of the 2500 mg of amlodipine were recovered, representing a yield of 83%.

Toxicity Modeling

The first pig developed hypotension and death within 15 minutes of the bolus infusion. Per our methods, in the subsequent

pigs, the dose was adjusted; we eliminated the bolus and initiated a more conservative drug infusion rate of 0.25 mg/kg/h, increasing the infusion every 20 min by 0.25–0.5-mg/kg/h increments. This dose adjustment was seemingly successful, in that it avoided reaching P_1 and death too quickly. However, this second animal survived until the end of the 5-hour protocol and only displayed mild evidence of amlodipine toxicity. Furthermore, toward the end of the monitoring period for this animal, it became clear that the DMSO solvent had depolymerized the polyethylene and polyvinyl tubing, catheters, and infusion bag and much of the drug leaked. To address this methodological flaw for the final pig in the pilot, the drug was infused through a more durable polytetrafluoroethylene (PTFE) line that was sewn directly into the animal's femoral vein. The same initial infusion rate was used, and the dose was increased in the same way. P_1 was reached at 2 mg/kg/h. Following the NS bolus, the infusion rate was increased every 10 minutes to a maximum dose of 5.5 mg/kg/h. The animal died at 160 minutes (2.67 h) at this maximum dose. Amlodipine infusion rate and hemodynamic parameters for the third pig can be seen in Fig. 2.

Discussion

This method development and feasibility study piloted a procedure to prepare amlodipine for intravenous administration from commercially available amlodipine tablets and provides guidance for an infusion protocol of a toxic model of amlodipine-induced hemodynamic shock. Through this process, we also discovered several unexpected and important considerations when using a similar approach. This pilot has admitted limitations (below), including the interpretation of results from an ultimate single successful animal; however, publishing feasibility work leading up to a follow-up study can be important [29]. As the work and resources necessary to determine feasibility become more intensive and potentially prohibitive for future researchers, as in this study, sharing this process is even more important [18].

Fig. 1 UV absorbance versus amlodipine concentration at 360 nm

Fig. 2 Hemodynamic parameters (MAP, cardiac output, heart rate) and amlodipine dose versus time in the third pig (weight = 37.6 kg). PoT is point of toxicity, defined as a 25% reduction from the baseline product of the cardiac output and mean arterial pressure

The toxicity-modeling portion of the study demonstrated that this amlodipine solution could be used in a model of amlodipine-induced hemodynamic shock. The first two pigs revealed several unforeseen problems with our initial approach. The first animal died very soon after the initial bolus. The reason for this is likely multifactorial—in large part due to the dose of the amlodipine and in small part due to the effect of the solvent, DMSO. Our decision to bolus the animals was based on our experience with propranolol in similar studies [16–19], in which the animals tolerated the bolus of the beta-blocker and exhibited expected hemodynamic shock characteristics relatively quickly and reliably. In an amlodipine rat model development study [24] and subsequent follow-up work [13], Jang and colleagues used intravenous amlodipine with a single infusion rate without a bolus. They demonstrated a toxic rat model with an infusion rate of 17 mg/kg/h. Therefore, we followed this infusion-only approach with the second two pigs and ultimately achieved a more satisfactory outcome in the third animal for the purpose of studying the animal's response to various interventions in future studies.

The effect of the DMSO bolus cannot be completely isolated from the amlodipine effect in this pilot given that no animal received a DMSO bolus alone without the study drug; DMSO has been associated with decreasing MAP in a previous swine model [25] so this effect cannot be discounted. This limitation will fall out with the completion of the ultimate two-arm study to follow that this present study is piloting for. All of the animals will be exposed to the same IV amlodipine solution, including the DMSO solvent, so differences in hemodynamics with the application of either the methylene blue or norepinephrine will have built in control.

Another flaw in the initial design, and an important learning point for any researcher looking to use this pilot to guide their work, was the depolymerization of the plastic components due to the DMSO solvent. The chemical compatibility of DMSO with plastics is not well reported in the literature, but has been reported in commercial reports [30]. This report notes that DMSO is incompatible with several plastics. The

intravenous tubing, syringes, and fluid bags used in our study were made from polyethylene or polyvinyl, which are not reported in this document [30]. When we switched to a compatible plastic (PTFE), we had no further depolymerization issues. We would therefore recommend using PTFE tubing and syringes when administering drug dissolved in DMSO.

After adjusting the amlodipine dose and moving to a compatible drug delivery system, the third pig demonstrated the expected hemodynamics we associate with poison-induced cardiogenic shock (Fig. 2)—with relative maintenance of blood pressure and increasing heart rate until the animal's ability to compensate for the increasing vasoplegic shock lead to a rapid hemodynamic decompensation. The initial point of toxicity was reached with a dose of 2.0 mg/kg/h and further titrated up to 5.5 mg/kg/h. This toxic dose is significantly lower than the dose required in the aforementioned rat study [13], which is expected given the increased metabolic rate of the rat as compared to the much larger pig.

Something worth mentioning in the pilot manuscript, as it is meant to provide some insight into this development process to benefit future research efficiency, is cost. The aforementioned amlodipine toxicity rat model demonstrated dissolved pure amlodipine powder in DMSO for direct infusion [24]. While this method is certainly less time consuming and less prone to impurities as our approach, it was determined that this approach would be cost prohibitive, primarily because of the amount of drug necessary for our large animal model (in addition to the high cost of the animal itself along with the instrumentation and laboratory time). Amlodipine powder, at the time of writing, is available from a commercial source (Sigma Aldrich, St Louis, MO) for \$14 per milligram versus commercially available amlodipine tablets which are available from \$0.017 per milligram [31]. Considering a 5 hour infusion at 2 mg/kg/h and an average pig weight of 40 kg, this translates to a cost savings of \$5593.20 per animal in drug cost alone using our approach.

Cost consideration played into the final important limitation of this pilot, which was the limited number of animals

used. For this reason, this pilot study was a true feasibility study as opposed to a powering study capable of indicating the statistical significance of our ultimate toxicity model comparing amlodipine infusion dose to any of the measured hemodynamic parameters. We can therefore say that this porcine model, including animal instrumentation, amlodipine preparation, and subsequent toxicity induction with amlodipine, is feasible; we cannot say with what certainty the particular dose we used will drive 100% repeatable toxicity.

Conclusion

We piloted a porcine model of amlodipine toxicity for the purpose of ultimately driving a future study comparing antidotes in this context. Amlodipine can be reliably extracted from tablets in a DMSO solution using a vacuum filtration procedure, and concentrations can be confirmed using UV/Vis spectroscopy. IV line and catheter material must be considered when using DMSO as a solvent, as several plastics used in many commercially available healthcare products are not compatible with DMSO infusion. An infusion rate of 2.0 to 5.5 mg/kg/h in a porcine model, without an initial bolus, can produce expected amlodipine toxicity. The preparation of amlodipine and ultimately determination of a toxic dose provide value to subsequent research on this drug, while the shared experience relating to the solvent could benefit a wide array of future pharmacotherapy research.

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Compliance with Ethical Standards

Conflict of Interest None.

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