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Main Article

Is halofantrine ototoxic? Experimental study on guinea pig cochlea model

N M Iskander, T F Youssef, M R Ahmed, A A K Mohamed

Abstract

Introduction: Halofantrine is a newly developed antimalarial drug used for the treatment of *Plasmodium falciparum* malaria. The introduction of this drug has been delayed because of its possible side effects, and due to insufficient studies on adverse reactions in humans. There have been no studies investigating its effect on hearing.

Methods: Thirty guinea pigs were divided into three groups: a control group, a halofantrine therapeutic dose group and a halofantrine double therapeutic dose group. One cochlea specimen from each animal was stained with haematoxylin and eosin and the other with toluidine blue.

Results: No changes were detected in the control group. The halofantrine therapeutic dose group showed loss and distortion of inner hair cells and inner phalangeal cells, and loss of spiral ganglia cells. In the halofantrine double therapeutic dose group, the inner and outer hair cells were distorted and there was loss of spiral ganglia cells.

Conclusion: Halofantrine has mild to moderate pathological effects on cochlea histology, and can be considered an ototoxic drug.

Key words: Halofantrine; Drug Toxicity; Ototoxicity; Cochlea; Guinea Pig

Introduction

Malaria is caused by the *Plasmodium falciparum* parasite, which is transmitted from person to person by the bite of an infected anopheles mosquito.[1] There are four types of malaria; of these, two are more common, with symptoms including fever, chills, headache, muscle aches, sweating and shivering, and malaise, and symptoms of progressive anaemia.[2]

Halofantrine is a newly developed antimalarial drug proposed to replace the older quinine group drugs.[3,4] It is effective in the treatment of acute malaria caused by single or mixed infections of *P falciparum* or *P vivax*.[2] It can also be used to treat mild to moderately severe chloroquine-resistant and multidrug-resistant malarial infections.[5,6]

The introduction of this drug has been delayed because of its possible side effects, with insufficient studies on adverse reactions in humans.[3]

Halofantrine acts by forming toxic complexes with ferritoporphyrin IX which damage the parasite membrane. Peak plasma concentrations are achieved 4–8 hours after oral intake.[6,7] Halofantrine is generally well tolerated, with a low incidence of gastrointestinal side effects.[3]

Halofantrine is administered as an 8 mg/kg treatment divided into three doses and repeated after 7 days.[6]

The known ototoxic effects of antimalarial drugs include: vertigo; tinnitus; and a transient, reversible, bilateral, symmetrical, sensorineural hearing loss with a characteristic audiographic notch at 4 kHz.[8,9] These side effects are probably secondary to decreased perfusion to and biochemical alterations in the cochlea, together with direct damage to the outer hair cells.[10,11] Most antimalarial drugs show marked, reversible ototoxic effects, but halofantrine appears to be less ototoxic than quinine.[6,7]

To our knowledge, however, no previous studies have investigated the effect of halofantrine on hearing. Thus, we conducted the present study to evaluate the effects of halofantrine on the cochlea of guinea pigs, using different histological preparations.

Materials and methods

This study was conducted in the ENT and histological units of Suez Canal University Hospital, Ismailia, Egypt. Healthy pigmented guinea pigs (male or female) of the same age and average weight (approximately 200 g) were used, selected according to the following criteria: a normal eardrum (as per otoscopic examination, to exclude infection) and a preserved pinna reflex (i.e. reflex contraction of the auricle in response to loud sounds, a rough indication of normal hearing).

Thirty guinea pigs were divided into three groups of 10: a control group, a halofantrine therapeutic

From the Department of Otolaryngology, Faculty of Medicine and the Suez Canal University, Ismailia, Egypt
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dose group, and a halofantrine double therapeutic dose group.

The 10 control group guinea pigs received 0.05 ml of physiological saline/100 g body weight, divided into three doses given orally at 6 hourly intervals.

The 10 guinea pigs in the halofantrine therapeutic dose group received 0.05 ml of halofantrine/100 g body weight, which corresponded to a therapeutic dose of 8 mg/kg body weight, divided into three doses given orally at 6 hourly intervals.[6]

The 10 guinea pigs in the halofantrine double therapeutic dose group received 0.10 ml of halofantrine/100 g body weight, which corresponded to twice the therapeutic dose of 8 mg/kg body weight, divided into three doses given orally at 6 hourly intervals.

