CHANGES IN THE HEART RATE OF RATS TREATED WITH VERAPAMIL OVERDOSE AND RESUSCITATED WITH LIPID EMULSION

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ABSTRACT

INTRODUCTION: Verapamil is a drug that is used often due to its wide spectrum of action. Many authors consider it to be the most dangerous of the calcium channel blockers due to its negative chronotropic and inotropic effect on the heart, leading to severe cardiodepression. Overdose is difficult to treat and is associated with high mortality despite existing treatment options.

Over the last 15 years, lipid emulsions (LEs) have been increasingly used for resuscitation after overdosing with lipophilic drugs such as verapamil. Despite the convincing results and the improved patient status, the association of the administration of LE with the recovery of patients may be questioned since LE was given in addition to standard therapy.

AIM: The aim of this article is to conduct an evaluation of the self-cardioprotective effect of LE in acute verapamil overdose (15 mg/kg) in rats by measurement of the heart rate and survival at the recommended LE dose of 1.5 mL/kg and 7 times the recommended LE dose (10 mL/kg).

MATERIAL AND METHODS: The experiment was performed on 30 male Wistar rats, provided by the Medical University of Varna. Instrumental methods included monitoring the heart rate of rats using an electrocardiographic monitor. The statistical analysis was performed using the statistical functions in Excel 2016 and Statistica 7.0.

RESULTS: A survival rate of 100% was observed in rats pretreated and treated with low and high LE dose. The high LE dose (10 mL/kg) showed a faster improvement in cardiac function as the highest mean heart rates were established.

CONCLUSION: Pretreatment and resuscitation with low or high LE dose reduce toxicity and prevent dose-dependent asystole induced by verapamil. The administration of a high LE dose (10 mL/kg) proved to be more effective in terms of heart rate in rats.

Keywords: *acute drug intoxication, lipid emulsion, resuscitation, verapamil*

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INTRODUCTION

Acute drug intoxication is a major problem worldwide, with antidepressants, neuroleptics, and cardiovascular drugs being the most commonly overdosed drugs. Most of these do not have a specific antidote and cause serious and sometimes fatal cardiovascular and neurological complications, necessitating the introduction into the routine practice of

more effective treatments such as intravenous lipid emulsions (LEs).

Initially, LEs were developed to meet the necessity for essential fatty acids in patients receiving parenteral nutrition. In the last 15 years, a new effect of LEs has been discovered, initially for the treatment of local anesthetic systemic toxicity (LAST), and subsequently, their ability to capture lipophilic drugs and extract them from target organs, thus reducing their toxicity. This phenomenon is known as "lipid sink". According to it, the rapid application of the LE into the bloodstream creates a lipid phase that absorbs lipophilic xenobiotics and prevents them from binding to target receptors. A concentration gradient is created, which allows toxins to be quickly removed from areas of high accumulation (brain, heart). Introduced by Weinberg in 1998, it is the most widely accepted mechanism of antidote action of LEs (1).

Over the last 15 years, LEs have been increasingly used in toxicological and intensive departments for resuscitation after overdosing with local anesthetics and lipophilic drugs. The dose determined by the American Society of Regional Anesthesia (ASRA) is a bolus intravenous dose of 1.5 mL/kg for one minute, followed by a continuous intravenous infusion of 0.25 mL/kg/min for 20–60 minutes until circulation is restored (2).

Despite the convincing results and the improved patient status, the association between the administration of LE with the recovery of patients can be questioned. Lipid emulson was given in addition to standard therapy and the effect of LE could not be associated with improving the clinical status of patients.

Verapamil is a calcium channel blocker that is used extremely often due to its wide spectrum of action: supraventricular arrhythmias, arterial hypertension, coronary heart disease, cerebral reperfusion, dysmenorrhea, migraine and others. Due to its strong cardiodepressive, negative chronotropic and inotropic effects, this drug is the most toxic calcium channel blocker and causes severe intoxication in case of overdose (3).

