БИОЛОГИЧЕСКИЕ НАУКИ

Kehayova G.K.
Doctor of Toxicology
Assistant professor in the Department of Pharmacology,
Toxicology and Pharmacotherapy
Medical University - Varna, Bulgaria
Zlateva Sn.Z.
Professor of Toxicology
Head of the Clinic for Intensive Treatment of Acute Poisoning
and Toxic Allergies at the Military Medical Academy - Varna, Bulgaria
Zgurova N.T.
Doctor of Pathology and Cytopathology
Assistant professor in the Department of General and Clinical Pathology,
Forensic Medicine and Deontology,
Medical University - Varna, Bulgaria

BIOCHEMICAL AND HISTOLOGICAL ANALYSIS OF RATS TREATED WITH VERAPAMIL OVERDOSE AND RESUSCITATED WITH LIPID EMULSION

Габриэла Красимирова Кехайова
Доктор токсикологии
Ассистент кафедры фармакологии,
токсикологии и фармакотерапии,
Медицинский университет - Варна, Болгария
Снежа Златева
Профессор токсикологии
Руководитель клиники интенсивного
лечения острых отравлений и токсикоаллергии,
Военно-медицинская академия - Варна, Болгария
Недялка Тодорова Згурова
Доктор патологии и цитопатологии
Ассистент кафедры общей и клинической патологии,
съедобной медицины и деонтологии,
Медицинский университет - Варна, Болгария

БИОХИМИЧЕСКИЙ И ГИСТОЛОГИЧЕСКИЙ АНАЛИЗ КРЫС, ПОЛУЧАВШИХ ПЕРЕДОЗИРОВКУ ВЕРАПАМИЛОМ И РЕАНИМИРОВАННЫХ ЛИПИДНОЙ ЭМУЛЬСИЕЙ

Summary. Since 2008, lipid emulsions have been used successfully as part of standard therapy for acute lipophilic drug intoxications at a bolus dose of 1.5 ml/kg lipid emulsion 20% for one minute, followed by an infusion of 0.25 ml/kg/min for 20 -60 minutes. The high dose of lipid emulsion carries a risk of serious side effects, which is why many doctors are afraid to use it, despite reports of its positive effect as an antidote. The study demonstrated the efficacy and safety of lipid emulsion administered as an antidote at the recommended and 7-fold higher dose in acute intoxication of rats with Verapamil.

100% survival was observed in rats treated with different doses of lipid emulsion. The high dose of lipid emulsion 10 ml/kg shows greater efficiency in terms of cardioprotection. Lipid emulsion at doses of 1.5 ml/kg and 10 ml/kg are safe in terms of fat metabolism, liver and kidney parameters, proven by laboratory and histological analysis.

Аннотация. С 2008 года липидные эмульсии успешно используются в рамках стандартной терапии острых липофильных лекарственных интоксикаций при болюсной дозе 1,5 мл/кг липидной эмульсии 20% в минуту с последующей инфузией 0,25 мл/кг/мин в течение 20-60 минут. Высокая доза липидной эмульсии несет риск серьезных побочных эффектов, из-за чего многие врачи боятся использовать её, несмотря на сообщения о её положительном действии в качестве антидота. Исследование продемонстрировало эффективность и безопасность липидной эмульсии, вводимой в качестве антидота при рекомендуемой и 7-кратной более высокой дозе при острой интоксикации крыс верапамилом.

100% выживаемость наблюдалась у крыс, получавших разные дозы липидной эмульсии. Высокая доза липидной эмульсии 10 мл / кг показывает большую эффективность с точки зрения кардиопротекции. Липидная эмульсия в дозах 1,5 мл / кг и 10 мл / кг безопасна с точки зрения жирового обмена, показателей печени и почек, подтвержденных лабораторными и гистологическими анализами.

Key words: lipid emulsion, Verapamil, rats

Ключевые слова: липидная эмульсия, верапамил, крысы.

Introduction. Acute drug intoxications are a serious problem worldwide. The most common overdoses are neuroleptics, antidepressants and cardiovascular drugs. Many of these drugs do not have a specific antidote in case of overdose and can cause fatal neurological and cardiovascular complications, which requires the introduction into routine practice of more effective treatments such as intravenous lipid emulsions (LE).

The dose determined by the American Society of Regional Anesthesia (ASRA) to treat local anesthetic systemic toxicity (LAST) and lipophilic drug intoxications is a bolus of LE 20% 1.5 ml/kg per minute, followed by a continuous intravenous infusion of 0.25 ml/kg/min for 20-60 minutes until circulation is restored [1].

