Avemar and Echinacea extracts enhance mobilization and homing of CD34+ stem cells in rats with acute myocardial infarction

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Acute myocardial infarction (AMI)

- Myocardial infarction (MI) is one of the major causes of cardiovascular diseases worldwide.
- AMI is responsible approximately for ¾ of all CHD deaths and accounts for about ½ of all CHD hospital admissions.
Acute myocardial infarction (AMI)

AMI results in:
- Loss of cardiomyocytes
- Scar formation
- Ventricular remodeling
- Heart failure

New approaches in Cardiovascular Therapy

- Although current pharmacological and surgical interventions have led to improved survival of patients, they failed to regenerate dead myocardium and/or prevent deterioration of cardiac function.
- In last decade, Stem Cell Therapy has emerged as a potential new strategy for incurable and life threatening MI.
- The ultimate goals of stem cell therapy are myocardial regeneration and neovascularization leading to clinical improvement without severe adverse effects.
Stem cell-based Myocardial Regeneration

- Stem cells are characterized by self-renewal, clonogenicity, and ability to differentiate into:

  - Cardiomyocytes
  - Endothelial cells
  - Vascular smooth muscle cells.

Stem cell-based Myocardial Regeneration

Various types of adult SCs that have demonstrated therapeutic potential with potential paracrine activities:

- **Bone marrow-derived SCs**: BM is the main reservoir of many types of SCs including: Mesenchymal SCs, Hematopoietic SCs, Very Small Embryonic-Like SCs, and BM-Derived Endothelial Progenitor Cells

- **Circulating SCs**: include hematopoietic, mesenchymal, endothelial, smooth muscle, and skeletal muscle precursors.

- **Resident cardiac progenitor cells**: are believed to represent self-renewing populations of cells confined to specific niches within the heart that may be stimulated to proliferate and differentiate as a result of paracrine effects
STEM CELL-BASED MYOCARDIAL REGENERATION

Three distinct treatment modalities of MI involving SCs can be recognized:

1- Stem Cell Transplantation

[Diagram showing bone marrow harvest, isolation, expansion, and delivery to the heart]

Adult stem cells are harvested from BM or peripheral blood and injected into the infarcted recipient.

STEM CELL-BASED MYOCARDIAL REGENERATION

2- Stem Cell Mobilization

[Diagram showing mobilization of stem cells from BM or tissue niches using cytokines or drugs]

Enhancing mobilization of endogenous stem cells from BM or tissue niches using cytokines or drugs.
Adult cells are reprogrammed into pluripotent state similar to ESCs which might provide a source of cardiomyocytes.
STEM CELL MOBILIZATION

The processes of mobilization and homing are guided by inflammatory and hematopoietic cytokines such as:

- **G-CSF**
- **GM-CSF**
- **SDF-1**
- **IL-8**
- **VEGF**

Inflammatory & hematopoietic cytokines

STEM CELL MOBILIZATION

- Stem cell mobilization technique **mimics the physiological release** of stem cells from BM in response to MI. However, unfortunately these mobilized cells are insufficient to promote myocardial repair.

- Although SC transplantation is the most common approach of Stem cell Therapy, its applications are restricted by **excessive cost, complexity and immune rejection**. On the other hand, stem cell mobilization is **less costly and less complex** approach.
In this study, we investigated the possible effect of two natural products namely Avemar and Echinacea extracts to enhance CD34⁺ SC mobilization and homing.
AVEMAR

- Avemar is a product of industrial fermentation of wheat germ.
- With a standardized content of benzoquinone and plant flavonoids

These activities are speculated to drive tissue repair and regeneration.
ECHINACEA

- Echinacea preparations are among the most popular herbal remedies worldwide.

- Active compounds of *Echinacea* include *lipophilic alkamides* and *caffeic acid derivatives*

ECHINACEA

1. Anti-inflammatory activity
2. Immunostimulatory activity
3. Antioxidant activity
4. Intensify the immunological angiogenesis
5. Improve the regeneration process of damaged tissues

*These effects could be of high benefit in wound healing and in future cardiology*
This study aimed to investigate the possible effect of Avemar and Echinacea on enhancing CD34+ SC mobilization and homing in relation to inflammatory and hematopoietic cytokines such as VEGF, IL-8 and GM-CSF in rat model of AMI.
EXPERIMENTAL DESIGN

- Animals used are Male Wistar rats weighing 170±20 g.

- AMI was induced by subcutaneous injections of isoprenaline (85 mg/kg) for two consecutive days.
EXPERIMENTAL DESIGN

Treatment regimens

Post-treatment (therapeutic)

Post-treated groups received drugs orally for 14 days after induction of MI

Pre- & post-treatment (prophylactic and therapeutic)

Pre- & post-treated groups received drugs orally for 14 days before and 14 days after induction of MI

EXPERIMENTAL DESIGN

Rats were randomly divided into six groups:

1. Normal
2. AMI Isoprenaline (85 mg/kg)
3. AMI post-treated with Avemar (121 mg/kg)
4. AMI pre- & post-treated with Avemar (121 mg/kg)
5. AMI post-treated with Echinacea (130 mg/kg)
6. AMI pre- & post-treated with Echinacea (130 mg/kg)
BLOOD SAMPLING

Animals were anesthetized with ip injection of Thiopental (50mg/kg) then blood was collected from the retro-orbital sinus at the following intervals:

- one day after last injection
- on day 1, 7 and 14 after induction of myocardial infarction
- on day 7 and 14 after induction of myocardial infarction

MEASURED PARAMETERS

- **Blood**
  - CD34+ stem cells (Flow cytometry)
- **Serum**
  - CK
  - VEGF
  - IL-8
  - GM-CSF
- **Heart tissue**
  - Immunohistochemical investigation of CD34+ cells
  - Histopathological analysis
  - Capillary density
RESULTS

EFFECT OF AVEMAR & ECHINACEA TREATMENTS ON THE NUMBER OF CD34+ STEM CELLS IN BLOOD OF RATS WITH AMI AT DIFFERENT TIME INTERVALS FROM THE INFARCTION DEVELOPMENT

Values are expressed as percentage of normal
a: significant difference from normal group at p < 0.05
b: significant difference from corresponding AMI control group at p < 0.05
c: significant difference from corresponding post-treated group at p < 0.05
EFFECT OF AVEMAR & ECHINACEA TREATMENTS ON SERUM CK ACTIVITY IN RATS WITH AMI AT DIFFERENT TIME INTERVALS FROM THE INFARCTION DEVELOPMENT

(Values are expressed as percentage of normal)

a: significant difference from normal group at p < 0.05
b: significant difference from corresponding AMI control group at p < 0.05
c: significant difference from corresponding post-treated group at p < 0.05

EFFECT OF AVEMAR & ECHINACEA TREATMENTS ON SERUM VEGF LEVEL IN RATS WITH AMI AT DIFFERENT TIME INTERVALS FROM THE INFARCTION DEVELOPMENT

(Values are expressed as percentage of normal)

a: significant difference from normal group at p < 0.05
b: significant difference from corresponding AMI control group at p < 0.05
c: significant difference from corresponding post-treated group at p < 0.05
EFFECT OF AVEMAR & ECHINACEA TREATMENTS ON SERUM IL-8 LEVEL IN RATS WITH AMI AT DIFFERENT TIME INTERVALS FROM THE INFARCTION DEVELOPMENT

Values are expressed as percentage of normal

EFFECT OF AVEMAR & ECHINACEA TREATMENTS ON SERUM GM-CSF LEVEL IN RATS WITH AMI AT DIFFERENT TIME INTERVALS FROM THE INFARCTION DEVELOPMENT

(Values are expressed as percentage of normal)
a: significant difference from normal group at p < 0.05
b: significant difference from corresponding AMI control group at p < 0.05
c: significant difference from corresponding post-treated group at p < 0.05
IMMUNOHISTOCHEMICAL DETECTION OF CD-34+ CELLS IN MYOCARDIAL TISSUE TWO WEEKS AFTER ACUTE MYOCARDIAL INFARCTION DEVELOPMENT

AVERAGE NUMBERS OF CD-34+ CELLS IN MYOCARDIAL TISSUE 14 DAYS AFTER AMI DEVELOPMENT

<table>
<thead>
<tr>
<th>Group</th>
<th>Average No. of cells per field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.50</td>
</tr>
<tr>
<td>AMI</td>
<td>1.00</td>
</tr>
<tr>
<td>Post-treatment with Avemar</td>
<td>1.50   (ab)</td>
</tr>
<tr>
<td>Pre- and post-treatment with Avemar</td>
<td>2.00 (ab)</td>
</tr>
<tr>
<td>Post-treatment with Echinacea</td>
<td>2.50   (ab)</td>
</tr>
<tr>
<td>Pre- and Post-treatment with Echinacea</td>
<td>2.00 (ab)</td>
</tr>
</tbody>
</table>

**a:** significant difference from normal group at \( p < 0.05 \)

**b:** significant difference from corresponding AMI control group at \( p < 0.05 \)
HISTOPATHOLOGICAL ANALYSIS OF MYOCARDIAL TISSUE AFTER AMI DEVELOPMENT (STAINED BY HEMATOXYLIN & EOSIN STAIN).

(A) normal. (B) AMI group. (C) AMI post-treated with Avemar. (D) AMI pre- and post-treated with Avemar. (E) AMI post-treated with Echinacea. (F) AMI pre- and post-treated with Echinacea. (1d) indicates 1 day after AMI; (14d) indicates 14 days after AMI. (h) hyalinization. (o) oedema. (m) inflammatory cells infiltration. (v) newly formed blood capillaries.

EFFECT OF AVEMAR AND ECHINACEA TREATMENTS ON CAPILLARY DENSITY IN THE MYOCARDIUM 14 DAYS AFTER AMI DEVELOPMENT (MEAN ± SE).

a : significant difference from AMI group at p < 0.05
CONCLUSION

- Avemar and Echinacea treatments effectively triggered the mobilization and homing of CD34+ stem cells to the ischemic myocardium.

- Thus, they may enhance Sc-based regeneration of myocardial tissue.

- Echinacea is superior to Avemar where treatment can be started after the development of MI to enhance stem cell mobilization.

- The potential of these safe and cheap products as additional complementary therapeutic agents holds great promise for MI.

- However, further studies are required to explore the exact mechanism of their role in enhancing Sc mobilization.
TAKE HOME MESSAGE

Avemar and Echinacea could be recommended for patients susceptible to myocardial infarction to promote stem cell-based myocardial regeneration.

Thank you