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Original article

Effect of Intravenous Lipid Emulsion on Clozapine Acute Toxicity in Rats

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Key Words
Clozapine, Intravenous Lipid Emulsion (ILE), acute toxicity, antidote, pathologic signs.

Abstract

Objectives: Many studies have been reported the efficacy of intravenous lipid emulsion (ILE) as an antidote on acute lipophilic drug toxicity. Clozapine, highly lipophilic dibenzodiazepine neuroleptics, is an important medication in the schizophrenia therapy regimen. Acute intoxication with antipsychotics is one of the main reasons for the referral of poisoned patients to the hospital. We expected that ILE could be used for the therapy of acute clozapine intoxicated patients.

Methods: We used two groups of consisting of six male rats. Both groups received a toxic dose of clozapine (40 mg/kg) intravenously, via the tail vein. After 15 minutes, they were treated with intravenous infusion of 18.6 mg/kg normal saline (NS group), or 18.6 mg/kg ILE 20% (ILE group). We evaluated blood pressure (BP) and heart rate by power lab apparatus through the tail artery, ataxia by a rat rotary circle, seizure scores and death in multiple times after starting clozapine administration. For biochemical and pathological evaluations the samples of tissue and blood were taken.

Results: Our results demonstrated that ILE 20% could return hypotension-induced clozapine better than normal saline. Furthermore, ataxia and seizure have rectified more rapidly and deaths reduced. Clozapine administration causes pancreatitis and lung injury but fat emulsion did not show an optimal effect on tissue damages caused by clozapine toxicity.

Conclusion: In conclusion, ILE can remove toxic signs of clozapine same as other lipophilic medicines, however, clinical uses of ILE for this intention requires more appraisement to determine the precise implication and safety.

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1. Introduction

AILE has been used to supply calories in the form of free fatty acids in patients who require parenteral nutrition. There are many reports on the efficacy of intravenous lipid emulsion (ILE) as an antidotal candidate in intoxication with medications [1-3]. Investigations and case reports about this novel benefit of ILE result in adding it into Guidelines for the Management of Severe Local Anesthetic Toxicity [4-6]. ILE has been shown to have positive impacts toxicity resulted in lipophilic drugs overdose through some case reports

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and animal studies [7, 8]. Acute antipsychotic drug poisoning is one of the most important reasons for referring to the hospital [9, 10]. Clozapine, highly lipophilic dibenzodiazepine neuroleptics, is one of the most effective for the therapy of schizophrenia and is increasingly being used to treat affective disorders, some neurological disorders, and aggression [11, 12]. Clozapine toxicity has been commonly seen in psychotic patients [13, 14], especially in patients who received different drugs simultaneously [13, 15].

Clozapine toxicity like other antipsychotic toxicity causes side effects in several organ systems that need emergency evaluation and treatment, like serious cardiovascular side effects include hypotension and myocarditis; the serious neurological side effects include seizures and ataxia; serious hematological side effect of agranulocytosis and tissue injuries [16-22]

The primary objective of this study is to determine if ILE would have beneficial effects in the setting of clozapine toxicity.

2. Materials and Methods:

2.1. Animals

Adult male Wistar rats (Bu-Ali Research Institute, Mashhad, I.R. Iran), weighing 250-300 g, were used for all experiments. These animals were housed in a pathogen-free facility on a 12 hour light/dark schedule and with ad lib access to food and water. All animal procedures were approved by the ethics committee of MUMS (Mashhad University of Medical Sciences).

2.2. Preparation of clozapine intravenous solution

5 mg of the clozapine powder (Exir Pharmaceutical Co., Iran) dissolved in HCl for preparing the clozapine intravenous (IV) solution. Then, normal saline was used for reaching working concentration. The neutralization was done with NaCl solution [23]. The sterile pyrpgen free normal saline (NS) and ILE (Fresenius Kabi AB, Spain) were also IV administered.

2.3. Procedures

2.3.1 Animal Groups

We used two groups of consisting six male rats. Both groups received a toxic dose of clozapine, 40 mg/kg intravenously, through tail vein through 60 seconds (time 0). After 15 minutes (time 1) the first group received 18.6mg/kg normal saline intravenously (NS group), and the second group received 18.6 mg/kg ILE 20% intravenously (ILE group) [24].

2.3.2. Animal Toxicity Tests

All toxicological evaluations and survival observations were performed at 0, 15, 30, 45, 60, 180, 360, 720 and 1440 minutes after starting clozapine administration.