The use of high doses of LE and the high infusion rate are associated with an increased risk of side effects such as fat overload syndrome, hypertriglyceridemia, pulmonary embolism, pancreatitis and others, which is why many doctors are afraid to use it, despite reports of its positive therapeutic effect in acute lipophilic intoxications. There is a lack of information about the mechanism of its action, the possible risks associated with its use, dosage, duration of administration, short-term and long-term effects.

Aims. Study of the efficacy and possible side effects of LE as an antidote to acute Verapamil intoxication in rats and demonstration of its safety with short-term use.

Materials and methods. The experiments were performed on 30 healthy male Wistar rats with an average weight of 243 g, provided by the Medical University "Prof. Dr. P. Stoyanov "- Varna. The animals are kept in standard plastic cages, in a well-ventilated room, under controlled environmental conditions (temperature 25 ± 2 oC), 12-hour light / dark cycle, with free and unrestricted access to food and water.

Biochemical, histological, instrumental and statistical methods were used to assess the effectiveness of LE safety.

The biochemical analysis of the plasma of the animals was performed in a Specialized Medical Diagnostic Laboratory. Histological methods included analysis of necropsy material from heart, liver and lung from each group of rats. Digital photos of the cases were taken using the Leica Aperio Scan Scope AT2 device (Aperio Technologies, Vista, CA) with subsequent analysis of the scanned images with ImageScope V12.1.0.5029 (Aperio).

Statistical methods include descriptive statistics, parametric variation analysis, Bernoulli method, two-way comparison of relative proportions of qualitative indicators, null (H0) and non-zero (alternative H1) hypothesis.

In all performed statistical analyzes, an acceptable level of confidence interval $P < 0.05$ is assumed, divided into three ascending classes: $P < 0.05$, $P < 0.01$ (high significance) and $P < 0.001$ (very high significance).

The following experimental substances were used:

Isocor® solution for injection 2.5 mg/ml - Verapamil hydrochloride - calcium channel blocker 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)

Design of the conducted laboratory experiment:

The animals are divided into six groups as follows:

Group I - healthy controls receiving only 1.5 ml/kg saline

II. group - rats treated with LE 1.5 ml/kg 5 minutes before injection of Verapamil overdose 15 mg/kg

III. group - rats treated with Verapamil overdose 15 mg/kg and treated with LE 1.5ml/kg

IV. group - rats treated with Verapamil overdose 15 mg/kg and treated with LE 10 ml/kg

V. group of rats treated with Verapamil overdose 15 mg/kg

All drugs were administered intraperitoneally. The blood needed for the biochemical examination is taken from the sublingual vein and through a heart puncture. An overdose of verapamil per kilogram body weight (15 mg/kg) was used to assess the severity of intoxication [2].

Verapamil is a drug that is used very often due to its broad spectrum of action: supraventricular arrhythmias, hypertension, ischemic heart disease, dysmenorrhea, migraine and others. A number of authors consider it to be the most dangerous of the group of calcium channel blockers due to its strong cardiodepressant effect [3]. Its overdose is difficult to treat and is associated with high mortality despite timely therapy.

In recent years, Verapamil toxicity has been successfully treated with the inclusion of LE in standard therapy [4]. LEs are already present in the latest guidelines and checklists as a mandatory component in the treatment of Verapamil toxicity [5]

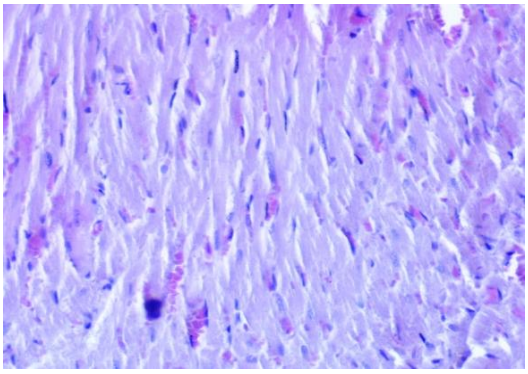
The dose of LE was selected as determined by Weinberg and approved by ASRA, used as an antidote for life-threatening lipophilic drug intoxications, namely a bolus of 1.5 ml/kg [6]. A group of rats treated with a toxic dose of Verapamil and treated with a high dose of LE 10 ml/kg was made to be able to compare the effects and survival at different dosages.

