Effects of Vitamin C on Liver Enzymes and Biochemical Parameters in Rats Anesthetized with Halothane*

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Abstract. Halothane is an important human and veterinary anesthetic, which produces free radicals during biotransformation. Occasionally, these free radicals may cause hepatic injury, especially in case of multiple halothane exposures over short periods. Vitamin C may protect cellular lipids and lipoproteins against oxidative damage by the free radicals. This study investigated the effects of vitamin C on liver enzymes and other biochemical parameters in rats anesthetized with halothane. One group of rats was used as a control, and saline (0.9 % NaCl) was injected intraperitoneally into these animals as a placebo. The second group of rats was used as an anesthesia control group and was only anesthetized by halothane for 2 h. The third group was anesthetized by halothane and injected vitamin C intraperitoneally. Activities of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase enzymes were significantly increased ($p < 0.05$, $p < 0.01$, $p < 0.05$, respectively) by halothane anesthesia, but decreased ($p < 0.05$, $p < 0.05$, $p < 0.05$, respectively) with administration of vitamin C. Concentrations of triglycerides, cholesterol, total bilirubin and creatinine were statistically affected ($p < 0.05$, $p < 0.01$, $p < 0.05$, and $p < 0.01$, respectively) by injection of vitamin C. Values of erythrocyte counts, packet cell volumes, hemoglobin concentration, leukocyte counts, rates of neutrophils and lymphocytes were significantly affected ($p < 0.01$, $p < 0.05$, $p < 0.05$, $p < 0.01$, $p < 0.001$ and p < 0.01, respectively) by halothane anesthesia. The values of erythrocyte counts, leukocyte counts, neutrophil and lymphocyte rates were significantly decreased ($p < 0.05$, $p < 0.05$, $p < 0.05$, $p < 0.01$ and $p < 0.01$, respectively) with administration of vitamin C. Based upon these results, vitamin C may play an important role in the prevention of hepatic cellular injury inflicted by halothane anesthesia.

Key words: Vitamin C — Halothane anesthesia — Rat

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Introduction

Halothane is one of the most important anesthetics commonly used for anesthetizing of humans and animals, and it is metabolized by the cytochrome P450 microsomal system in the liver (Younes et al. 1988; Lind et al. 1989; Durak et al. 1996; Spracklin et al. 1996). Halothane is transformed *via* either oxidative or reductive pathways leading to generation of volatile metabolites (Spracklin et al. 1996). The metabolites produced by the two pathways may damage cellular macromolecules and other components in the liver (Lind et al. 1989; Durak et al. 1996; Spracklin et al. 1996). In some studies, hepatic injury was occasionally induced in animal models as a result of interaction of halothane exposure, induction of liver enzymes, and hypoxia, especially after multiple halothane exposures over short periods (Knights et al. 1987; Gil et al. 1988; Nomura et al. 1988; Lind et al. 1989; Hassal et al. 1990; Ray and Drummond 1991).

Volatile anesthetics may increase the plasma activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), and affect some other biochemical and hematological parameters in laboratory animals (Fee et al. 1979; Comporti 1985; Younes et al. 1988; Lind et al. 1989; Durak et al. 1996; Spracklin et al. 1996; Naziroglu 1999). Accumulation of volatile metabolites and other free radicals in hepatic cellular components may increase cellular degeneration of the liver. High levels of these enzymes are usually indicative of hepatic damage in humans (Gil et al. 1988; Hassal et al. 1990) and experimental animals (Mitra et al. 1991; Parola et al. 1992; Harvey et al. 1994; Durak et al. 1996; Netke et al. 1997; Ölmez and Karakilçik 2004). Several procedures have been used to protect the liver from damage by administrations of antioxidants such as β-carotene (Ölmez and Karakilçik 2004), vitamin C (Mitra et al. 1991; Netke et al. 1997), vitamin E (Parola et al. 1992; Harvey et al. 1994; Durak et al. 1996; Naziroglu 1999) and selenium–vitamin E combination (Brucato et al. 1986; Sies and Stahl 1992; Naziroglu 1999).

Vitamin C may protect lipids and lipoproteins in cellular membranes against oxidative damage caused by toxic free radicals at early stage. The antioxidant function of vitamin C is related to its reversible oxidation and reduction characteristics. Thus, vitamin C may partially prevent certain types of hepatic cellular damage (McDowell 1989; Parola et al. 1992; Sies and Stahl 1992; Burtis and Ashwood 1994; Netke et al. 1997). Therefore, the aim of the present study was to investigate the protective effects, if any, of intraperitoneally injected vitamin C on activities of functional liver enzymes and some biochemical parameters in the plasma of rats anesthetized with halothane.