Drugs were administered orally dissolved in saline, 2 hours after food (as dosing with food, especially fatty food, has been shown to increase the rate of absorption, with an increased risk of drug side effects such as irregular heart rate).[6]

The guinea pigs were sacrificed 12 hours after the last drug dose. All animals received an intraperitoneal injection of 100 mg sodium pentobarbital, followed if necessary by a 50 mg intracardiac injection after loss of consciousness.

Approval for similar experimental studies had previously been obtained from the animal care and use committee of our institution.[12]

The animals' temporal bones were placed in ethylene diamine triacetic acid solution for 25 days. Specimens were processed into 6 mm thick sections.

Histological techniques

One cochlea from each animal was stained with haematoxylin and eosin (H&E) and the other with toluidine blue.

In order to visualise the general architecture of the inner ear, Harris's haematoxylin was prepared with the following: 2.5 g haematoxylin, 2.5 ml absolute ethyl alcohol, 50 g potassium alum, 500 ml distilled water, 1.25 g mercuric oxide and 20 ml glacial acetic acid.

Haematoxylin was dissolved in absolute alcohol and added to the alum, which had previously been dissolved in warm distilled water. The mixture was rapidly brought to the boil and mercuric oxide added. The solution was rapidly cooled by plunging the flask into cold water. When the solution was cold, acetic acid was added, and the stain was ready for immediate use. Glacial acetic acid was used to give more precise, selective staining of nuclei.

Eosin was prepared as a 1 per cent solution in distilled water.

The H&E staining technique proceeded as follows: (1) dewaxing of paraffin sections in xylol and then hydration through graded alcohol–water mixtures; (2) haematoxylin staining for 5 minutes; (3) washing under running tap water until specimens became bluish; (4) differentiation in 1 per cent acid alcohol (5–10 seconds); (5) washing with tap water until bluish again; (6) eosin counter-staining for 2 minutes; (7) rapid rinsing; and (8) dehydration with ascending grades of alcohol, clearing in xylene and mounting in distyrene-plasticiser-xylene mixture.[13]

Toluidine blue stain was prepared with 0.2 per cent aqueous toluidine blue and 95 per cent ethyl alcohol.

This staining technique proceeded as follows: (1) dewaxing of paraffin sections in xylene and progressive hydration down to distilled water (through graded alcohol–water mixtures); (2) toluidine blue staining for 6 hours at room temperature; (3) washing in distilled water; (4) rinsing in 90 per cent alcohol and differentiation in 95 per cent alcohol, controlled microscopically until Nissl's substances were seen in the cell bodies of large neurons; and (5) dehydration in alcohol, clearing in xylene and mounting in distyrene-plasticiser-xylene mixture.[14]

Statistical analysis

Histological data were collected using both staining techniques for all three animals groups, and were then analysed and compared with normal images.

Results and analysis

The guinea pig cochlea is normally greyish-yellow in colour and approximately 3×4 mm in size. It has a wide base and tapering apex. It has the appearance of a spiral formed of three and a half turns encircling a bony core (the modiolus) visible on longitudinal sections.

The guinea pig cochlear duct appears triangular; its roof consists of a thin membrane (Reissner's membrane), while its floor is composed of an outer bony spiral lamina from the modiolus and an inner basilar membrane, which carries the organ of Corti on its upper surface.

Control group

No gross changes were detected in control group cochlear specimens.

Haematoxylin and eosin. In specimens treated with this stain, the cochlea appeared as a cone-like structure with a pointed apex and wide base. Each turn was divided into three components, the scala vestibuli, scala media and scala tympani, as seen in Figure 1. The normal structures appeared as follows: nuclei stained blue; cytoplasm stained varying shades of pink; muscle fibres stained a deep pink-red; and collagen stained a pale pink-red. The three rows of outer hair cells appeared as tall cells with acidophilic cytoplasm and basal, basophilic nuclei. One row of the inner hair cells showed acidophilic cytoplasm but more globular cell bodies, as seen in Figure 2.

Toluidine blue. Spiral ganglia sections from control group specimens treated with this stain revealed aggregates of dark blue granules (Nissl's granules), together with pale-staining rounded areas (representing nuclei) containing deep blue stained nucleoli, as seen in Figure 3.

