Levosimendan Does Not Improve Survival Time in a Rat Model of Verapamil Toxicity

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ABSTRACT

Objective: Calcium channel blocker (CCB) toxicity, in particular that induced by verapamil and diltiazem, presents clinical challenges with no true antidote. Levosimendan, a calcium sensitizer, improves cardiac contractility in patients with heart failure. We tested the hypothesis that calcium channel sensitization will prolong survival in a rat model of severe verapamil poisoning.

Methods: This was a blinded, randomized, controlled animal study. Wistar rats (mean weight, 371 ± 50 g) were used. Verapamil (2.5 mg/ml) was infused at a rate of 37.5 mg/kg per hour. Bolus doses of levosimendan (5 µg/mL) were given at 0 min (12 µg/kg) and 5 min (18 µg/kg); saline control was of equal volume. The rats were intubated and maintained under general anesthesia with isoflurane. Electrocardiographic activity and core temperature were monitored during the poisoning and treatment phases. Each rat underwent femoral vein cannulation and was then randomized, in blinded fashion, to receive either levosimendan or an equal volume of saline at 0 and 5 minutes. Death, defined as 1 minute of asystole, was used as the primary endpoint.

Results: Rats treated with levosimendan died before the control group (7.37 ± 0.7 min [n = 7] vs. 16.4 ± 4.2 [n = 7] [$p = .053$]). All animals experienced bradycardia prior to asystole.

Discussion: Although levosimendan has the ability to sensitize and enhance binding of troponin C to Ca^{2+}, this study did not show an improvement in survival time in the setting of verapamil toxicity. This may be attributed to levosimendan's inhibition of phosphodiesterase, which possibly exacerbated the CCB-induced hypotension.

Conclusion: In this rat model, levosimendan as a solitary antidotal treatment for verapamil toxicity was not beneficial.

INTRODUCTION

Calcium channel blocker (CCB) poisoning is an increasing cause of morbidity and mortality resulting from drug overdose. In 1983, the American Association of Poison Control Centers (AAPCC) received 96 reports of exposures to CCBs [1]. By 2005, the number of toxic exposures had risen to 2,828; of that total, 176 caused major outcomes and 32 caused death. Twenty percent of the exposures occurred in children younger than 6 years old [2]. Thus, pediatricians have grouped CCB exposures into the "one-pill killer" category. The typical toxic effects of these medications are hypotension, bradycardia, and, in the most severe cases, profound circulatory collapse. Verapamil and diltiazem have one of the highest case-fatality rates among the antihypertensive medications [1,2].

The initial treatment for CCB poisoning employs IV fluids, glucagon, calcium, vasopressors, and hyperinsulinemia-euglycemia. Unfortunately, there is no antidotal therapy that has shown the ability to consistently restore cardiovascular function, which leads to some confusion in the clinical setting.

Keywords: verapamil, toxicity, levosimendan

Acknowledgment: The manuscript was copy edited by Linda J. Kesselring, MS, ELS, technical editor and writer in the Department of Emergency Medicine, University of Maryland School of Medicine.

Notes: This study was funded by the Maryland Emergency Medicine Grant Network.

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The study described in this article investigated the in vivo effects of a new drug, levosimendan, in the setting of CCB poisoning. Levosimendan is a calcium sensitizer that is actively being studied for the treatment of congestive heart failure, stunned myocardium, and ischemic heart disease [3]. Its proposed mechanism of action involves enhanced binding of cardiac troponin C to calcium [4], which, in theory, allows the calcium released by the sarcoplasmic reticulum to work more efficiently. The calcium-sensitizing effect holds promise for mitigating the deadly cardiovascular effects of CCB poisoning. CCBs, in particular verapamil and diltiazem, antagonize the L-type calcium channels, preventing the influx of calcium into the cardiac myocyte, which makes less calcium available to bind cardiac troponin C and yields less activation of the actin-myosin filaments. Since the L-type calcium channels are located in skeletal and smooth muscle cells, there is also a vasodilatory effect that further exacerbates the negative inotropic and chronotropic effects on the heart. This leads to the bradydysrhythmias and hypotension seen in overdose. Levosimendan, initially studied in patients with congestive heart failure, may allow the cell to better utilize the remaining intracellular calcium and enhance cardiac output in CCB exposures. This investigation will help isolate levosimendan's effects in a rat model of CCB poisoning and indicate whether the calcium-sensitizing effects are prominent enough to allow levosimendan to be deemed a viable antidote for CCB overdose.

