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Original Research Article

Therapeutic effects of Laser and L-carnitine against amiodarone-induced pulmonary toxicity in adult male rats

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Abstract

Purpose: *To compare the effects of laser and/or L-carnitine (LC) on amiodarone (AM)-induced lung toxicity in adult male rats.*
Methods: *Lung toxicity was induced in 50 healthy male albino rats (150-180 g) by AM for 8 weeks. The rats were divided into 4 groups (7 per group): AM recovery, LC, laser, and laser + LC. After a 4-week exposure to laser and/or LC, key antioxidant enzymes, nitric oxide (NO), angiotensin II, cyclooxygenase-II (COX-II) and lipoxygenase (LOX) as well as oxidative stress and inflammation parameters were determined.*
Results: *Administration of LC and/or laser exposure led to significant increases in reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and angiotensin II; and significant decreases in nitric oxide (NO) level and glutathione-S-transferase (GST) activity in the AM-treated rats when compared with the AM recovery group ($p < 0.05$). The LC and/or laser exposure also significantly inhibited COX II and LOX activities in the lungs, and brought about significant reduction in levels of TNF-α and lipid peroxidation (LPO), and significant increases in cytochrome levels ($p < 0.05$).*
Conclusion: *These results indicate that AM induced pulmonary fibrosis in rats. However, AM withdrawal and treatment by LC and/or exposure to laser exerted mitigating effects against the AM-induced fibrosis.*

Keywords: *Amiodarone, Lung toxicity, Laser; L-carnitine*

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INTRODUCTION

The lung is the primary organ of respiration in humans and many lower animals. Lung tissue may be affected by many diseases such as pneumonia, lung cancer and lung fibrosis, due to exposure to harmful substances. Pulmonary fibrosis is a lung disease characterized by scarring of lung tissue, leading to breathing problems and other symptoms. The fibrotic scar and excess accumulation of fibrous connective tissue thicken the walls of the lung tissue and reduce oxygen supply to the blood, leading to shortness of breath [1].

Pulmonary toxicity is considered a life-threatening complication of amiodarone usage. In medicine, most of pulmonary toxicity diseases are caused by side effects of drugs. Drug-induced pulmonary toxicity can lead to fibrosis [2].

Amiodarone (AM) is a derivative of iodine benzofuran HCl (marketed as Pacerone or Cordarone). It is considered the strongest and most effective anti-arrhythmic drug because it

surpasses other agents in its ability to prevent and treat ventricular and supraventricular dysrhythmias. However, the use of AM is often limited by adverse reactions involving many different organ systems [3]The toxicity of AM which manifests in pneumonia can be seen as adult respiratory distress syndrome (ARDS); it has a high rate of mortality (about 50% of patients) [4], and can lead to severe pulmonary fibrosis [5].

The carnitine system consists of L-carnitine (C7H15NO3), acetyl-carnitine (C9H17NO) and the cellular proteins which are required for metabolism and transport. L-carnitine consists of amino acids naturally produced in humans and lower animals. It plays a role as a carrier for translocation of long-chain fatty acids from the cytosol into the mitochondria for β-oxidation [6].

The use of low levels of visible light to reduce inflammation-related pain, promote healing of deep wounds in tissues and nerves, and prevent tissues from damage have been documented These applications are based on the fact that that low level (soft-cold) lasers do not have thermal effects on tissues: lasers that stimulate biological function have an output below 10 milliwatts [7]. The effect of laser on tissue depends on the absorption of monochromatic visible rays by the components of the cellular respiratory chain of mitochondria. Evidence shows that treatment by laser light (low-level) induces the mitochondria to increase ATP production [8].

The present study focused on the potential therapeutic effects of L-carnitine and laser, either singly or synergistically, on lung toxicity induced by AM.

