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Therapeutic effects of intralipid and medialipid emulsions in a rat model of verapamil toxicity

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Background/aim: Lipid emulsions are promising as a potential new therapy for severe verapamil overdose. Our purpose is to draw attention to the choice of solution by investigating the efficacy of intralipid 20% or medialipid 20% in verapamil overdose.

Materials and methods: Eighteen adult Sprague Dawley rats were randomly divided into three groups: control (saline; Group C), intralipid 20% (Group I), and medialipid 20% (Group M). Rats were anesthetized with ketamine. Blood gas analysis, baseline heart rate (HR_b), and mean arterial pressure (MAP_b) were evaluated. Verapamil at 2.5 mg kg^{-1} min^{-1} was infused until the HR_b and MAP_b decreased by 50% and the times to HR_0 and MAP_0 were recorded. Treatment solutions of the groups were administered as 12.4 mL kg^{-1} in 5 min.

Results: While HR did not show a difference, MAP showed statistically significant differences among the groups. Intralipid 20% was more efficient than the other two treatments at an early stage; however, as the administration time progressed, medialipid 20% also turned out to be more efficient than the control treatment.

Conclusion: Our findings indicate that in a toxicity model of rats produced with verapamil, intralipid 20% and medialipid 20% solutions partially eliminate cardiac-depressant effects and increase the survival rate.

Key words: Verapamil, toxicity, cardiovascular collapse, intralipid, medialipid

1. Introduction

Verapamil is a calcium channel blocker with a potent negative inotropic effect used in the treatment of cardiac disorders such as hypertension, cardiac arrhythmias, heart rate control, and angina pectoris, as well as for cluster headaches and migraines. Toxicity due to verapamil overdose is generally fatal. Dysrhythmia, bradycardia, and hypotension due to sinoatrial node depression are common toxic symptoms. Supportive approaches, inotropic agents, and drugs such as calcium, insulin, and glucagon are standard treatment approaches (1).

Intravenous lipid emulsions are an important part of total parenteral nutrition. Protective and life-saving effects of intralipid 20% against the cardiotoxic effects of bupivacaine have been proved by experimental studies (2–5) and case presentations (6–8), and those effects have been applied in treatment protocols (9). In studies regarding overdose of verapamil, having a highly lipophilic characteristic, it has been reported that intralipid 20% might contribute to treatment. Lipid emulsions are promising as a potential new therapy for severe verapamil overdose (10,11).

This experimental study was designed to compare the therapeutic efficacy of intralipid 20% (long-chain triglyceride, LCT) and medialipid 20% (long- and medium-chain triglyceride, LCT/MCT) emulsions on verapamil toxicity. Our objective is to determine the efficacy of these two lipid emulsions when cardiotoxicity symptoms develop in a verapamil toxicity model in rats, without administering standard treatment medication, and to discuss the importance of solution choice.

2. Materials and methods

This study was performed at the Gazi University Faculty of Medicine Experimental Animals Research Center, Ankara, Turkey, with the approval of the Local Animal Ethics Committee (GU.ET-166-24523, 20, 2012).

Eighteen adult Sprague Dawley rats weighing between 300 and 400 g were divided into three groups. The rats were adapted to the environment by being kept at 20

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± 2 °C for 7 days, with 12 h in daylight and 12 h in the dark. They were fed on standard pellet rat feed and water. Anesthesia was performed with 50 mg/kg IM ketamine (Ketalar, Pfizer, İstanbul, Turkey). Head and abdominal fur were shaved, and rats were placed supine on a heating blanket and their extremities were fixed. Rectal temperature was monitored to maintain normothermia. Needle electrodes were placed on the forefeet bilaterally and the left rear foot. Electrocardiograms were recorded in D2 derivation (MINDRAY Patient Monitor PM-8000 Express, Shenzhen MINDRAY Bio-Medical Electronics Co. Ltd.). The tail vein was cannulated and the drugs and liquids involved in the study were administered via an infusion pump (Perfuser, B. Braun Melsungen AG, Spase, Germany). Surgical tracheostomy was performed by 16-G or 18-G IV cannula. For neuromuscular block, 0.1 mg kg^{-1} vecuronium (Blok-L, vecuronium bromide, 4 mg, Mustafa Nevzat İlaç Sanayi A.Ş., İstanbul, Turkey) was administered as a bolus. Respiration was maintained at 100% O_2, 4 L/min fresh gas flow, 12 mL kg^{-1} tidal volume, and 40–55 breaths per minute via an automatic ventilator (Harvard Rodent Model, Inspira ASV, Hollstone, MA, USA). Invasive blood pressure was monitored though cannulation of the abdominal aorta (SASAN Medical Disposable Products Pressure Set, Ankara, Turkey).