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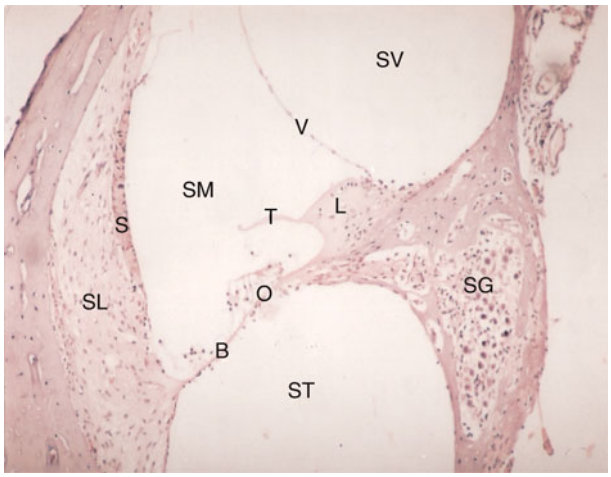

FIG. 1

Photomicrograph of cochlea of a control group guinea pig, showing the structure of the organ of Corti. B = basilar membrane; O = organ of Corti; S = stria vascularis; L = limbus; T = tectorial membrane; V = vestibular or Reissner's membrane; SG = spiral ganglion; SL = spiral ligament; SV = scala vestibule; SM = scala media; ST = scala tympani. (H&E; ×100)

Halofantrine therapeutic dose group

This group received 0.05 ml of halofantrine/100 g body weight, which corresponded to the therapeutic dose of 8 mg/kg body weight, divided into three doses and given orally at 6 hourly intervals.[6]

Haematoxylin and eosin. Specimens stained with H&E showed loss and distortion of inner hair cells and inner phalangeal cells, rupture and distortion of pillar cells, and rupture and vacuolation of Hensen's cells, as seen in Figure 4. These specimens also showed atrophy of the stria vascularis, observed as cell loss and distorted cellularity, together with separation of the stria vascularis from the underlying spiral ligament, and congestion and fusion of stria vascularis blood vessels, as seen in Figure 5.

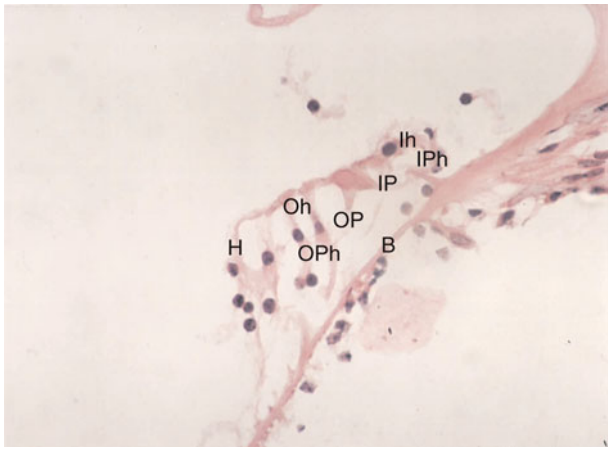

FIG. 2

Photomicrograph of cochlea of a control group guinea pig, showing the detailed structure of the organ of Corti. IP = inner pillar cell; IPh = inner phalangeal cell; Ih = inner hair cell; OP = outer pillar cell; OPh = outer phalangeal cell; Oh = outer hair cell; H = Hensen's cell; B = basilar membrane. (H&E; ×400)

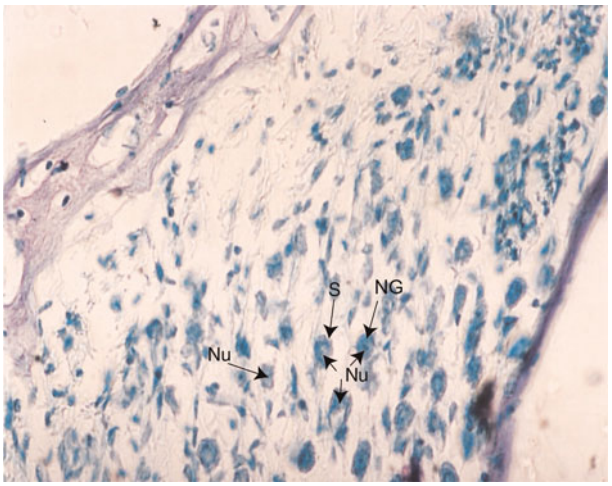

FIG. 3

Photomicrograph of cochlea of a control group guinea pig, showing spiral ganglion cells (S), nuclei (Nu) and Nissl's granules (NG) filling the cytoplasm. (Toluidine blue; ×400)

Toluidine blue. Toluidine blue stained spiral ganglia sections showed chromatolysis, giving the cytoplasm a pale appearance due to reduced Nissl's granules, with moderate loss of spiral ganglia cells, as seen in Figure 6.

