

The hepatotoxic effect of halofantrine in guinea pigs

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Received: 21.10.2003

Revised: 10.2.2004

Accepted: 23.2.2004

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ABSTRACT

Objective: To investigate the hepatotoxic effects of halofantrine in guinea pigs.

Material and Methods: Halofantrine (30-105 mg/kg) was administered orally to three groups of guinea pigs thrice a week for 4 weeks. One group of animals which received distilled water served as control. The relative weight of the liver was measured. The biochemical parameters like alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and also total and conjugated bilirubin were measured. Liver tissues were subjected to histopathological examination.

Results: There was a significant ($P<0.05$) increase in the relative weight of the liver in all the treated groups compared to control. There was also a significant ($P<0.05$) increase in all the liver enzymes and total and conjugated bilirubin. Histopathological examination of the liver revealed moderate portal triaditis to severe hepatic degeneration in the halofantrine-treated groups.

Conclusion: Halofantrine exhibits hepatotoxic effect in guinea pigs.

KEY WORDS: Antimalarial, live functions, liver damage

Introduction

Halofantrine hydrochloride is a highly lipophilic phenanthrenemethanol, belonging to the aryl-amino-alcohol family. This drug is prescribed as standby treatment in travelers to the tropics who develop febrile illness and has been proposed as a radical cure to avoid the constraints of prolonged chemophylaxis.¹ Numerous studies indicate the cardiotoxicity of halofantrine.^{2,3} The observed cardiotoxicity may be due to its quinidine-like effect.⁴ The effect of other antimalarials like chloroquine on biochemical liver functions and liver tissue have been studied.⁵ Farver and Lavin (1999) reported an increase in alanine transaminase (ALT) and aspartate transaminase (AST) in quinine-induced hepatotoxicity.⁶ The effects of older antimalarials on the liver have been appreciably studied.⁵ The information is scanty on the effect of new antimalarials, especially of halofantrine on the liver.

The present study was based on biochemical parameters and pathological changes as indices for assessing liver dysfunction as a result of halofantrine toxicity.

Material and Methods

Animals: Dunkin–Hartley guinea pigs weighing 500-600 g

obtained from the Veterinary Institute, Vom, Nigeria were acclimatized to housing conditions for one week prior to the commencement of the experiment. They were housed under standard housing conditions of temperature ($22\pm3^{\circ}\text{C}$), and a 12 h light 12 h dark cycle. The animals were housed singly in small partitioned cubicles and provided with water and food (elephant grass) *ad libitum*.

Experimental design

Halofantrine (Halfan) (100 mg/5 ml suspension) was used for the experiment. Four experimental groups of ten animals each were used. The first group received 30 mg/kg of halofantrine (low dose). The second group had 50 mg/kg of halofantrine (medium dose), the third group received 105 mg/kg of halofantrine (high dose)⁷ and the fourth group received only distilled water (control). The drug was administered orally thrice a week for 28 days using oral cannula. The animals were allowed free access to food and water till the end of the experiment.

Any mortality that occurred was recorded. At the end of the experiment, blood samples were collected by cardiac puncture for measurement of biochemical parameters and the animals were sacrificed under chloroform anesthesia. The liver tissue was excised and transferred into ice-cold containers

for biochemical estimations.

Histology

Small pieces of liver tissues were collected in 10% formal saline for fixation. They were processed in an automatic tissue processor, and embedded in paraffin wax. Sections of 5 μ m were cut on a rotary microtome by serial sectioning until the entire thickness of the liver was sectioned. Staining was done by haematoxylin and eosin (H&E) staining method, and microscopy and photomicrograph carried out, using a leitz light microscope.

Determination of serum aspartate and alanine transaminases (AST, ALT), alkaline phosphatase (ALP) and bilirubin

The transaminases, alkaline phosphatase and total and

conjugated bilirubin were measured using the Randox laboratories (UK) Kit procedures.

Statistical analysis

All parameters were analyzed using one-way analysis of variance (ANOVA) while the differences between treatment groups were tested using the Scheffe multiple comparison method with 0.05 representing the level of significance. Results were expressed as mean \pm SEM.

Results

There was a significant ($P < 0.05$) increase in the levels of serum transaminase, alkaline phosphatase activity and total and conjugated bilirubin in all the treated groups compared to control (Table 1). There was also a significant ($P < 0.05$) in-

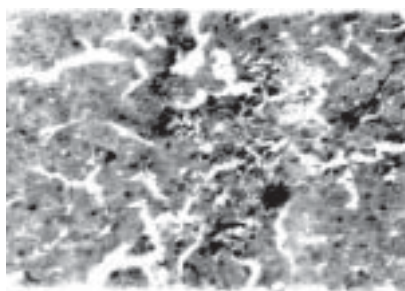
Table 1

Liver enzymes, bilirubin levels and relative weight of the liver in the halofantrine treated and control guinea pigs

