Histopathological monitoring of cardioprotective effects of MgSO₄, pioglitazone and omega-3 fatty acids in rabbits induced with myocardial infarction.

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Abstract
Myocardial infarction (MI) is one of the leading causes of morbidity and mortality all over the world.

In a trial of developing more effective and safe management of this problematic form of ischemic heart disease, that has several cardioprotective mechanisms, pioglitazone, Omega-3, and magnesium sulfate have been assessed in a rabbit model of induced myocardial infarction (MI).

A thirty local domestic adult male rabbits were divided into 5 groups include healthy control, induced untreated, pioglitazone treated, magnesium treated, Omega-3 treated group. These drugs were given in a dose of 1 mg/kg, 2 mg/kg and 2 mg/kg respectively single per day for 60 days with clinical and histopathological assessment along the treatment course.

All of the tested agents caused significant cardioprotective and cardioregenerative effects in comparison to the untreated group by improving the autopsy histopathological figure causing left graphic shift in regard to reducing the infarct thickness and inflammatory cells infiltration at the end of treatment course P<0.05. In this study omega-3 fatty acids have caused significant reduction in the mean infarct size from 2000 +/- 147 micrometer to 1400 +/- 35 micrometer at the end of treatment course in
comparison with the control untreated group. In regard to anti-inflammatory reduction by these drugs, MgSO4 caused reduction in the mean PMN, MN cells and fibrocytes from 250 +/- 45 per high power field at start of treatment to 90 +/- 23 at the end of the treatment course. In conclusion, omega-3 fatty acids and MgSO4 had a promising cardioprotective effects that could be further evaluated as a supportive treatment for human myocardial infarction.

Introduction

Ischemic heart disease:

Ischemic heart disease is one of the major cause of mortality and morbidity allover the world. It was estimated that in 2002, 12.6 percent of deaths worldwide were from ischemic heart disease [1]. It is estimated that more than 1 million of Americans will have a new or recurrent coronary attack, and about one-third of them will die [2].

Supply ischemia or low flow ischemia which is responsible for many attacks of acute myocardial infarction (AMI) and many episodes of unstable angina [3].

Consequences of myocardial ischaemia:

The effect of coronary occlusion on myocardial contraction was previously believed as transient severe ischaemia causes either irreversible cardiac injury i.e. infarction, or prompt recovery [4]. However, later on, it becomes clear that regional contraction remains depressed for more than 3 hours after 5 minutes coronary occlusion and more than 6 hours after 15 minutes occlusion in conscious dogs [5].

Myocardial stunning was observed later in patient with coronary artery disease in a variety of clinical conditions after exercise induced ischaemia [6,8].

The cellular and molecular mechanisms of myocardial stunning include:

1- Generation of Oxygen free radicals.
2- Calcium overload.
3- Reduced sensitivity of myofilaments to calcium and loss of myofilaments [6,7].

Histopathological parameters:

1- Inflammatory cells pattern:

Classically the irreversibility of Prominent I bands is established only after 4-8 hours when polymorph nuclear cells first migrate within the vessels to the periphery of affected area and when penetrate centripetally into the necrotic tissues lead to "Wavy" fibers or "undulating myocardial fibers" which is not associated, usually, with a marked exudates of fibrin and red cells and it disappears in approximately 1 week [11].

2- Infarct size parameter:

In all AMI, a rim of coagulative myocytolysis of varying widths is observed at the periphery in a continuity with the infarct and, in 85%, the infarction not seen in associated with hemorrhage (as foci or confluent areas) in non ischemic myocardium surrounds the infarct [9,10].

Materials and Methods

The used animals:

A forty local, domestic, males rabbits of four-six months age old and 1200-1400 gm body weight were used as animal model to demonstrate the cardioprotective effects of the tested agents. They were divided into 6 groups and housed in a standard breeding metal
cages separately in a room with constant temperature of 25 degree and constant lighting cycle (12 hours light\dark). They were given standard oxoid (130 gm/day) with water ad libitum. All groups were monitored with prior clinical and ECG examination to exclude any underlying health and cardiovascular abnormalities.

Grouping of animals:
The used rabbits were divided into the following groups:

(1) Group A:
As a control group of 6 healthy rabbits (n=6). They had been given 1 ml of distilled water (DW) 1 hour prior to histopathological examination.