Halofantrine double therapeutic dose group

Ten guinea pigs received 0.10 ml of halofantrine/100 g body weight, which corresponded to twice the therapeutic dose of 8 mg/kg body weight, divided into three doses given orally at 6 hourly intervals.

Haematoxylin and eosin. This staining shows distortion and loss in inner and outer hair cells and inner and outer phalangeal cells. Pillar cells showed rupture and distortion, with vacuolation of Hensen's cells. Congestion of blood vessels was observed, together with atrophy of the stria vascularis

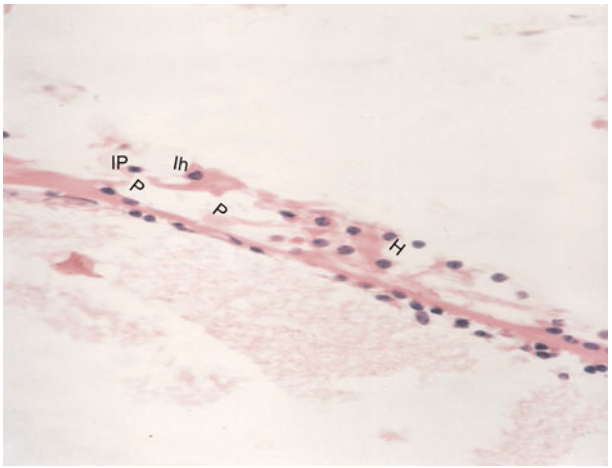

FIG. 4

Photomicrograph of cochlea of a guinea pig in the halofantrine therapeutic dose group, showing rupture of Hensen's cells (H) and pillar cells (P), in addition to loss of inner phalangeal cells (IP) and inner hair cells (Ih). (H&E; ×400)

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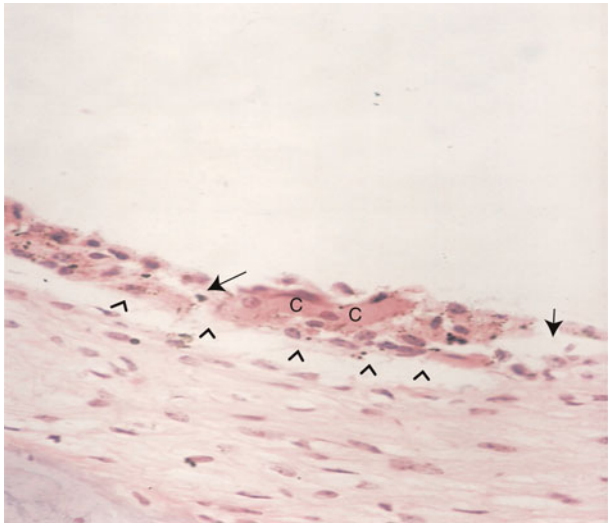

FIG. 5

Photomicrograph of cochlea of a guinea pig in the halofantrine therapeutic dose group, showing congestion and fusion of blood vessels in the stria vascularis (C), distorted cellularity, and loss of cells (→), in addition to separation from the underlying spiral ligament (^ ^ ^). (H&E; ×400)

in the form of distorted cellularity, cell loss and pyknosis, as seen in Figure 7. Spiral ganglia sections showed a decrease in cell numbers and light staining of spiral ganglion cell cytoplasm, as seen in Figure 8.

Toluidine blue stain. Spiral ganglia sections stained with toluidine blue showed chromatolysis, giving the cytoplasm a pale appearance due to reduced number of Nissl's granules, and severe loss of spiral ganglia cells, as seen in Figure 9.

