Histopathological Changes with Acute Pioglitazone Intoxication and the Effects of Intralipid Emulsion Treatment in Rats

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Abstract

Introduction: The primary aim of this study is to investigate the histopathologic effects of acute pioglitazone intoxication and the effects of intralipid emulsion treatment in reversing these possible unwanted effects in a rat model. Methods: For the study, 32 Spraque-Dawley rats weighing between 250-300 g were randomly divided into 4 groups. The first group was the control group and 12.4 ml/kg normal saline was given by intravenously (IV). 12.4 ml/kg intralipid emulsion was given to the second group by IV route. ½ LD50 pioglitazone (1000 mg/kg) per oral (PO) followed by 12.4 ml/kg normal saline IV was given to the third group. ½ LD50 pioglitazone (1000 mg / kg) PO followed by 12.4 ml / kg intralipid emulsion IV was then given to the fourth group. After 24 hours, rats were decapitated and tissue samples were taken for histopathological examination. Results: The histopathological scores of liver tissue in the third and fourth groups were significantly higher than the other groups. The histopathological scores of the kidney tissue were significantly higher in the second, third and fourth groups compared to the control group. The histopathological scores of the heart tissue were examined, no statistically significant difference was found in our model. ILE itself were found toxic to kidney cells. Conclusion: Although toxic effects can be observed in the liver and kidney tissues due to pioglitazone usage, intralipid emulsion treatment is insufficient to reverse these toxic effects. Toxic effects can be seen in the kidney tissue due to intralipid treatment and studies on different doses and timing are needed. Keywords: Pioglitazone, intravenous lipid emulsion, intoxication, animal model

HISTOPATHOLOGICAL CHANGES WITH ACUTE PIOGLITAZONE INTOXICATION AND THE EFFECTS OF INTRALIPID EMULSION TREATMENT IN RATS

ABSTRACT

Aim: The primary aim of this study is to investigate the histopathologic effects of acute pioglitazone intoxication and the effects of intralipid emulsion treatment in reversing these possible unwanted effects in a rat model.**Methods:** For the study, 32 Spraque-Dawley rats weighing between 250-300 g were randomly divided into 4 groups. The first group was the control group and 12.4 ml/kg normal saline was given by intravenously (IV). 12.4 ml/kg intralipid emulsion was given to the second group by IV route. ½ LD50 pioglitazone (1000 mg/kg) per oral (PO) followed by 12.4 ml/kg normal saline IV was given to the third group. ½ LD50 pioglitazone (1000 mg / kg) PO followed by 12.4 ml / kg intralipid emulsion IV was then given to the fourth group. After 24 hours, rats were decapitated and tissue samples were taken for histopathological examination.**Results:**The histopathological scores of liver tissue in the third and fourth groups were significantly higher than the other groups. The histopathological scores of the kidney tissue were significantly higher in

the second, third and fourth groups compared to the control group. The histopathological scores of the heart tissue were examined, no statistically significant difference was found in our model. intralipid emulsion itself were found toxic to kidney cells.**Conclusion:**Although toxic effects can be observed in the liver and kidney tissues due to pioglitazone usage, intralipid emulsion treatment is insufficient to reverse these toxic effects. Toxic effects can be seen in the kidney tissue due to intralipid treatment and studies on different doses and timing are needed.**Keywords:** Pioglitazone, intravenous lipid emulsion, intoxication, animal model**What's already known about this topic?**

In the literature there are no studies about the reversibility and treatability of the toxic effects of pioglitazone. Only supportive treatment modalities are advised to use in the management of pioglitazone overdose cases. Effectiveness of intralipid emulsion treatment in lipophilic drug toxicities have been studied in literature.

What does this article add?

Our study found that intralipid emulsion is not effective in reversing effects of pioglitazone toxicity. Also, that intralipid emulsion itself may be toxic for renal tissues.