Despite the treatment, about 5% of the cases die (4). The parameters of cardiotoxicity include change in blood pressure, change in heart rate, rhythm and conduction disorders (5, 6, 7).

Verapamil is a lipophilic drug that suggests a good effect of the LE in case of overdose (1). For this reason, we conducted a laboratory experiment on rats with verapamil overdose to which we administered only 20% LE in low and high doses as resuscitation therapy to demonstrate its cardioprotective effect.

AIM

The aim of the study is to evaluate the self-cardioprotective effect of LE in acute verapamil intoxication in rats by measurement of the heart rate and survival at the recommended dose by ASRA (1.5 mL/kg) and 7 times the recommended dose (10 mL/kg) LE.

MATERIALS AND METHODS

The experiment was performed on 30 healthy male Wistar rats with an average weight of 243 g provided by the vivarium of the Medical University of Varna. The procedures for the treatment of animals were carried out with the permission and in accordance with the rules for working with experimental animals of the Ethics Committee of the Bulgarian Food Safety Agency (permission for the use of animals in experiments No. 214, valid until 12.10. 2023).

Experimental Drugs Used

- Isokor® solution for injection, 2.5 mg/mL—verapamil hydrochloride—a blocker of calcium channels to cause arrhythmias and conduction disorders (Sopharma AD, Bulgaria)
- Intralipid® lipid emulsion 20% 500 mL (Fresenius Kabi AB)
- Saline 0.9% 500 mL (B. Braun Melsungen AG)
- Midazolam Panpharma solution for injection, 5 mg/mL (Panpharma Laboratories, France)

Methods Used in the Experimental Part with Animals

- **Instrumental methods:** monitoring the heart rate of rats using an electrocardiographic monitor Bionett Co, Ltd, model BM3.
- **Statistical methods for the laboratory experiment**: the statistical analysis was performed using the statistical functions in Excel 2016 and Statistica 7.0. For all statistical analyses, a plausible confidence level of $P<0.05$, divided into three ascending classes, was assumed: $P<0.05$,

P<0.01 (high significance) and P<0.001 (very high significance).

Design of the Laboratory Experiment

The animals were divided into five groups as follows:

- **Group I**—healthy controls receiving only saline at a dose of 1.5 mL/kg
- **Group II**—rats pretreated with LE at a dose of 1.5 mL/kg 5 minutes before injection of verapamil overdose 15 mg/kg
- **Group III**—a group of rats poisoned with an overdose of verapamil at a dose of 15 mg/kg and 5 minutes later treated with LE at a dose of 1.5 mL/kg (recommended dose)
- **Group IV**—a group of rats poisoned with an overdose of verapamil at a dose of 15 mg/kg and 5 minutes later treated with an LE at a dose of 10 mL/kg (much higher than the recommended dose)
- **Group V**—rats poisoned with 15 mg/kg verapamil overdose

All drugs were administered intraperitoneally. Heart rate was measured using an electrocardiographic monitor at the 5th, 10th, 15th, 20th, 25th, 30th, and 40th minute during drug administration. Survival was measured in all groups of rats. Verapamil overdose of 15 mg/kg was used to assess the severity of intoxication (8). Midazolam 4 mg/kg was used at the beginning of the study as a sedative agent because of its minimal cardiovascular effects (9).

The dose of LE was selected as determined by Weinberg and approved by ASRA to be used as an antidote for life-threatening lipophilic drug intoxications, namely a bolus of 1.5 mL/kg (10).

A group of rats with a verapamil overdose of 15 mg/kg and such with 7 times the recommended LE dose of 10 mL/kg was selected to compare the effects at different dosages (11).

A group of animals pretreated with LE was also selected, as according to Weinberg pretreatment with LE prolongs survival and increases the LD_{50} of bupivacaine in rats (1).

