

The Effect of Systemic Amantadine Sulfate on Malondialdehyde and Total Thiol Levels in Rat Corneas

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Abstract

Purpose: To evaluate the malondialdehyde (MDA) and total thiol (sulfhydryl, SH) levels in rat corneas after intraperitoneal injection of amantadine sulfate.

Methods: A total of 12 Wistar albino rats were divided into two groups: control group ($n = 6$) and amantadine group ($n = 6$). Balanced salt solution (1 mL, 0.9% NaCl, twice/day) was injected into rats in control group. Amantadine sulfate (2 mg/1 mL, twice/day) was injected into rats in amantadine group. In each group, two rats were injected for 1 week, two received injections for 1 month, and two rats received injections for 3 months. The corneas were homogenized and MDA and SH levels were measured spectrophotometrically.

Results: In control group, median MDA and SH levels were 2.37 (range, 0.92-3.60) and 25.35 (range, 6.30-54.0) nmol/mg, respectively. In amantadine group, median MDA and SH levels were 3.57 (range, 1.25-5.92) and 32.65 (range, 3.30-48.3) nmol/mg, respectively. The difference between this two groups regarding MDA ($P = 0.14$) and SH ($P = 1.0$) levels was statistically insignificant.

Conclusion: Systemically administered amantadine sulfate seems not to cause MDA and SH imbalance in rat corneas.

Keywords: Amantadin Sulfate; Anti-Oxidant; Cornea; Malondyaldehyde; Oxidant, Rats; Total Thiol

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INTRODUCTION

Amantadine, an M2 proton channel inhibitor^[1] and N-methyl-D-aspartate-glutamate receptor antagonist,^[2] is used for prophylaxis and treatment of influenza A,^[3] to treat Parkinson's disease,^[4] tardive dyskinesia,^[5] multiple sclerosis associated fatigue,^[6] and attention deficit/hyperactivity disorder.^[7] Blanchard, for the first time, found a relationship between amantadine and corneal epithelial edema with clear stroma and argued that rapid improvement of the edema after discontinuing amantadine indicates corneal endothelial toxicity.^[8] Since then, 15 cases (3 irreversible, 12 reversible) of amantadine-associated bilateral corneal edema have been reported in peer-reviewed journals. While most of these cases improved after cessation of the drug, some were irreversible and required surgical management

with lamellar or penetrating keratoplasty.^[9-11] Thus, ophthalmologic assessment was suggested not only after the occurrence of visual impairment, but also before initiation and during an uneventful amantadine treatment course.^[12,13]

It has been demonstrated by specular, light and electron microscopic examinations that amantadine causes morphological damage to corneal endothelial cells.^[9] However, the literature lacks reports explaining the mechanism. Whether amantadine accelerates preexisting dysfunction or causes new damage is still unknown. Although there is no evidence for oxidant/antioxidant imbalance induced by amantadine, it has been demonstrated that oxidative status leads to

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apoptosis and necrosis in corneal endothelial cells.^[14] In the present study, malondialdehyde (MDA) and total thiol (SH) have been selected to investigate the oxidant/antioxidant balance of the cornea as these two agents have been used widely for the determination of oxidant/antioxidant balance.^[15-17]

Malondialdehyde is a reactive aldehyde produced by degradation of polyunsaturated lipids by reactive oxygen species and causes toxic stress in cells forming covalent bonds with amino groups of proteins^[15] and phospholipids,^[16] which are advanced lipoxidation end products. Thus, as a biomarker, high levels of MDA indicate oxidative stress in organisms.^[15-17]

Thiols are organic compounds that contain sulfhydryl (SH) groups, also are referred to as thiol groups. They are a major antioxidant component in the body and protect tissues from reactive oxygen species. Total thiol exhibits free or protein-bound intracellular and extracellular thiols. High levels of SH indicate anti-oxidative status.^[17] MDA and SH levels reflect the oxidant/antioxidant balance in tissues or organisms.^[15-17]

Thiol groups are components of Na⁺- and K⁺-dependent ATPase, an endothelial ionic pump that transports excess fluid from within the corneal stroma to the aqueous to maintain corneal transparency. Thus, reduction of thiol levels due to oxidative stress may lead to dysfunction of the pump and consequently corneal edema. Herein, the occurrence of oxidative stress due to systemic amantadine sulfate in rat corneas is evaluated through measuring MDA (oxidant) and SH (anti-oxidant) levels.

