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TOXICOLOGY INVESTIGATION

Comparative Hemodynamic Effects of Levosimendan Alone and in Conjunction with 4-Aminopyridine or Calcium Chloride in a Rodent Model of Severe Verapamil Poisoning

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Abstract Levosimendan (Levo) increases sensitivity of troponin-C to calcium, thus increasing myocardial contractility. It is also a vascular K+-ATP channel agonist producing peripheral vasodilation. Previous research with levosimendan revealed an increase in cardiac output (CO) but not blood pressure (BP) in experimental verapamil poisoning. Levosimendan's K+-channel agonist properties may augment verapamil's vasodilatory effects. 4-Aminopyridine (4-AP) is a K+-channel antagonist. It has successfully reversed hypotension in experimental verapamil poisoning. We hypothesized that coadministration of these agents may improve BP and CO in verapamil poisoning. Anesthetized, ventilated, and canulated male Wistar rats were poisoned with verapamil. Animals received one of six treatments, which are as follows: (1) n-saline infusion (control), (2) Levo 6.25 μg/kg loading dose and 36 μg/kg/h infusion, (3) 4-AP 2 mg/kg loading dose and infusion (4-AP), (4) Levo+4-AP, (5) $CaCl_2$ loading dose and infusion, and (6) Levo+$CaCl_2$. Hemodynamic parameters were recorded for 60 min. Outcome measures were changes in CO, BP, and heart rate (HR) compared to control. All groups had similar pretoxicity and peak toxicity CO (50% of pretoxicity value), BP (50% or pretoxicity value), and HR. Control group CO, BP, and HR progressively dropped during the verapamil infusion. Levosimendan produced a statistically significant improvement in CO (75% of pretoxicity level) but not BP in comparison to control. 4-AP produced a significant improvement in CO (110% of pretoxicity) and BP (78% of pretoxicity). Levo+4-AP and Levo+$CaCl_2$ groups improved CO (100% of pretoxicity) and BP (77% and 50% of pretoxicity, respectively), but there was no additive increase in CO or BP in animals compared to 4-AP or $CaCl_2$ alone. Levosimendan moderately improved CO but not BP in verapamil poisoning. The hypotensive effects of levosimendan were not overcome by coadministration of either 4-AP or $CaCl_2$. Levosimendan may not be an appropriate agent to use in the treatment of verapamil poisoning.

Keywords Poisoning · Overdose · Calcium channel blockers · Verapamil · Levosimendan · Aminopyridine · Rodent

Introduction

Verapamil is regarded as potentially the most toxic of the calcium-channel-blocking agents in overdose, producing both negative inotropic and chronotropic effects on the myocardium as well as peripheral vasodilatation with resultant severe hypotension from a combination of reduced cardiac output and fall in systemic vascular resistance. To date, there has been no single antidotal agent that reliably reverses shock in verapamil poisoning. Patients often require more than one agent to maintain blood pressure.

Levosimendan donated for research purposes by Abbott Australasia, Pty Ltd.

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This may include a combination of inotropic and pressor drugs to produce a positive hemodynamic response. Levosimendan (Levo) is a novel cardiac inotropic agent that exerts its effects by sensitizing troponin-C to calcium, thus increasing myocardial contractility without increasing intracellular calcium and adenosine triphosphate concentrations or myocardial oxygen demand [1, 2]. It also possesses mild inhibitory actions on phosphodiesterase-III and relaxes peripheral vascular smooth muscle by agonism of vascular smooth muscle potassium channels [3]. As a result, levosimendan increases cardiac output and reduces after load. These effects may be beneficial in other forms of heart failure. Given the effects of levosimendan on modulation of myocardial calcium, we theorized that it may be a useful agent in reversal of verapamil-induced cardiac toxicity and have previously reported on the effects of this agent in a rodent model of verapamil poisoning [4]. Levosimendan produces a moderate but significant improvement in cardiac output in verapamil poisoned rats but does not result in a concomitant rise in systemic blood pressure suggesting that its vasodilatory properties may antagonize any benefits seen in improvement in cardiac output. Additionally, the coadministration of other potentially vasoactive agents with levosimendan, such as calcium chloride and norepinephrine, do not improve blood pressure or result any additional increase in cardiac output compared either agent infused on its own [4, 5]. As a result, we were unable to overcome the hypotension seen in verapamil poisoning despite moderate improvements in cardiac output seen with infusion of levosimendan. The current study aims to determine whether the administration of a specific K+-channel antagonist in conjunction with levosimendan overcomes the lack of improvement in blood pressure seen when levosimendan is administered as a single agent in verapamil poisoned rodents. 4-Aminopyridine (4-AP) is a potent K+-channel antagonist that has been shown to improve hemodynamic instability in experimental and in a small number of human cases of verapamil poisoning [6, 7]. This agent acts by an indirect influence on calcium channels. 4-AP blocks $K^{+}1$ potassium channels on the cytoplasmic side of the cellular membrane, blocking the efflux of intracellular potassium, resulting in depolarization of voltage-dependent calcium channels and influx of calcium into the cell [7].

Method

This was a randomized open-label study utilizing male adult albino Wister rats. Blinding was not performed as a single investigator performed the experiments once surgery was completed. The method was similar to that described previously [4].

Animals

Consent was obtained from the University of New South Wales Animal Care and Ethic Committee. Male adult albino inbred Wister rats (300 to 500 g) were used to avoid problems associated with the oestrus cycle of the female rat.

