α-enolase and caveolin alterations in the heart of rats having undergone gentamicin-induced toxicity

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Abstract. Gentamicin (GM)-induced heart damage is associated with alterations in expression levels of various enzymes and proteins. The aim of the present study was to investigate GM-induced alterations in cardiac α-enolase and caveolin after GM administration. Male Wistar rats were randomly divided into two groups: a control group and a GM group. The GM group intraperitoneally received GM at a single dose of 7 mg/kg for 8 days, while the controls were given the same amount of saline via the same route. On Day 9, the rats were anesthetized and a thoracotomy was performed in all animals. Immunohistochemical analysis was performed to evaluate protein expression of α-enolase and caveolin. Sections were analyzed by digital image analysis. Our results revealed that cardiac protein expression of α-enolase and caveolin was altered after GM-induced toxicity in the rat. The expression of α-enolase and caveolin was significantly increased after GM-induced toxicity, as determined by integrated optical density analysis, when compared with the control (P<0.05). The current findings indicate that such changes in protein expression may be reflected in abnormal cardiac function, and the proteins identified in this study may be useful for elucidating the mechanisms underlying GM-induced toxicity and may also provide various clues for further investigations.

Introduction

Gentamicin (GM) is considered to be an important antibiotic against life-threatening, severe gram-negative bacterial infections (1), yet it also exhibits severe side effects such as nephrotoxicity (2) and inner ear injury (3,4). Nephrotoxicity is

the main side effect of GM (5). Thus, its clinical use is partially limited due to its nephrotoxic effect (6).

Tested hypotheses concerning the mechanisms involved in GM-induced nephrotoxicity are as follows. Evidence indicates that free radicals are important mediators of GM nephrotoxicity (7,8). It has been shown that GM-induced renal dysfunction was attributable to enhanced oxidative stress (9), as revealed by decreased superoxide dismutase and catalase activities, glutathione depletion and increased lipid peroxidation (8). In addition, it has been suggested that the c-Jun N-terminal kinase (JNK) signaling pathway may be involved in the secondary apoptosis of spiral ganglion cells (SGCs) induced by intracochlear GM injection (9), and the formation of aminoglycoside-iron complexes have been proposed to be the major mechanisms in the development of GM-induced acute renal failure (ARF) (10). Moreover, it has been demonstrated that GM-induced nephrotoxicity leads to increased urinary losses of carnitine, and carnitine deficiency should be viewed as a mechanism during the development of GM-induced ARF (11). GM-induced nephrotoxicity has also been associated with marked upregulation of Kim-1, TIMP1 (12) and mitogen-activated protein kinase (MAPK) (2), but downregulation of MMP-9 (13).

Extended use or excessive dosages of GM have been found to result in irreversible damage to the inner ear such as inner ear hair cell loss and sensorineural hearing loss. Apoptotic cell death is considered to play a key role in this injury (3,4).

It has been shown that the GM-induced toxicity is involved in changes in superoxide dismutase and catalase activities, glutathione and lipid peroxidation (8) and respiratory components (cytochrome c, NADH) (14). However, little is known concerning the changes in α-enolase and caveolin expression in the heart having undergone GM-induced toxicity.

The present study used immunohistochemistry to evaluate the expression of α-enolase and caveolin in the heart, and determine whether the protein expression is altered upon GM-induced toxicity.

Materials and methods

Sixteen healthy adult male Wistar rats, weighing 210-250 g, were used in this study. All animals were housed individually in stainless-steel wire-bottomed cages in an air-conditioned

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room at 22˚C with 50% relative humidity and a 12-h light/dark cycle. 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 2 groups, each with 8 rats. GM group animals were induced by an intraperitoneal injection of GM at a dose of 100 mg/kg/day for 8 days, and the control group animals were treated with normal saline for 8 days in equal volumes as for the experimental groups (7,15).

After 8 days of treatment with GM (100 mg/kg/day), control and experimental animals were anesthetized with xylazine (10 mg/kg) and ketamine HCl (50 mg/kg) and sacrificed. A thoracotomy was then 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 for histopathological evaluation. The heart tissues were fixed in 10% formalin, embedded in parafin, sectioned at 4 μm, and then stained with hematoxylin and eosin. The slides were coded and semi-quantitative analysis of the sections was performed without knowledge of the treatment protocol. Pathologic changes were evaluated in these tissues (16,17).

Tissue sections and immunohistochemical staining. All rat hearts were immersed in 4% formaldehyde buffered with phosphate-buffered saline (PBS, pH 7.2), before being embedded in paraffin and sectioned coronally with a microtome into 4-μm sections. 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-α-enolase, or anti-caveolin (Santa Cruz Biotechnology, USA) overnight at 4˚C, tissue sections were washed three times in phosphate-buffered saline (PBS) and incubated with biotin-conjugated secondary antibody for 1 h at room temperature according to the manufacturer's instructions. The specimens were then 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). PBS was substituted for the primary antibody as the negative control.