METHODS

Study Design

This is a randomized, blinded, placebo-controlled trial in a rat model designed to test the ability of levosimendan to prolong survival in the setting of verapamil overdose. It was approved by the Institute for Animal Care and Use Committee (IACUC) and was conducted in accordance with all pertinent IACUC guidelines.

Animals

Male Wistar rats weighing a minimum of 300 g were purchased through the Veterinary Resource Department at the University of Maryland. They were acclimatized for a minimum of 1 week prior to their use in the experiment.

Study Protocol

We based our experimental design on a model developed by Tebbutt et al. [5]. Briefly, the animals were placed in a container with 5% isoflurane gas for 5 minutes for sedation. The isoflurane was reduced to 3% for 1 minute. Following this sedation, the animals were intubated using a $1^1/_2$-inch, 16-gauge catheter tip. Animals (n = 7 for both the experimental and control groups) were placed on small-mammal ventilators and maintained on 1% isoflurane. Endotracheal intubation was confirmed by observing condensation in the catheter, bilateral chest wall expansion, and changes in breathing patterns with ventilation. Depth of anesthesia was monitored by testing the eyelash reflex, toe pinch, and palpebral reflexes.

Following intubation, each animal was attached to a Mennen Series 742 portable 3-lead electrocardiograph (Mennen Medical Inc., Clarence, New York). A rectal temperature probe was placed, and the rats were prepared for cannulation of the femoral vein. A femoral cutdown was performed and a 24-gauge angio-cath (Becton, Dickinson and Company, Franklin Lakes, New Jersey) was inserted into the vein for the purpose of administering verapamil and levosimendan. The catheter was sutured into place and attached to a 3-way stopcock. One port was used to deliver levosimendan or saline; the other was attached to a Medfusion 2010 IV infusion pump (Smiths Medical MD, Inc, St. Paul, Minnesota), which was set to deliver verapamil (2.5 mg/ml) at a rate of 37.5 mg/kg per hour. Levosimendan was obtained as an active pharmaceutical ingredient from ACC Corporation (San Diego, California), and its composition was verified by mass spectrometry. Five milligrams of the yellow powder was dissolved in 1 liter of normal saline, which was stored in aluminum foil to block exposure to light.

We based our verapamil dosing on the previously mentioned study by Tebbutt et al. [5], which demonstrated verapamil toxicity at a mean time of 51.1 ± 7.1 minutes when an infusion rate of 37.5 mg/kg per hour was used. At time 0, the rats were randomized to receive an equal volume of either IV levosimendan (12 µg/kg) or 0.9% sodium chloride (control). The infusion of verapamil was started immediately after the bolus of levosimendan or saline. Five minutes after the initial dose, a second dose of levosimendan (18 µg/kg) was administered. During the delivery of levosimendan, the verapamil infusion was paused. Control animals received an equal volume of saline. The amount of levosimendan given was extrapolated from human studies demonstrating mild efficacy at these doses [3,6–8]. Thus, the total experimental time was a maximum of 200 minutes. Surviving animals were euthanized with a rapid IV bolus of pentobarbital, 100 mg/kg.

Measurements

Heart rate was recorded at 5-minute intervals from the electrocardiograph monitor. Core temperatures, total dose of verapamil, and time of death were also noted. Death, the primary endpoint, was defined as 1 minute of asystole confirmed on 2 electrocardiographic leads.

Data Analysis

Data are described using means and the standard error of the mean (SEM), with appropriate confidence intervals. The groups were compared using student's 2-tailed T-test (SigmaStat, Systat Software, San Jose, California), with $p \leq 0.05$ considered significant.

RESULTS

The time from beginning of verapamil infusion to asystole for the levosimendan and control groups (n = 7) was 7.4 ± 0.7 min (95% CI, 5.7–9) and 16.4 ± 4.2 min (95% CI, 6.2–26.2) ($p = .053$),