Pre-experiment Procedures

Animals were anesthetized initially with halothane in an enclosed Perspex anesthesia box. Once unconscious, 120 mg/kg thiobutabarbital, a long-acting barbiturate agent, was intraperitoneally administered (Inactin, Sigma Chemicals, MO, USA). Before the start of surgical procedure, animals were administered 0.04 mg/kg fentanyl citrate (Mayne Pharma, Melbourne, Australia). Animals were placed on a heating pad and under a heating lamp to maintain body temperature between 36°C and 38°C. The trachea was canulated, and the animals were ventilated (10 ml/kg and 45 breaths per minute) using a Ugo Basile model 7025 Ventilator (Comerio, VA, Italy). The left carotid artery was canulated, and a MLT1402 T-type Ultra Fast Thermocouple probe (AD Instruments, Castle Hill, Australia) was passed through the canula for aortic blood temperature measurement for cardiac output estimation by the thermodilution technique. The left and right jugular veins were canulated with double lumen polyethylene catheters (North Rocks, Australia) for infusion of drugs and fluids. The left femoral artery was canulated with a single lumen polyethylene tubing (BD Diagnostics, Sparks, MD, USA) for arterial blood pressure monitoring via an ADInstruments MLT844 physiological pressure transducer and ML110 bridge amp (ADIstrusments, Castle Hill, Australia). A rectal temperature probe was inserted for tissue temperature monitoring. Cutaneous ECG electrodes were placed for single lead III recording of heart rate and rhythm. Continuous data collection for ECG, arterial blood pressure, and rectal and central temperature was performed using a PowerLab 4/30 data acquisition system and Chart Version 5.0 software (ADInstruments, Castle Hill, Australia). Cardiac output was estimated sequentially with the PowerLab cardiac output system and cardiac output Module Software (ADInstrument, Castle Hill, Australia). Briefly, 200 µl of cold Hartmann's solution was injected via the left internal jugular vein, and a thermodilution curve was recorded from the carotid thermistor probe on PowerLab Chart Software. Cardiac output was calculated using the area-under-the-curve by Cardiac Output Module found in PowerLab Chart Software. Cardiac output is reported in milliliters per minute per 100 g. Average cardiac output for healthy rats is reported as 20–25 ml/min/100 g [8].

Once surgery was completed, animals were allowed 30 min for equilibration. Prior to commencement of

experiment baseline heart rate (HR), mean arterial blood pressure (MAP), systolic blood pressure (SBP), and cardiac output (CO) were recorded. Hemodynamic parameters were then recorded at peak toxicity (time 0) and every 10 min during treatment for 1 h or until death. Two or sometimes three syringe pumps were used for drug infusion during the experiment (Graseby 3100; Terumo model STO 523). Animals were euthanized at the end of the experiment.

Experimental Protocol

Animals were administered verapamil hydrochloride (isoptin injection, 2.5 mg/ml, purchased from Abbott Australasia) by continuous infusion of 6 mg/kg/h until MAP fell to 50% of baseline (time 0). The verapamil infusion was then reduced to 4 mg/kg/h to maintain toxicity. Once toxicity was established, HR, MAP, SBP, and CO were recorded, and animals were randomly assigned to one of six treatment groups (ten rats in each group).

The treatment groups were:

Group 1 (Control)

Rats received normal saline loading dose of 10 ml/kg infused over 10 min followed by normal saline infusion of 10 ml/kg/h for 50 min.

Group 2 (Levo)

Rats received levosimendan 6.25 μg/kg in 10 ml/kg of n-saline as loading dose infused over 10 min followed by 36 μg/kg/h as an infusion dose in 10 ml/kg/h n-saline for 50 min.

Group 3 (4-AP)

Rats received 2 mg/kg loading dose of 4-aminopyridine over 10 min in 10 ml/kg n-saline followed by 2 mg/kg/h in 10 ml/kg/h n-saline for 50 min.

Group 4 (Levo and 4-AP)

Rats received levosimendan loading dose of 6.25 μg/kg/min in 5 ml/kg n-saline and 4-aminopyridine loading dose of 2 mg/kg/min in 5 ml/kg/h n-saline over 10 min. After this, levosimendan 36 μg/kg/h and 4-aminopyridine infusion at 2 mg/kg/h were each administrated in 5 ml/kg/h n-saline for 50 min.

Group 5 ($CaCl_2$)

Rats received 0.2 mmol/kg loading dose of calcium chloride over 10 min in 10 ml/kg n-saline followed by 0.2 mmol/kg/h in 10 ml/kg/h n-saline for 50 min.

Group 6 (Levo and $CaCl_2$)

Rats received levosimendan loading dose of 6.25 μg/kg in 5 ml/kg n-saline and calcium chloride loading dose at 0.2 mmol/kg in 5 ml/kg n-saline over 10 min. After this, levosimendan (36 μg/kg/h) and calcium chloride (0.2 mmol/kg/h) were each administered in 5 ml/kg/h n-saline for 50 min.

Treatment protocols lasted a total of 60 min. Ten minutes for loading dose followed by 50 min for maintenance infusion. HR, CO, SBP, and MAP were measured every 10 min for the duration of treatment. All animals received 10 ml/kg loading dose of fluid and 10 ml/kg/h as an infusion with their respective treatments.

Data were analyzed and plotted graphically as mean± SEM for the variables every 10 min until the end of the 60 min treatment protocol or death using Graph Pad Prism Version 4.03 Software (GraphPad Software, Inc.).