The rats were randomly assigned to one of three groups (six rats each): control (saline; Group C), intralipid 20% (Group I; Intralipid 20%, Fresenius Kabi AB, Uppsala, Sweden), and medialipid 20% (Group M; Lipofundin MCT/LCT 20%, B. Braun Melsungen AG).

Following the completion of all the invasive procedures, electrocardiograms and invasive blood pressure of the rats were monitored for 10 min and the data belonging to the 10th minute (T_b) heart rates (HR_b) and mean arterial pressure (MAP_b) were recorded as baseline values. Blood gas analysis was evaluated before the experiment (NOVA Biomedical, Stat Profile, Critical Care Xpress, Waltham, MA, USA). The experiment was then started by administering verapamil (Isoptin, 5 mg in 2 mL, Abbott Laboratories, Abbott Park, IL, USA) in a 2.5 mg kg^{-1} min^{-1} infusion in the groups. The times to reach 50% of HR_b and MAP_b were marked as T_0 and the data were recorded as HR_0 and MAP_0. When MAP decreased by 50%, verapamil infusion was stopped and treatment solutions, saline, intralipid 20%, or medialipid 20%, were infused at 12.4 mL kg^{-1} in 5 min. Effects of treatment solutions on HR_0 and MAP_0 were recorded once every 5 min for the first 20 min (T_0, T_5, T_{10}, T_{15}, T_{20}) and once every 10 min following the first 20 min ($T_{30,}$ T_{40}, T_{50}, T_{60}). Time of asystole was recorded until the 60th minute, and if the rats did not die at this time, intracardiac blood was removed.

Statistical analysis was performed using SPSS 18.0 for Windows. Numerical variables are presented as mean ± standard deviation. Whether there were differences among the groups in terms of weight, pH, CO_2, O_2, and HCO_3 was evaluated using the Kruskal–Wallis test. Differences among the groups and within-group changes in terms of HR and MAP were evaluated using a linear mixed model. The level of statistical significance was determined as $P < 0.05$.

3. Results

The body weights (g) of the rats were similar. No statistically significant differences were observed in baseline control blood gas values (pH, $PaCO_2$ mmHg, PaO_2 mmHg, and $HCO3^-$ mEq/L) ($P > 0.05$). MAP_b was significantly higher in Group I than in Group C and Group M, while MAP_b was lower in Group C than in Group M ($P < 0.004$) (Table 1). Duration of infusion and the infused doses of verapamil were similar among the groups ($P > 0.05$) (Table 2).

HR recorded at T_5, T_{10}, T_{15}, T_{20}, T_{30}, T_{40}, T_{50}, and T_{60} did not show a significant difference ($P = 0.304$) according to HR_0 among the groups (Table 3). MAP and HR values decreased significantly when compared to HR_0 and MAP_0 values within the groups ($P < 0.001$) (Tables 1 and 3). When the values were evaluated at different time stages, Group I was found to be significantly higher in all stages between MAP_{10} and MAP_{60} than Group C. Group I was only significantly higher in MAP_{10} when compared to Group M. Group M was found to be significantly higher than Group C in MAP_{15}, MAP_{20}, and MAP_{30} stages (Table 1). When the experiment ended (T_{60}), the surviving number of rats was 4, 2, and 3 and the rate of death was 66.6%, 33.3%, and 50% ($P = 0.507$) in Group C, Group I, and Group M, respectively.