Integrated optical density (IOD), a parameter representing expression levels of α-enolase and caveolin 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 a magnification of x400, five images were captured in each immunostained section and the average was calculated (19,20).

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

Results

Histological examination. Routine histological examination revealed little 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 α-enolase protein. Positive staining for the α-enolase antibody was detected mainly in the extracellular matrix, or was manifested as fine brown cytoplasmic granularity or surface membrane expression. Two observers examined the sections independently.

Positive expression of α-enolase in the control (Fig. 1A) and GM-injured (Fig. 1B) rat hearts was noted. Total IOD expression of α-enolase in the rat hearts from each group is shown in Table I. Total IOD expression of α-enolase in hearts from rats after GM-induced toxicity was significantly higher than that in the control (Table I) (P<0.05).

Expression of caveolin protein. The caveolin staining was detected mainly in the extracellular matrix, or was manifested as fine brown cytoplasmic granularity or surface membrane expression.

The distribution of caveolin in the rat hearts of the control (Fig. 2A) and GM (Fig. 2B) groups is shown. Total caveolin IOD in the heart of animals subjected to GM-induced toxicity was significantly higher than that of the control hearts (Table I) (P<0.05).

Discussion

Although therapy with GM is widely used for the treatment of severe gram-negative bacterial infections, the undesirable side effects associated with it markedly decrease the quality of life of patients.

α-enolase, also called non-neuronal enolase, belongs to a family of cytoplasmic and glycolytic enzymes (21). It is a glycolytic enzyme that also acts as a surface plasminogen receptor (22). α-enolase plays a functional role in several physiological processes depending on the cellular localization (23). It is a metabolic enzyme involved in the synthesis of pyruvate (24), and it has been suggested that increased α-enolase may contribute to acceleration of glycolysis in the preterm heart (25).

α-enolase plays an important role in pathophysiological processes (26). It has been reported that α-enolase is an important target for oxidative and nitrative modifications in diabetic cardiac proteins (27), and tyrosine nitration of α-enolase may contribute to impaired glycolytic activity in diabetic cardiomyopathy (27). It was found that α-enolase was dramatically upregulated in hypertrophic hearts (28). α-enolase is known to be bound to neutrophils and interacts in the immune response, and thus may play a role in inflammation associated with human labour (29). It is also recognized as a virulence factor of various pathogenic bacteria facilitating plasminogen activation and host cell invasion (30).

Apart from its enzymatic function in the glycolytic pathway, α-enolase has been implicated in numerous diseases,
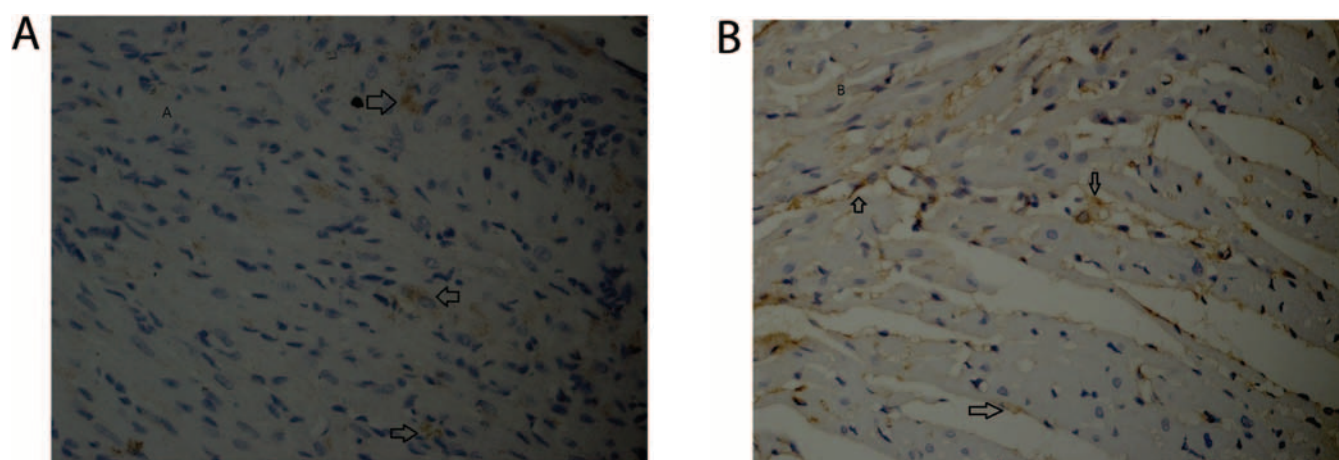
Figure 1. Effect of gentamicin-induced toxicity on α-enolase expression in rat heart. Photomicrographs display a representative distribution of α-enolase-positive expression in hearts from the (A) control and (B) gentamicin-induced group. All photomicrographs were captured at a magnification of x400. Positive immunostaining appears as brown staining (arrows).
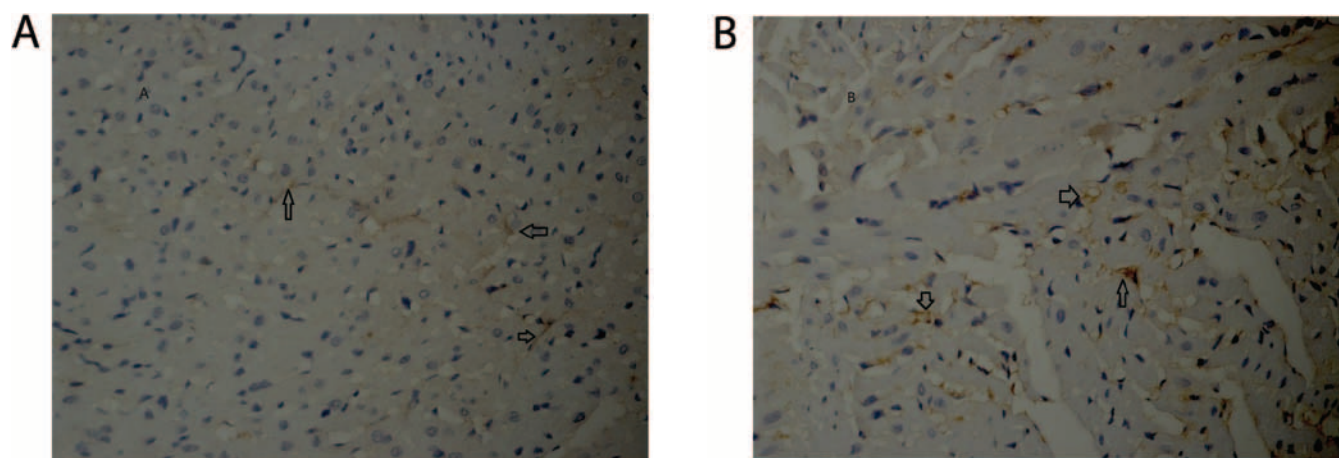