Statistical Method

Continuous hemodynamic measures were examined using one-way analysis of variance and Dunnett's posttest comparing each treatment group to control. Mortality at 60 min was assessed by Fisher's exact test comparing each treatment groups to the control group. Results were considered statistically significant at $p<0.05$ (two-tailed). All statistical analyses were carried out using GraphPad InStat 3.01 statistical software (GraphPad Software, Inc.).

Results

Prior to the administration of verapamil, baseline SBP, CO, HR were similar in all groups (Table 1), and there was also

Table 1 Summary of baseline hemodynamic parameters prior to verapamil administration expressed as mean±SEM

t=−10	Control	Levo	4-AP	Levo+4-AP	Calcium	Levo+Calcium	
CO (ml/100 g)	18.9±0.6	19.2±0.9	21.5±0.8	19.2±1.2	18.2±1.2	20.4±0.9	NS
SBP (mmHg)	158.4±3.1	129.8±4.09	148.6±6.5	135.5±4.1	139.6±7.2	141.5±7.5	NS
HR (bpm)	334.5±13.0	345.3±11.8	328.8±18.6	336.5±10.3	345.8±11.3	358.0±6.8	NS
Weight (g)	417.5±12.4	386.8±8.7	413.0±10.3	415.4±7.6	425.4±8.6	401.8±11.8	NS

NS not significant

Table 2 Summary of hemodynamic parameters at the time of peak toxicity (t=0) expressed as mean±SEM

t=0	Control	Levo	4-AP	Levo+4-AP	Calcium	Levo+Calcium	
CO (ml/100 g)	8.3±1.2	11.9±1.1	13.8±1.4	12.4±1.3	11.4±1.1	11.8±1.0	NS
SBP (mmHg)	58.4±3.1	61.9±1.7	61.5±4.3	58.7±3.9	56.3±4.4	61.0±3.3	NS
HR (bpm)	304.8±23.6	308.5±19.8	313.3±12.5	290.6±11.4	286.2±20.2	322.3±14.5	NS

NS not significant

no significant difference in peak toxicity hemodynamic parameters (time 0) in all groups (Table 2). Animal weights were also comparable between groups.

Survival

Only four of ten (40%) animals survived the protocol in control group. Death resulted from a combination of bradycardia and hypotension. In the group receiving calcium infusion, there was a 100% survival till the end of the experiment. This was statistically significant compared to control (p=0.0108) by Fisher's exact test. The 4-AP and Levo+$CaCl_2$ groups had survival in nine out of ten animals at 60 min (90%; p=0.05 vs control). The Levo and Levo+4-AP groups showed survival in eight out of ten animals at 60 min (80%). This was not statistically significant compared to control for these two groups (Fig. 1).

Hemodynamic Effects of Levosimendan, 4-Aminopyridine, and Calcium Chloride

i) Response of cardiac output to treatment

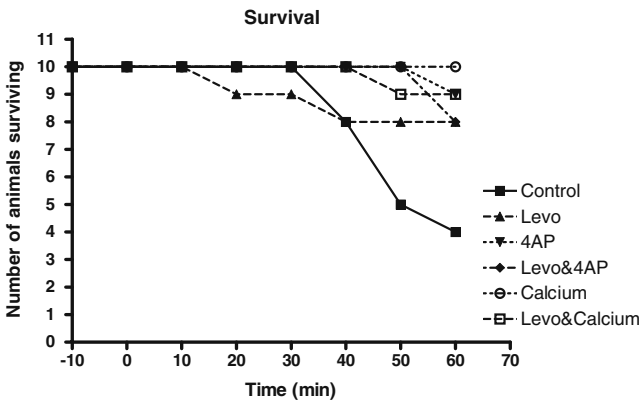


Fig. 1 Comparison of survival of animals in five treatment groups and control over the 60-min time course of study. Calcium chloride-treated group (p=0.012) significant survival difference compared with control by Fisher's exact test. 4-Aminopyridine and levosimendan+calcium groups significant survival compared with control (p=0.05). Levosimendan and 4-AP+levosimendan did not have statistically significant survival difference compared with control

Cardiac output was significantly higher than untreated control animals for all treatment groups (Fig. 2). The levosimendan treatment group showed a statistically significant improvement in cardiac output compared to control at t=30 and 40 min (p<0.05).

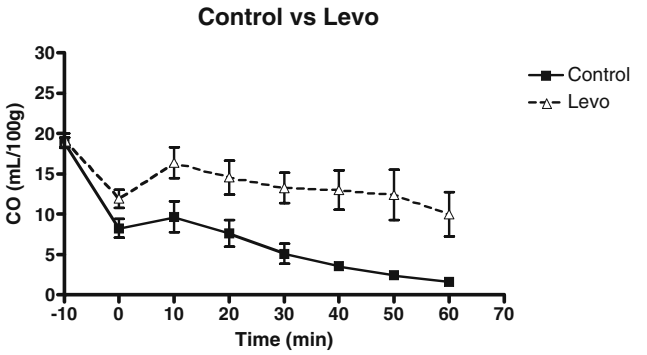
4-AP produced the most rapid improvement in cardiac output compared to control from 10 min after the start of the therapy (p<0.01). Levo+4-AP and $CaCl_2$ also produced a statistically significant improvement in cardiac output compared to control from t=20 min onward (p<0.01). Administration of Levo+$CaCl_2$ also resulted in an improvement in cardiac output compared to control particularly at the beginning of the treatment (p<0.05 at 10 min, p<0.01 from 20 min onward). Analysis of area-under-the-curve data for the treatment groups compared to control also showed significant differences for all treatments (Table 3). Notably, there did not appear to be any additive improvement in CO with coadministration of levosimendan with the other agents.