4. Discussion

In the present experimental study, lipid emulsions having the same concentration but different formulations were evaluated with regard to verapamil toxicity. When assessed in terms of survival rate within 60 min, although the intralipid 20% (33.3%) treatment seemed to be more advantageous then medialipid 20% (50%) and saline (66.7%), the expected benefit was not fully provided.

Verapamil is the most serious poisoning calcium channel blocker that causes overdoses due to its cardiodepressive effect. In the literature, there are experimental studies in which different doses were used to develop verapamil toxicity in rats (10,12–14). In this study, the dose of verapamil was selected as 2.5 mg kg^{-1} min^{-1} based on the study by Magdalan (14). Tebbutt et al. (10) showed the rats treated with intralipid 20% lived longer than those in the control group and they stated that intralipid 20% at 12.4 mL kg^{-1} was the minimum dose for a mean length of life of 63 min in verapamil infusion. Similarly, in the present study, the chosen volume of lipid emulsions was 12.4 mL kg^{-1} in 5 min as soon as verapamil infusion was stopped.

Table 1. Mean arterial pressure values (mean ± standard deviation).

mmHg	Groups Control (n = 6)	Intralipid (n = 6)	Medialipid (n = 6)	P among groups
MAP_b	73 ± 24.9	111.5 ± 26.6†	101 ± 12.2*	0.004
T_0	34 ± 15	53.7 ± 14	45.7 ± 7.1	0.224
T_5	10.3 ± 3.7	28.7 ± 22.6	18.3 ± 7	0.273
T_{10}	14.3 ± 13.7	47.2 ± 31.6†	16 ± 14.1	0.007
T_{15}	20 ± 22.1	52.3 ± 37.2‡	53 ± 34*	0.009
T_{20}	19 ± 23.6	52 ± 37.7‡	59.7 ± 12.9*	0.007
T_{30}	18.7 ± 23.1	63 ± 34.8‡	58.7 ± 15.6*	0.003
T_{40}	20 ± 20.3	77 ± 8.8‡	52 ± 18.5	0.003
T_{50}	21.7 ± 20.6	74.5 ± 10.5‡	46 ± 15.7	0.009
T_{60}	28 ± 12.7	77.3 ± 13.1‡	44.3 ± 14.6	0.008
Within-group P	< 0.001	< 0.001	< 0.001	

P < 0.05 is significant; MAP, Mean arterial pressure; T, time, b, baseline.
†Intralipid is higher than control and medialipid; ‡Intralipid is higher than control; *Medialipid is higher than control.

Table 2. Duration and doses of infusion of verapamil in groups (mean ± standard deviation).

Groups	Time (s)	Dose (mg)
Control (n = 6)	105.0 ± 45.5	1.4 ± 0.5
Medialipid (n = 6)	95.8 ± 27.3	1.3 ± 0.4
Intralipid (n = 6)	79.2 ± 16.9	1.1 ± 0.3

P > 0.05 for all parameters in all groups.

Table 3. Heart rate values in groups (mean ± standard deviation).

beats/min	Groups Control (n = 6)	Intralipid (n = 6)	Medialipid (n = 6)	P among groups
HR_b	232.8 ± 17.8	238.3 ± 33.7	251.3 ± 42.3	0.727
T_0	159.8 ± 49.2	148.3 ± 18	175.3 ± 58	0.525
T_5	125.3 ± 58.3	140 ± 29.4	126.5 ± 33.8	0.791
T_{10}	118 ± 38.5	119 ± 44.7	95.3 ± 44.2	0.606
T_{15}	104.3 ± 44	123 ± 43.4	86.8 ± 46.2	0.135
T_{20}	90 ± 36.1	122 ± 52.7	116 ± 17.4	0.205
T_{30}	96 ± 17.8	117.6 ± 57.2	115 ± 4	0.581
T_{40}	83.7 ± 31,8	134 ± 35.4	102 ± 8.5	0.207
T_{50}	70 ± 40.1	129.8 ± 35,3	88.7 ± 9	0.104
T_{60}	80 ± 38.2	126.3 ± 36.7	82.3 ± 4.6	0.195
Within-group P	<0.001	<0.001	<0.001	

P < 0.05 is significant; HR, Heart rate; T, time; b, baseline.