EXPERIMENTAL

Materials

Amiodarone (AM, empirical formula C_{25} H_{29} I_2 NO_3) and L-carnitine (3-hyroxy, 4-trimethylamino butyric acid) were obtained from Sigma Chemical Company (USA). Therapeutic low-intensity red laser module with more patterns projector was purchased from Shenzhen Light Technology Co. Ltd (China).

Experimental animals

Healthy 50 adult male albino rats (150 – 180 g, aged 6 - 7 weeks were used in this study. They were obtained from Medical and Bilharzia Research Center, Faculty of Medicine, Ain Shams University, Egypt.

The animals were housed in separate cages at room temperature (22 ± 4 ° C), with free access to water and feed. Blood parasitological tests and clinical examination were performed to ensure the animals were healthy. Amiodarone and L-carnitine were administered at doses of 30 and 100 mg/kg body weight, for 8 weeks and 30 days, respectively by oro-gastric tube [9, 10].

The study procedures were performed in line with the ethical guide for the care and use of laboratory animals of theU.S. National Research Council of the National Academics [11].

The experimental procedures were approved by theInstitutional Animal Care and Use Committee (IACUC), Faculty of Science, Menofia University, Egypt (approval no. MNSH174).

Animal groups

The rats were randomly assigned to five groups, each containing 7 animals: (i) control group: rats received 0.5 ml distilled water through oro-gastric tube; (ii) AM recovery group: rats in this group did not receive any treatment for 30 days after exposure to AM (30 mg / kg/ BWT) for 60 days [10]; (iii) AM+LC group: after exposure to AM (30 mg/kg BWT) for 60 days, the rats received LC (100 mg/kg BWT) for 30 days [9], and (iv) AM + laser group: after AM exposure, the rats were exposed to laser [12] and (v) LC + laser: after AM treatment, the rats exposed to laser and received L-carnitine for 30 days.

Exposure of animals to laser light

The rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg/ BWT) [12]. Before exposure to laser light, hair was removed from 4 cm^2 of abdominal area of each rat. Then, 1cm^2 of the lung-facing area was exposed to laser light (650 nm) for 2 minutes. On the 30th day of exposure, the rats were sacrificed then serum and lung tissue samples were collected for biochemical and histological assays.

Comet assay

The comet assay was done under alkaline conditions, including the unwinding of DNA to detect combination of DNA (single, double) strand breaks and alkali-labile sites in the DNA [3].

Statistical analysis

Data on lung parameters were expressed with mean ± standard deviation. Statistical analysis was performed using ANOVA to analyze data in

SPBS version 10. Values of $p< 0\ 05$ were taken as statistically significant [13].

RESULTS

Antioxidant levels

Treatment of the rats with AM induced oxidative stress as demonstrated by significant decreases in GSH, SOD, CAT and Angiotensin II, which were accompanied by significant elevations in NO and GST contents when compared with control group ($p< 0.05$; Table 1). Oral administration of LC and/or exposure of the AM-treated rats to laser led to significant elevations in GSH and angiotensin II levels, and in the activities of SOD and CAT, with concomitant significant decreases in NO and GST contents, relative to AM-treated rats ($p < 0.05$).

Lung COX II and LOX activities

The administration of AM resulted in significant elevation in COX II and LOX activities in lung tissue when compared with the control group ($p < 0.05$; Table 2). However, the activities of these two enzymes were significantly decreased in the AM-treated rats by LC and/ or laser exposure, relative to AM-treated rats ($p < 0.05$; Table 2).

Oxidative stress and inflammation status

The results also showed that AM induced significant increases in serum TNF-α and LPO product when compared with control group ($p < 0.05$; Table 2). However, LC and/or laser exposure led to significant reductions in TNF-α and in the degree of lipid peroxidation in AM-treated rats ($p < 0.05$; Table 2).