RESULTS

Acute intoxication with verapamil. After 15 minutes of adaptation and 20 minutes after administration of midazolam, six rats were poisoned with a verapamil overdose 15 mg/kg. Table 1 shows the heart rate of rats poisoned with verapamil. In poisoned rats, the heart rate began to slow down 5 minutes after administration of verapamil (P<0.05 vs. midazolam heart rate) until it dropped to critical levels below 44 beats per minute after 25 minutes (P<0.001 vs. verapamil overdose heart rate). In all six rats poisoned with verapamil, asystole occurred in an average of 43.33 minutes.

Lipid emulsion pretreatment. Six sedated with midazolam rats received intraperitoneal LE Intralipid 20% 1.5 mL/kg 5 minutes before verapamil poisoning. A significant increase in heart rate was observed as early as the 15th minute of acute intoxication (P<0.01 vs. verapamil overdose heart rate) and

Table 1. Heart rate of rats poisoned with verapamil

Rats	With Midazolam	5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min
№1	398	351	227	180	86	29	25	18	10	death
№2	431	412	215	165	91	54	22	15	death	
№3	435	374	230	159	95	49	21	17	12	death
№4	419	369	229	177	90	51	29	16	death	
№5	425	370	218	153	82	37	19	19	11	death
№6	433	354	221	160	79	43	23	15	9	death
Average heart rate	424 ±13.8	**372** ±21.8 P<0.05	**223** ±6.22 P<0.001	**166** ±10.7 P<0.001	**87** ±6.0 P<0.001	**44** ±9.5 P<0.001	**23** ±3.5 P<0.001	**17** ±1.6 P<0.001	10.5 ±1.15 P<0.001	-

stabilization within the normal range began after 25 minutes (P<0.05 vs. verapamil overdose heart rate) (Fig. 1). The survival rate in this group was 100%.

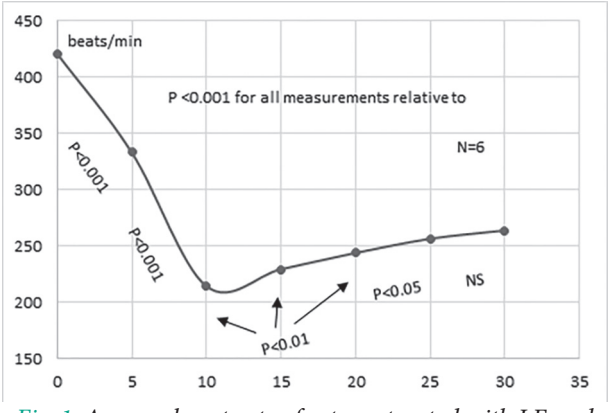

Fig. 1. Average heart rate of rats pretreated with LE and poisoned with verapamil; interval 0–30 min.

Resuscitation with LE at a dose of 1.5 mL/ kg. Six rats received a toxic dose of verapamil, 5 minutes later a bolus dose of 1.5 mL/kg LE was administered as the only medication for resuscitation. Animals responded to LE as early as the 15th minute when the mean heart rate was 233±6.34 beats per minute (P<0.05 vs. verapamil overdose heart rate) (Fig.2). The pulse rose to a safe value by the 25th minute (P<0.05 vs. verapamil overdose heart rate) and all animals were rescued. The survival rate in this group was 100%. The mean heart rate at the 30th minute was 270±6.69 beats per minute (P<0.01 vs. verapamil overdose heart rate).

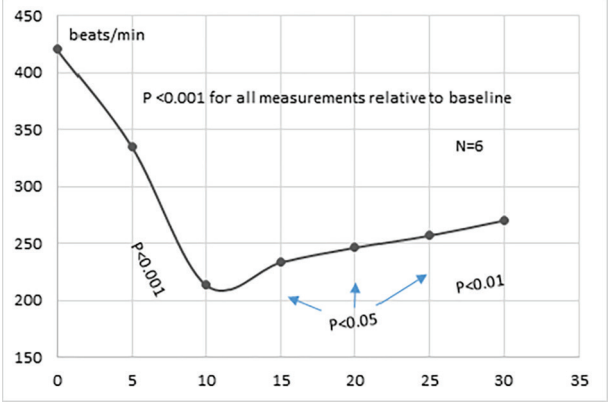

Fig. 2. Average heart rate of rats, poisoned with verapamil and treated with LE 1.5 mL/kg; interval 0–30 min.