Discussion

Halofantrine is a phenanthrene methanol derivative related to mefloquine and quinine.[5] This newly developed antimalarial drug is proposed as a replacement for the older quinine group drugs, due to increasing reports of side effects from the latter drugs.[3] The US Food and Drug Administration approved halofantrine for use in 1992.[15]

The introduction of halofantrine has been delayed because of fears of side effects and insufficient studies on adverse reactions in humans.[3] Older antimalarial drugs have been shown to have marked, reversible ototoxic effects.[16] However, there are currently no studies investigating the effects of halofantrine on the cochlea. However, the clinical experience of the otolaryngology staff of Suez Canal University

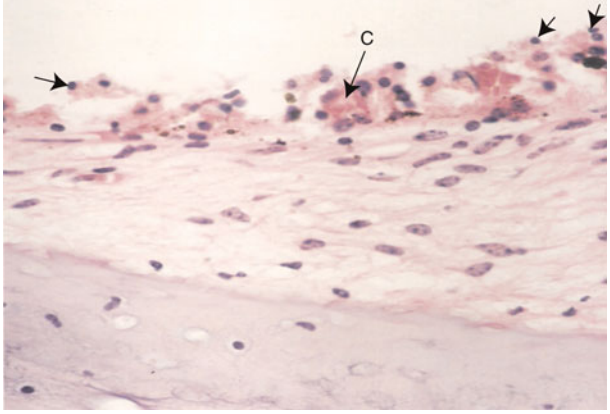

FIG. 7

Photomicrograph of cochlea of a guinea pig in the halofantrine double therapeutic dose group, showing congestion of blood vessels (C) and distorted cellularity with pyknotic nuclei (→) and cell loss. (H&E; ×400)

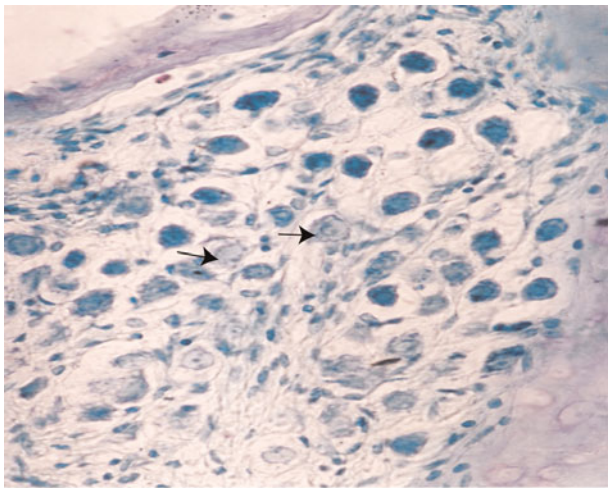

FIG. 6

Photomicrograph of cochlea of a guinea pig in the halofantrine therapeutic dose group, showing mild loss of spiral ganglion cells in addition to chromatolysis (loss of Nissl's granules) in some cells (→). (Toluidine blue; ×400)

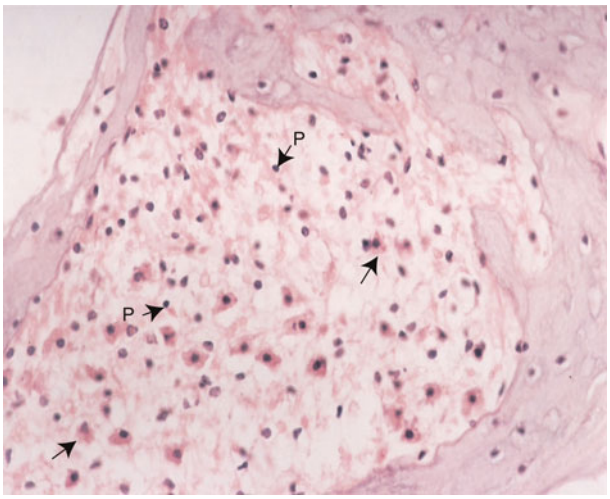

FIG. 8

Photomicrograph of cochlea of a guinea pig in the halofantrine double therapeutic dose group, showing moderate to severe loss of spiral ganglion cells, reduced staining (→) and pyknotic nuclei (P). (H&E; ×400)

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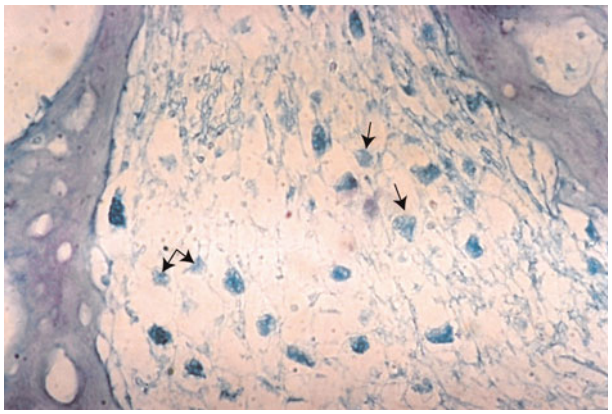

