E-cadherin and 5-HT alterations in the heart of rats having undergone atropine-induced toxicity

QUAN-YONG HUANG[1], XUE-FANG LI[2] and SHUI-PING LIU[3]

[1]Department of Pathology, School of Basic Medical Sciences, Dali University; [2]School of Basic Medical Sciences, Dali University, Dali, Yunnan 671000; [3]Department of Forensic Pathology, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou 510080, P.R. China

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Abstract. Atropine-induced heart damage is associated with changes in the expression of various enzymes and proteins. The purpose of this study was to investigate atropine-induced alterations in cardiac E-cadherin and 5-hydroxytryptamine (5-HT) after atropine administration. Male Wistar rats were randomly divided into two groups: a control group and an atropine group. The atropine group intraperitoneally received atropine at a single dose of 15 mg/kg for 7 days; the controls received the same amount of saline via the same route. On Day 8, the rats were anesthetized, and a thoracotomy was performed in all animals. Immunohistochemical analysis was performed to evaluate protein expression of E-cadherin and 5-HT. Sections were analyzed by digital image analysis. Cardiac protein expression of E-cadherin and 5-HT was altered after atropine-induced toxicity in the rat. The expression levels of E-cadherin and 5-HT were significantly decreased after atropine treatment, supported by IOD analysis, when compared with the control ($P<0.05$). The current findings indicate that such changes would be reflected in abnormal cardiac function, and these proteins may be useful for revealing the mechanisms underlying atropine-induced toxicity and may also provide various clues for further research.

Introduction

Atropine sulphate, a competitive antagonist for the muscarinic acetylcholine (ACh) receptors, was isolated from *Atropa belladonna*. It has been suggested that high potencies of atropine sulphate may have an influence on ACh-induced contraction of smooth muscles (1). Atropine is a competitive antagonist of the action of ACh and other muscarinic agonists, and it is shown to extract Na(+), K(+), Mg(2+) and Ca(2+) ions from an aqueous phase into an organic one with a preference for Ca(2+) ions (2).

Correspondence to: Dr Quan-Yong Huang, Department of Pathology, School of Basic Medical Sciences, Dali University, Xiaguan Wanhua Road, Dali, Yunnan 671000, P.R. China
E-mail: hqy0726@126.com

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Atropine exhibits diverse effects on the cardiovascular system. It prevents α2-agonist-induced bradycardia and increases the severity and duration of hypertension (3). Atropine was found to reduce discharge rates and reverse the increase in spontaneous activity induced by ACh (4). Patients who received an overdose of atropine had worse signs and symptoms of cardiomyopathy (5). It has also been suggested that chronic atropine administration affects vasoactive intestinal polypeptide synthesis in rat heart atria and consequently modifies regulation of the heart rate (6). Additionally, routine prophylaxis with an anticholinergic agent was found to aid in the prevention of sinus bradycardia during urological laparoscopic surgery (7). Furthermore, it has been demonstrated that early i.v. administration of high-dose atropine can prevent neurogenic pulmonary edema development on the basis of the prevention of baroreflex-induced bradycardia (8).

Atropine, a non-selective muscarinic receptor antagonist, is currently the most potent agent used to prevent myopia in animal models and children (9). It has been reported that regular topical administration of 0.025% atropine eye drops can prevent myopia onset and myopic shift in premyopic school children for a 1-year period (10).

However, numerous side effects attributed to atropine are commonly reported. Atropine has the potential to cause central toxicity which may complicate the management of cardiomyopathy (11). It has been reported that atropine may cause aspiration pneumonia through induction of the swallowing disorder and inhibition of the cough reflex (12), and it is associated with a higher incidence of a transient rash (13).

It has been shown that atropine-induced toxicity is involved in alterations in enzyme activities and nitric oxide (14); however, little is known concerning the changes in E-cadherin and 5-hydroxytryptamine (5-HT) expression in the heart having undergone atropine-induced toxicity.

The present study used immunohistochemistry to evaluate the expression of E-cadherin and 5-HT in the heart, and determine whether protein expression is altered upon atropine-induced toxicity.

Materials and methods

Sixteen healthy adult male Wistar rats, weighing 210-250 g, were used in this study. All animals were accommodated in

cages in an environmentally controlled room at an ambient temperature of 20-26˚C and relative humidity of 30-70%, respectively, under a 12-h light/dark cycle (lights on from 6:00-18:00 h). Rats were fed with a standard pellet chow and water throughout the experimental period. All procedures described in this study were approved by the Ethics Committee of Sun Yat-Sen University.