Changes in cytochromes $P450_1A_2$ (CYP_1A_2) and $P450_2E_1$ (CYP_2E_1)

Table 2 also shows that serum levels of cytochromes CYP1A2 and CYP2E1 were significantly higher in the AM-treated rats when compared with the normal control ($p < 0.05$). On the other hand, treatment of the AM rats with LC and/or exposure to laser (Figure 1) significantly reduced the levels of CYP1A2, CYP2E1 relative to AM rats ($p < 0.05$).

Table 1: Effect of L-carnitine or/and laser on antioxidants (GSH, GST, SOD, and CAT), NO and angiotensin II levels in lung tissue of AM-treated rats (mean ± SEM*)*

Group & treatment	GSH mg/ml	GST u/ml	SOD u/ml	CAT mu/ml	NO n/mol	Angiotensin II pg/ml
Control	13.489 ±0.209	165.343 ±0.399	5.534 ±0.041	1.591 ±0.018	17.843 ±0.094	1.357 ±0.008
AM recovery	5.550 ±0.029	515.490 ±5.206	2.339 ±0.040	0.693 ±0.006	66.557 ±0.554	0.462 ±0.004
AM+ L-carnitine	10.364 ±0.134	301.570 ±6.027	4.071 ±0.050	1.330 ±0.017	29.408 ±0.760	1.106 ±0.007
AM + laser	9.169 ±0.093	342.541 ±5.543	3.320 ±0.022	1.065 ±0.023	35.724 ±0.259	0.945 ±0.012
AM + L-carnitine + laser	11.368 ±0.200	216.747 ±4.619	4.927 ±0.037	1.541 ±0.029	22.620 ±0.352	1.249 ±0.016

Values are expressed as mean ± SEM (n = 10, p < 0.05)

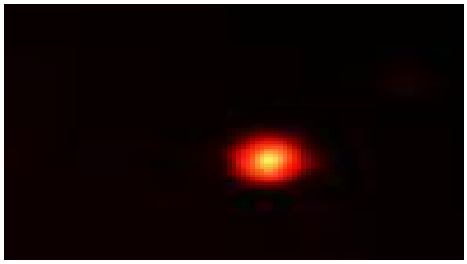
Table 1: Effect of L-carnitine or/and Laser on the lung enzymes (COX, LOX), oxidative stress &inflammatory status (TNF-α, MDA) and cytochromes (CYP 1A2, CYP 2E1) contents in Amiodarone treated rats

Group & treatment	Lung enzyme		Oxidative stress and inflammatory status		Cytochrome	
	COX ng/ml	*LOX pg/ml*	*TNF-α pg/ml*	*LPO µm*	*CYP_1A_2 pg/ml*	*CYP_2E_1 ng/ml*
Control	4.607 ±0.019	12.600 ±0.023	1.526 ±0.003	6.517 ±0.012	4.650 ±0.028	13.754 ±0.078
AM Recovery	17.929 ±0.463	70.334 ±0.319	5.382 ±0.053	16.177 ±0.091	24.152 ±0.317	90.343 ±0.616
AM + L-carnitine	8.044 ±0.198	36.686 ±0.495	2.442 ±0.068	9.194 ±0.073	10.318 ±0.114	39.795 ±0.849
AM + laser	10.433 ±0.250	48.772 ±0.579	3.227 ±0.071	11.333 ±0.044	13.944 ±0.273	55.717 ±0.596
AM + L-carnitine + laser	6.549 ±0.134	25.722 ±0.785	1.910 ±0.030	8.125 ±0.089	6.794 ±0.099	24.104 ±1.023

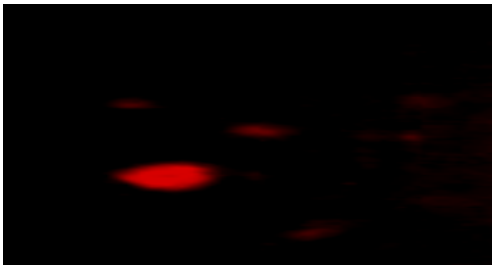
Values are expressed as mean ± SEM (n = 10, $p < 0.05$)