FIG. 9

Photomicrograph of cochlea of a guinea pig in the halofantrine double therapeutic dose group, showing severe loss of spiral ganglion cells in addition to chromatolysis (with loss of Nissl's granules) in some cells (→). (Toluidine blue; ×400)

within endemic areas such as the Republic of Yemen (where malaria is a more common health problem) has indicated that the drug may cause tinnitus and transient hearing loss.

Different doses of halofantrine were used in our study. The recommended therapeutic dose of 8 mg/kg/day was used in the halofantrine therapeutic dose group to investigate cochlear histopathological changes, while double the therapeutic dosage was used in the third animal group to demonstrate dose-dependent effects of halofantrine on cochlear structures. Both doses were adjusted according to the animal's body weight.[15]

The halofantrine therapeutic dose group showed inner hair cell loss with distortion, inner phalangeal cell loss, rupture of pillar cells and Hensen's cells, and decreased numbers of spiral ganglia cells. In their study of the effect of halofantrine on rat testes, Diadia *et al.* found a reduction in the number and level of maturity of spermatogenic cells, associated with damage to the seminiferous tubules, following animal exposure to therapeutic doses of halofantrine.[17]

In addition, we observed degeneration of the spiral ganglia cells, in the form of chromatolysis. Halofantrine has also been shown to have hepatotoxic effects; Obi *et al.* found pathological hepatic degeneration following exposure of guinea pigs to halofantrine.[18]

The halofantrine double therapeutic dose group showed atrophy of the stria vascularis, in the form of cell loss, pyknosis and distorted cellularity. The stria vascularis also showed congestion of blood vessels. These findings are in accordance with those of Obi *et al.*, who found portal triaditis (i.e. inflammation of the portal tract triad including the portal vein, hepatic artery and bile duct) following exposure of guinea pigs to halofantrine.[18]

In the halofantrine double therapeutic dose group, we also noted inner and outer hair cell distortion and loss, inner and outer phalangeal cell distortion and loss, rupture and distortion of pillar cells, and rupture of Hensen's cells. These severe effects, with massive cell loss, are in accordance with Diadia and colleagues' findings in spermatogenic cells: following exposure to halofantrine at double the therapeutic dose, these authors found disturbed seminiferous tubule cellularity, in the form of cell number reduction, loss of maturity and degeneration.[17]

Our findings of outer hair cell distortion and loss are similar to those of Berninger and Gustafsson, who observed damage to the outer hair cells of the organ of Corti following exposure to quinine.[19] Halofantrine and quinine share many side effects, including neurological, vestibular, visual and abdominal problems.[15] The correlation between the observed histopathological effects of halofantrine and quinine on outer hair cells may be due to their structural similarity.[19]

- **Halofantrine is a new antimalarial drug proposed to replace the older quinine group of drugs**
- **Introduction of this drug has been delayed due to fears of its side effects and insufficient studies on adverse reactions in humans**
- **This study demonstrated histological signs of ototoxicity following halofantrine exposure, in a guinea pig cochlea model**

In our sections, the stria vascularis showed congestion of blood vessels and atrophy, in the form of distorted cellularity, cell loss and pyknosis. These features could be explained by the findings of Diadia *et al.*, who reported tubular atrophy and degeneration of seminiferous tubules following exposure to double-dose halofantrine in an animal model.[17]

In our histological preparations, spiral ganglia sections showed decreased cell numbers and cell degeneration in the form of chromatolysis.

In addition, all of our above-mentioned findings are in agreement with those of Bassi *et al.*, who found that halofantrine exhibited cardiotoxic and hepatotoxic effects.[20]

Based on the above data, it was impossible to determine whether the ototoxic effects of halofantrine were reversible or not.

Conclusion

In a guinea pig model, the recommended therapeutic dose of halofantrine results in mild to moderate histological changes in the cochlea; at double the therapeutic dose, marked histological changes occur. Thus, halofantrine may be considered an ototoxic drug.