ii) Response of blood pressure to treatment

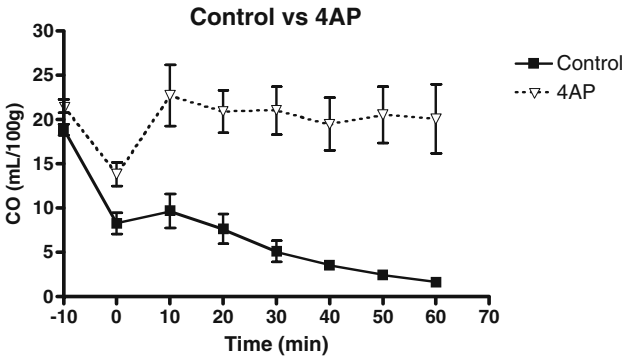
Treatment with levosimendan resulted in no improvement in SBP in this study compared to control group. Blood pressure deteriorated progressively till the end of the experiment (Fig. 3). Treatment with 4-AP alone resulted in a statistically significant improvement in systolic blood pressure as compared to control group (p<0.01) from t=+10 min until the end of the experiment. Levo+4-AP also produced a similarly statistically significant improvement in SBP (p<0.01). $CaCl_2$, in comparison to control group, also resulted in a significant improvement in SBP from t=+10 min (p<0.05) although SBP did not return to pretoxicity levels. Treatment with Levo+$CaCl_2$ only produced a significant improvement in SBP at t=30 min (p<0.05) and t=40 min (p<0.01). There was no additive effect on blood pressure with coadministration of levosimendan with the other agents.

iii) Response of heart rate to treatment

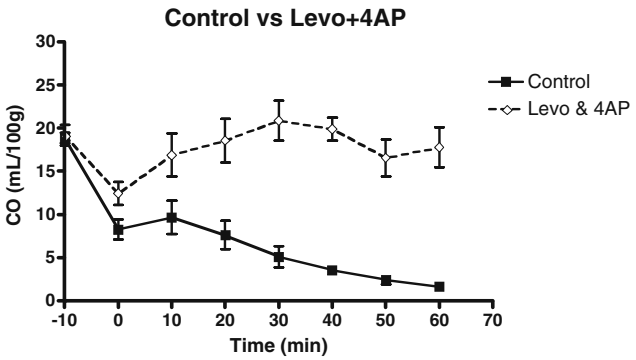
In the control group, a gradual decline in heart rate was observed (Fig. 4). Treatment with 4-AP maintained heart rate at the pretoxicity level compared to control throughout the period of verapamil poisoning at t=+30 min (p<0.05) and t=+40 and +60 min (p<0.01). Levosimendan, Levo+4-AP,



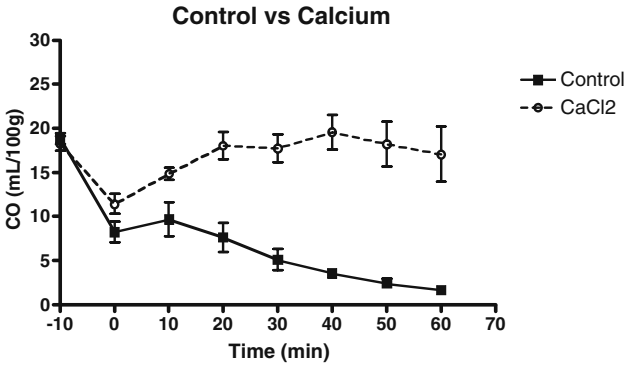
1. At t=-10, 0, 10,20,50 and 60, Control vs Levo not significant
2. At t = 30 and 40, Control vs Levo (P<0.05)



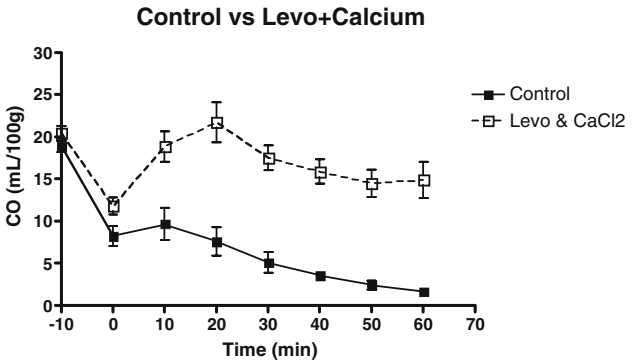
1. At t=-10 and 0, Control vs 4AP not significant
2 At t = 10, 20, 30, 40, 50 and 60, Control vs 4AP (P<0.01)