Table 3: DNA damage in rats

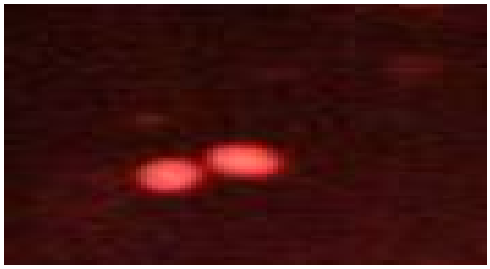
Group & treatment	Head/1000(mean ± SEM)	DNA in head (%)	Tail/1000(mean ± SEM)	DNA in tail
Control	2.1 ±0.20	33.8	20.61 ±2.1	62.2
AM recovery	5.9 ±0.20	20.6	30.51 ±3.5	79.4
AM + L-Carnitine	4.5 ±0.52	27.7	28.51 ±3.1	72.3
AM + Laser	4.01 ±0.55	29.6	26.1 ±2.5	70.4
AM + L-carnitine + laser)	3.5 ±0.61	31.9	25.0 ±5.1	66.2



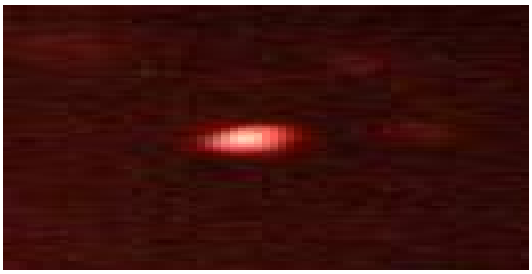
Control Group



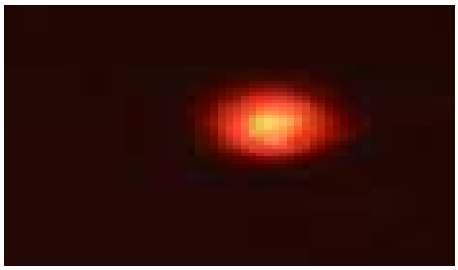
AM Recovery group (30 days)



AM + L-Carnitine group (30 days)



AM + Laser group (30 days)



AM + L-carnitine + laser group (30 days)

Figure 1: Images of fragment and pattern of DNA migration by comet assay evaluated with a fluorescence microscopy of lung cells from the various groups.

DISCUSSION

Results from several studies have suggested that oxidant-antioxidant imbalance in the lower respiratory system plays a crucial role in pulmonary toxicity. Amiodarone administration results in interstitial alveolar inflammation [10]. In the present study, the administration of AM to rats induced marked elevations in NO and GST, and significant reductions in GSH, SOD, CAT and Angiotensin II when compared with control rats. These changes can be attributed to AM-induced pulmonary toxicity. Several mechanisms are involved in the accumulation of iodine-rich AM in pneumocytes [14]. The mechanisms involve direct toxic effect that disrupts cellular membranes through the activation of protein kinase C, release of toxic reactive oxygen species (ROS), decreases in GSH, mitochondrial dysfunction, necrosis and ultimately apoptosis [15]. Amiodarone treatment has been reported to increase generation of free radicals, and production of mitochondrial hydrogen peroxide which could be metabolized to an aryl radical that

may give rise to other ROS [16]. The strong oxidizing potential of ROS can lead to generation of advanced oxidation products and induce damage to cellular structures within the lungs. In addition, GSH plays an important role in a variety of detoxification processes. It has been reported that AM significantly reduced GSH levels in rat lung homogenate, suggesting its oxidation-induced role in lung toxicity [17].