Materials and Methods

Animals and treatments

This study was carried out on thirty male rats (Wistar albino). The rats weighed 180–200 g and were the same age (3–3.5 months old). The experimental animals

were randomly divided into three groups of ten animals and were housed in cages at 15–17℃ in a dark-light cycle. During the study, the animals received water and pellet food *ad libitum*; the composition of food is shown in Table 1.

Table 1. Diet composition of experimental animals

Ingredient	%
Wheat	10
Corn	22
Barley	15
Wheat bran	8
Soybean	26
Fish flour	8
Meat-bone flour	4
Pelted	5
Salt	0.8
Vitamin-mineral mix*	0.2

* Vitamins A, E, K_3, B_1, B_2, B_3, B_6, and B_{12}, nicotinamid, folic acid, biotin, butylhydroxytoluol and minerals – Ca, Co, Cu, Fe, I, Mn, Zn, and Se.

The first group was used as a control, and only a placebo (saline, 0.9% NaCl) was intraperitoneally injected. The second group (halothane) was exposed to an anesthetic gas mixture (100% oxygen and 1.6% halothane, v/v) with halothane in the vaporizer (Boyle) using the semi-closed method. The third group (halothane plus vitamin C) was exposed to the anesthetic gas mixture (100% oxygen and 1.6% halothane, v/v) with halothane and intraperitoneally injected vitamin C (L-ascorbic acid, 100 mg/kg-body weight). The gas mixture was administered for 2 h from eight to ten o'clock in the morning, and then the vitamin C administrations were done twice at eleven every other day.

Obtaining of blood and plasma

The blood of all animals was taken by cardiac puncture, 12 h after the latest application. Whole blood was collected into tubes (Beckon Dickinson Vacutainer System, France) containing the disodium salt of ethylenediaminetetraacetic acid (EDTA) as anticoagulant. The blood (0.5 ml) in the tubes was used for analysis of hematological parameters; another portion of the blood was centrifuged ($1500 \times g$, 15 min; Heraeus Inst., Mega Fuge 1.0) and plasma was removed using disposable pipettes.

Biochemical and hematological parameters

In the plasma samples, the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), triglycerides (TG), cholesterol (CHOL), total protein (TP), albumin (ALB), total bilirubin (T-BIL) and creatinine (CR) were determined using an Technicon RA-XT autoanalyzer (Technicon Corp., New York, USA). The values of erythrocyte counts (RBC), packet cell volumes (PCV), mean corpuscular volumes (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentrations (MCHC), leukocyte counts (WBC), neutrophil (NEU, per cent) and lymphocyte rates (LYM, per cent) were determined according to the methods described in Schalm's Veterinary Hematology of Jain (1986). Hemoglobin (Hb) concentrations in whole blood were measured spectrophotometrically using the cyanmethemoglobin method of Cannon (1958).

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) for multiple comparisons of all groups. Means and their standard errors (SE) were calculated using the StatView^{TM} 512 program (Brain Power Inc., Calabasas, CA, USA).

Results

Biochemical and hematological parameters and their comparisons among all groups are presented in Tables 2 and 3. In comparisons with the control and anesthesia groups:

The activities of AST, ALT and ALP enzymes were significantly increased ($p < 0.05$, $p < 0.01$, and $p < 0.05$, respectively) by halothane anesthesia, but decreased ($p < 0.05$ in all parameters) with the administration of vitamin C. The concentrations of TG, CHOL and T-BIL in the vitamin C group were statistically lower ($p < 0.05$, $p < 0.01$, $p < 0.05$, and $p < 0.01$, respectively) than in the anesthesia group. CHOL and CR in the anesthesia plus vitamin C group was higher ($p < 0.01$, respectively) than in the control and anesthesia groups. TP in the anesthesia group was also lower ($p < 0.05$) than in controls. ALB in the anesthesia and anesthesia plus vitamin C groups was not statistically affected (Table 2).

The values of RBC, PCV, Hb, and neutrophil rates were significantly increased ($p < 0.01$, $p < 0.05$, $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.01$, respectively) by halothane anesthesia. WBC counts and lymphocyte rates were significantly decreased ($p < 0.01$ and $p < 0.01$, respectively) by halothane anesthesia, but the values of RBC, WBC, LYM and NEU rates were significantly decreased ($p < 0.05$, $p < 0.05$, $p < 0.05$, $p < 0.01$ and $p < 0.01$, respectively) with administration of vitamin C. However, the MCV, MCH, MCHC were not significantly influenced ($p > 0.05$) by halothane anesthesia or administration of vitamin C (Table 3).