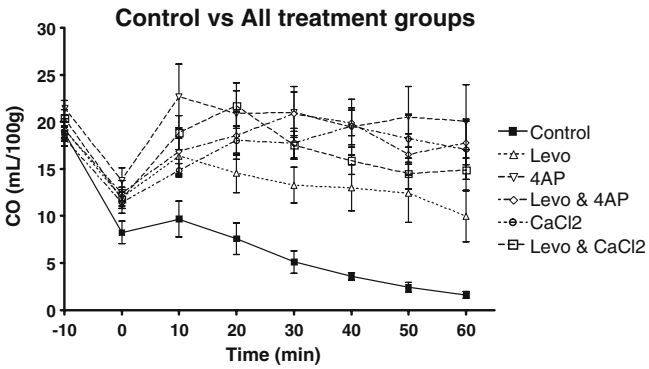


1. At t=-10, 0 and 10, Control vs Levo+4AP not significant
2. At t = 20, 30, 40, 50 and 60, Control vs Levo+4AP (P<0.01)



1. At t=-10, 0 and 10, Control vs $CaCl_2$ not significant
2. At t = 20, 30, 40, 50 and 60, Control vs $CaCl_2$ (P<0.01)





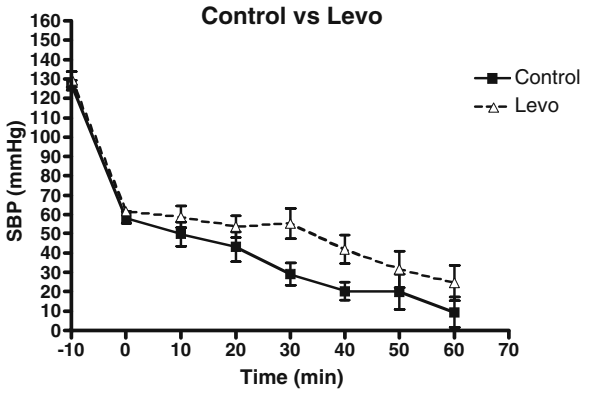
1. At t=-10and 0, Control vs Levo+$CaCl_2$ not significant
2. At t = 10, 50 and 60, Control vs Levo+$CaCl_2$ (P<0.05)
3. At t = 20, 30 and 40, Control vs Levo+$CaCl_2$ (P<0.01)

Fig. 2 The effects of experimental treatments on cardiac output (mean+SEM) in rats intravenously poisoned with verapamil

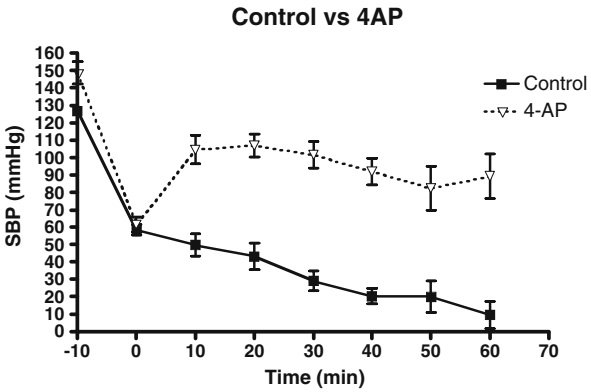
Table 3 A comparison of cardiac output area-under-the-curve for animals in each of the treatment groups compared with control

	Control	Levo	4-AP	Levo+4-AP	$CaCl_2$	Levo+$CaCl_2$
Mean AUC (ml/h/100 g)	420	861	1,356	1,210	1,084	1,155
95% CI	296–543	589–1,132	996–1,717	1,012–1,408	857–1,311	949–1,360

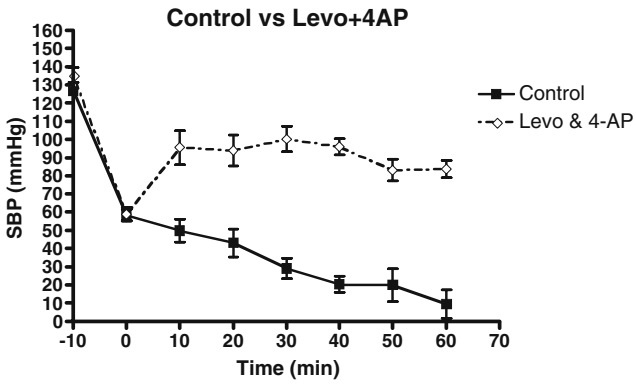
All groups area-under-the-curve (AUC) significant compared with control $p<0.01$ except Levo, $p<0.05$ (Dunnett's posttest). Levo AUC also significantly less than 4-AP, $p<0.01$ (Dunnett's posttest)



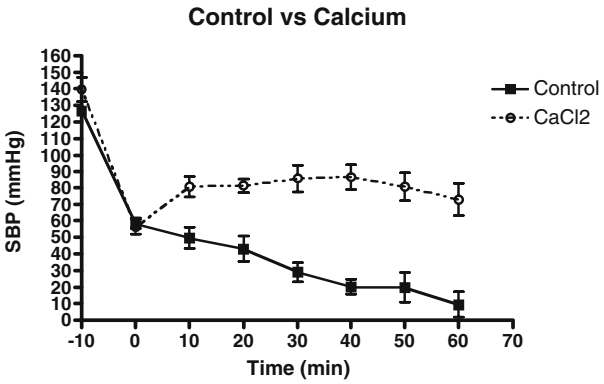
1. At t = -10,0, 10, 20, 30, 40, 50 and 60, Control vs Levo not significant



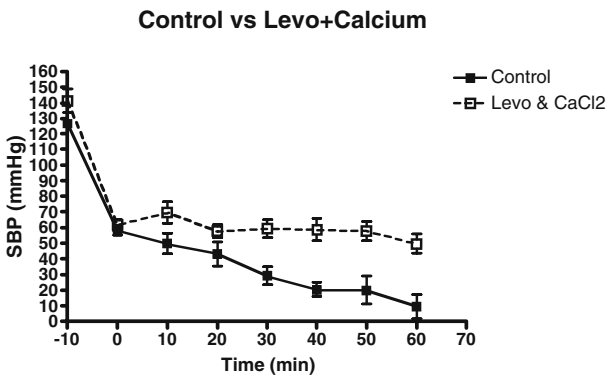
1. At t = -10 and 0, Control vs 4AP not significant
2. At t= 10, 20, 30, 40, 50 and 60, Control vs 4AP (P<0.01)