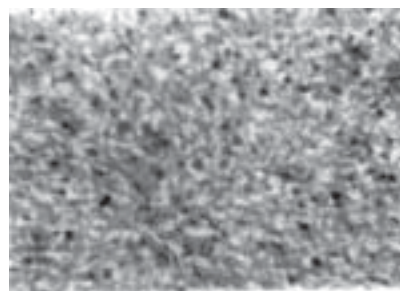
| Treatment with halofantrine (mg/kg) P. O. | | Mean activity in serum | | | Total bilirubin (mg/dl) | Conjugated bilirubin (mg/dl) | Relative weight (%) |
|---|----|------------------------|------------------|-------------------|-------------------------|------------------------------|---------------------|
| | | ALT (IU/L) | AST (IU/L) | ALP (IU/L) | | | |
| Control | | 15.1 \pm 4.02 | 18.6 \pm 4.48 | 62.1 \pm 5.27 | 12.7 \pm 3.40 | 3.2 \pm 0.75 | 2.40 \pm 0.46 |
| 30 | | 19.6 \pm 0.80* | 21.9 \pm 1.40* | 108 \pm 2.79* | 20.5 \pm 3.42* | 5.1 \pm 1.95* | 4.33 \pm 0.53* |
| 50 | | 33.9 \pm 1.35* | 38.6 \pm 0.92* | 134.1 \pm 2.51* | 22.3 \pm 1.36* | 5.6 \pm 0.33* | 4.34 \pm 0.44* |
| 105 | | 48.7 \pm 1.64* | 59.3 \pm 6.60* | 237.0 \pm 6.38* | 27.5 \pm 1.31* | 6.4 \pm 0.50* | 3.71 \pm 0.43* |
| One-way | F | 54.3 | 26.17 | 88.40 | 7.04 | 29.64 | 20.35 |
| ANOVA | df | 3, 36 | 3, 36 | 3, 36 | 3, 36 | 3, 36 | 3, 36 |
| | P | 0.0001 | 0.0001 | 0.0001 | 0.003 | 0.0001 | 0.0001 |

Values are expressed as mean \pm SEM for n=10.

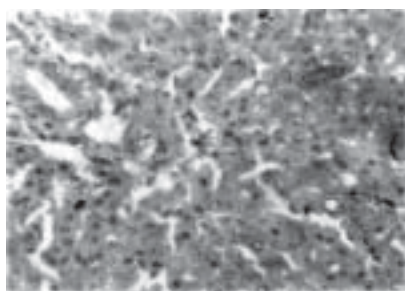
* $P < 0.05$ Significantly different from control



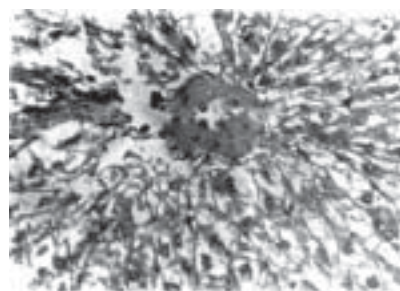
(a)



(b)



(c)



(d)

Figure 1: Photomicrographs of HE-stained sections of guinea pig liver.

(a) Control showing normal hepatic structure,

(b) Group treated with 30 mg/kg of halofantrine showing moderate portal triaditis,

(c) Group treated with 50 mg/kg of halofantrine showing moderate hepatic degeneration,

(d) Group treated with 105 mg/kg of halofantrine showing severe hepatic degeneration. Magnification X 250.

crease in the relative weight of the liver, in all the treated groups compared to control (Table 1). Five animals died in the 105 mg/kg dose group and one animal died in the 50 mg/kg whereas there was no death in the 30 mg/kg dose group. Histological examination showed that the control group exhibited normal architecture of the liver (Figure 1a) and liver cells in the treated group showed moderate portal triaditis to severe hepatic degeneration (Figures 1b, 1c and 1d).

Discussion

More than 1000 xenobiotic substances are potentially hepatotoxic.⁸ The ability of the chemical to produce liver damage *in vivo* often results from the interaction of a series of complex cellular processes involved in the uptake, biotransformation and elimination of these potentially toxic compounds.

The observed increase in the relative weight of the liver in all the treated groups is in agreement with the work of Simons *et al*, 1995 who reported that increased organ weight (whether absolute or relative) is a sensitive indicator of organ toxicity.⁹

Bilirubin and all the enzymes measured in this study were significantly increased in all the treated groups. Liver enzymes are usually raised in acute hepatotoxicity, but tend to decrease with prolonged intoxication due to damage to the liver cells.¹⁰ Increase in liver enzymes such as alanine transaminase (ALT) and aspartate transaminase (AST) are common findings in liver toxicity^{5,11} while increased activity of these enzymes may be found in damaged tissues.¹² The present study has shown that there was a significant increase in all the measured liver enzymes indicating liver damage. Smith *et al*, 1998 reported an increase in ALT in oral acetaminophen-induced hepatotoxicity in rats indicating a biochemical evidence of significant liver damage.¹³ Mizutani and co-workers, 1999 reported an increase in serum ALT activity in methimazole-induced hepatotoxicity in mice.¹⁴

Exposure to halofantrine caused pathologic changes in guinea pigs, which included moderate portal triaditis to severe hepatic degeneration. These changes were dose-dependent. Whatever the agent responsible for injury, the reaction of the liver involves a common sequence of events that can be analyzed at the tissue, cellular and molecular levels. It is however difficult *in vivo* to distinguish the primary effects of a compound from those induced secondarily because liver functions are under the influence of various endogenous and exogenous factors that result from complex interactions with other organs.⁹ The interaction of halofantrine with biological membranes is potentially significant as membrane-related processes might be implicated in the antimalarial action and toxicity of the drug.¹⁵

Most of the understandings of liver injury induced by chemi-

cals remain confined to animal models, and data obtained in animals cannot be extrapolated with certainty to the human situation. Because of the drawbacks of *in vivo* studies of drug and chemical induced hepatotoxicity, *in vitro* liver systems may be approached to investigate mechanisms by which halofantrine induces liver damage. In conclusion, we are aware that the dose used in the present study is high in comparison to the therapeutic dose levels in humans; since small laboratory animals eliminate drugs at a higher rate than humans,¹⁶ higher doses were used in order to maintain an appreciable quantity of the drug in the system.

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