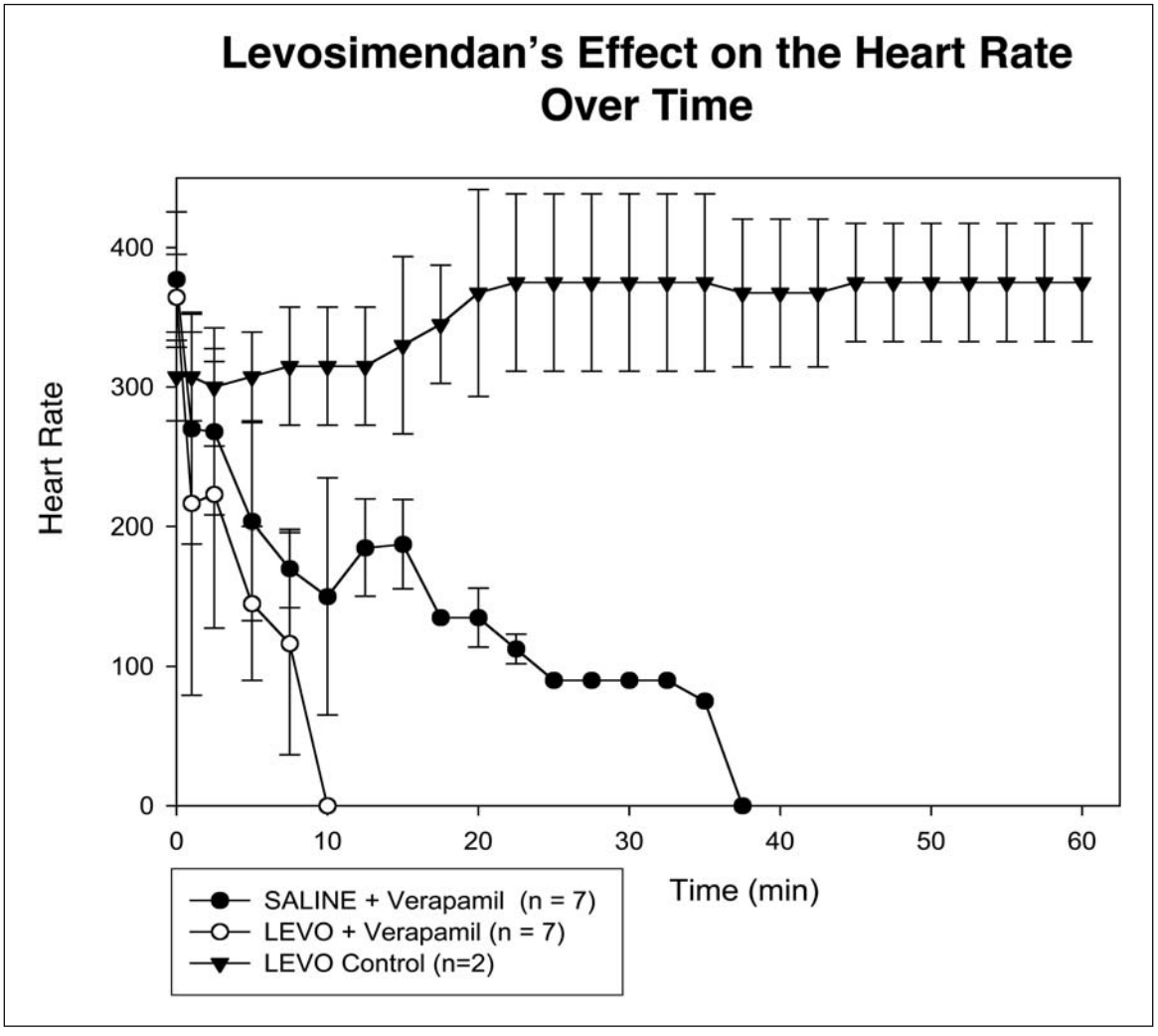

Figure 1: Function of heart rate vs. time. Data are shown as mean ± SD.

respectively (*Figures 1 and 2*). All animals experienced bradycardia prior to asystole. Two animals were given only levosimendan (no verapamil) to ensure that the dose of levosimendan itself was not toxic. Neither animal experienced a significant decrease in heart rate; both were euthanized after 1 hour (see *Figure 1*).

DISCUSSION

Although there are no formal studies on the subject, the advent of a class of Ca^{2+} sensitizers was thought to afford a novel approach to CCB poisoning. The physiological mechanism of CCB overdose has dual action in that it affects both the cardiac myocyte and the smooth muscle of the vasculature, resulting in bradycardia and hypotension, usually a lethal combination. We hypothesized that levosimendan would increase the binding of available intracellular calcium to troponin C, thus increasing activation of the actin-myosin complex, resulting in a positive inotropic effect and reversing the vasodilation caused by blockade of the calcium channels. This experiment demonstrated that the combination of verapamil and levosimendan actually decreased survival. It is possible that there is too little intracellular calcium for levosimendan to make a difference in inotropy or chronotropy. It is also likely that levosimendan has other effects that exacerbate CCB toxicity.

