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Effects of Enflurane on Histology of Liver and Kidneys in Rats

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Abstract: The aim of this study was to assess the probable hepatic and renal histopathological changes in rats following a 2 h of enflurane administration, which is still used for anesthesia. A total of 20 Wistar rats were used in the study. The rats were classified randomly into 2 groups as control group and experimental group. The rats in the experimental group were anesthetized with 2% inhalational enflurane for 2 h. Following the anesthesia, sacrificed rats were subjected to necropsy and liver and kidneys were examined histopathologically. Histopathological changes in the experimental group included degenerative and necrotic changes and inflammatory cell infiltration. The kidneys were more affected compared to liver. Consequently, it was assessed that enflurane had significant effects on the histology of liver and kidneys. It was suggested that renal function should be taken into consideration while using this anesthetic agent.

Key words: Enflurane, rat, kidney, liver, histopathology

INTRODUCTION

Halogenated inhalational anesthetic agents undergo biotransformation in varying rates and as a result, metabolic products are formed and some of these products are excreted via kidneys (Eger *et al*., 2007; Martin, 2005; Njoku *et al*., 1997; Reichle and Conzen, 2003). Biotransformated agents cause hepatic injury by immunological reactions of reactive metabolites. The metabolites produce serious microsomal lesions by raising lipid peroxidation and malonyldialdehyde (Martin, 2005; Njoku *et al*., 1997; Reichle and Conzen, 2003). Enflurane (2-chloro-1, 1, 2,-trifluoroethyl-difluoromethyl ether) is a halogenated ether that is metabolized about 2% (Reichle and Conzen, 2003; Eger *et al*., 2007) and still used for inhalational anesthesia, especially in North America (Eger *et al*., 2007). Inorganic fluoride formed due to metabolism of halogenated anesthetics, especially in long inhalation anesthesia in humans and animals, is the main cause for the changes in the kidneys. Inhalation anesthetics are more metabolized in rats when compared to humans (Conzen *et al*., 1995; Eger *et al*., 2007). However, experimental studies about the effects of enflurane on histology of liver and kidneys in rats are limited (Njoku *et al*., 1997). The aim of this study was to assess the probable hepatic and renal histopathological changes in rats following a 2 h of administration of enflurane which has been still used for anesthesia.

MATERIALS AND METHODS

A total of 20 Wistar rats were used. The rats were 4 months old and weighed approximately 350-400 g. The rats were kept at temperature 21±2°C with humidity 50±9% and were fed with a standard diet for 10 days. Animals subjected to daily lightening regime of 12 h light followed by 12 h dark. Rats received standard rat chow and water *ad libitum*. The rats were classified randomly into 2 groups as control group and experimental group, as 10 rats per group. The control group was administered with 50-50% oxygen-dry air for 2 h in 50×50×40 cm glass mechanism using an anesthesia device (Drager Cato Edition model, Germany). The rats in experimental group were anesthetized with 2% inhalational enflurane and 50-50% dry air-oxygen for 2 h with same mechanism. Following the anesthesia, rats were killed by decapitated by cervical dislocation in conformity with the animal welfare law. Then, necroscopy were made and tissue samples of liver and kidneys were fixed in 10% buffered formalin solution. Following the routine process, cross sections of paraffin blocks were stained with Heamtoxylin and Eozin (H and E) and examined under light microscope.

Histopathological changes in the liver were assessed with the modified scoring criteria of Tsimoyiannis *et al.* (1993). Briefly, 0- no histopathological changes 1-minimal degenerative changes, 2-moderate centrilobular degnerative and necrotic changes, 3-serious centrilobular

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cellular changes 4-centrilobular and moderate midzonal cellular changes, 5-severe centrilobular and midzonal cellular changes, 6-widespread and severe degenerative and necrotic changes in liver. Changes in kidneys were assessed with the modified scoring criteria (0-3) of Shigematsu (1997). Accordingly, 0-No histopathological change, 1-minimal; glomerular mesangial proliferation and congestion, 2-moderate; thicking in basal membrane, intratubular mass and interstitial cell infiltration 3-severe; thicking in basal membrane and widespread tubular nephrosis.

RESULTS

The kidneys were more affected compared to liver in the rats exposed to enflurane anesthesia. In this study, histopathological findings were not detected in 2 of 10 cases in the liver. Histopathological changes in 6 cases were assessed with score 1. Observed findings in the liver were as follows: degeneration, sinusoidal congestion and Kuppfer cell activation. Hydropic and vacuolar degenerations were seen in the liver, generally located on centrilobular area (Fig. 1). In 2 cases, assessed with score 2, were detected with focal liver cell necrosis and mononuclear cell infiltration generally on portal zones in addition to hydropic and vacuolar degeneration.

In all rats of experimental group, histopathological changes were detected in kidneys. In terms of scoring criteria, 6 cases were assessed with score 1, 4 cases were assessed with score 2. In cases with score 1, glomerular congestion and mesangial cellular proliferations were observed. Because of congestion, glomeruli filled the Bowman's capsule (Fig. 2). In 4 cases with score 2, widespread proximal tubular necrosis with desquamated necrotic epithelial cells in the lumen was observed following enflurane exposure (Fig. 3). Remnants of cellular debris were observed in the lumen of both proximal and distal tubules (Fig. 4). Epithelial cell nuclei showed pyknosis, karyolysis and karyorhexsis, indicating that they were in the process of necrose. In addition, inflammatory cell infiltrations consisting of neutrohpil and mononuclear cells were seen in interstitial area in the kidneys. There was not any histopathological abnormalities for the liver and kidneys of control group.