2.3.3. Evaluation of cardiovascular toxicity, blood pressure, and heart rate measurement

Blood pressure (BP) and heart rate were evaluated by power lab apparatus (Data Acquisition Systems, US) through the tail artery, in the conscious animals. For this purpose, rats were restricted in the clear restraint tube. Their tails were warming up to 28-29°C. After allowing 15 min for each animal to acclimatize in the tube, an 11-mm cuff was placed around the tail. The pulses were recorded at a rate of 3 mm Hg/s. The systolic pressure was incorporated as the pressure at the point when the first tail pulse was discovered. By calculating the number of pulses registering over a 2-s period, the heart rate was calculated (beats/min). [25].

2.4. Evaluation of neurotoxicity, ataxia, and seizure

2.4.1. Rotarod performance

Rotarod test (Borj Sanat, Iran), as ataxic criteria, measured the ability of the animal to maintain itself on the revolving rod. Four days before the onset of tests, the rats were trained (three 2-min trials/day). Only animals able for remaining 120 seconds on the rod without any fall, were selected for experiments [26].

2.4.2. Seizure scores

Seizure scores were classified according to a modified scale [27].

2.5. Biochemical and Histopathological Studies

For biochemical and pathological evaluations the samples of tissue and blood were taken from all animals after 24h or at the death point. All tests were blindly carried out with a single pathologist.

2.6. Statistical Analysis:

Statistical analysis of all variables was performed using Statistical Package for Social Sciences software version 11.5 (SPSS Inc., Chicago, Illinois, USA). Two-way repeated measures analysis of variance (ANOVA) was used to evaluate the differences in BP of different groups. Kruskal–Wallis ANOVA or Mann–Whitney test was used, if necessary. The alpha level was set at 0.05.

3. Results

3.1. Survival

After Clozapine administration, animals were assessed for their survival and 2 deaths were recorded in the NS group before 24 hours.

According to Fisher Exact test, there were no significant differences with regard to the number of deaths between the 2 groups receiving either NS or ILE. (P = 0.4545)

3.2. Seizur

According to seizure assessment, seizure scores during a 24-hour observation were recorded. Seizures initiated as soon as Clozapine injection was completed in all groups. ILE significantly decreased (P < 0.05) seizure scores in comparison with NS (Fig. 1).

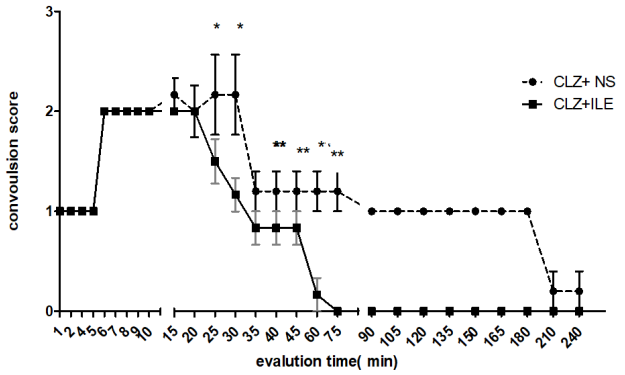

Figure 1 Effect of infusion of 18.6 ml/kg intravenous lipid emulsion (ILE) on seizure scores induced by acute clozapine (40 mg/kg) poisoning in comparing with normal saline (NS).* P < 0.5, ** P<0.01 data were reported by Mean ± SE, analyzed by ANOVA, post test = Tuky, (N = 6)

3.3. Ataxia

ILE significantly rectified ataxia more rapid than NS, and ILE treated rats were able to stay and walk on the rat rotarod at the 3rd hour (P < 0.01), however, the NS-treated group was ataxic more than 24 hours (Fig. 2).

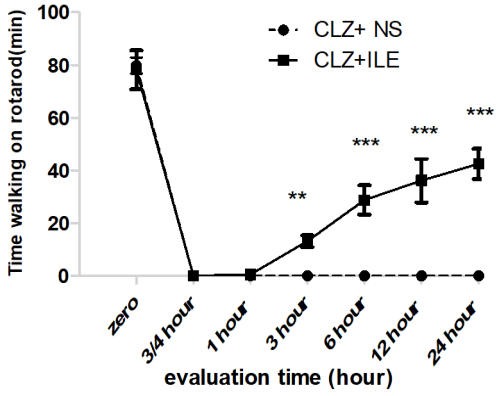

Figure 2 Effect of infusion of 18.6 ml/kg intravenous lipid emulsion (ILE) on acute clozapine (40 mg/kg) induced an ataxia in comparing with normal salin (NS).**= (p < 0.01); ***= (p < 0 .001) data were reported by Mean ± SE, analyzed by ANOVA, post test = Tuky, (N = 6)

3.4. Blood pressure

As shown in figure 3, administration of 18.5 ml/kg of NS or ILE to clozapine poisoned hypotensive rats raised their blood pressure to near normal baseline, but NS-treated group become hypotensive again, while the BP of ILE treated group remained near normal up to the end of measurement (P < 0.001).