Figure 2. Effect of gentamicin-induced toxicity on caveolin expression in rat hearts. Photomicrographs reveal a representative distribution of positive caveolin expression in hearts from the (A) control and (B) gentamicin-induced toxicity group. All photomicrographs were captured at a magnification of x400. Positive immunostaining appears as brown staining (arrows).

Table I. The integrated optical density (IOD) of α-enolase and caveolin in heart of rats.

Groups	α-enolase	Caveolin
Control group	0.0071±0.00035	0.0077±0.00064
Gentamicin group	0.0149±0.00027	0.0189±0.00078

The integrated optical density (IOD) per field is proportional to the total amount of staining. Total α-enolase IOD expression in rat hearts having undergone gentamicin-induced toxicity was significantly higher compared with control hearts ($P<0.05$). Total caveolin IOD expression in rat hearts subjected to gentamicin-induced toxicity was significantly higher compared with the control ($P<0.05$).

including metastatic cancer, autoimmune disorders, ischemia and bacterial infection (31). α-enolase is a key glycolytic enzyme that has been used as a diagnostic marker to identify human lung cancers (32). Increased α-enolase in the heart noted in our results suggests that the tissues were extensively damaged when undergoing GM-induced toxicity. The present study indicates that GM-induced toxicity causes increased α-enolase expression that is related to impaired heart function.

Caveolin, a member of the membrane-anchoring protein family, accumulates various growth receptors in caveolae and function in a variety of biological processes (33). It has been reported that caveolae are organelles abundant in the plasma membrane of many specialized cells including endothelial cells, epithelial cells and adipocytes (34). Previous research has demonstrated that caveolae are small, flask-like invaginations of the plasma membrane, and caveolin are structural proteins found in caveolae that have scaffolding properties to allow the organization of signaling (35). Caveolin and caveolae have been implicated as being involved in the signal transduction of many receptors, including the EGF, PDGF, LPA and β3-adrenergic receptors (36). It has been suggested that the ATP-sensitive K^+ channels in the heart are localized in caveolae (37). Caveolins consist of three different membrane

scaffolding proteins that play a role in a variety of processes in different tissues (38). It has been reported that upregulation of caveolin attenuates cellular proliferation and growth (33). It has also been shown that the caveolins are the major structural proteins of caveolae and play a role in tumor surveillance (39). The increased levels of caveolin in the heart noted in our results suggests that the tissues were greatly damaged when undergoing GM-induced toxicity. The present study revealed that GM-induced toxicity caused increased caveolin expression related to impaired heart function.

Our results demonstrated that cardiac protein expression of α-enolase and caveolin is altered after GM-induced toxicity in the rat. After undergoing GM-induced toxicity, the protein expression of α-enolase and caveolin was significantly elevated. The present study indicated that GM-induced toxicity caused different alterations in heart proteins that may be related to cardiac-function deterioration.

In conclusion, the current findings indicate that such alterations in the protein expression of α-enolase and caveolin would be reflected in abnormal cardiac function. These proteins may be useful in elucidating the mechanisms underlying GM-induced toxicity and may also provide certain clues for further research.

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