Table 2. Mean levels and comparison of enzymes levels and biochemical parameters in all groups[1]

	Control	Anesthesia	Anesthesia + vitamin C
AST ($IU \cdot l^{-1}$)	138.15 ± 7.7	157.14 ± 6.0[a]	142.42 ± 7.5[d]
ALT ($IU \cdot l^{-1}$)	28.00 ± 0.9	48.40 ± 4.2[b]	36.42 ± 3.3[a,d]
ALP ($IU \cdot l^{-1}$)	360.20 ± 6.7	370.40 ± 7.7[a]	347.30 ± 6.9[d]
TG ($mg \cdot dl^{-1}$)	163.52 ± 6.0	172.14 ± 5.4	154.42 ± 5.3[d]
CHOL ($mg \cdot dl^{-1}$)	57.10 ± 0.8	54.00 ± 2.2	74.14 ± 3.1[b,e]
TP ($g \cdot dl^{-1}$)	6.02 ± 0.3	4.50 ± 0.3[a]	5.18 ± 0.3
ALB ($g \cdot dl^{-1}$)	2.80 ± 0.0	2.57 ± 0.1	2.60 ± 0.1
T-BIL ($mg \cdot dl^{-1}$)	0.35 ± 0.0	0.31 ± 0.0	0.22 ± 0.0[a,d]
CR ($mg \cdot dl^{-1}$)	0.28 ± 0.0	0.27 ± 0.0	0.51 ± 0.02[b,e]

[1]Data are expressed as means ± SE; statistical significance with respect to the control group: [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$; statistical significance with respect to the anesthesia group: [d]$p < 0.05$, [e]$p < 0.01$.

Table 3. Mean levels and comparison of some hematological parameters in all groups[1]

	Control	Anesthesia	Anesthesia + vitamin C
RBC ($\times 10^6 \cdot \mu l^{-1}$)	7.08 ± 0.1	8.02 ±0.3[b]	7.30 ± 0.3[d]
PCV (per cent)	45.10 ± 0.6	46.70 ± 0.2[a]	46.00 ± 0.5
Hb ($g \cdot dl^{-1}$)	16.41 ± 0.4	17.75 ± 0.4[a]	17.77 ± 0.3[a]
MCV (fl)	59.85 ± 1.1	58.30 ± 1.4	61.67 ± 2.2
MCH (pg)	22.31 ± 0.6	20.05 ± 1.0	22.32 ± 0.9
MCHC ($g \cdot dl^{-1}$)	36.70 ± 0.6	37.50 ± 0.4	38.97 ± 0.5
WBC ($\times 10^3 \cdot \mu l^{-1}$)	7.70 ± 1.7	5.44 ± 3.3[b]	5.26 ± 2.7[b]
NEU (per cent)	14.70 ± 0.5	20.14 ± 0.6[c]	15.00 ± 0.4[e]
LYM (per cent)	79.20 ± 0.8	73.40 ± 1.0[b]	78.22 ± 1.0[e]

[1] Data are expressed as means and SE; statistical significance with respect to the control group: [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$; statistical significance with respect to the anesthesia group: [d]$p < 0.05$, [e]$p < 0.01$.

Discussion

Vitamin C may play an important role in physiological reactions such as mixed function oxidation involving incorporation of oxygen into a biochemical substrate. In addition, this vitamin is considered the most important antioxidant in extracellular fluids and its antioxidant function has been shown to efficiently scavenge superoxide, hydrogen peroxide, hydroxyl, peroxyl and singlet oxygen radicals. Deficiency in vitamin C causes damage of collagen synthesis in cellular basal membranes, de-

struction of mucosal epithelium and increased capillary fragility (McDowell 1989; Sies and Stahl 1992; Burtis and Ashwood 1994). In some cases, halothane may cause liver injury, especially after multiple exposures over short periods (Gil et al. 1988; Nomura et al. 1988; Lind et al. 1989; Hassal et al. 1990; Ray and Drummond 1991; Durak et al. 1996). Halothane is metabolized by the cytochrome P450 microsomal system in the liver (Younes et al. 1988; Lind et al. 1989; Durak et al. 1996; Spracklin et al. 1996). However, the mechanism that leads to halothane hepatotoxicity is not completely clear, though it has been generally accepted that halothane is transformed by either oxidative or reductive pathways, depending on tissue oxygen concentrations (Lind et al. 1989; Johnson et al. 1993; Durak et al. 1996; Spracklin et al. 1996). As halothane is metabolized by one of the two pathways, it generates volatile metabolites such as 2-chloro-1,1-difluoroethene and 2-chloro-1,1,1-trifluoroethane (Spracklin et al. 1996). These intermediary metabolites, responsible for the hepatotoxic effect of halothane, may bind to cellular macromolecules and react with free amino groups of proteins (Younes et al. 1988; Lind et al. 1989; Durak et al. 1996; Spracklin et al. 1996; Teppema et al. 2002) or hepatocytes to produce more toxic metabolites (Durak et al. 1996; Spracklin et al. 1996) hence the macromolecules may lose their physiological functions.

