

# Elevated enolase and caveolin-1 in the heart of rats following dexamethasone-induced toxicity

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Received December 8, 2011; Accepted February 28, 2012

DOI: 10.3892/mmr.2012.817

**Abstract.** Dexamethasone (DEX)-induced heart damage is associated with enzyme and protein alterations. The purpose of this study was to investigate DEX-induced alterations in cardiac enolase and caveolin-1 (cav-1) following DEX administration. Male Wistar rats were randomly divided into two groups: a control and a DEX. The DEX group intraperitoneally received DEX at the single dose of 10 mg/kg for 7 consecutive days, and the control was given 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 enolase and cav-1. Sections were analyzed by digital image analysis. Our results demonstrated that cardiac protein expression of enolase and cav-1 was altered following DEX-induced toxicity in the rat. The expression of enolase and cav-1 was significantly increased after DEX treatment, supported by integrated optical density compared with the control ( $P < 0.05$ ). In conclusion, following DEX-induced toxicity, protein expression of enolase and cav-1 was significantly elevated. The current findings indicate that such alterations would be reflected in abnormal cardiac function, and the proteins identified in this study may be useful in revealing the mechanisms underlying DEX-induced toxicity and also in providing various clues for further research.

## Introduction

Corticosteroids are hormonal substances widely used in human and veterinary medicine for their anti-inflammatory properties. Among the numerous existing artificial corticosteroids, dexamethasone (DEX) remains the most commonly used, mainly through esterified forms such as acetate or

phosphate (1). DEX is a potent, synthetic member of the glucocorticoid class of steroid drugs with pleiotropic effects on multiple signaling pathways, and has been widely used in numerous disorders during the last 50 years (2). Recent studies have demonstrated that DEX is routinely prescribed to reduce cerebral oedema and is used increasingly as an anti-emetic (3). It has been reported that nausea, fatigue, and pain scores were all reduced using DEX treatment (4), and a single administration of a DEX implant significantly reduced inflammation in an animal model (5). DEX pretreatment arterializes venous endothelial cells by inducing MAP kinase phosphatase-1 and may protect the veins from inflammation (6).

However, it has also been reported that a number of side effects are attributable to DEX (7). DEX has been shown to cause hyperglycaemia (3), and it has been suggested that it increases the risk of infection in multiple myeloma patients (8). Moreover, intratympanic DEX injection may be a simple and effective treatment for patients with sudden sensorineural hearing loss (9). Yet, recent studies have demonstrated that DEX led to growth retardation, increased mortality and severe kidney damage (10). In addition, DEX treatment leads to a decreased number of sialic acids on the surfaces of human macrophages promoting recognition and uptake of apoptotic cells (11).

It has been shown that DEX-induced toxicity is involved in changes in tropoelastin (12),  $Ca^{2+}$  and  $Na^+$  (13). However, little is known concerning the changes in enolase and caveolin-1 (cav-1) expression in the heart following DEX-induced toxicity.

The present study utilized immunohistochemistry to evaluate the expression of enolase and cav-1 in the heart, and to determine whether protein expression is altered following DEX-induced toxicity.

## Materials and methods

Sixteen healthy adult male Wistar rats, weighing 210-250 g, were used in this study. All animals were kept in individual stainless steel cages under standardized conditions (constant temperature and humidity, 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.

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**Key words:** enolase, caveolin-1, dexamethasone-induced heart damage

**Animals and study design.** Animals were divided randomly into 2 groups (n=8). The experimental group received intraperitoneal injections of 10 mg/kg DEX (dexamethasone phosphate dissolved in 0.9% saline) for 7 consecutive days. The control group was injected with 0.9% saline in equal volumes as the experimental group (14). One day after the last injection, animals were sacrificed by decapitation under ether anesthesia. A thoracotomy was subsequently performed, and the heart was harvested, fixed in phosphate-buffered 10% formalin, embedded in paraffin wax, and sectioned (4  $\mu$ m) for light microscopy and immunohistochemical examination.

**Histopathological examination.** Heart specimens from each group were examined histopathologically. After the heart tissues were fixed in 10% formalin solution for 48 h and embedded in paraffin wax, they were sectioned (4  $\mu$ m), 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. Pathological changes were evaluated in the tissues as previously described (15,16).

**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- $\mu$ m sections. The tissue sections were then deparaffinized in xylol and hydrated in decreasing series of ethanol. Endogenous peroxidase activity was blocked by incubation in methanol containing 0.3% H<sub>2</sub>O<sub>2</sub> for 10 min at room temperature, followed by a rinse in 0.1 M PBS (pH 7.4) for 5 min. Before application of specific primary antibodies, nonspecific background staining was prevented by incubation with goat serum diluted 1:10 v/v in PBS for 50 min. After incubation with either rabbit anti-enolase or anti-cav-1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, 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. After washing, tissue sections were incubated for 10 min in streptavidin-peroxidase and then washed three more times in PBS. Positive signals were visualized with diaminobenzidine, followed by counter-staining with methyl green as previously described (17-19). PBS was substituted for the primary antibody for the negative control.