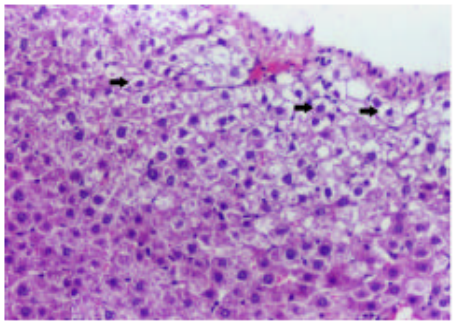

Fig. 1: Liver from a rat exposed to enflurane shows hydropic degeneration (arrows) in the centrilobular area. H and E ×400

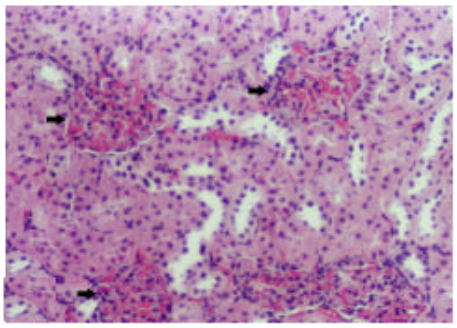

Fig. 2: Kidney from a rat exposed to enflurane shows glomerular congetions (arrows). H and E ×400

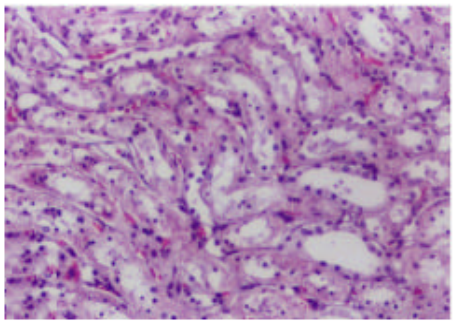

Fig. 3: Kidney from a rat exposed to enflurane shows tubular degeneration and necrosis in the cortical area. H and E ×400

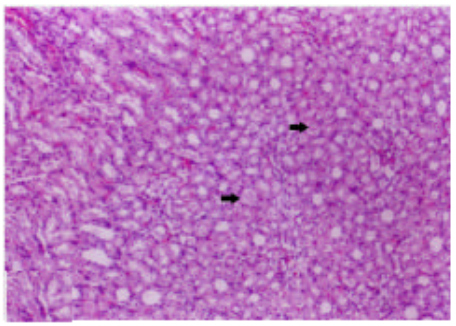

Fig. 4: Kidney from a rat exposed to enflurane shows tubular degeneration and necrosis in the corticomedullar area and intra tubular cellular debris (arrows). H and E ×200

DISCUSSION

Since, the initiation of administration of halogenated inhalational anesthetics, their possible negative effects on parenchyma organs have been discussed. While, halothane comes to the forefront in discussions, the negative effects of its alternatives have widely been discussed (Baden and Rice, 2000; Cook *et al*., 1978; Eger *et al*., 2007; Plummer *et al*., 1986). The new alternatives bring forward the effects of them on personnel and environment (Cook *et al*., 1978; NIOSH, 1977). A study by Frink *et al.* (1992) on dogs stated observed that as a result of the change in the oxygen balance of hepatic blood flow and hepatic drug metabolism, there were negative effects. Ghantous *et al.* (1992) stated that enflurane, isoflurane and sevoflurane have less effect on protein synthesis than halothane in similar concentrations in isolated rat livers. It has been reported that the degree of metabolism of a volatile anaesthetic depends on, among other factors, the amount of the anaesthetic absorbed by the organism and therefore, the metabolism correlates with the solubility in blood and other tissues (Reichle and Conzen, 2003). It has been assessed that there is a relationship between metabolism rates of halogen containing inhalation agents and hepatic and renal injury. When, the enflurane is used as alternative of halothane, it is metabolized with a rate of 2% (Carpenter *et al*., 1986; Plummer *et al*., 1986; Reichle and Conzen, 2003). The fact that inhalation agents are more metabolized in rats than humans (Conzen *et al*., 1995; Eger *et al*., 2007) is the reason for usage of rats in the present study. It was reported that the liver and the kidneys are the major organs affected by inhalational anaesthetic toxicity (Reichle and Conzen, 2003). Observed histopathological changes in liver and kidneys are formed with reactive metabolites due to enflurane biotransformation (Njoku *et al*., 1997; Reichle and Conzen, 2003). Although, some researches (Lewis *et al*., 1983; Paull and Fortune, 1987) reported deaths in accordance with enflurane exposure period and personal sensitivity, it has been claimed that as seen in this study, severe hepatic injury duo to enflurane does not generally occur (Njoku *et al*., 1997). In the present study, of the 10 rats administered with enflurane for 2 h, in 2 cases no findings was observed in liver, 6 cases had minimal histopathological changes (score 1) and 2 cases had moderate histopathological changes (score 2).

Enflurane has nephrotoxic effects as well as hepatotoxic effects and it has been reported that the reason of renal injury duo to enflurane is the inorganic fluoride in blood circulation (Conzen *et al*., 1995; Malan *et al*., 1993). In this study, degenerative and necrotic changes in kidneys of experimental groups were seen. These changes were minimal in six cases (score 1) and moderate in four cases (score 2). When, the number of cases and histopathological changes are taken into consideration, it is obvious that kidneys are more affected than liver.

Consequently, the present study assessed the histopathological effects of 2% enflurane in terms of hepatic and renal toxicity. Enflurane caused significant changes in liver and kidneys. It was suggested that renal function should be taken into consideration while using this anesthetic agent.

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