METHODS

This experimental study was conducted in Ankara Training and Research Hospital Hüsni Sakal Experimental and Clinical Practice Center. The study was approved by the Local Ethics Committee of Animal Experiments (Ankara Training and Research Hospital, Ankara, Turkey), and conducted in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Visual Research.

Animals

A total of 12 healthy Wistar albino rats (Saki Yenilli Experimental Animal Production Laboratory, Ankara, Turkey) weighing 200-250 g were used. The animals were housed at mean temperature of 21 ± 2°C, with a 14-hour light and 10-hour dark cycle and fed with standard chow and water *ad libitum*.

Twelve rats were divided into two groups: control group ($n = 6$) and amantadine group ($n = 6$). Balanced salt solution (BSS) (1 mL, 0.9% NaCl, twice/day) and amantadine sulphate (2 mg/1 mL, twice/day) (PK-Merz Infusion, Merz Pharma GmbH and Co., Frankfurt, Germany) were injected into rats in the control and

amantadine groups, respectively. In each group, two rats received injections for one week, two rats received injections for one month, and two were injected for 3 months. All injections were performed intraperitoneally. The animals were euthanized with an overdose of xylazine.

Outcome Measures

The outcome measures were MDA and SH levels measured with spectrophotometric methods. The corneas were homogenized in 2 mL of physiologic saline and stored at -80°C.

MDA levels were measured with the method described by Wasowicz et al, through which MDA was reacted with thiobutiric acid, and the reaction product was extracted in butanol. The measurements were performed at 525 nm of excitation wavelength and 547 nm of emission wavelength. As a standard, 0-25 µmol/L 1,1',3,3'-tetraethoxypropane solutions were used.^[18]

For SH determination, samples were treated with 2,2-dithiobisnitrobenzoic acid to form a chromogen compound, which was measured at 412 nm.^[19]

Data Analysis

Data analysis was performed using Statistical Package for Social Sciences for Windows software (SPSS version 16.0, SPSS Inc. Chicago, USA). Although P values, calculated using Shapiro-Wilk test, were higher than 0.05, depending on the small sample size, P-P plots and histograms, it was accepted that data were distributed abnormally. Thus, comparisons between the groups regarding MDA and SH levels were performed using Mann-Whitney U-test, and values were expressed as median, minimum, and maximum. $P < 0.05$ was accepted as a level of significance.

RESULTS

The levels of MDA and SH in the control group and amantadine group are listed in Table 1 and Figure 1. Concerning MDA ($P = 0.14$) and SH ($P = 1.0$) levels, the difference between the control group and amantadine group was not statistically significant [Table 1].

DISCUSSION

Current literature contains 15 amantadine-associated corneal edema cases occurring from 2 weeks to 8 years after initiation of the drug.^[9-12,20-22] The improvement of corneal edema following cessation of amantadine and recurrence of the edema after restarting this drug suggests that the corneal edema is amantadine-induced.^[20]

Histopathologic characteristics of amantadine-associated corneal damage were described as endothelial