Resuscitation with LE at a dose of 10 mL/kg. A group of six sedated rats received an overdose of verapamil, 5 minutes after a LE at a high dose of 10 mL/kg was administered as the only medication for resuscitation.

After the 10th minute, a gradual increase in the animal's pulse began: 236±10.5 beats per minute at the 15th minute (P<0.01 vs. verapamil overdose heart rate), and after the 20th minute, it returned to stabilization within the normal range (P<0.001 vs. verapamil overdose heart rate) (Fig. 3). Full recovery is also observed in this group. The survival rate in this group was 100%. The highest mean heart rate values were found in this group: 300±8.67 beats per 30 minutes (P<0.001 vs. pretreatment and low dose of LE).

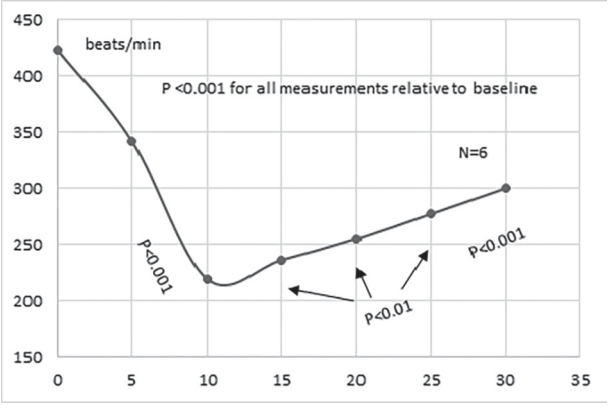

Fig. 3. Average heart rate of rats, poisoned with verapamil and treated with LE 10 mL/kg; interval 0–30 min.

DISCUSSION

The analysis shows that the best results are observed in rats treated with a high dose of LE 10 mL/kg, which is 7 times higher than the recommended for human resuscitation, where the highest mean values of the heart rate are found, which confirms its positive inotropic effect.

The results coincide with those of leading scientists in the field. First Weinberg (1998) demonstrated that the cardioprotective effect of LE at a dose of 3 mL/kg resulted in the alleviation of the cardiac toxicity induced by bupivacaine in rats and prevented asystole. Later, these findings were also confirmed in dogs (12). According to Moshiri (2014) in a haloperidol neurotoxicity model in rabbits, the best results were obtained at a dose of 12 mL/kg, while Har-

vey and Cave (2008) recommended 6 mL/kg LE as an antidote to local anesthetic toxicity in rabbits (13, 14). Perez (2008) reported that 18.6 mL/kg LE was the optimal dose as an antidote to verapamil toxicity in rats (3).

In an experiment with rats, Tebbutt demonstrated the positive effects of LE on acute intoxication with drugs that directly exert a cardiotoxic effect, such as calcium channel blockers, beta blockers, and antiarrhythmic drugs. Intralipid 20% resulted in a doubling of verapamil LD_{50} in rats and a reduction in bradycardia induced by verapamil (15). In the propranolol toxicity model, pretreatment with LE resulted in the restoration of an expanded QRS complex and extended the QT interval, as well as led to a reduction in the severity of bradycardia in rats. No similar therapeutic effect has been observed in atenolol poisoning, which is explained by the hydrophilic nature of the drug (16).

Different authors suggest different doses, and these variations in the dosage of LE are probably due to peculiarities in different species of animals (rodents and mammals) as well as differences in fat content in healthy and intoxicated tissues such as brain and heart from which LE should pull the toxin.

CONCLUSION

The present study shows that both pretreatment and resuscitation with low or high dose of LE could reduce toxicity and prevent dose-dependent asystole induced by the lipophilic drug verapamil. The administration of a high dose of 10 mL/kg LE proved to be more effective in terms of heart rate in experimental animals.

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Conflict of interest

The authors declare that they have no conflict of interest.

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