LIGHT AND ELECTRON MICROSCOPE STUDY OF THE DIABETIC RAT MYOCARDIUM AFTER TRIMETAZIDINE (VASTAREL) TREATMENT

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ABSTRACT
Diabetic patients with ischaemic heart disease have a greater liability of myocardial ischaemia, and an increased incidence of heart failure compared to the non-diabetic ones. The goal of this study was to clarify the effects of trimetazidine on the architecture of the myocardium of diabetic rats. Thirty adult male albino rats (200-250 gm) were used in this investigation. They were divided into three equal groups; control, diabetic non-treated and diabetic TMZ-treated. At sacrifice, small pieces of the myocardium of left ventricle were processed for histological, histochemical and immunohistochemical study. Myocardium of diabetic rats showed an apparent increase of endomysium. The muscle fibers showed areas of degeneration. Ultrastructurally, the cardiac myocytes of diabetic rats showed distortion of cardiac myofibrils with loss of cross banding in many areas. The nucleus had a corrugated nuclear membrane and the mitochondria were swollen and distorted. Histochemically, myocardium of diabetic rats exhibited a weak succinic dehydrogenase reaction and a strong positive immunoreaction for NF-kappa B and caspase-3 in myocardial sarcoplasm. On the other hand, TMZ-treated diabetic rats showed an improvement in the histological architecture and in both histochemical and immunohistochemical reactions. So, TMZ should always be advised for diabetic patients to alleviate the cardiac hazards.

INTRODUCTION
Diabetes mellitus (DM) is a chronic metabolic disorder characterized
levels (more than 300 mg/dl) (Aoki et al., 2001; Evelson et al., 2004). Eight weeks after injection of streptozoto- cin, group I and II animals were given normal saline (1ml/ day orally for six months). Diabetic animals of group III were given TMZ (Blister of modified release film coated 35 mg-tablets of trimetazidine dihydrochloride manufactured by Servier Egypt Industries Limited, 6th October City, Egypt, in a dose of 70 mg per day orally, dissolved in 1 ml normal saline). Doses were given orally daily by a modified plastic syringe for six months (Qiu et al., 2005). The human dose of TMZ was corrected according to formula of Paget and Barnes, (1964). All animals were housed under the same conditions and allowed food and water ad-libitum.

Histochemical study:

Twenty-four hours after the last dose of TMZ, rats of all groups were anaesthetized by ether and sacrificed. Small pieces of the myocardium of left ventricle were immersed in 10 % formalin, dehydrated, cleared and embedded in paraffin. Paraffin sections (6 μm) were prepared and stained with haematoxylin & eosin (H&E) to study the general histological architecture of the ventricular myocardium (Drury and Wallington, 1980).

For electron microscopy, fine fragments of the left ventricle were fixed in glutaraldehyde (2%) in 0.1 M phosphate buffer at pH 7.4. They were, then, transferred to 1% osmium tetroxide in the same buffer, dehydrated in ascending grades of alcohol and propylene oxide, embedded in epon (Hayat, 1989). Ultrathin sections (40-50nm) were cut, using a glass knife, stained with 4% uranyl acetate, 2% lead citrate and examined by JEOL 100S electron microscope.

Histochemical study:

Fresh frozen cryocut sections (10μm) were processed for nitro-blue tetrazolium (NBT) staining to estimate the succinic dehydrogenase enzyme (SDH) activity (Kiernan, 1999)

Immunohistochemical study

Paraffin sections of left ventricle (5 μm) were stained by peroxidase antiperoxidase enzymatic immunohistochemical method(PAP) (Sternbargar et al., 1970), using anti nuclear factor-
between myofibrils (Fig.6).

*Histochemical changes:*

The myocardium of control rats (group I) showed an intense succinic dehydrogenase (SDH) reaction, which appeared as small purple granules scattered in the sarcoplasm of the muscle fibers (Fig. 7). In diabetic rats (group II), the reaction was mostly moderate (Fig. 8). TMZ treated diabetic rats (group III) exhibited an intense reaction in most fibers and a moderate one in some areas (Fig. 9).

*Immunohistochemical changes:*

1) **Nuclear factor kappa B (NF kappa B):** Group I rats showed a weak expression of NF kappa B in the sarcoplasm of cardiac myocytes (Fig. 10). In group II rats, there was a strong positive immune reaction to NF kappa B, which appeared as brownish dots in the cytoplasm (Fig.11). In TMZ-treated diabetic rats (group III), the level of NF-Kappa B reaction in most of cardiac myocytes returned nearly to the control level (Fig.12).

2) **Caspase-3:** Group I rats showed a negative immune reaction to caspase-3 in their myocardial sarcoplasm (Fig. 13). A strong positive immune reaction to caspase-3, was encountered in the cardiac myocytes of group II rats in the form of brownish punctate in the cytoplasm (Fig. 14). Group III animals exhibited a very weak reaction to caspase-3 in most of their cardiac myocytes and strong reaction in the degenerated ones (Fig.15).
Fig.(5) : An electron micrograph of a cardiac myocyte of group II rat showing distortion of cardiac myofibrils with loss of cross banding in many areas. The nucleus (N) has corrugated nuclear membrane and the mitochondria (M) are swollen and distorted. (Uranyl acetate / lead citrate X 10000).