Introduction

Pioglitazone, an oral antidiabetic agent in thiazolidinedione group, was first introduced in 2000 as a single dose per day use. Then, in 2002, it was also approved for use in oral monotherapy for overweighed diabetic patients whose blood sugar could not be controlled by diet and exercise (1). Thiazolidinediones, also called peroxisome proliferating activating receptor gamma (PPAR-γ) agonists, exert their effects mainly by the activation of these receptors. Thiazolidinediones, which provide glycemic control by improving insulin sensitivity, are lipophilic and can enter to the cell nucleus and bind to PPAR-γ (2). Thiazolidinediones also increase transcription of insulin-sensitive genes by targeting nuclear PPAR-γ (3). The active substance pioglitazone is used frequently today either alone or in combination with other drugs (4). Other thiazolidinediones, troglitazone and rosiglitazone, were withdrawn from the market because of their hepatotoxic and cardiotoxic effects (4–6). The most important side effects of pioglitazone are heart failure and peripheral edema. The congestion caused by pioglitazone is resistant to diuretic therapy (7). Ventricular hypertrophy, congestion of kidney and liver tissues, deterioration of liver and cardiac enzymes have been demonstrated in animal model studies on pioglitazone intoxication (8,9). Because it lowers blood glucose by improving target cell response to insulin, and without increasing pancreatic insulin secretion, it is highly preferred for obesity control by the physicians besides antidiabetic effects. It is an easy-to-find and frequently used drug, therefore intoxications with pioglitazone alone or in combination with other drugs are not rare (10,11). However, to our knowledge in the literature there are no studies about the reversibility and treatability of the toxic effects of pioglitazone. Supportive treatment modalities are advised to use in the management of overdose cases. Intralipid emulsions (ILE) are present in the form of medium chain triglycerides, long chain triglycerides or combinations thereof. They are primarily used for nutritional support. The effects of intralipid emulsion treatment in poisoning cases were first tested in cardiac arrest models studied with bupivacaine, a local anesthetic, in animals (12). Then, with the positive results reported consecutively, it was recommended to be used in humans if cardiac arrest developed due to local anesthetic poisoning (13). Finally, the researchers focused on the effectiveness of ILE as a rescue agent in other lipophilic drug intoxications (12,14,15). American Heart Association declared in 2015 guidelines that it may be reasonable to administer ILE to patients with other forms of drug toxicity who are failing to recover from standard resuscitative measures (13). In this study, the histopathologic effects of pioglitazone which is a lipophilic drug and changes due to acute poisoning on tissues were investigated (2). Also, the effectiveness of ILE in reversing these possible unwanted effects was tested in a rat model.

2. Materials and Methods

The study was approved by the Ethical Committee of Yeditepe University, Medical Faculty, Experimental Animals Research Laboratory (Atasehir, Istanbul, Turkey 34755) on 27th of August in 2018 with the registry number 675. Authors declare here that all applicable international, national and/or institutional guidelines for the care and use of animals were followed. All procedures and practices involving animals in the study were in compliance with the ethical standards of the respective institution at which the study was conducted and is in accordance with the ARRIVE guidelines (16).**2.1. Experiment:**32 adult male Spraque-Dawley rats weighing 250-300 g were used for the study. All experimental animals were adapted to the environment by keeping 50-70% relative humidity at ambient temperature at 22 ± 2 ° C for 12-hour night and 12-hour daytime until the beginning of the experiment. Rats were given standard diet and water during follow-up. For the study, 32 Spraque-Dawley rats were randomly divided into 4 groups; Group 1 (Control): 12,4 ml/kg normal saline IV (n=8) Group 2 (ILE): 12,4 ml/kg intralipid emulsion IV (n=8) Group 3 (PIO): $\frac{1}{2}$ LD50 (1000mg/kg) pioglitazone PO + 12,4 ml/kg normal saline IV (n=8) Group 4 (PIO+ILE): $\frac{1}{2}$ LD50 (1000mg/kg) pioglitazone PO + 12,4 ml/kg intralipid emulsion IV (n=8) Before all drug applications and interventional procedures, inhaler isoflurane (Izofluran, Isofludem 100 ml, Dem Ilac, Istanbul, Turkey) was provided by anesthesia machine (Animal Anesthesia Machine) to all rats. After providing sufficient depth of anesthesia, tail vein cannulation was performed with 26 G catheter (Bicakcilar -B-CAT2, İstanbul, Turkey) to each group to administer the drugs used in the study. Pioglitazone tablets (Glifix tablet 45 mg, Bilim Ilac /Turkey) were crushed in mortar and mixed with normal saline in vortex to prepare the solution. The prepared solution was given to the rats in the 3rd (PIO) and 4th (PIO+ILE) groups by a gastric tube at a dose of 1000 mg/kg pioglitazone. Toxicity was induced by administering pioglitazone at a dose of $\frac{1}{2}$ LD50 with an average lethal dose (LD50) of 2000 mg/kg (8,17). ILE (ClinOleic 20% lipid 500 ml, Eczacibasi, Baxter/Belgium) was administered to the 2nd (ILE) and 4th (PIO+ILE) groups at the dose of 12.4 ml/kg IV from the tail vein of the rat. 20% intralipid emulsion dose was applied with reference to previous studies (18–20). All rats were anesthetized prior to decapitation using inhaler isoflurane (Izofluran, Isofludem 100 ml, Dem Ilac, Istanbul, Turkey). After decapitation liver, heart and kidney tissue samplings were performed. Tissue specimens were taken for histopathological examination.**2.2. Histopathological studies:**The kidney, liver and cardiac tissues of each rat identified in 10% formol and they were sampled by pathologist. Tissue specimens were fixed in 10% neutral buffered formalin and embedded in paraffin by rising alcohol series (70%, 90%, 96% and 100%) and xylene. 5 μm thick sections were taken with microtome and stained with Hematoxylin & Eosin method for histopathological evaluation. Tissues were examined by light microscope (Carl Zeiss, AxioZoom). Liver tissue morphology was evaluated microscopically for vacuolization, vascular dilatation, inflammatory cell infiltration, necrosis and hemorrhage. Heart tissue morphology was evaluated microscopically for vascular dilatation, connective tissue edema, inflammatory cell infiltration, hemorrhage and transverse streaking in cardiomyocytes. Renal tissue morphology was evaluated microscopically for glomerular structure, vacuolization, hemorrhage, tubular dilatation and inflammatory cell infiltration. Each evaluation criterion was scored as 0 = normal, 1 = mild, 2 = moderate, and 3 = high. Scores of these parameters were combined on the basis of organs and histopathological scores of each animal were formed.**2.3. Statistical Examinations**: Nonparametric Kruskal-Wallis test and Dunn's multiple comparison test were used to compare histopathological scoring results between the groups. Statistical significance level was taken as $p < 0.05$. Statistical analysis of histopathological findings was performed using GraphPad (Prism 6.0, GraphPad Software, San Diego, California, USA).