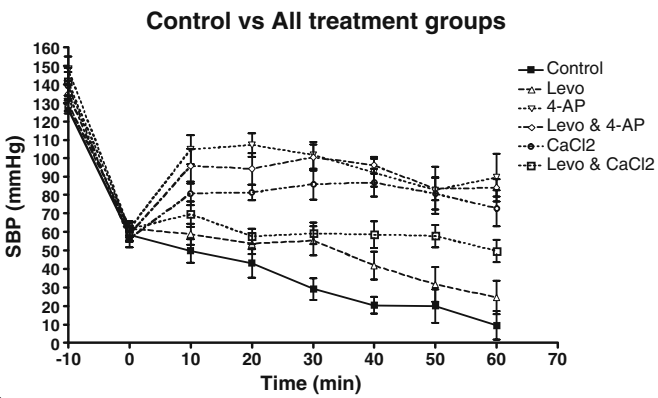


1. At t = -10 and 0, Control vs Levo+4AP not significant
2. At t= 10, 20, 30, 40, 50 and 60, Control vs Levo+4AP (P<0.01)



1. At t = -10 and 0, Control vs $CaCl_2$ not significant
2. At t=10, Control vs $CaCl_2$ (P<0.05)
3. At t= 20, 30, 40, 50 and 60, Control vs $CaCl_2$ (P<0.01)





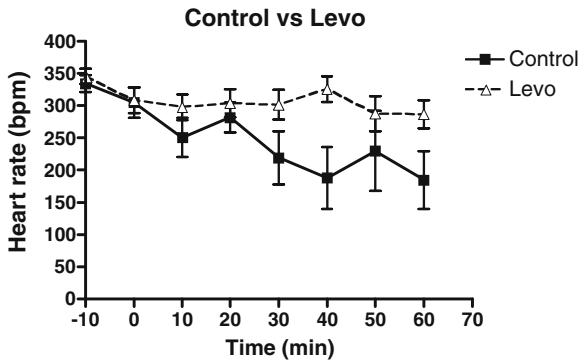
1. At t = -10, 0, 10, 20, 50and 60, Control vs Levo+ $CaCl_2$ not significant
2. At t=30, Control vs Levo+ $CaCl_2$ (P<0.05)
3. At t= 40, Control vs $CaCl_2$ (P<0.01)

Fig. 3 The effects of experimental treatments on systolic blood pressure (mean+SEM) in rats intravenously poisoned with verapamil

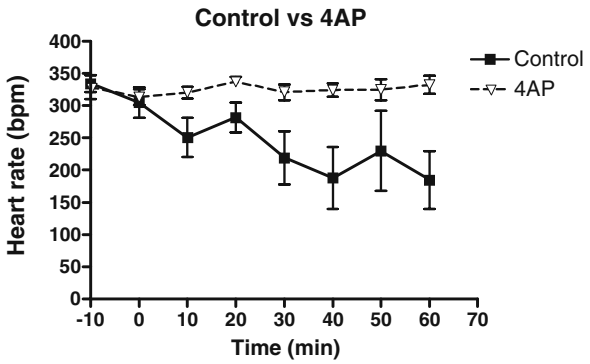
$CaCl_2$, and Levo+$CaCl_2$ treatment groups also maintained heart rate at the pretoxicity levels throughout the experiment.

iv) Complications of 4-aminopyridine

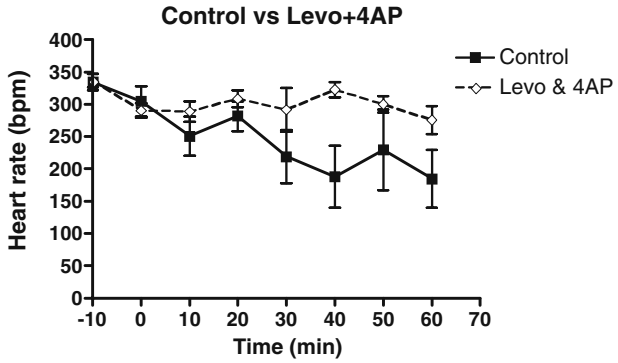
Treatment with 4-AP resulted in onset of generalized muscle fasciculation in all animals in the 4-AP group and the Levo+4-AP group after the 2 mg/kg loading dose. During the subsequent continuous infusion of 4-AP, intermittent muscle fasciculation persisted and was also associated with intermittent myoclonic jerking of all four limbs, which was either spontaneous myoclonic or epileptogenic activity, as well as hypersalivation in the animals in these treatment arms.