The intermediary metabolites produced during biotransformation of volatile anesthetics are held responsible for hepatotoxicity and the increase of plasma activities of liver enzymes. They may cause cellular damage by covalent binding to cellular components such as enzymes, nucleic acids and proteins or by another mechanism. Damage of cellular components may play an important role in death of liver cells (Fee et al. 1979; Comporti 1985; Knights et al. 1987; Lind et al. 1989; Durak et al. 1996; Teppema et al. 2002), hence, AST, ALT and ALP may be released to plasma, and serum levels of these enzymes would increase. High serum levels of AST and ALT are usually indicative of liver damage in animals (Knights et al. 1987; Nomura et al. 1988; Younes et al. 1988; Lind et al. 1989; Durak et al. 1996) and humans (Gil et al. 1988; Hassal et al. 1990; Ray and Drummond 1991). Liver-ALP is mobilized most rapidly into blood and its levels in plasma may increase at early periods of liver damage. High ALP serum level is usually indicative of cholestasis. Cholestasis may also result in a progressive liver disease – biliary cirrhosis (Murray et al. 1988; Burtis and Ashwood 1994).

Vitamin C can efficiently scavenge free radicals before they can initiate lipid peroxidation, and contribute to stability of cellular and basal membranes. The antioxidant vitamin C may suppress the hepatotoxic effects of halothane by interference with intermediary metabolites in the cytochrome P450 microsomal system. If vitamin C deficiency occurs in body tissues, cellular membranes may be damaged (Murray et al. 1988; McDowell 1989; Sies and Stahl 1992; Burtis and Ashwood 1994). Practical and effective methods of prevention of early-stage liver injury are quite important. Antioxidants may prevent the harmful effects of free radicals, and may suppress the formation of reactive intermediary metabolites of halothane (Mitra et al. 1991; Parola et al. 1992; Harvey et al. 1994; Durak et al. 1996; Netke et al. 1997; Naziroglu 1999; Karakilçik et al. 2003; Asha and Indira 2004; Ölmez and

Karakilçik 2004). The results of our study (Table 3) are in agreement with the reports of another studies performed using antioxidants and hepatotoxic substances (Durak et al. 1996; Huang et al. 1996; Naziroglu 1999). The activities of AST, ALT and ALP enzymes were statistically affected ($p < 0.01$, $p < 0.05$, respectively) by halothane anesthesia and the administration of vitamin C (Table 2). Those findings are essentially in agreement with the results of other similar investigations (Murray et al. 1988; Parola et al. 1992; Harvey et al. 1994; Durak et al. 1996; Huang et al. 1996; Netke et al. 1997; Naziroglu 1999; Ölmez and Karakilçik 2004). In addition, the values of ALB, TG, CHOL, TP, T-BIL and CR are similar to those reported in similar studies (Naziroglu 1999).

Halothane anesthesia significantly increased the values of RBC ($p < 0.05$) and decreased the values of WBC ($p < 0.05$) (Table 3). There is hypoxia during halothane anesthesia and production of free radical species may increase *via* the reductive metabolism of halothane in hypoxia (Johnson et al. 1993; Asha and Indira 2004). Antioxidants may prevent depression of acute hypoxic ventilatory response (Asha and Indira 2004). In addition, hypoxia stimulates secretion of renal erythropoietin (EPO), and thus erythropoiesis increases in hypoxic conditions because of the increasing secretion of EPO (Murray et al. 1988; Johnson et al. 1993; Burtis and Ashwood 1994). High CHOL levels in the anesthesia plus vitamin C group (Table 2) may be attributed to decreased uptake of CHOL from the plasma by less WBC-derived macrofages. This consideration is confirmed by the results of Huang et al. (1996).

In conclusion, we determined that halothane could increase the liver enzyme levels and affect some hematological parameters. Increase in these parameters may occur due to peroxidation reactions, arising in halothane biotransformation during anesthesia, and these reactions may inflict oxidative injury to cellular components. In the light of these results, vitamin C may play a role in the prevention of hepatic cellular injury produced by volatile anesthetics. However, there is a need for more detailed studies in order to assess the possible relationships between antioxidants and halothane hepatotoxicity. We have been considering the possibility that administration of other antioxidants (like β-carotene, vitamin E and selenium) may have a prophylactic effect in the prevention of halothane hepatotoxicity complications. Therefore, we have been presently carrying out further studies investigating the effects of hepatotoxic chemicals.

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