In addition to the calcium-sensitizing effects, levosimendan also has an inhibitory effect on phosphodiesterase III (PDE III) [9], which causes vasodilation through activation of potassium and calcium channels [10]. In the setting of congestive heart failure, these effects may be beneficial. But in the setting of CCB overdose, the resultant hypotension and bradycardia seem to potentiate the effects of the verapamil. This synergistic effect may have decreased survival time in our experimental group. When the animals were treated with levosimendan alone, their heart rate did not change. Since this experiment was designed to test survivability with this single agent, we did not attempt any adjunctive measures to counteract the presumed hypotension. Another variable that should be taken into consideration is the effect of isoflurane on the myocardium. We used isoflurane as the

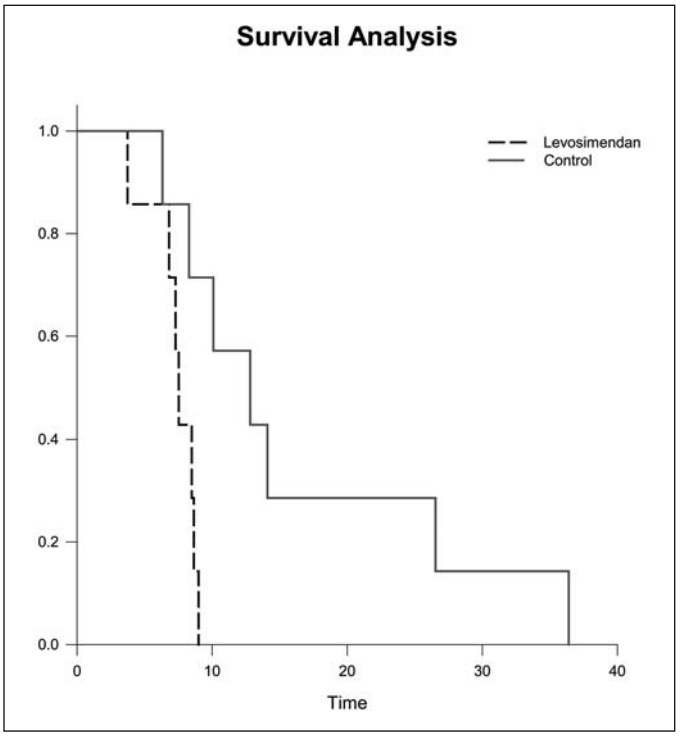

Figure 2: Kaplan-Meier graph of levosimendan and control group survival times.

anesthetic of choice due to the availability in the laboratory. There is, however, data that shows it has adverse effects on myocardium. Isoflurane in certain settings can induce a rise in intracellular calcium, especially during hypoxia [11], although this rise in intracellular calcium has not been shown to have lusitopic effects [12]. In combination with levosimendan, this effect may account for the fact the experimental animals expired relatively shortly after the administration of the second dose of levosimendan. The addition of a peripheral vasoconstrictor, such as norepinephrine, may prove beneficial; however, the results of this study showed such a dramatic negative effect on the animals that we are not optimistic that adding other interventions will be advantageous.

LIMITATIONS

Limitations of this study begin with the general limitations of using a rat model to evaluate cardiac physiology and then extrapolating to a human effect. This model was chosen since it was the smallest and most economical that allowed us to monitor physiologic variables and give infusions. The effect of verapamil on rat myocardium had been examined in previous studies [5], thus the rat seemed to be a reasonable model for testing the feasibility of using levosimendan to counteract verapamil overdose.

In vivo and in vitro studies showing levosimendan's effect on rat myocardium have been conducted, but a precise efficacious dose or dosing regimen is currently being debated. The dose chosen for this experiment was extrapolated from human data; theoretically, other doses could be beneficial. Our decision to administer the levosimendan as a bolus at time 0 and the 5-minute mark was arbitrary. Levosimendan is typically administered in a single bolus followed by infusion, which may offer a different outcome [3].

Perhaps the most salient limitation, however, is the fact that levosimendan is not available in the United States as an active pharmaceutical. Abbott Pharmaceuticals (Abbott Park, IL) refused to sell the drug to us for this study; therefore, it had to be manufactured specifically for this experiment. The material was supplied by ACC Corporation (San Diego, CA) as a lyophilized powder that had to be reconstituted. It was not the identical formulation that is infused into human patients. During reconstitution, a small amount of solid is usually lost, thus the precise concentration of levosimendan administered to the animals could not be verified without expensive testing, which we did not pursue for financial reasons.

CONCLUSION

Levosimendan did not prolong the time to asystole in verapamil-poisoned rats. In fact, it accelerated the time to asystole in the treatment group. Levosimendan is not an effective treatment for verapamil poisoning in this rat model.

The authors have no potential financial conflicts of interest to report.

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