The most important antioxidant enzymes in the lung are SOD and CAT. These antioxidant enzymes are the first lines of defense against oxidative damage [10]. Alveolar macrophages isolated from AM-treated rats released large amounts of TNF-α [18]. Pulmonary toxicity by AM is an immuno-mediated process involving an imbalance in T-helper cells and over-production of cytokines which are reported to significantly increase serum TNF-α [14].In addition, the oxidative capacity of ROS leads to generation of oxidation products and cellular damage within the lungs. Malondialdehyde (MDA) is an index of membrane LPO which damages pulmonary endothelium [17].

L-carnitine is a very effective antioxidant agent. In this study, LC significantly increased the levels of GSH, GST, SOD and NO. This is in agreement with a previous report on the antioxidant and free radical-scavenging properties of LC [19]. It has been suggested that LC is involved in ATP synthesis through its role in mobilization of mitochondrial fatty acids during β-oxidation. During ATP synthesis, massive oxygen consumption depletes oxygen and ROS levels. The anti-oxidant effect of LC is thought to be due its ability to increase the activity of antioxidant enzymes [20,21].
Laser therapy restores the balance between oxidants and antioxidants [22]. This is in agreement with the laser-induced significant increases in antioxidant parameters seen in the present study. The synergistic effects of laser and LC led to significant increases in GSH, SOD, CAT, GST and angiotensin II, as well as significant decreases in NO. Thus the laser restored the balance between oxidant and antioxidants [23].

Special laser light near the infrared region (NIR) is used to reduce pain, inflammation, and edema. It is also used to promote healing of deep tissue wounds, and to protect the nerves and prevent tissue damage. These uses of laser light are based on its properties and its ability to interact with living tissue to produce energy (ATP). Interestingly, mammalian cell cytochrome c oxidase has uptake peaks in the NIR region. Mitochondria are the primary sites of light effects, production of ATP, modification of reactive oxygen species, and induction of transcription factors. These events, in turn, lead to increases in cell proliferation and migration, as well as increases in cytokine levels, growth factors, inflammatory mediators and tissue oxygenation. Changes in biochemical and cellular outcomes in animals and patients include benefits such as increased healing of chronic wounds, carpal tunnel syndrome, pain reduction in arthritis, neuropathy, as well as reduced damage after heart attacks, stroke and nerve injury [24]

Laser light

The effect of laser light at the cellular level (Figure 2) is based on the absorption of monochromatic visual radiation from by the components of the mitochondrial respiratory chain. The initial photoreceptor of red light is cytochrome C oxidase. Furthermore, absorption spectra obtained for cytochrome C oxidase and recorded in various oxidation states are similar to the spectra reflecting the biological responses to light [8]. Thus, laser light can stimulate biological processes in the mitochondria to produce energy, which is similar to what occurs within the mitochondria in the alveolar cells of the lung tissue.

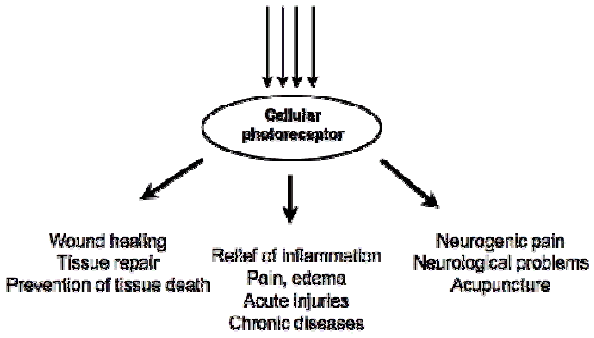


Figure 2: Therapeutic effect of laser light on tissues

Studies have accounted for how the absorption of photon produces laser energy [25]. Absorption of photons by molecules causes electron excitation, resulting in accelerated electron transfer. This transport increases ATP production, and also increases light-induced ATP synthesis through increased proton gradients.

CONCLUSION

The findings of this study demonstrate that prolonged administration of AM in rats induces lung fibrosis. Thus, withdrawal of AM and treatment by LC and/or exposure to laser can potentially produce significant amelioration of AM-induced fibrosis.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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