Total integrated optical density (IOD), a parameter representing the expression levels of enolase and cav-1 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 for each immunostained section and the average IOD value was calculated as previously described (18,19).

**Statistical analysis.** Statistical analysis was performed using the SPSS 11.0 software. The results are presented as the mean  $\pm$  SEM. The sources of variation were analyzed by the unpaired Student's t-test. P<0.05 was considered to indicate a statistically significant difference.

Table I. IOD of enolase and caveolin-1 in the rat hearts.

Groups	Enolase	Caveolin-1
Control	0.0053 $\pm$ 0.00041	0.0057 $\pm$ 0.00053
Dexamethasone	0.0121 $\pm$ 0.00021	0.0133 $\pm$ 0.00062

IOD, integrated optical density per field, which is proportional to the total amount of staining. Total enolase and caveolin-1 IOD expression in rats subjected to dexamethasone-induced toxicity was significantly higher compared to that in the control hearts (P<0.05, respectively).

## Results

**Histological examination.** Routine histological examination revealed little morphological changes 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 enolase protein.** Positive staining for the enolase antibody was detected mainly in the extracellular matrix, and the enolase antibody was manifested as fine brown granularity. Two observers examined the sections independently.

The photomicrographs in Fig. 1A and B show the positive expression of enolase in the control (Fig. 1A) and DEX-injured (Fig. 1B) rat hearts. Total IOD expression of enolase in the rat hearts from each group is shown in Table I. Total IOD expression of enolase in the rat heart following DEX-induced toxicity was significantly higher compared to the control (Table I, P<0.05).

**Expression of caveolin-1 protein.** Cav-1 staining was detected mainly in the extracellular matrix, and positive staining for cav-1 was manifested as fine brown granularity.

The distribution of cav-1 in the rat hearts of the control and DEX groups is shown in Fig. 2A and B, respectively. Total cav-1 IOD in the heart of animals subjected to DEX-induced toxicity was significantly higher compared to that in the control (Table. I, P<0.05).

## Discussion

Although therapy with DEX is widely used for anti-inflammation treatment, its associated undesirable side effects markedly decrease the quality of life of patients.

Enolase is a multifunctional protein that participates in glycolysis and gluconeogenesis and acts as a plasminogen receptor on the cell surface of several organisms (20). It is a glycolytic enzyme that catalyzes the interconversion of phosphoenolpyruvate and 2-phosphoglycerate (21). It has been reported that enolase is a key glycolytic enzyme that catalyzes the dehydration of 2-phosphoglycerate to phosphoenolpyruvate (22). Furthermore, it has long been considered an enzyme of the glycolytic pathway ubiquitously occurring in the cytosol

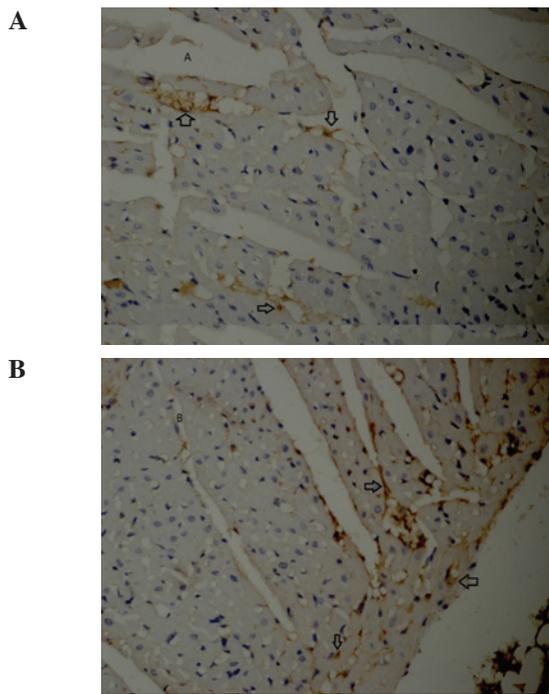


Figure 1. Effect of dexamethasone (DEX) exposure on enolase expression in the rat hearts. Photomicrographs display a representative distribution of enolase-positive expression in the (A) control and (B) DEX-exposed hearts. All photomicrographs were captured at a magnification of x400. Positive immunostaining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

