American Journal of Emergency Medicine 34 (2016) 1112–1116

Contents lists available at ScienceDirect

American Journal of Emergency Medicine

journal homepage: www.elsevier.com/locate/ajem

Brief Report

Intravenous lipid emulsion prolongs survival in rats intoxicated with digoxin☆,☆☆

Bülent Serhan Yurtlu, MD [a,*], Şule Özbilgin, MD [a], Derya Arslan Yurtlu, MD [b], Nilay Boztaş, MD [a], Gonca Kamacı, DVM [c], Mahmut Akaltun [a], Volkan Hancı, MD [a], Osman Yılmaz, PhD [c]

[a] Dokuz Eylül University, School of Medicine, Department of Anesthesiology and Reanimation
[b] Katip Celebi University, School of Medicine, Department of Anesthesiology and Reanimation
[c] Dokuz Eylül University, Institute of Health Science, Department of Laboratory Animals Science

ARTICLE INFO

Article history:
Received 17 February 2016
Accepted 14 March 2016

ABSTRACT

Background: Intravenous lipid emulsion eliminates the toxicity-related symptoms of several drugs. We hypothesized that intravenous lipid emulsion prolongs the survival time in digoxin-intoxicated rats.
Methods: Electrocardiograms of 14 anesthesized Wistar rats were monitored. All of the rats received digoxin infusion at a rate of 12 mL/h (0.25 mg/mL). Five minutes after the start of digoxin infusion, animals were treated either with 12.4 mL/kg intravenous lipid emulsion (group L) or saline (group C). The primary outcome variable was time elapsed until asystole development. Cumulative dose of digoxin required to induce asystole was also recorded.
Results: Mean time until asystole development in groups C and L were 21.28 ± 8.61 and 32.00 ± 5.41 minutes, respectively ($P < .05$). The mean lethal doses of digoxin in the groups C and L were 3.97 ± 1.54 and 6.09 ± 0.96 mg/kg, respectively ($P < .05$).
Conclusion: Intravenous lipid emulsion prolonged the time until asystole development and increased cumulative lethal dose in rats intoxicated with digoxin.

1. Introduction

Treatment of local anesthetic toxicity with the aid of intravenous lipid emulsion (ILE) became a standard of care in recent years [1]. At the first years of the discovery, it was thought that ILE forms a "lipid sink" in the intravenous compartment and that this lipid sink incorporates highly lipid-soluble bupivacaine inside itself so that bupivacaine is removed from the tissues where it exerts its toxic effects [2]. This hypothesis has led to an idea that ILE therapy could have similar therapeutic effect at the toxic conditions caused by highly lipid-soluble drugs. Thereafter, successful treatment reports of cardiovascularly collapsed or central nervous system disoriented patients owing to drug intoxications have appeared in the literature [3–7]. Experimental animal models of cardiac arrest due to drug toxicity with verapamil, clomipramine, and amitriptyline have also yielded supporting evidence for ILE therapy [8–10]. Thus, ILE has become a promising therapy for drug toxicities [11,12].

Digoxin is one of the oldest drugs used for the treatment of heart failure. Although digoxin is prescribed frequently, its therapeutic margin of safety is narrow. It is a lipid-soluble cardiac glycoside that is readily absorbed from the gastrointestinal tract [13]. Because its therapeutic index is narrow, dose adjustment is a major concern in the elderly [14]. Patients with digoxin overdose can be seen at emergency and intensive care unit services. Mortality rate during the hospital course can be as high as 7% in patients intoxicated with digoxin [14]. Because digoxin is lipid soluble, we have hypothesized that ILE therapy may delay cardiac arrest due to digoxin intoxication. To test this hypothesis, digoxin-intoxicated rats were treated with either ILE or saline. The primary outcome variable was determined as time until asystole.