Animals and study design. Animals were divided randomly into two groups, each with 8 rats. Atropine group animals were treated by an intraperitoneal (i.p.) injection of atropine at a dose of 15 mg/kg/day for 7 days; the control group animals were treated with normal saline for 7 days in equal volumes as for the experimental groups (15).

After 7 days of treatment with atropine (15 mg/kg/day), the control and experimental animals were anesthetized with xylazine (10 mg/kg) and ketamine HCl (50 mg/kg). Anesthetized rats were sacrificed and a thoracotomy was performed. The heart was harvested, fixed in phosphate-buffered 10% formalin, embedded in paraffin wax, and sectioned (4 μm) for light microscopy and immunohistochemical examination.

Histopathologic examinations. Heart specimens from each group were removed to be examined histopathologically. After the heart tissues were fixed in 10% formalin and embedded in parafin, tissue sections were coronally sectioned to a thickness of 4 μm, and stained with hematoxylin and eosin. The slides were coded, and semi-quantitative analysis of the sections was performed without knowledge of the treatment protocol. In these tissues, pathological changes were evaluated (16,17).

Tissue sections and immunohistochemical staining. All rat hearts were immersed in 4% formaldehyde buffered with phosphate-buffered saline (PBS, pH 7.2), and then embedded in paraffin. Tissue sections (4 μm) were prepared for immunohistochemical staining. After deparaffinization, all sections were immersed in 0.3% H_2O_2-PBS for 10 min and incubated with PBS containing 1% normal goat serum to reduce non-specific reactions. After incubation with either rabbit anti-E-cadherin or anti-5-HT (Santa Cruz Biotechnology, USA) overnight at 4˚C, tissue sections were washed three times in PBS and incubated with biotin-conjugated secondary antibody for 1 h at room temperature according to the manufacturer's instructions. Then the specimens were incubated with peroxidase-conjugated avidin-biotin for 30 min at room temperature. After washing, the sections were incubated with diaminobenzidine (DAB) as chromogen, and counterstained with hematoxylin (18-20). Negative control sections were performed using PBS buffer instead of the primary antibody.

Total integrated optical density (IOD), a parameter representing the expression levels of E-cadherin and 5-HT in cardiac tissue, was determined using a cast-grid microscope (MetaMorph/DP10/Bx41, UIC/Olympus, US/JP) together with an image-analysis program (MetaMorph offline, version 4.65). Under magnification of x400, five images were captured in each immunostained section, and the average was calculated (19,20).

Statistical analyses. Statistical analyses were performed using SPSS 11.0 software. The results were presented as the mean ± SEM. The sources of variation were analyzed by unpaired Student's t-test. A P-value of <0.05 was considered to denote statistical significance.

Table I. Integrated optical density (IOD) of E-cadherin and 5-HT in the heart of rats.

Groups	E-cadherin	5-HT
Control group	0.0136±0.00057	0.0145±0.00026
Atropine group	0.0069±0.00028	0.0056±0.00068

The integrated optical density (IOD) per field is proportional to the total amount of staining. Total E-cadherin IOD expression in rats having undergone atropine intoxication was significantly lower compared with the control hearts (P<0.05). Total 5-HT IOD expression in rat hearts subjected to atropine intoxication was significantly lower when compared to the control (P<0.05).

Results

Histological examination. Routine histological examination revealed slight morphologic change in the rat hearts from each group (data not shown).

Immunohistochemical staining. Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded sections from all parts of the heart including the conduction system.

Expression of E-cadherin protein. Positive staining for the E-cadherin antibody was detected mainly in the extracellular matrix. The E-cadherin antibody was manifested as fine brown granularity. Two observers examined the sections independently.

The photomicrographs in Fig. 1 display the positive expression of E-cadherin in the control (Fig. 1A) and atropine-injured (Fig. 1B) rat hearts. Total IOD expression of E-cadherin in the rat hearts from each group is documented in Table I. Total IOD expression of E-cadherin in the hearts from rats having undergone atropine intoxication was significantly lower than that in the control (P<0.05).

Expression of 5-HT protein. The 5-HT staining was detected mainly in the extracellular matrix, and positive staining for 5-HT was manifested as fine brown granularity.