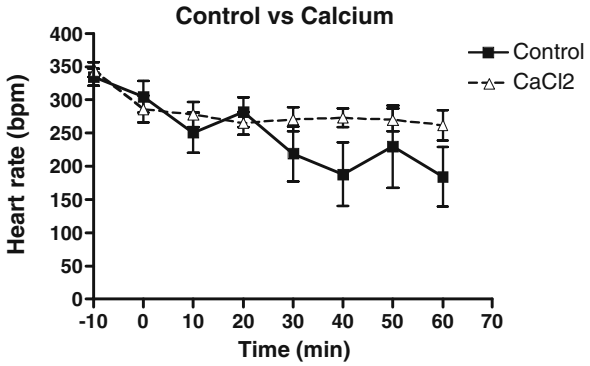
1. At t= -10, 0, 10, 20, 30 and 50, Control and Levo not significant
2. At t= 40, Control and Levo (P<0.01)
3. At t=60, Control and Levo (P<0.05)



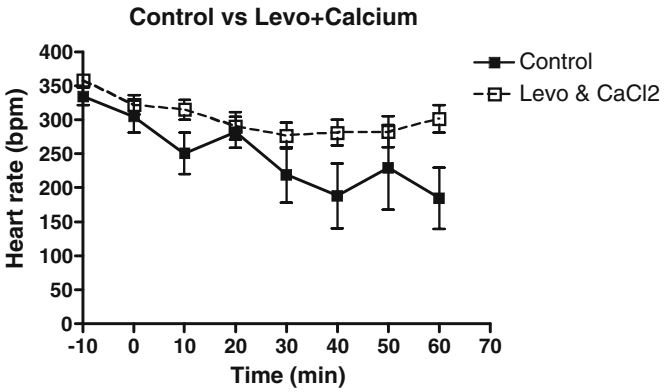
1. At t= -10, 0, 10, 20 and 50, Control and 4AP not significant
2. At t= 30, Control and 4AP (P<0.05)
3. At t= 40 and 60, Control and 4A (P<0.01)

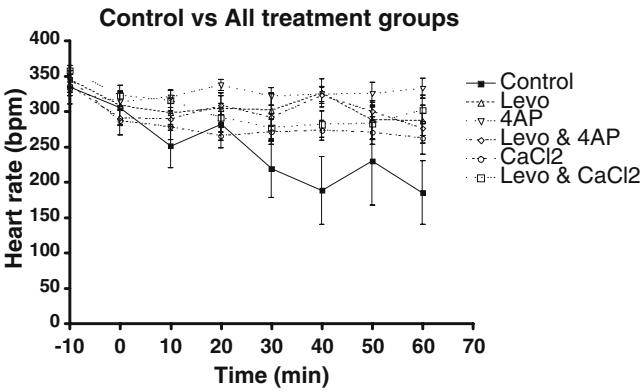


1. At t= -10, 0, 10, 20 and 50, Control and Levo+4AP not significant
2. At t= 30 and 60, Control and Levo+4AP (P<0.05)
3. At t= 40, Control and Levo+4AP (P<0.01)



1. At t= -10, 0, 10, 20, 30, 40, 50 and 60, Control and $CaCl_2$ not significant





1. At t= -10, 0, 10, 20, 30 and 50, Control and Levo+ $CaCl_2$ not significant
2. At t= 40, Control and Levo+$CaCl_2$ (P<0.05)
3. At t= 60, Control and Levo+$CaCl_2$ (P<0.01)

Fig. 4 The effects of experimental treatments on heart rate (mean+SEM) in rats intravenously poisoned with verapamil

Discussion

The present study confirmed the previous observations of Graudins et al. that levosimendan infusion alone showed a significant improvement in cardiac output when compared to control animals without a significant improvement in blood pressure [4]. Levosimendan is a positive inotropic agent. It has a short half-life of about an hour but is metabolized to an active metabolite, OR-1896, with an elimination half-life of 70–80 h. Levosimendan is currently used for treatment of decompensated heart failure caused by chronic cardiac failure, acute myocardial infarction, and post-cardiac bypass surgery [3, 9]. Several studies have demonstrated the benefits of levosimendan in patients after

cardiopulmonary bypass to enhance their cardiac performance [10]. Levosimendan is also a vascular smooth muscle ATP-potassium channels agonist, which reduces after load and peripheral vascular resistance [11]. From our observations, it appears that the vasodilatory properties of levosimendan may antagonize any benefit in blood pressure that might result from an increase in cardiac output. This suggests that levosimendan might not be an effective inotropic agent in treating verapamil poisoning.