2. Methods

We used a model that is an established way of studying drug toxicity [8,15]. The study protocol was approved by the Animal Ethics Committee of the School of Medicine, Dokuz Eylul University (date 04/03/2014, number: 03/05). The study was carried out at the Dokuz Eylul University Faculty of Medicine, Department of Laboratory Animals. All of the rats included in the study were obtained from institution's Laboratory Animals Department, all fed with standard rat pellets and housed in temperature- and humidity-controlled (22°C-24°C and 60% relative humidity) rooms that were lit on a daily schedule (12:12 hours light/dark) until the day of experiment. During the experimental period, the care of the laboratory animals was in accord with international guidelines.

☆ Financial support: none.
☆☆ Conflict of interest: none.
* Corresponding author at: Dokuz Eylül Universitesi, Tıp Fakültesi, Dokuz Eylül Universitesi Hastanesi, Ameliyathane, Kat: 1, Narlıdere, İzmir, Turkey. Tel.: +90 535 887 17 23, +90 232 412 28 01.
E-mail addresses: syurtlu68@gmail.com, syurtlu@hotmail.com (B.S. Yurtlu).

http://dx.doi.org/10.1016/j.ajem.2016.03.038
0735-6757/

Fourteen Wistar rats weighing between 245 and 291 g were anesthesized with intraperitoneal injection of 60 mg/kg ketamine. Electrocardiogram was applied to monitor the heart rate, and 2 intravenous cannulas were placed at the lateral tail veins. A venous blood sample from the tail vein was drawn to control blood pH and gas tensions. One liter per minute of oxygen was supplied to all the rats. Digoxin (0.25 mg/mL), at a rate of 12 mL/h, was started to be infused to all of the rats at time zero (T0). Digoxin infusion continued until rat's *death* (defined as asystole lasting 1 minute together with absence of respiratory efforts). This infusion rate of digoxin was taken from a previous experiment to enable death of animals within a reasonable time frame [15]. At the fifth minute of infusion (T5), rats were administered either 12.4 mL/kg 20% ILE (Clinoleic 20% Lipid Emülsiyonu, Eczacıbaşı-Baxter, İstanbul) or saline at an equal volume over 5 minutes through the second venous route [8]. Duration of the time elapsed between start of digoxin infusion and death of the rat was defined as *time until asystole* and recorded in minutes. Times of first appearance of dysrhythmia (atrioventricular conduction delay) and widening of QRS complex were recorded in minutes. The total dose of digoxin infused until asystole development was recorded.

2.1. Statistical analysis

Data were analyzed by a statistician who was blinded to treatment groups. SPSS 15.0 program was used for the analysis of data. Data were expressed as mean ± SD. Mann-Whitney *U* test was used for the analysis of baseline and toxicologic characteristic properties of the groups. A *P* value less than .05 was considered as statistically significant.

3. Results

The mean age and weight of the rats in all the groups included in the study and their mean heart and respiratory rates before drug infusion were similar (Table 1). In control blood gases analyses, none of the animals had developed respiratory acidosis, hypoxia, or hypercarbia. In all animals, the terminal event was respiratory arrest, most often preceded by primary apnea and then gasping respirations. The T5 heart rates of groups C and L before lipid infusion were determined as 372.42 ± 45.69 and 361.00 ± 50.57, respectively, and there were no statistical difference between them ($P > .05$).

Time until asystole at groups C and L were determined as 21.28 ± 8.61 and 32.00 ± 5.41 minutes, respectively. Time until asystole in group L was significantly longer than that in group C ($P < .05$) (Table 2, Figs. 1 and 2). Whereas 4 rats in the group L survived until the 30th minute, only 1 rat survived longer than 30 minutes in group C (Figs. 1-3).

The mean lethal doses of digoxin in control and lipid groups were 3.97 ± 1.54 and 6.09 ± 0.96 mg/kg, respectively. The mean lethal dose of digoxin was significantly higher in group L than in group C ($P < .05$).

Heart rate tended to decrease in both groups during the digoxin infusion; however, the rate of decrease was faster in group C in comparison with group L (Fig. 4).

Electrocardiographic analysis showed that the dominant rhythm was sinus bradycardia together with atrioventricular conduction block, and widening of the QRS complex followed that later on. Some of the rats had junctional, idioventricular rhythms or varying degrees of heart block before asystole.