3. Results

32 rats in the 4 groups in our study were followed 24 hours after the drug and fluid administrations. All the animals had free access to food and water. After 24 hours, mice were sacrificed by cervical dislocation. No rats were lost during this period. Heart, liver and kidneys were dissected and subjected to histopathological examination. Group 1 was determined as the control group. Histopathological scoring results were

obtained according to microscopic examination of liver tissues. Figure 1 represents the microscopic views of the hepatic tissues. Comparison of histopathological scores of liver tissues between groups are shown in figure 2. There was no statistically significant difference between 1st (control) and 2nd (ILE) groups in terms of histopathological scoring. 3rd (PIO) and 4th (PIO+ILE) groups showed a statistically significant increase in histopathological scoring compared to 1st (control) group in terms of increased tissue damage ($p < 0.001$). There was no statistical difference between the 3rd (PIO) and 4th (PIO+ILE) groups. Histopathological scoring results obtained by microscopic examination of cardiac tissues are shown in Figure 3. Histopathological scores were higher in group 3 and 4 than in other groups but there was no statistically significant difference between the groups in terms of histopathological scoring. Figure 4 shows the comparisons of the results in terms of cardiac histopathological scoring. Histopathological scoring results obtained according to microscopic examination of kidney tissues are shown in Figure 5. The 2nd (ILE), 3rd (PIO) and 4th (PIO+ILE) groups showed a statistically significant difference in terms of histopathological scoring compared to the 1st group (control) ($p < 0.001$). There was no statistically significant difference between 2nd (ILE), 3rd (PIO) and 4th (PIO+ILE) groups. Figure 6 represents the comparisons among the groups.