References

1 Batey AJ, Lightbown ID, Lambert JP, Edwards G, Coker SJ. Comparison of the acute cardiotoxicity of the antimalarial drug halofantrine in vitro and in vivo in anaesthetized guinea-pigs. *Br J Pharmacol* 1997;**122**:563–9

2 Baune B, Flinois J, Furlan VFG, Taburet A, Becquemont L, Farlnotti R. Halofantrine metabolism in microsomes in man: major role of CYP 3A4 and CYP 3A5. *J Pharm Pharmacol* 1999;**51**:419–26

https://doi.org/10.1017/S0022215110001301 Published online by Cambridge University Press

3 Ter Kuile FO, Dolan G, Nosten F, Edstein MD, Luxemburger C, Phaipun L. Halofantrine versus mefloquine in treatment of multidrug-resistant falciparum malaria. *Lancet* 1993;**341**:1044–9
4 White NJ. Delaying antimalarial drug resistance with combination chemotherapy. *Parassitologia* 1999;**41**:301–8
5 Connell MB. History of treatment and prophylaxis of malaria. In: Emilio Pampana. *RPH Laboratory Medicine*, 5th edn. New York, NY: Oxford University Press, 1998; 643–51
6 Nosten F, McGready R, d'Aleessandro U, Nonell A, Verhoeff F, Menenez C *et al.* Antimalarial drugs in pregnancy: a review. *Curr Drug Saf* 2006;**1**:1–15
7 Nyunt MM, Plowe CV. Pharmacologic advances in the global control and treatment of malaria: combination therapy and resistance. *Clin Pharmacol Ther* 2007;**82**:601–5
8 Jung TTK, Rhee CK. Ototoxicity of salicylate, nonsteroidal anti-inflammatory drugs and quinine. *Otol Clin* 1993;**26**: 791–810
9 Winstanley P, Ward S. Malaria chemotherapy. *Adv Parasitol* 2006;**61**:47–76
10 Miller JJ. *Handbook of Ototoxicity*. Boca Raton, Florida: CRC Press, 1985
11 Makanga M, Premji Z, Falade C, Karbwang J, Mueller EA, Andriano K. Efficacy and safety of the six dose regimen of artemether lumefantrine in pediatrics with uncomplicated *Plasmodium falciparum* malaria: a pooled analysis of individual patient data. *Am J Trop Med Hyg* 2006;**74**:991–8
12 Yung MW, Wright A. An improved morphological technique for the study of cochleotoxicity in guinea pigs. *J Laryngol Otol* 1998;**100**:1235–44
13 Steven A. The haematoxylins: In: Bancroft JD, Stevens A, eds. *Theory and Practice of Histological Techniques*, 3rd edn. Edinburgh: Churchill Livingstone, 1990;107–11
14 Bancroft JD, Cook HC. Central and peripheral nervous system. In: Sterling RW, Turner DP. *Manual of Histological Techniques and their Diagnostic Application*. Edinburgh: Churchill Livingstone, 1994;343–72
15 Touze JF, Fourcade L, Peyrone F, Heno P, Deharo JC. Is halofantrine still advisable in malaria attack? *Ann Trop Med Parasitol* 1997;**91**:867–73
16 Horton J. Halofantrine in the treatment of malaria. *Drug Saf* 1995;**13**:271–2
17 Diadia BC, Gbigbi PS, Dapper DV. Preliminary study on the effect of halofantrine on the testes of mature Wister rats. *Journal of Applied Sciences and Environmental Management* 2002;**6**:45–8
18 Obi E, Orisakwe OE, Asomugha LA, Udemezue OO, Orish VN. The hepatotoxic effect of halofantrine in guinea pigs. *Indian J Pharmacol* 2004;**36**:303–5
19 Berninger E, Gustafsson LL. Changes in 2f1-f2 acoustic distortion products in humans during quinine-induced cochlear dysfunction. *Acta Otolaryngol* 2000;**120**:600–6
20 Bassi PU, Buratai BL, Kuchali W. Effect of halofantrine administration on some liver and heart enzymes in healthy human volunteers. *Afr J Biomed Res* 2006;**9**:31–5

Address for correspondence:
Dr M R Ahmed,
Faculty of Medicine,
Suez Canal University,
Ismailia, Egypt.

Fax: +20 66 3415603
E-mail: M_rifaat@hotmail.com

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