The distribution of 5-HT in the rat hearts of the control and atropine groups is shown in Fig. 2A and B, respectively. Total 5-HT IOD in the heart of animals subjected to atropine intoxication was significantly lower than that of the control hearts (Table I) (P<0.05).

Discussion

Although atropine is widely used for fiberoptic bronchoscopy and helps by drying secretions, producing bronchodilatation, or preventing vasovagal reactions, the undesirable associated side effects may markedly decrease the quality of life of patients.

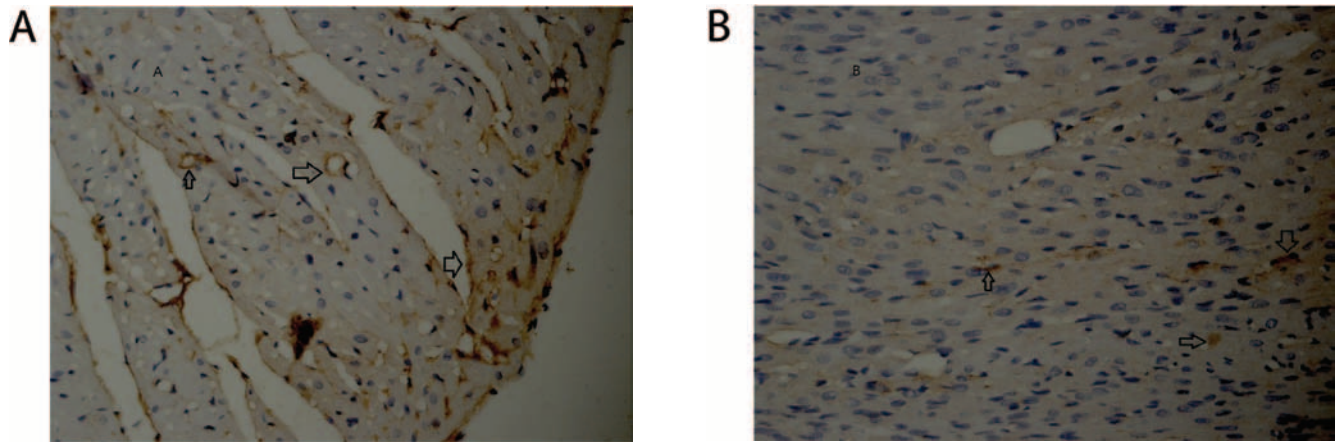

Figure 1. Effect of atropine exposure on E-cadherin expression in rat hearts. Photomicrographs display the representative distribution of E-cadherin-positive expression in rat hearts from the (A) control and (B) atropine-exposed group. All photomicrographs were captured at a magnification of x400. Positive immunostaining appears as brown staining (arrow).