Results of the histological analysis. The rats treated with Verapamil died on average 43.33 minutes after acute Verapamil intoxication, after which organs were taken for histological analysis. All other animals treated with different doses of LE had 100% survival.

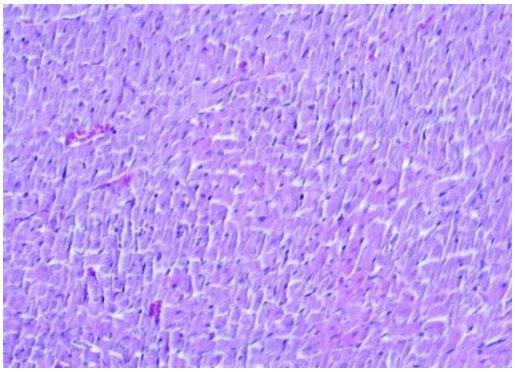
On the fourth day after Verapamil poisoning, from each group rats were euthanized and necropsy material from heart, lung, and liver was taken.

Picture 1 shows damaged myocardium after administration of a toxic dose of Verapamil to rats. Cardiomyocytes with loss of striation, nuclei with

karyorexis, erythrodiapedesis and karyopyknosis have been observed. Picture 2 shows the myocardium of rats treated with Verapamil overdose and treated with LE 10 ml/kg. Histological analysis showed cardiomyocytes with centrally located nuclei, transverse striation, preserved histological structure, no evidence of necrosis, confirmed by normal values of cardiac enzymes after resuscitation with LE 10 ml/kg.



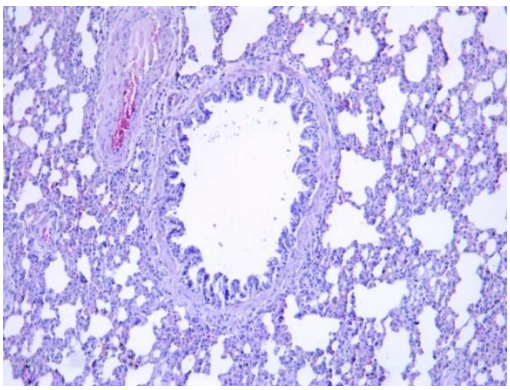
Picture 1 Damaged myocardium in acute intoxication with Verapamil in rats. Coloration with Hematoxylin and eosin (H&E) x20.



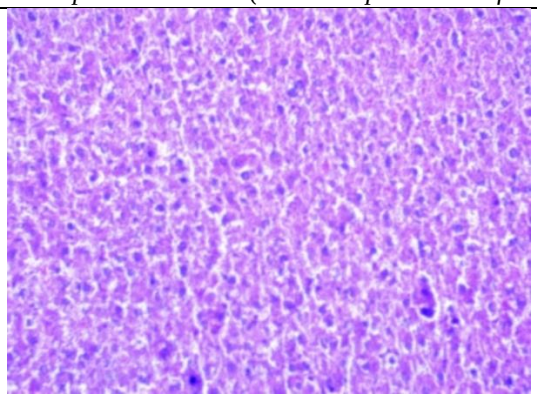
Picture 2 Normal myocardium after resuscitation with LE 10 ml/kg. Coloration with H&E x20.

Pictures 3 and 4 show the lungs and liver of rats treated with Verapamil after administration of a high dose of LE 10 ml/kg. Liver analysis showed hepatocytes with preserved histological structure, no evidence of steatosis. The lung has a preserved histological structure, alveoli are seen, centrally located bronchioles, no evidence of acute respiratory distress syndrome (ARDS) - no hyaline membranes are observed and no areas of atelectasis.

Both organs have preserved structure and no pathological changes in the tissues, which confirms the safety of LE even when administered in high doses of 10 ml / kg.



Picture 3 Lung with normal structure after administration of LE 10 ml/kg in rats. Coloration with H&E x20.



Picture 4 Liver with normal structure after administration of LE 10 ml/kg in rats. Coloration with H&E x20.

Results of the biochemical analysis. To assess the safety profile and clinical efficacy of LE, total bilirubin, total cholesterol, triglycerides, alkaline phosphatase, creatine phosphokinase (CPK), creatine phosphokinase MB-fraction (CPK-MB), urea, creatinine, gamma-glutamyl transferase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

In rats poisoned with Verapamil, elevated values of early markers of myocardial damage CPK, CPK-MB were found compared to healthy controls, respectively 295.66 U/L and 118.80 U/L (see Table 1) (P <0.001).