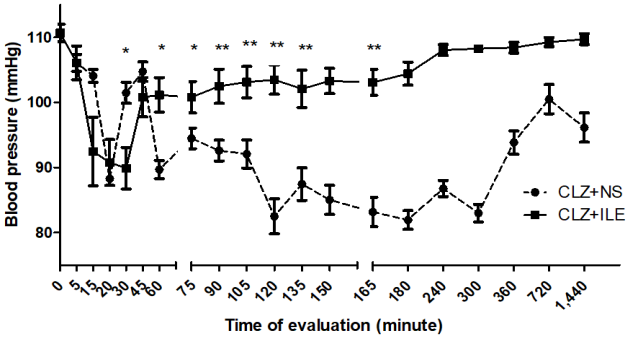

Figure 3 Effect of lipid emulsion on blood pressure after acute clozapine poisoning in different groups of animals. Significant difference *= (P < 0.05), **= (p < 0.01); ***= (p < 0.001), (N = 6)

3.5. Histopathological Studies

The histopathological evaluation of the lung of the rats showed similar educative secretions and alveolar wall Inflammation in both groups (Fig. 4). However, the conjugation of vessels and fat necrosis of pancreatic tissue slides were reported equal in both groups, the scoring of pancreatic parenchyma inflammation in ILE group was lower than NS treated group (1 ± 0.089 and 1.67 ± 0.51; P < 0.05) (Fig. 5).

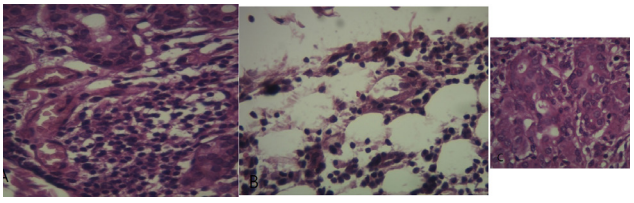

Figure 4 Pancreas slides of rats treated by Clozapine and intravenous lipid emulsion (ILE) or normal saline (NS). Magnification = ×400. (A) Moderate inflammation: Clozapine+NS (B) Sever inflammation: Clozapine+NS. (C) Mild inflammation: Clozpine+ILE , (N=6)

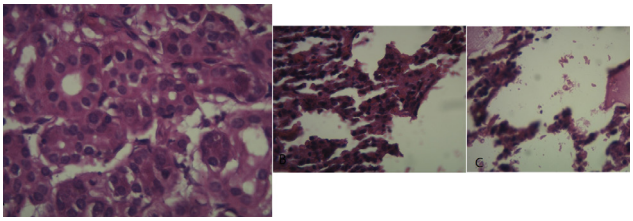

Figure 5 Lung slides of rats treated by Clozapine and intravenous lipid emulsion (ILE) or normal saline (NS). (A) Magnification = ×400, Inflammation of the lining of the lung alveoli in both groups (Clozapine+NS and Clozapine+ILE). (B) Magnification = ×100 Pulmonary alveolar wall with hemorrhage, inflammation and vessels Conjugated in both groups (clozapine+NS and Clozapine+ILE). (C) Magnification = ×100 Exudative secretions and edematous wall of the alveoli in both groups (Clozapine+NS and Clozapine+ILE).

3.6. Biochemical tests

As demonstrated in table 1, following clozapine intoxication, lipase and amylase increased in both groups, and there was no significant difference between the NS group and the ILE group in the amount of the amylase and lipase. The number of blood platelets and white blood cell in rats receiving NS compared with ILE after clozapine intoxication significantly reduced ($P < 0.001$). Other cell count criteria were almost similar in both groups.

Table 1 The result of comparing the biochemical tests in both groups receiving normal saline or intralipid after clozapine poisoning. (Significant difference *: $P < 0.05$)

Laboratory Tests	CLZ + NS	CLZ + ILE
Lipase U/L	254±0.52	248±0.44
Amylase U/L	2744.8±104.26	2276.5±205.12
WBC/103µl	8.1097±0.319	11.458±0.5*
RBC/103µl	7.313±6.2582	7.568±0.1848
PLT/103µl	390±5.164	687±17.860*

4. Discussion

As the result revealed fat emulsion could reduce toxic signs of clozapine same as other lipophilic medicines.

After clozapine administration, animals were assessed for survival and 2 deaths were recorded in the NS group during the 24 hours evaluation. Although the mortality rate of ILE treated group was lower than NS, there was no significant difference between the two groups, and it may be due to small sample size. Therefore, further study is needed to evaluate the effects of ILE on clozapine mortality.

