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EFFECTS OF FLUNIXIN MEGLUMINE ON KIDNEY FUNCTION AND MUSCLE INJURY IN EXPERIMENTAL RAT RHABDOMYOLYSIS MODEL

J. TAJIK[1], S. NAZIFI[2], M. SHAKIBAEINIA[2] & M. BAHADORI[2]

[1]Department of Clinical Studies, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran; [2]Department of Clinical Studies, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

Summary

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Rhabdomyolysis is associated with extensive muscle injury accompanied by the secondary renal failure due to myoglobin deposition in the kidney. It has been shown that administration of acetaminophen in a rat model of rhabdomyolysis was effective in improving renal function and decreased renal damage. In this study, the effects of flunixin meglumine administration in a rat model of rhabdomyolysis were investigated. Four groups of rats (8 rats in each group) were employed in this study. Group 1 served as control, the glycerol (Gly) group was given 50% glycerol (7 mL/kg, i.m.), saline-NSAID group received saline injection in place of glycerol and flunixin meglumine daily (2 mg/kg, i.p.), and Gly-NSAID group was given glycerol and flunixin meglumine. Ninety-six hours after glycerol injection, blood samples (2–3 mL) were collected by heart puncture. Serum concentrations of creatinine, blood urea nitrogen (BUN) creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were measured in serum samples. Gly and Gly-NSAID groups had higher serum concentrations of creatinine, BUN, ALT, AST and CK than the control and saline-NSAID groups ($P<0.05$). The Gly-NSAID group had higher serum creatinine and BUN levels than the Gly group ($P=0.022$ and $P=0.04$, respectively). It seemed that flunixin meglumine administration decreased the muscle injury, however, it increased the renal damage and hence administration of flunixin meglumine in rhabdomyolysis cases needs more consideration.

Key words: flunixin meglumine, rat, rhabdomyolysis

INTRODUCTION

Rhabdomyolysis is associated with extensive muscle injury accompanied by the release of myoglobin into the circulation and secondary renal failure due to myoglobin deposition in the kidney. It occurs in human and animals such as horses. The causes of rhabdomyolysis are various: trauma, malignant hyperthermia, seizures, muscle ischaemia, drug overdose, intense exercise, heat stroke, metabolic disorders and genetic disorders. In humans, it is estimated that renal failure occurs in 30% of rhabdomyolysis cases and rhabdomyolysis has been implicated as the cause of 7% of acute renal failure cases (Moore

et al., 1998; Boutaud *et al.,* 2010; Boutaud & Roberts, 2011).

A rat model of rhabdomyolysis, using intramuscular injection of glycerol, has been used in different studies as a popular model for renal damage formation following rhabdomyolysis (Zurovsky, 1993). Tubular obstruction and necrosis due to direct heme protein-induced cytotoxicity, and renal vasoconstriction have been introduced as the main pathophysiologic mechanisms of rhabdomyolysis-associated renal damagese (Boutaud *et al.,* 2010). Deposition of the released Mb in the kidney causes lipid peroxidation, which play an important role in the rhabdomyolysis-associated renal tubular cells injuries (Boutaud & Roberts, 2011). Lipid peroxidation also produces very potent renal vasoconstrictors, which have been proposed as the main cause of renal vasoconstriction during myoglobinuria (Moore *et al.,* 1998).

It has been shown that administration of acetaminophen, a nonsteroidal anti-inflammatory drug (NSAID), in a rat model of rhabdomyolysis was effective in improving renal function and decreased renal damage (Boutaud *et al.,* 2010). Hepatic necrosis and renal damage occur following acetaminophen overdose in both human and laboratory animals and it is believed that the use of the same drugs that are more potent than acetaminophen and have less hepatic and renal toxicity can provide a new approach in more effective treatment of rhabdomyolysis and prevention of renal damages (Tripathy & Grammas, 2009; Boutaud *et al.,* 2010). Flunixin meglumine is a very potent NSAID with analgesic, anti-inflammatory and antipyretic activities, which is approved for use in veterinary medicine. There is no clinical case report of flunixin meglumine overdoses (Plumb, 2002) indicating that it may be a safe drug. On the other hand, renal crest necrosis has been reported as an adverse effect of NSAIDs administration, as its occurrence increases in patients predisposed to renal damage (MacAllister *et al.,* 1993; Stokes & Bartges, 2006;).

Administration of flunixin meglumine has been recommended in horse rhabdomyolysis to decrease pain and inflammation (Valberg, 2002) and there are some reports of its administration in horse cases of rhabdomyolysis (Harris, 1991; Sponseller, 2005; Estill & Valentine, 2007). However, there is no previous study about the evaluation of the effects of flunixin meglumine in rhabdomyolysis cases. This study was undertaken to investigate the effects of flunixin meglumine administration in a rat model of rhabdomyolysis.