Treatment with the recommended dose of LE 1.5 ml/kg showed a slight increase in CK-MB (20.85 U/l), while treatment with a very high dose of LE 10 ml/kg, CPK-MB showed normal values (19.65 U/l), compared with a human rate of 20 U/l. (No norm for rats).

Table 2 presents the laboratory parameters in all studied groups of animals. Triglycerides, total bilirubin, cholesterol, alkaline phosphatase, urea, creatinine, gamma-glutamyl transferase (GGT) AST and ALT in all groups remain normal. Normal values are observed even in the group treated with a high dose of Intralipid 20% 10 ml/kg.

Table 1

Laboratory parameters: results - rats

Laboratory indicators	Healthy	+Verapamil	Pretreated with LE 1.5 ml/kg	Verapamil + LE 1.5 ml/kg	Verapamil+ LE 10 ml/kg
Total bilirubin	2.09	0.52	3.53	4.88	2.40
Total cholesterol	1.72	1.57	2.13	1.64	1.72
Triglycerides	1.15	0.94	1.20	1.25	1.45
Alkaline phosphatase	108.79	125.75	123.87	121.53	118.32
CPK	157.79	295.66	192.43	189.76	179.49
CPK-MB	18.92	118.80	21.21	20.85	19.65
Urea	6.93	10.41	7.34	6.76	7.38
Creatinine	39.59	45.92	38.32	33.25	43.39
GGT	2.27	1.85	2.34	4.21	0.69
AST	78.45	80.82	78.23	79.98	76.04
ALT	66	62.50	79.82	75.35	81.99

Discussion of the results of the histological analysis. Histological analysis showed that LE protected both cardiomyocytes and liver and lung function and did not cause side effects. The myocardium of rats treated with Verapamil showed severe areas of necrosis, while in the group of rats treated with low and high doses of LE 10 ml/kg the myocardium had a preserved histological structure.

The experiment demonstrated that the treatment of rats with a high dose of LE 10 ml/kg did not have adverse effects on liver function. Hepatocytes with preserved histological structure without evidence of steatosis were observed microscopically. The highest dose of LE tested was 4 g/kg. Its effects have been studied on rabbits. Liver steatosis has been reported, but without biochemical signs of liver dysfunction [7].

The lack of toxicity of LE on the internal organs is confirmed by other authors. Hiller demonstrated that high doses of LE 20, 40, 60 and 80 ml/kg did not cause pathological abnormalities in the normal histology of the myocardium, brain, pancreas and kidneys. Microscopic abnormalities in the lung and liver have only been observed at doses of 60 and 80 ml/kg, which are significantly higher than those in humans and support the safety of LE [8].

Administration of LE in high doses may induce dose-dependent pulmonary fat embolism and ARDS. The reason is that the high dose and rapid administration of LE lead to an increase in the concentration of free fatty acids due to the activation of lipoprotein lipase. The high concentration of free fatty acids causes vasoconstriction due to impaired endothelium-dependent vasorelaxation. Fatty acid infusion stimulates an increase in prostaglandin concentration and endothelial dysfunction [9].

In our study, we found no lung damage at either low or high dose LE. LE in doses of 1.5 ml/kg and 10 ml/kg are safe. In support of the safety of LE, Levine [10] hypothesized that pulmonary complications were much more likely to be due to the disease itself than to the administration of LE.

Based on our results, the best and at the same time safe dose of LE as an antidote in an animal model of Verapamil cardiotoxicity is 10 ml/kg. These findings coincide with other leading researchers that high doses of LE are more effective as an antidote. According to Moshiri [9], in a Haloperidol neurotoxicity model in rabbits, the best results were obtained at a dose of 12 ml/kg, while Harvey and Cave recommended 6 ml/kg LE as an antidote to local anesthetic toxicity in rabbits [11]. Perez reported that 18.6 ml/kg LE was the optimal dose as an antidote to Verapamil toxicity in rats [12].

Discussion of the changes in laboratory parameters. The present experiment showed that both low and high doses of LE had a cardioprotective effect in rats, as evidenced by the normalization of elevated cardiac enzymes. CPK-MB is slightly elevated at a low dose of LE 1.5 ml/kg (recommended by ASRA), but is normal at a high dose of LE 10 ml/kg.

The lipid profile, liver and kidney parameters in all groups remain normal. Normal values were observed in the group of animals treated with a high dose of Intralipid 20% 10 ml/kg, which confirms its safety because there are no data on suspected liver, lung and renal toxicity.