(2) Group B: (n=6)
They were induced with myocardial infarction as a control untreated group and were given 1 ml of distilled water prior to induction of infarction and thereafter along with 2 months course.

(3) Group C: (n=6)
MgSo4 treated group. They were given 5mg\kg of MgSo4 orally prior to induction of AMI and 2 mg\kg of MgSo4 for 60 days at the morning once per day along with 2 months of treatment course.

(4) Group D: (n=6)
Omega-3 mixed fatty acids treated group of types; decosahexonomic and eicospentamic acid given 2 mg\kg (1mg from each) 1 hour prior to induction and same dose daily along 60 days (once daily at morning).

(5) Group E: (n=6)
Pioglitazone was given 1 mg\kg 1 hour prior to induction of AMI and 0.5 mg\kg daily once per day at morning along 60 day thereafter.

Induction of myocardial infarction in the rabbits.
The rabbits were anesthetized under general anesthesia with 1 ml of 5% Phenobarbital. Then left parasternal thoracotomy was made along the left sternocostal junction (7th-1st). Exposure of the heart after application of tissue and lung traction was made. The left coronary artery and its branches were identified through a small excision of pericardial membrane, a fine catgut 40 gauge stitch was tied around the apical sub-branch of the anterior branch of left coronary artery.

Histopathology
Histopathological setting:
It was the most important tool for both detection of myocardial cell damage and for evaluation of the efficacy of the tested agents by estimation of structural changes, which occur under the effect of both ischemia and cardio protective treatment [9,12]. The used device was a base sledge microtome (Leitz, Germany). It can gives slices of 1-30 micrometer thickness by special fine adjustment. This instrument is heavy in weight so that it can be used to get slices from solid specimen. The used stains were haematoxyin -Eosin.

Results
Autopsy histopathological examination:
Sectioning of the heart 60 days after induction of acute myocardial infarction depending on pilot study.
Figure (1) this figure shows the mean reduction in infarct size (in micrometers) when pioglitazone, MgSo4, and EPA were used. Most significant size reduction was with MgSo4 and with EPA. The figure

Figure (2) shows the mean reduction of inflammatory response of the infarct area. Omega 3 and MgSo4 were the most significant testing agents when they were used.
Discussion

Many factors can modify the size of the ischemic myocardial area including collateral circulation, vasodilatatory substances, lysozymes activity, cytokines level in addition to local free radicals and inflammatory cells activity [13,14]. These factors could be valuable targets for cardio regenerative therapy. On the other hand, the size of ischemic cardiac area and inflammatory cells activity could be of highly predictive value in assessment of regenerative response to different treatments since infarct size has the greater prognostic role in myocardial infarction.

Different therapeutic modalities were conducted in this study namely, the coronary vascular relaxants MgSO4 and omega-3 fatty acids have also antiplatelets and immunemodulatory activity in addition to the scavenering for free radicals [18,19].

Similar studies on human and animals have agreed with this study in that

In this study omega-3 fatty acids have reduced the mean infarct size from 2000 micrometer at the start of the treatment to 1400 micrometer at the end of treatment course which was the highly significant infarct size reduction potency in comparison with the control untreated group at P< 0.05. whereas MgSO4 showed the second significant value and the oral insulin sensitizer, pioglitazone came the last.

In regard to anti-inflammatory reduction by these drugs, MgSO4 caused reduction in the mean PMN, MN cells and fibrocytes from 250 per high power field at start of treatment to 90 at the end of the treatment course in comparison with untreated group. Which could indicate a significant anti-inflammatory effect of these tested agents at P< 0.05 that can modify the course of myocardial damage in regard to protecting the heart quantitatively and qualitatively by shifting inflammatory picture from necrotic to apoptotic type [15,16]. The later is characterized microscopically by more percentage of intact myocardial cells although many are still lacking intracellular constituents [17]. The clinical significance of this cardio protective mechanism of omega-3 fatty acids and MgSO4 is an expected reduction of development of arrhythmia and improving ventricular ejection fraction after treatment course in addition to minimizing post-MI remodeling effects. Many agents are now assessed for their cardio protective effects with high promising outcomes and some have agreed with enhancing collateral coronary vessels and scavenger strategy in treating myocardial infarction [20,21,22]. In conclusion, omega-3 fatty acids and MgSO4 had a promising cardio protective effects that could be recommended for further evaluation as a supportive treatment for human myocardial infarction.

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