MATERIALS AND METHODS

Thirty two male Sprague–Dawley rats (180–200 g) were obtained from the Razi Serum Research Institute, Shiraz, Iran. The animals were acclimatized to the animal room conditions 14 days before the beginning of the experiment. The rats were kept under constant conditions of temperature (25–27 °C), relative humidity (20–30%), and a 12 h light/dark cycle. There was free access to food (standard laboratory rodent pellet diet, Razi, Iran) and water. The experiment was performed according to the suggested European ethical guidelines for the care and use of laboratory animals in experimental investigations.

The animals were randomly divided into 4 groups (8 rats for each group) as follows: control, saline-NSAID, Gly and Gly-NSAID. Experimental rhabdomyolysis in the Gly and Gly-NSAID groups was induced by a single intramuscular injec-

tion of 50% glycerol (7 mL/kg) divided into both lower hind limbs (Homsi *et al.,* 2010). The control and saline-NSAID groups received saline injection in place of glycerol. The saline-NSAID and Gly-NSAID groups also received flunixin meglumine (Flunex®, Razak, Iran) daily by intraperitoneal injection (2 mg/kg) concomitant with glycerol and on subsequent days.

Ninety-six hours after glycerol injection, the animals were sacrificed using ether anaesthesia. Blood samples (2–3 mL) were collected by heart puncture. The blood serum was separated after centrifugation at 750 *g* for 10 min and stored at –18 ^{o}C until analysis.

Serum biochemical analysis was done for measuring creatinine concentrations with the modified Jaffe method, blood urea nitrogen (BUN) by diacetyl monoxime method (Burtis & Ashwood, 1994), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities by the modified method of Reitman & Frankel, lactate dehydrogenase (LDH) activity by the Sigma colorimetric (Cabaud Wroblewski) method, creatine kinase (CK) by the Sigma colorimetric method (modified by Hughes) and alkaline phosphatase (ALP) activity by the modified method of Bowers & McComb (Thrall *et al.,* 2004). All the enzyme activities were measured at 37 °C and the results have been presented in U/L (Burtis & Ashwood, 1994).

Statistical analysis was performed using SPSS12 (Illinois, Chicago). One-way analysis of variance (ANOVA) tests with a *post hoc* Bonferroni test were used for comparison of the measured factors between different groups. Differences were considered significant at $P<0.05$.

RESULTS

Appearance of myoglobinuria and *post mortem* evaluation of glycerol injection sites confirmed the induction of rhabdomyolysis in the Gly and Gly-NSAID groups. Table 1 shows the results of the measured serum factors in the control and treatment groups. No significant difference in the serum concentration of LDH was found between the different groups. Serum BUN in the Gly and Gly-NSAID groups was significantly higher than in the control group (P=0.004 and P=0.006, respectively). These groups also have higher serum BUN than the saline-NSAID group (P=0.01 and P=0.009, respectively). Similarly, the Gly and Gly-NSAID groups had higher creatinine concentrations than the control group (P=0.004 and P=0.006, respectively) and than the saline-NSAID group (P=0.006 and P=0.008, respectively). The Gly-NSAID group had higher serum creatinine (P=0.022) and BUN (P=0.04) than the Gly group.

Evaluation of serum ALT revealed that the Gly group had higher activities than both control and saline-NSAID groups (P=0.006 and P=0.019, respectively). The Gly-NSAID group had significantly higher serum ALT than these groups (P=0.04 and P=0.05, respectively). The Gly group also had higher serum AST than the control and saline-NSAID groups (P=0.011 and P=0.014, respectively), and serum AST in the Gly-NSAID was significantly higher than these groups (P=0.02 and P=0.025, respectively).

Measurement of serum CK showed that the Gly and Gly-NSAID groups had higher serum concentration than the control and saline-NSAID groups (P=0.018 and P=0.014, respectively).

Table 1. The serum concentrations (mean±SEM; n=8) of blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine phosphokinase (CK) and lactate dehydrogenase (LDH) in control and treated rats

Group	Creatinine (μmol/L)	BUN (mmol/L)	LDH (U/L)	CK (U/L)	ALP (U/L)	ALT (U/L)	AST (U/L)
Control (saline)	63.65± 3.54^{a}	8.33± 0.37^{a}	2969± 563.8^{a}	170± 18.4^{a}	728± 69.4^{a}	102.5± 20.2^{a}	330.8± 43.3^{a}
Saline+NSAID	65.42± 3.54^{a}	9.05± 0.53^{a}	3833± 602.5^{a}	168± 15.6^{a}	679± 54.1^{a}	95.2± 11.2^{a}	298.2± 22.6^{a}
Glycerol	101.66± 8.84^{b}	17.46± 3.7^{b}	4015± 309.6^{a}	1629± 140.1^{b}	521± 31.5^{a}	317.6± 60.4^{b}	651.2± 60.79^{b}
Glycerol+NSAID	173.27± 22.1^{c}	36.80± 6.3^{c}	3318± 728.4^{a}	1500± 108.6^{b}	601± 65.1^{a}	290.8± 59.6^{b}	569.4± 76.64^{b}

^{abc} Different letters show significant differences ($P<0.05$).