4. Discussion

Intravenous lipid emulsion is presented as a lifesaving treatment of lipophilic drug intoxications (12,14,15). 'Lipid sink' theory is the most emphasized theory explaining how ILE works, was first introduced by Weinberg et al. in 1998 (12). According to this theory, by administering ILE intravenously, expansion in the lipid compartment of blood is provided. The drug, which is at a toxic level, first migrates to the aqueous part of the plasma and then to the lipid compartment, which is subsequently dissolved, thereby reducing the amount of active drug in the target tissue. Because of these properties, it is suggested that lipid emulsions trap oil-soluble toxic substances in circulation and cause to sink in the emulsion (12). Another theory is the 'Ion Channel Theory'. In this theory, it has been suggested that free fatty acids in lipid therapy have been shown to activate voltage-gated calcium channels and increases intracellular calcium level. These mechanism has positive inotropic effect on myocytes (21–23). The other theory is that the lipid emulsion is used as a source of cardiac energy. Fatty acids are used for myocardial ATP synthesis in normal resting cardiac tissue (24). In recent literature it is said that this cleaning effect is not only a static waste effect, but also a dynamic shuttle effect (14). The lipid compartment in the blood creates a shuttle effect by removing lipophilic drugs from organs. In this way, organs with high blood flow are detoxified from the drugs. Although the mechanism of action is not fully understood, it is thought that the effectiveness of ILE in the intoxication of local anesthetic and other lipophilic drugs depends on the combined effects of these mechanisms (15). Based on these theories we had investigated the effectiveness of ILE in reversing the unwanted effects of pioglitazone at a single dose of $\frac{1}{2}$ LD50. We had used histopathological evaluation in order to test and compare the changes. Our results showed that toxic effects may occur in the liver and kidney in an acute intoxication model with a given dose of pioglitazone, and intralipid emulsion therapy is not fully effective in reversing these toxic effects. Besides, ILE itself may have nephrotoxic effects. In the literature, studies and case reports about pioglitazone generally showed that it has hepatotoxic effects both in acute or subacute overdoses which is also consistent with our study. Farley Hills et al. reported a patient who developed and died of acute hepatitis due to pioglitazone use (25). May et al. reported a case in which increased liver transaminase levels and histopathological changes in the liver due to pioglitazone use and these effects improved after discontinuing pioglitazone (26). Chase et al. reported a patient presenting with fulminant liver failure due to pioglitazone use and liver failure was recovered after discontinuing the drug (27). The mechanism for hepatotoxicity it is thought to be related with inhibition of ATP production by pioglitazone which causes cytotoxicity and oxidative stress. Reactive metabolite formation and hepatocyte mitochondrial dysfunction may occur by this way (28). On the contrary, El Gawly et al. reached a different result with their experimental studies. In their study, streptozotocin was used to generate diabetes mellitus in the animal model. For the pioglitazone dose, the maximum daily dose for humans, 4mg/kg, was used. The study showed that pioglitazone may have positive effects on the liver (29). This different result might be

related with low dose of pioglitazone in this study. Our result showed a significant hepatotoxicity with $\frac{1}{2}$ LD50 dose of pioglitazone. The answer to the question of whether ILE can reverse this toxic effect was negative. Elimination of pioglitazone and its excretion of metabolites are mainly from the liver. Renal clearance of pioglitazone is very low (30). Chinnam et al. showed that the acute toxicity of the pioglitazone in an animal model caused congestion in the kidney besides changes in the liver and heart tissue (8). In the study of Sai Elshama et al., subchronic toxicity was created for 90 days in an animal model of diabetes mellitus. They showed similar results with the Chinnam et al in the kidney tissues of the subjects (9). Our results about renal toxicity are consistent with the literature. ILE did not have a positive effect on reversing the toxic effects of pioglitazone on kidney according to our results. Besides, we observed toxic effects of ILE on the kidney cells in the 2nd group which we had administered only ILE. Even if the underlying mechanism is not fully understood studies showing that ILE itself is renal toxic, our findings are also consistent with the literature (31,32). Some experimental studies also showed that pioglitazone also has toxic effects on heart tissue. Yang et al. reported that pioglitazone causes cardiac muscle hypertrophy and subsequent ventricular hypertrophy in an animal model. They gave daily pioglitazone at a dose of 200 and 540 mg/kg/day for 12 weeks (33). Therefore, we had also investigated the histopathological changes on our subjects' heart tissues. Although some changes observed in the 3rd and 4thgroup which we had administered pioglitazone, those were not found statistically significant. We thought that more long-term use might be needed in order to see a significant cardiac muscle hypertrophy. ILE did not change the heart tissue evaluations in our study.

5. Limitations

This is an experimental animal study and small number of subjects is the main limitation due to ethics considerations. Although we had used pioglitazone and ILE at doses used in the previous animal studies, they may not be effective in order to demonstrate our hypothesis. We had evaluated the tissue effects after 24 hours. This time period may not be enough for histopathological changes to be observed especially for the heart. As also mentioned in the previous studies, ILE administration may prevent biochemical parameters from being measured accurately. Therefore, we could not say anything about molecular based changes in the blood in terms of organ functions.

6. Conclusion

According to our results, acute pioglitazone intoxication with 1000mg/kg may cause histopathological changes in renal and liver tissues in rats and ILE is not effective in reversing these unwanted effects. Our results also showed that acute intoxications with that given dose may not cause cardiac myocyte changes. Another important result of our study is that ILE itself may be toxic for renal tissues.**Conflicts of Interest:** The authors declare that they have no conflict of interest.**Funding:** This work was supported by the University of Health Science, Istanbul, Turkey (grant number 2018/084).

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