Fig.(6) : An electron micrograph of the cardiac myocyte of group III rat showing regular cross banding of sarcomeres (S). The dark bands are bisected by H zone and the light bands appear very narrow. The nucleus (N) is oval with smooth nuclear membrane. Some myofibrils are still degenerated (d). Notice the mitochondria (M) arranged in rows between myofibrils. Uranyl acetate / lead citrate x10000).

Fig.(7) : A photomicrograph of a control rat myocardium showing intense SDH enzyme activity of muscle fibers with no staining of intercalated disc (arrows). (NBT X 200).

Fig.(8) : A photomicrograph of a diabetic rat myocardium showing mostly a moderate SDH enzyme activity. (NBT X 200).
Fig. (13): A photomicrograph of the control rat myocardium showing negative reaction to caspase-3 in the cytoplasm of cardiac myocytes. (PAP X 400).

Fig. (14): A photomicrograph of the myocardium of diabetic rat showing strong reaction to caspase-3 (PAP X 400).

Fig. (15): A photomicrograph of the myocardium of TMZ-treated diabetic rat showing a very weak reaction to caspase-3 in the majority of cardiac myocytes with area of strong reaction (arrow) in the degenerated fibers. (PAP X 400).

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TZM in the current investigation, it is

In light of the beneficial effects of

TZM on the maintenance of the metabolic po-
microvascular integrity, thus allowing
the rapid production and increase
of oxygen- protective effect. TZM could decrease o2
-attack on the cells, which contains a key enzyme in the activation of cas-
slowly中关的cytochrome C release
microvascular myocytes. Green
Montero et al. (2004) declared that
the stress, also, Roy (2000)
and
-oxidative stress, such as oxida-
apoptosis under a variety of pro-
-oxidant play an important role in
the stressed cardiac myocytes. Green
and Feen (1998) showed that mi-
the stressed cardiac myocytes. Green
and Feen (1998) showed that mi-
the stress, which could be owed to the effect of
TZM on these

In the current study, immun-
-ease in the current study, in TZM-treated diabetic
-diabetic patients, it was owing to the incidence of
-F-kinase phosphorylation it and
which prevents it from entering the
hibernation molecule in the cytoplas
in unstimulated cells, it is bound to in-
esterified by a wide range of stimuli.
NF-Kappa B has been found to be

-oxidative, stress, strain, and ischemia.
Involving in the cellular response to
(F-kinase) is also
-oxidant, including apoptosis, growth, division,
ion of several biological phenomena.
NF-Kappa B is implicated in the regula-
Jones et al. (2003) reported that NF-
myocardial degeneration by DM.
pression of NF-Kappa B was in-

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was negative in the cardiac myocytes

cells.

This could be due to the

crease. This could be due to the
advisable to widen the scale of its use for patients at high risk of diabetes mellitus to alleviate the diabetic undesirable cardiac hazards.

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دراسة بالساعة الضرورية والالكترونية لعِضلة قلب الفأر المصاب بالسكر بعد علاجها بعقار ترايميتازيدين (فستاريل)

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أجري هذا البحث لكشف أثر تعاونا عقار ترايميتازيدين (فستاريل) على قلب الفئران المصابة بمرض السكر. وقد استخدم في البحث ثلاثين من ذكور الفئران البيضاء البالغة تراوح وزنها بين 200 - 250 جم. قسمت بالتساوي إلى ثلاثة مجموعات. استخدمت فئران المجموعة الأولى كضابطة وفئران المجموعة الثانية تم إصابتها بمرض السكر وفئران المجموعة الثالثة تم إصابتها بمرض السكر واعطيت عقار ترايميتازيدين (فستاريل) بعد ثمانية أسابيع من الإصابة بالسكر واستمر العلاج ستة أشهر متتالية من طريق الفم مرة يومياً.

وقد أظهرت الدراسة الهيستولوجية والهيستوكيماوية والمناعية الهيستوكيماوية التغييرات المصاحبة للاصابة بمرض السكر على عضلة القلب من فقد ترتيب الألياف العضلية مع اتساع في الغشاء البيتيني بينها ووجود تحلل في بعض هذه الألياف. وأصبحت الألياف صغيرة داكنة وجدارها ممتورجة. وحدث تحلل في الميتوكوندريا كذلك. ولاحظ أيضاً نقص واضح في نشاط أنزيم السكينيك ديهيدروجينيز وزيادة في التفاعل الناعم للثلاجة والكسباس. 3 وقد حدد تحسن ملحوظ وغير ثام على عضلة القلب بعد إعطاء عقار ترايميتازيدين (فستاريل) أدلى إلى استعادة الألياف العضلية لمعظم تركيبها الطبيعي كما قلل التفاعل الناعم والكيماوي الدم على تحلل الخلايا.

ويعود استعراض النتائج السابقة يمكن الاستنتاج أن عقار ترايميتازيدين (فستاريل) مفيد لعضلة القلب التي تتأثر بمضاعفات مرض السكر.