DISCUSSION

Although administration of flunixin meglumine has been recommended in treatment of rhabdomyolysis affected horses (Valberg, 2002), to the best of our knowledge, there is no previous study regarding the probable adverse effects of flunixin meglumine administration in these cases. Recent studies have revealed the causative role of myoglobin-mediated oxidative injury in renal pathogenesis during rhabdomyolysis. The release of free iron from the myoglobin and myoglobin redox-cycling induced lipid peroxidation has been proposed as the probable mechanisms (Moore *et al.,* 1998; Boutaud & Roberts, 2011). On the other hand, it has been shown that renal lipid peroxidation during myoglobinuria produces very potent renal vasoconstrictors that has been proposed as the main cause for the intense renal vasoconstriction that occurs in myoglobinuria (Moore *et al.,* 1998).

In vitro and *in vivo* studies have shown that acetaminophen decreases the rhabdomyolysis induced lipid peroxidation and renal histopathologic damage, and improves the renal function in experimental rhabdomyolysis (Boutaud *et al.,* 2010). Flunixin meglumine is a very potent blocker of the cyclooxygenase acitivity and restricts the production of inflammatory molecules, which are responsible for a part of the renal pathogenesis during rhabdomyolysis (Soma *et al.,* 1992; Galbraith & McKellar, 1996).

Renal toxicosis and renal crest necrosis are the adverse effects of NSAIDs administration, which are more common in patients with concurrent renal disease or patients with conditions predisposing them to renal damage (Stokes & Bartges, 2006). The diminished synthesis of protective prostaglandins and reduction in their protective effects on renal function in the presence of systemic vasoconstrictors has been introduced as the cause of the NSAID-associated renal damage (Levenson *et al.,* 1982). Our results showed that rhabdomyolysis caused the expected rise in serum creatinine and BUN. Serum creatinine and BUN had no significant difference between the control and the saline-NSAID groups, which showed

that administration of flunixin meglumine in healthy animals did not affect renal function. However, comparisons of creatinine and BUN between the Gly-NSAID and Gly groups revealed that concurrent administration of flunixin meglumine clearly exacerbate the renal function. However, Boutaud *et al.* (2010) reported that acetaminophen treatment significantly attenuated the creatinine rise in the rat model of rhabdomyolysis, which is in discrepancy to our findings regarding the flunixin meglumine administration. It seems that the additional effects of acetaminophen rather than flunixin meglumine in blocking the redox cycling of myoglobin and preventing the myoglobin-induced lipid peroxidation cause its different reported effects in rhabdomyolysis cases. Aggressive fluid therapy to restore renal perfusion is the basic treatment for prevention of rhabdomyolysis renal failure (Bagshaw *et al.,* 2010). It is possible that concurrent administration of flunixin meglumine and aggressive fluid therapy have a different therapeutic result.

Another beneficial effect of NSAIDs administration in rhabdomyolysis cases may be a decrease in muscle injury, and for this reason some authors have recommended the administration of flunixin meglumine in rhabdomyolysis affected horses to decrease muscular pain and inflammation (Valberg, 2002). According to our results, flunixin meglumine causes a numerical decrease in serum CK and AST in the Gly-NSAID group in comparison to the Gly group and it seems that flunixin meglumine decreased the muscle injury. In the experiment of Boutaud *et al.* (2010), although the decrease in muscle injury by acetaminophen was not examined by measurement of CK levels, it is believed that the same levels of myoglobin deposited in the kidney suggested that muscle injury was not significantly affected by acetaminophen. In contrast, it is generally believed that acetaminophen has no anti-inflammatory effect (Rahusen *et al.,* 2004), which may be the cause of the observed difference between flunixin meglumine and acetaminophen.

ALT and AST activities in liver, cardiac and skeletal muscle are greater than in other organs (Hoffmann & Solter, 2008). Increased serum concentrations of these enzymes in the Gly group were possibly due to the muscle injury and the observed decrease in their serum concentrations in Gly-NSAID treated rats may confirm the muscle injury decrement.

Although more sophisticated work is required on a larger number of animals from different species before the importance of these findings can be assessed, the results of the current study revealed that flunixin meglumine administration in rhabdomyolysis cases may decrease the muscle injury. However, administration of NSAIDs, except acetaminophen, may increases the renal damage and needs further consideration.

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Correspondence:

Dr. S. Nazifi
Professor of Veterinary Clinical Pathology
Department of Clinical Studies,
School of Veterinary Medicine,
Shiraz University, P.O. Box: 1731-71345,
Shiraz, Iran
Tel: +98-711-2286940
Fax: +98-711-2286950
E-mail: nazifi@shirazu.ac.ir