Table 1
Baseline characteristics of groups (mean ± SD)

Group	Control (n = 7)	Lipid (n = 7)	*P*
Age (d)	126.00 ± 9.89	127.00 ± 10.24	.902
Weight (g)	266.28 ± 15.46	262.57 ± 16.02	.710
Heart rate (beat/min)	388.28 ± 35.59	387.14 ± 62.37	.649
Respiratory rate (breath/min)	86.14 ± 5.08	85.00 ± 4.32	.700

Mann-Whitney *U* test.

Table 2
Toxicological characteristics of groups (mean ± SD)

Group	Control (n = 7)	Lipid (n = 7)	*P*
Dysrhythmia duration (min)	14.28 ± 6.99	22.28 ± 8.69	.165
QRS change duration (min)	16.71 ± 8.63	21.57 ± 7.95	.259
Time until asystole (min)	21.28 ± 8.61	32.00 ± 5.41	.026*
Lethal dose of digoxin (mg/kg)	3.97 ± 1.54	6.09 ± 0.96	.017*

* $P < .05$ compared with control group, Mann-Whitney *U* test.

4. Discussion

The results of the current study demonstrated that administration of ILE before a catastrophic cardiac event increases the dosage of digoxin required to produce asystole. In addition, ILE therapy increases the time until asystole development. To the best of our knowledge, these results are the first findings in the literature giving clues about the potential role of ILE therapy for digoxin intoxication.

Digoxin has a long history in the treatment of chronic heart failure. Although new therapeutic options have emerged for the treatment of heart failure during the last decades, digoxin remains as an important tool, and intoxication reports with this drug are still frequently encountered in the literature. Because the therapeutic index of digoxin is small and overdose is a common problem, digoxin-binding antibodies were developed as an antidote. Thus, most of the previous studies focus on the effects of digoxin-binding antibodies. The other examples focus on the effect of glucose-insulin infusion, anticalin administration, or nanomagnet-based electrochemical immunosensor technology to remove digoxin molecules from the plasma [16–19]. Unfortunately, it is not possible to make direct comparisons between the results of this study and those previous ones because of methodologic heterogeneities.

The improvement in survival with ILE therapy in drug toxicity other than a local anesthetic was first demonstrated by Krieglstein et al [20]. They had shown that rabbits which have been intoxicated with chlorpromazine had improved survival if they were pretreated with ILE [20]. Two decades later, the therapeutic efficacy of ILE for local anesthetic systemic toxicity was established both with experimental studies and with case reports. The first experimental evidence of ILE therapy came up with the lipophilic local anesthetic bupivacaine, and clinical reports have confirmed this finding later on [21–23]. Because the drug's lipophilicity is suggested as a key point for the efficacy of ILE, it is thought that the same therapy could have beneficial effects on toxidromes with other lipophilic agents. However, accumulated experimental evidences about drugs other than local anesthetics are limited at the moment. Most of the other drugs investigated for ILE therapy's effect belong to the cardiovascular group of drugs such as β-blockers and calcium channel blockers. One of these studies was conducted with verapamil. Tebbutt et al [8] administered intravenous 37.5-mg/(kg h) verapamil infusion to the rats, and then at the fifth minute of infusion, rats were treated with either ILE or saline. They found that the survival time in ILE-treated rats was almost twice longer than that in the saline-treated rats (44 ± 21 vs 24 ± 9 minutes). They also found out that there was also a similar difference in the mean lethal dose (27.4 vs 14.7 mg) of verapamil for both groups. The results of the current study demonstrate a similar increase in both survival time (32.00 vs 21.28 minutes) and the mean lethal doses of digoxin (3.97 vs 6.09 mg) for saline- or ILE-treated rats, respectively. The findings of Tebbutt et al [8] were later replicated by another study in which dogs were used and resuscitated [24]. In the later study, it was demonstrated that while resuscitation has survived 100% of animals which were treated with ILE, resuscitation was successful only in 14% of animals which received saline [24]. The results of our study gave the initial clues about the role of ILE for digoxin intoxication. In case of life-threatening toxidromes, it does not seem ethical to conduct a randomized controlled study on volunteers, so our findings need a similar confirmation and advancement with a resuscitation model.