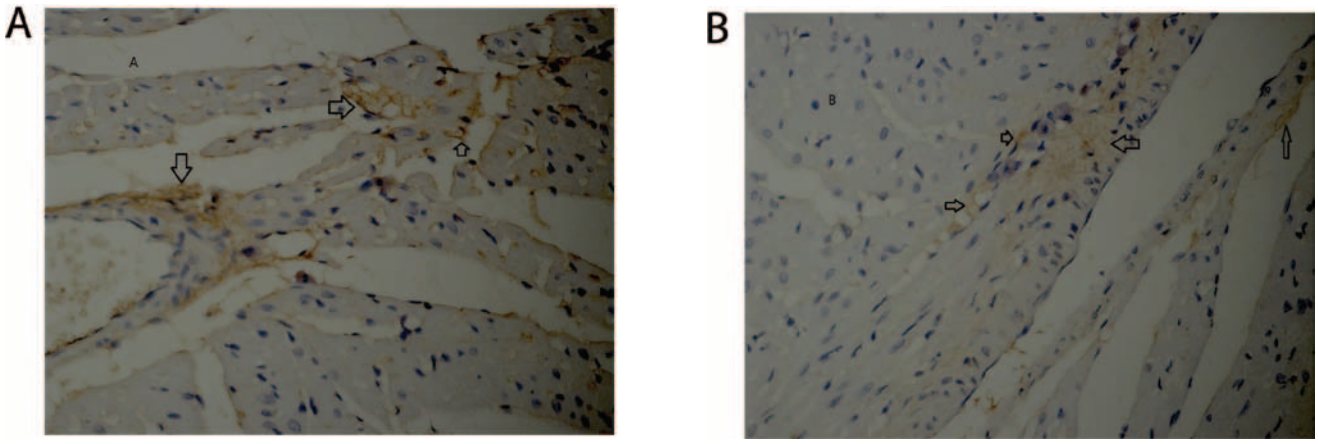

Figure 2. Effect of atropine exposure on 5-HT expression in rat hearts. Photomicrographs reveal a representative distribution of positive 5-HT expression in rat hearts from the (A) control and (B) atropin-exposed group. All photomicrographs were taken at a magnification of x400. Positive immunostaining appears as brown staining (arrow).

It has been suggested that dysregulation of E-cadherin is involved in carcinoma development (21). E-cadherin is a cell-cell adhesion protein and tumor suppressor that is silenced in many malignancies, and it is thought to suppress tumor cell growth by antagonizing β-catenin signaling (22). Alterations in E-cadherin are associated with certain types of cancer. Regulated adhesion between cells and their environment is critical for normal cell migration, and it has been suggested that mediated reduction in H_2O_2 is required to maintain E-cadherin protein levels in the early embryo (23).

E-cadherin has been proven to be widely downregulated and tightly associated with tumor invasion and metastasis in multiple human cancer types (24). Previous research has indicated that loss of E-cadherin induces possible pre-cancerous lesions in the gastric mucosa but may not be sufficient for its malignant conversion (25). However, research has shown that downregulation of E-cadherin plays important roles in tumor progression and metastasis. It has been shown that loss of E-cadherin contributes to both mammary tumor initiation and metastasis (26), and the expression of E-cadherin correlates with the progression and metastasis of gastric cancer (27). Meanwhile, E-cadherin is a tumor-suppressor gene involved in epithelial cell-cell interactions and plays important roles in the etiology of gastric cancer (28). Similarly, it has been reported that reduced levels of E-cadherin in nasopharyngeal carinoma may play an important role in invasion and metastasis (29). Decreased E-cadherin levels in the heart noted in our results suggest that the tissues were extensively damaged after undergoing atropine-induced toxicity. The present study indicates that atropine-induced toxicity causes decreased E-cadherin expression that is related to impaired heart function.

The biogenic amine serotonin 5-HT plays a key role in the regulation and modulation of various physiological and behavioral processes (30). It has been recognized that 5-HT plays important roles in peripheral tissues and vascular cells and is involved in coagulation, hypertension, inflammation, healing and protection (31). It also has been shown that descending serotonergic 5-HT system is shown to be plastically altered under pathological conditions, for example, the enhancement of 5-HT-induced contraction in mesenteric arteries (32). Furthermore, it has been suggested that 5-HT is involved in the fine adjustment at several brain centers including the

core of the mammalian circadian timing system (33). It has been reported that 5-HT is a major factors in the pathogenesis of pulmonary arterial hypertension (34). In addition, the endogenous brain serotonin 5-HT system is believed to have an important influence in mediating drug reward and seeking mechanisms (35). Furthermore, previous research has shown that a variation in expression of the 5-HT transporter (5-HTT) is linked to the risk of psychiatric illness (36).

It has been suggested that stress plays a key role in the modulation of 5-HT. For example, stress is known to activate the central 5-HT system, and this is probably part of a coping response involving several 5-HT receptors (37). The serotonin 5-HT is capable of reducing the oxidative burst of phagocytes (38). Melatonin may act as a modulator of excess 5-HT release from colonic mucosa (39). Moreover, decreased 5-HT in the heart noted in our study suggests that the tissues were extensively damaged after undergoing atropine-induced toxicity. The present study indicates that atropine-induced toxicity causes decreased 5-HT expression that is related to impaired heart function.

In the present study, our results demonstrated that cardiac protein expression of E-cadherin and 5-HT is altered after atropine-induced toxicity in the rat. After undergoing atropine-induced toxicity, protein expression of E-cadherin and 5-HT was significantly reduced. The present study indicates that atropine-induced toxicity causes different alterations in heart proteins that may be related to cardiac-function deterioration.

In conclusion, the present findings indicate that such alterations would be reflected in abnormal cardiac function, and the studied proteins may be useful for revealing the mechanisms underlying atropine-induced toxicity and may also provide certain clues for further research.

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