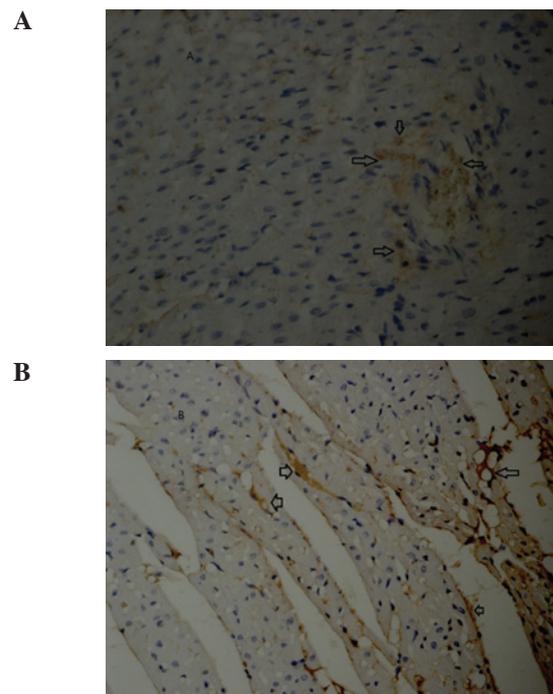


Figure 2. Effect of dexamethasone (DEX) exposure on caveolin-1 (cav-1) expression in the rat hearts. Photomicrographs reveal a representative distribution of positive cav-1 expression in the (A) control and (B) DEX-exposed hearts. All photomicrographs were captured at a magnification of x400. Positive immunostaining appears as brown staining (arrow).

of prokaryotic and eukaryotic cells (23,24). Recently, enolase has been shown to possess a variety of different regulatory functions, such as glycolysis, gluconeogenesis, hypoxia, ischemia and Alzheimer's disease (25). Aside from its classic role in carbohydrate metabolism, enolase was recently found to localize to membranes, where it binds host plasminogen and functions as a virulence factor for several pathogens (26). Also it has been demonstrated that enolase is an essential protein in fungal metabolism (27), and its lack of expression may cause delays in larval development (28).

Apart from its glycolytic function, enolase has also been shown to possess antigenic properties and to be present on the cell wall of certain invasive organisms, such as *Candida albicans* (29). In addition, it has been found that enolase levels are highly elevated in malaria parasite-infected red blood cells (30). Recently, enolase was revealed to be an important protein in many pathophysiological and disease processes (31). It is expressed on the surface of eukaryotic cells such as macrophages, neutrophils, endothelial, neuronal and tumor cells (32). Moreover, it is important in myogenesis, tumorigenesis and angiogenesis (32), as well as for pathogen invasiveness and the development of tumors (33). It has been shown that enolase mediates the activation of enzymes involved in the invasion of tissues by pathogens and tumor cells, as well as in the evasion of host immune responses (34), and that it is sensitive to fluoride (35). The increased amount of enolase in the heart noted in our study, suggests that the tissues were greatly damaged following DEX-induced toxicity. The present study indicates that DEX-induced toxicity causes an increase in enolase expression, which is related to impairment of the heart function.

Cav-1, a 21- to 24-kDa integral membrane protein, is a major structural coat protein of caveolae which are specialized plasma membrane invaginations involved in multiple cellular functions (36), including molecular transport, cell adhesion and signal transduction (37). In addition, cav-1 regulates critical cell functions including proliferation, apoptosis, cell differentiation and transcytosis via diverse signaling pathways (38). In addition, cav-1 is important for cell transformation, cell migration, metastasis and angiogenesis (39,40). Furthermore, it is a membrane protein that forms the coat structure of plasma membrane caveolae and regulates caveola-dependent functions (41).

Cav-1 plays a crucial role in the regulation of various physiological and pathophysiological processes such as oncogenic transformation, tumorigenesis, tumor invasion and metastasis (42). Furthermore, it is a key protein involved in anoikis tumor metastasis (43), as well as a promising biomarker for identifying clinically significant cancer (37). It has been suggested that cav-1 may contribute to certain steps in carcinogenesis (44). It regulates multiple cancer-associated processes related to malignant tumor progression (45). Cav-1, which has been proposed as a candidate tumor suppressor, plays a regulatory role in several signaling pathways (46) and in the invasion of pathogenic microbes into the host cells (47). The increased cav-1 in the heart noted in our study suggests that the tissues were greatly damaged following DEX-induced toxicity. The present study indicates that DEX-induced toxicity causes an increase in cav-1 expression, which is related to impaired heart function.

In this research, our results demonstrate that cardiac protein expression of enolase and cav-1 is altered after DEX-induced toxicity in the rat. Following DEX-induced toxicity, protein

expression of enolase and cav-1 was significantly elevated. The present study indicates that DEX-induced toxicity caused different alterations in heart proteins that are related to reduced cardiac function.

In conclusion, the present findings indicate that such alterations are reflected in abnormal cardiac function. Moreover, the proteins identified in this study may be useful for revealing the mechanisms underlying DEX-induced toxicity and also for providing various clues for further research.

### Acknowledgements

This study was supported by a grant from the Natural Science Foundation of Guangdong Province, China (4203003) and the Guangdong Medical College (no. XQ0426).

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