The safe profile of LE was demonstrated on 20 sedated rabbits in a Clomipramine toxicity model treated with a dose very close to ours, namely LE 12 ml/kg. This is a dose that is much higher than the dose of LE recommended by ASRA [13].

LD50 of 20% LE studied in an experiment with rodents and mammals. It was found to be 67.72 ml/kg in rats and 135 ml/kg in dogs. Such a dose is significantly higher than the doses used for resuscitation of patients, which supports the safety of LE in therapeutic doses [8].

Conclusion. The present analysis showed that both pretreatment and resuscitation with low or high dose LE could reduce toxicity and prevent dose-dependent asystole induced by Verapamil. Laboratory parameters and histology of internal organs remain normal, even at a high dose of LE 10 ml/kg which proves its safety with short-term use as an antidote. LE in doses of 1.5 ml/kg and 10 ml / kg are safe. These results suggest that lipid emulsions can be used as an innovative method for the treatment of acute exogenous intoxications in humans, especially with hemodynamic instability, when standard resuscitation protocols prove ineffective.

Acknowledgments. I am grateful to my supervisor Professor Snezha Zlateva for the support. Thanks to the clinical pathologist Dr. Zgurova for the performed histological analysis.

References:

1. Weinberg G.L. Treatment of local anesthetic systemic toxicity (LAST). Reg Anesth Pain Med. 2010;35:188–193. Doi: 10.1097/AAP.0b013e3181d246c3.

2. Gul F, Duman N.C, Arslantas M.K, et al. The Effect of Low Dose Sildenafil On Verapamil - Induced Cardiovascular Toxicity in Rats. Intensive Care Med Exp. 2015; 3 (1): A500. Doi:10.1186/2197-425X-3-S1-A500

3. Seyffart G. Poison index. The Treatment of Acute Intoxication. 4 Edition, 1997;166.

4. Vuković-Ercegović G, Perković-Vukčević N, Đorđević Sn, et al. Successful usage of intravenous lipid emulsion in treatment of acute verapamil poisoning: A case report. Vojnosanitetski Pregled, Volume 74, Number 3, 2017, pp. 278-281(4). Directory of Open Access Journals. https://doi.org/10.2298/VSP150901010V

5. St-Onge M, Anseeuw K, Cantrell F.L., et. al. Experts Consensus Recommendations for the Management of Calcium Channel Blocker Poisoning in Adults. Crit Care Med. 2017 Mar;45(3):e306-e315.

1. doi: 10.1097/CCM.0000000000002087.

6. Neal J.M., Mulroy M.F., Weinberg G.L. American Society of Regional Anesthesia and Pain Medicine. American Society of Regional Anesthesia and Pain Medicine checklist for managing local anesthetic systemic toxicity: 2012 version. Reg Anesth Pain Med 2012;37:16–18.

7. Morán Penco J.M., Maciá Botejara E, Salas Martinez J, et al. Liver lipid composition and intravenous, intraperitoneal, and enteral administration of intralipid. Nutrition. 1994 Jan-Feb; 10 (1): 26-31.

8. Hiller D.B., Di Gregorio G, Kelly K, et al. Safety of high volume lipid emulsion infusion: a first approximation of LD50 in rats. Reg Anesth Pain Med. 2010;35(2):140-144. Doi:10.1097/aap.0b013e3181c6f5aa

9. Moshiri M, Mohammadpour A.H., Vahabzadeh M, et al. Evaluating the Effects and Safety of Intravenous Lipid Emulsion on Haloperidol-Induced Neurotoxicity in Rabbit. BioMed Research International, 2014 (2014): 9; Article ID 949262, https://doi.org/10.1155/2014/949262.

10. Levine M, Skolnik A.B., Ruha A.M., et al. Complications following antidotal use of intravenous lipid emulsion therapy. J Med Toxicol. 2014 Mar; 10(1):10-4.

11. Harvey M, Cave G. Intralipid infusion ameliorates propranolol- induced hypotension in rabbits. J Med Toxicol. 2008; 4: 71–76. Doi: 10.1007/BF03160958

12. Perez E, Bania T.C., Medlej K, et al. Determining the optimal dose of intravenous fat emulsion for the treatment of severe verapamil toxicity in a rodent model. Academic Emergency Medicine.2008; (15) 12: 1284–1289, 2008.

13. Harvey M, Cave G, Hoggett K. Correlation of plasma and peritoneal diasylate clomipramine concentration with hemodynamic recovery after intralipid infusion in rabbits. Acad Emerg Med. 2009 Feb; 16 (2): 151-6.