There are many standard treatment approaches for verapamil toxicity in humans; among these are calcium, atropine, adrenaline, glucagon, amrinone, 4-aminopyridine, Bay K 8644, and insulin (13–16). French et al. (17) reported that sufficient hemodynamic recovery could not be obtained in a patient in shock due to verapamil overdose although all supportive methods were used. They observed that serum verapamil level decreased and MAP increased following the administration of intralipid 20%. It was also stated that the recovery of the patient cannot be related only to intralipid.

Liang et al. (11) concluded that intravenous lipid treatment was found to be effective in massive calcium channel blocker overdose, and its early start in the case of hemodynamic compromise was recommended.

In another study, it was reported that when intravenous lipids were used along with standard treatment methods such as atropine and calcium chloride, the increases in MAP and survival rate were higher. The authors also pointed out that drugs that do not interact with lipid solutions should be selected as they do not affect intracellular fatty acids and carbohydrate metabolism (18).

Jamaty et al. (19) stated that lipid emulsions decrease mortality in cases of poisoning due to lipophilic agents; however, since their reliability has not been proven yet, they cannot replace common antidotes or supportive special treatments in cardiotoxic agent poisoning.

The "Lipid Rescue" protocol recommended that in cases of local anesthetic toxicity lipid rescue treatment should be started as early as possible along with basic life support, that epinephrine should not be administered at more than 10 μg kg^{-1}, and that vasopressin should not be used (16). The question is whether this protocol is suitable for cases of lipophilic drug toxicity. We are of the opinion that lipid rescue should be done as soon as possible together with known standard treatment approaches such as calcium or insulin and glucose in verapamil toxicity.

Because of their cardiovascular effects depending on their lipid components, lipid emulsion should be used with caution. When compared with LCTs, MCTs have the same superior qualifications. MCTs are absorbed, hydrolyzed, and transported easily and make energy available rapidly, and they have a smaller molecular size and greater water solubility with very low tendency to be deposited as body fat (20).

Contradictory views are also found in different studies. Li et al. (21) reported that long-chain triglyceride emulsion in rats for local anesthetic-induced cardiovascular collapse showed superior hemodynamic recovery compared to long- and medium-chain triglyceride emulsion. In another study, the authors concluded that intravenous infused lipid emulsion increased left ventricular pressure via blockading the nitric oxide released from endothelial cells and the positive inotropic effect of medialipid 20% was found superior to intralipid 20% (22). However, in cases where rapid administration of lipids at high volumes would cause a risk, medialipid could be considered as an alternative, and it could be administered in higher doses than intralipid due to its molecular structure. In cases where higher doses are required, medialipid 20% may be considered.

The main limitation of this study is the small sample size (six rats in each group). Another limitation is the potential effects of ketamine, including stimulation of the sympathetic tone, positive inotropic effects, and ion channel blocking action (23). The occurrence of differences in baseline MAP values among the groups is another limitation of this study. Heavy blood loss during the preparation of the some rats for the study might be an explanation for this limitation. The similarity among the groups in times and doses required in decreasing MAP values by 50% eliminated this limitation. All data during the treatment period were compared with this time (T_0).

In conclusion, although the benefits of lipid rescue in bupivacaine toxicity have been proved, the benefits of a similar rescue protocol for verapamil should be discussed and further studies are required to determine efficient doses and combinations. Our findings indicate that in a toxicity model of rats produced with verapamil, the supportive hemodynamic effects of intralipid 20% and medialipid 20% emulsions do not show a significant difference. Both of them partially eliminate cardiac-depressant effects and increase the survival rate. We agree that lipid emulsions also have supportive treatment effects in addition to the standard treatment drugs for verapamil toxicity.

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