4-Aminopyridine is an investigational drug, which has been suggested as a treatment in a number of neuromuscular disorders including multiple sclerosis, spinal cord injury, Alzheimer's disease, and myasthenia gravis. 4-Aminopyridine is a potassium channel antagonist with an indirect influence on calcium channels. It blocks voltage-dependent potassium channels on the cytoplasmic side in excitable membranes, leading to accumulation of K+ in the cytoplasm, resulting in rapid depolarization and opening of voltage gated calcium channels [6]. As a result, it is mechanistically appealing in reversal of verapamil poisoning. In a small number of human cases of verapamil intoxication where patients did not respond to calcium, atropine and vasopressor therapy, blood pressure and cardiac rhythm were improved quickly with the addition of 4-aminopyridine infusion [6]. The present study demonstrated that 4-aminopyridine (2 mg/kg) infusion significantly improved cardiac output and corrected verapamil-induced hypotension compared to control and Levosimendan alone in verapamil poisoned rats. These observations are consistent with previous animal studies assessing the effectiveness of this agent in verapamil poisoning. In a rat model of verapamil overdose, high dose of 4-aminopyridine (2 mg/kg) infusion improved blood pressure and heart rate, however, low dose 4-aminopyridine (1 mg/kg) infusion did not significantly increase the HR and MAP at 60 min and proved to be ineffective in the treatment of experimentally induced verapamil poisoning [12]. In another rat model study, Magdalan demonstrated similar results [6]. 4-Aminopyridine (2 mg/kg) increased blood pressure and heart rate. Moreover, 4-aminopyridine (2×0.5 mg/kg IV) reversed cardiodepression and hypotension completely within 50 min in a cat model of verapamil poisoning [13]. Similarly, 4-aminopyridine (1 mg/kg) produced improvements in heart rate, MAP, and cardiac output in verapamil poisoned dogs [14]. None of the previous studies utilizing rodent models measured the effect of 4-aminopyridine on cardiac output in verapamil poisoning. The response to treatment with 4-aminopyridine in animals appears to be dose-dependent. Animals exhibited adverse effects in the above studies as well as in our own study. Muscle twitching and convulsions are known complications of 4-aminopyridine use and despite its beneficial hemodynamic effects in CCB poisoning, these effects may preclude its safe use in humans. Our initial dose finding study, at 1 mg/kg, revealed that this lower dose of 4-aminopyridine did not produce a hemodynamic response in our model of verapamil poisoning. Although 4-aminopyridine has shown favorable effects on the cardiovascular system in this animal model of verapamil poisoning, significant side effects were observed. These included muscle fasciculation, myoclonus, possible seizure activity and increased oral secretions. These side effects commonly developed after the loading dose and continued during the infusion of 4AP. The epileptogenic action of 4-aminopyridine results from stimulation of glutamic acid release in the central nervous system. Glutamic acid stimulates NMDA receptors to enhance calcium ion influx to neurons, consequently resulting in convulsions. In humans, it has been reported that doses of 0.5–1 mg/kg may produce hyperexcitability and higher doses may trigger seizure activity [15]. These adverse effects are a significant drawback of using this agent in treatment of clinical verapamil overdose.

Coadministration of levosimendan with 4-aminopyridine did not reveal any added improvement in either cardiac output or blood pressure when compared to 4-aminopyridine alone.

As was seen in our previous study, intravenous calcium chloride infusion produced a significant increase in cardiac output and blood pressure compared to control in this study [4]. However, in the clinical setting of verapamil poisoning, calcium chloride does not result in consistent improvements in hemodynamic function. In verapamil poisoning, L-type calcium channels are blocked and increasing the extracellular calcium concentration may not help to increase the calcium concentration in the cytoplasm [6]. This may explain the transient effect of calcium chloride in the treatment of clinical verapamil poisoning. Alternatively, a lack of response to calcium may be a reflection of an inadequate dose of calcium being administered. In our study, rats received 0.2 mmol/kg. This is a relatively large dose of calcium in a clinical context. We did not measure serum calcium concentrations, but human case reports suggest that increasing serum calcium above physiological reference ranges may improve the response of blood pressure in CCB poisoning by increasing the extracellular to intracellular calcium concentration gradient [16]. In our study, when levosimendan was coadministered with calcium chloride, cardiac output improved significantly in the first 20 min and reached the preverapamil toxicity level, but SBP values gradually declined. Similar effects were seen with calcium alone.

Limitations

The present study was conducted in anesthetized rats, and there is an obvious limitation in application in human subjects. Notably, animals did not develop bradycardia during verapamil infusion despite a drop in blood pressure

of 50% from the baseline at the time of initiate treatment. Bradycardia is a significant early feature of human verapamil poisoning but only developed in rats as a preterminal finding. Consequently, the response to levosimendan or 4-aminopyridine in verapamil poisoned rats may differ to that seen in humans. Hence, the result from this study may not truly reflect the actual physiological or pharmacological response seen in humans. Nevertheless, levosimendan has improved cardiac output in both human and animal models of nontoxicological severe heart failure [17]. Moreover, in this poisoning model, rats were administered a continuous infusion of verapamil to simulate continuous GI absorption of the drug from a sustained-release ingestion. As a result, a severe degree of toxicity was evoked which may not mimic the pathophysiology of sustained-released ingestion in a real clinical setting. We did not examine the metabolic effects of verapamil poisoning and metabolic responses to the various treatment arms. An assessment of acid-base status, blood glucose and lactate concentrations may have provided additional information on the physiological response to each therapeutic intervention. This was a nonblinded experimental study. Since the investigator knew the treatment groups being administered in every experiment, observer bias may have been introduced into data collection. However, all the data collected in this study was objective hemodynamic data with little opportunity to make subjective interpretations of any response to the various therapies.

Thiobutabarbital is a long-acting barbiturate animal anesthetic, with minimal effects on hemodynamics in cardiovascular animal research [18]. In high doses, it may result in myocardial depression and lower the blood pressure in animals due to central inhibition of the vasomotor center. The doses used in this study were similar to those cited in previous research examining cardiovascular physiology in verapamil poisoned rodents [4, 5, 18].

Conclusion

Levosimendan produced moderate improvements in cardiac output in this animal model of verapamil poisoning. The benefits of its positive inotropic effects were negated by a lack of improvement in blood pressure with this agent, most likely related to levosimendan's peripheral vasodilatory effects. We were unable to show any additive effects on cardiac output or blood pressure by coadministration of 4-aminopyridine or calcium in the presence of levosimendan. Based on this animal research, extreme caution should be exercised if considering the use of levosimendan in human verapamil poisoning. The vasodilatory effects of this agent may compound the hemodynamic instability seen with calcium channel blocker poisoning and result in worsening of shock in the clinically poisoned patient.

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