Using plasma expanders is an effective way for the treatment of clozapine-induced hypotension. In our investigation, clozapine-induced hypotension has responded to a volume expander solution like normal saline. Normal saline could raise reduced BP of rats after administration, although its effect was not long-lasting and BP reduced again at the first hour (Fig.3). A persistent rise in BP after ILE administration suggests that it may also act through mechanisms other than volume expansion. It seems that ILE could redistribute clozapine, which is a high lipid soluble medicine from peripheral or central receptors into the new neutral compartment and resolves the signs of toxicity. Previous animal tests support the effect of ILE in reveling lipophilic drugs induced hypotension. In a study ILE has shown significant positive effects for primary or secondary hemodynamic endpoints in metoprolol, verapamil, tramadol and haloperidol intoxication [2, 7, 28-30]. There is a suggested mechanism for ILE in revealing drugs-induced hypotension, the lipid sink theory. Through lipid sink theory, lipophilic drugs redistribute from the site of toxicity into a new lipid compartment following ILE infusion. Lipid sink theory justifies the failure of ILE to improve intoxication of less lipid-soluble drugs such as nifedipine [31]. Clozapine volume of distribution is lower than that of other antipsychotic drugs, but, is nonetheless considerable (2.0 – 5.1 L/kg) [32].

Although the lipid sink theory could explain the antihypotensive effect of ILE in lipophilic drug toxicity, it is suggested that there are other important mechanisms. Free fatty acids increase the calcium influx into myocytes and smooth muscles [33]. It not only improves impaired cardiac function but also raises blood pressure [34, 35]. Although we did not evaluate the cardiac function of intoxicated rats, it is possible that some of the antidotal effects of ILE on resolving clozapine-induced hypotension are related to its cardiac effects, as well.

The toxic level of clozapine causes neurotoxicity symptoms, such as ataxia and seizure. In addition to blocking dopamine receptors, clozapine antagonizes a number of other receptor sites, including the norepinephrine, histamine, acetylcholine and serotonin system and partially reverses the inhibitory effect of GABA on 35S-t-butyl bicycle phosphorothionate (35S-TBPS) binding [36]. According to previous studies, clozapine affects excitatory amino acid release in nucleus accumbens [37], alters glutamate-dependent excitatory postsynaptic potentials in hippocampal slices [38] and glutamate- mediated evoked field potentials in rat striatum [39]. Clozapine-induced ataxia may relate to its serotonergic effect [40]. However, clozapine administration induced ataxia and some levels of the seizure (time 0), at time 1 these signs have rectified more rapidly in the ILE group than in the NS group. The most important hypothesis that explains the mechanism of the antidotal effect of ILE against CNS intoxication of xenobiotics is the lipid sink theory [31]. It suggests that lipophilic medicines or xenobiotics, when administered at toxic levels, were redistributed from their site of action to a new inner compartment made by ILE in the vessels. Also, there is another antidotal mechanism for ILE in neurotoxicity caused by clozapine, ILE and high chain free fatty acid are capable of increasing intracellular calcium concentrations in neurons, and also in ventricular muscles cells. Thus, it is suggested that ILE not only reduces clozapine concentration in target organs based on the lipid sink theory but also restores the clozapine-induced neurotoxicity through increasing the intraneuronal calcium concentration.

Most patients who take clozapine, especially in the first month of use, symptoms of inflammation of the pancreas and increasing enzymes like amylase and lipase are visible [41]. In this study after pathological tests, pancreatitis was observed in both groups receiving normal saline and ILE after clozapine intoxication, but the parenchymal inflammation of pancreas tissue was significantly higher in the NS group compare with the ILE group. But other pathological factors explored in the study did not show a significant difference between the two groups. On the other hand, there were no significant differences in the amount of amylase and lipase in both groups.

One of the important body organ that clozapine was stored and causes toxicity is the lungs [42]. In this study, ILE also neither reduce nor raised clozapine intoxication lung injuries. Perhaps no decrease in lung injury is due to the fat emulsion potential to cause pulmonary injury [43].

Therefore, we can conclude that these results represent the little effect of ILE in resolving tissue damages caused by clozapine toxicity.

Jagadheesan, et al., in a study showed that 17.8% of clozapine-treated people at least once affected with thrombocytopenia or a reduction in the number of platelets [44]. Administration of clozapine can cause drug-induced leukopenia and neutropenia, especially in the patient with low WBC or Co- administration with other medicines [45-49]. In this study, platelet and leukocyte count of NS-treated group significantly was lower than ILE-treated group, which reflects the ability of ILE to prevent of clozapine-induced leukopenia and neutropenia.

5. Conclusion

In conclusion, ILE can remove toxic signs of clozapine as same as other lipophilic medicines. But ILE has little effect in relieving tissue damage induced by clozapine. However, clinical uses of ILE for this purpose needs more evaluation to determine the exact indication and safety.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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