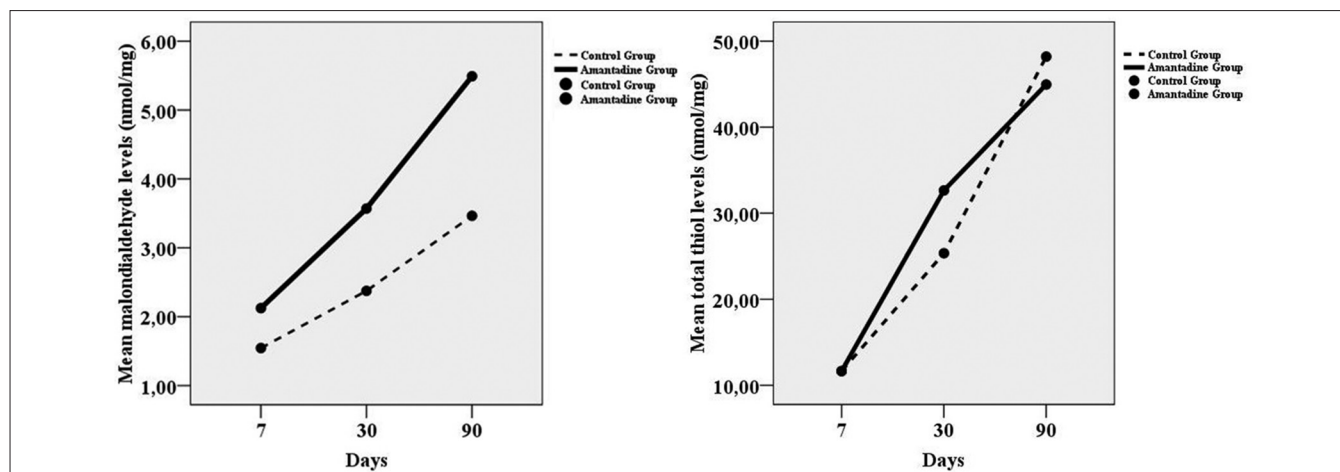


Figure 1. Mean malondialdehyde (MDA) and total thiol (SH) levels.

Table 1. Malondialdehyde (MDA) and total thiol (SH) levels in control group and amantadine group

	Median (min-max) (n=6)		P value ^b
	Control group	Amantadine group	
MDA (nmol/mg)	2.37 (0.92-3.60)	3.57 (1.25-5.92)	0.14
SH (nmol/mg)	25.35 (6.30-54.0)	32.65 (3.30-48.3)	1.0

^bP value: Mann-Whitney U-test. min-max: Minimum-maximum

attenuation, loss of endothelial cells, and areas of denuded endothelial cells with no sign of inflammation;^[9,10] and low density of endothelial cells was demonstrated using specular microscopy.^[20] Although the histopathologic characteristics were described, an accepted mechanism explaining the pathogenesis is still absent. Two suggested mechanisms are dose-dependent toxicity and delayed idiosyncratic hypersensitivity.^[20] In Chang et al study, the reduced density of endothelial cells in 169 eyes of 169 patients, treated longer and with higher cumulative amantadine doses was interpreted as dose-dependent endothelial damage.^[13] In contrast, the occurrence of corneal edema from 2 weeks to 8 years after the initiation of amantadine opposes the idea of dose-dependent damage.^[9-12,20-22]

It was shown that amantadine potentiates the effects of L-dopa^[23] and that rabbit corneal endothelium contains D1 and D2 dopamine receptors.^[24] These findings suggest a third possible mechanism; endothelial damage can occur via dopamine receptor stimulation.

The current survey was designed to evaluate the oxidative stress as a fourth possible mechanism.

Thiol groups are components of Na⁺- and K⁺-dependent ATPase, an endothelial ionic pump that transports excess fluid from within the corneal stroma to the aqueous to maintain corneal transparency. Thus, reduction of thiol levels may lead to dysfunction of the pump and consequently can cause corneal edema. As oxidative stress reduces thiol levels, we investigated

MDA and SH levels in rat corneas to determine the oxidant/antioxidant balance after intraperitoneal amantadine sulfate injections and ultimately, the MDA and SH levels were not statistically different between the amantadine group and control group. Despite the small sample size, according to the results of this preliminary study it seems that amantadine-associated endothelial damage does not occur via MDA/SH imbalance. The vast range of SH levels is another indicator requiring larger sample size.

According to the results of postmarketing surveillance of amantadine use, only 0.27% of 13,137 patients developed corneal edema or Fuch's dystrophy.^[25] These results can be interpreted such that some eyes might be susceptible to endothelial damage or that the pathogenesis may be multifactorial.^[26] In addition, these results may not represent the accurate ratios since amantadine-induced corneal edema could be misdiagnosed as idiopathic corneal edema or Fuch's corneal dystrophy.

In summary, despite the small sample size of current study restricted by the Local Ethics Committee of Animal Experiments, it can be concluded that the amantadine-induced corneal damage does not seem to occur via MDA and SH imbalance. Furthermore, a potential risk for corneal damage should not be overlooked.

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