Investigation of the protective and treatment effects of vinpocetine in myocardial infarctional with isoprotenol in rats

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Abstract
As aim of this study, the knowledge of whether vinpocetine (VINPO) is cardioprotective or not following isoprotenol (ISO)-induced cardiac ischemia in rats. In myocard infarction, the one of the responsible mechanisms of injury is oxidative damage and inflammation. The effect of VINPO which is the potent antioxidant and anti inflammatory agent aimed to reveal as the biochemical, electrophysiological, and histopathologic parameters. In this study, thirty- two Wistar-Albino male rats were divided into four groups, each consisting of eight rats. The group 1 named as sham, no any drug used in this group. Group 2 named as iso group, only ISO was administered, the group 3 named as VINPO and iso group, here initially vinpocetine then ISO were used, and the group 4 named as iso and VINPO, here initially ISO then VINPO were used. For the rats in group 2, in first and second day ISO administered at a dose of 120mg/kg using an intraperitoneal injection. At third and fourth day no any drug used. At fifth day the experiment terminated. At fifth day experiment was terminated. Lastly for the rats in group4, first and second day ISO administered at a dose of 120 mg/kg using an ip injection, at third and fourth day no any drug was used. And at fifth day experiment was terminated. For the rats in group 3, at the first and second day VINPO administered at a dose of 20 mg/kg using an intraperitoneal (ip) injection, after 30 minute isoprotenol administered at adose of 120 mg/kg using an ip injection. At third and fourth day no any drug was used. And at fifth day experiment terminated. Prior to termination of experiment the pulse rate and ECG changes were recorded. After completion of experiment protocols blood samples and cardiac tissue samples were recieved. For the knowledge of effects of VINPO experimental myocardial ischemic injury induced in rats, and the serum myoglobulin, total creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanin aminotransferase (ALT), measured. In myocardial tissue as an antioxidative system and an oxidative stres markers; malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathine (GSH), total oxidant status (TOS), total antioxidant status (TAS), oxidative stres index (OSI) were studied. As a result, VINPO showed positive impact on cardiac functions in the light of electrophysiographic, biochemical and histopathological. According to our results, it is possible to say that it exerts these beneficial effetc via its powerfull antioxidant, antiinflammatory and free radical scavenger effects.

Keywords: Isoproterenol, vinpocetin, rat, heart

Introduction
Acute myocardial infarction (AMI), which is one of the most common diseases in recent years, has been affected by several adverse living conditions of advancing technology. Myocardial infarction occurs as a result of a sudden interruption of blood flow with thrombotic occlusion of the previously narrowed coronary artery due to atherosclerosis. The amount of myocardial damage caused by coronary occlusion depends on the extent of vessel occlusion, the duration of coronary artery occlusion, the area fed by the damaged vessel, the amount of blood supplied to the damaged area with collateral circulation, the factors providing spontaneous thrombus lysis, myocardial oxygen requirement, and the adequacy of myocardial perfusion in the infarct zone when the flow is reestablished in the occluded coronary artery [1].

Isoproterenol (ISO) is a synthetic non-selective β-adrenoceptor agonist that produces acute myocardial infarction at high doses. ISO-induced lesion is defined as myocardial necrosis and shows the features which are seen in hypoxic/ischemic heart disease [2,3]. ISO-induced myocardial necrosis involves the changes which are characterized by the loss of function and integrity of myocardial membranes. The occurrence of changes in lipid metabolism is also one of the characteristic effects of ISO [4,5]. ISO also stimulates free radical formation and lipid peroxidation, which leads to irreversible damage of the myocardial membranes [4, 6]. Free oxygen radicals are reactive molecules containing an unsubstituted electron. Antioxidants are substances which prevent the effects of free oxygen radicals on target tissues or repair the damage that occurs. Oxidative stress, which arises as a result of an imbalance between free radical production and antioxidant defenses, has been reported to increase in atherosclerosis. Increased free radicals in the medium leads to increased lipid peroxidation [7]. Malondialdehyde (MDA), which occurs as a result of the oxidation of membrane polyunsaturated fatty acids, is one of the most important indicators of lipid peroxidation [8].

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VINPO has been documented that it has powerful antioxidant, anti-inflammatory and free radical scavenger effects. Therefore, this study is to examine the effect of vinpocetine on serum, tissue and cardiac function levels in experimental myocardial infarction induced by isoproterenol in rats and to reveal the role of oxidative cell damage observed in myocardial infarction.

Material and Methods

Our study was performed after having received approval from the Inonu University Medical Faculty Ethics Committee (Date: 20.01.2015 and Protocol No: 2015/A-12). Our study was also supported by the Scientific Research Project Fund of Inonu University (Project No: BAP-2015/42). The study was performed on 32 Wistar Albino rats produced by the Experimental Animal Research Laboratory of Inonu University Faculty of Medicine. They had the same biological and physiological properties and were randomized into 4 groups consisting of 8 rats. They were kept under standard laboratory conditions (22±10°C, 60% humidity, 12 hours light/12 hours dark cycle) and were fed ad libitum. The removal of animal wastes, the supply of water and feed, the cleaning and control of the cages were made by the veterinarian and staff of the center. All animal procedures were performed in accordance with “The Principles for the Care of Laboratory Animal” published by the National Health Research Community and “The Guide for the Care and Use of Laboratory Animals” published by the Institute of Laboratory Animal Resources and the National Institute of Health. The study was carried out at the Experimental Animal Research Laboratory of Inonu University Faculty of Medicine, the Central Laboratory of the Turgut Ozal Medical Center, the Department of Pharmacology of Inonu University Faculty of Medicine, the Department of Histology of Inonu University Faculty of Medicine, and the Department of Biochemistry of Inonu University Faculty of Medicine.

Experimental Groups

Group 1) SHAM group; (n=8): No medication was applied. ECG was taken on the 5th day. Heart rate and mean blood pressure were measured. Then, the experiment was terminated.

Group 2) ISO group; (n=8): 120 mg/kg of ISO was given intraperitoneally (i.p.) at first and second day. No medication was given at third and fourth day. ECG was taken on the 5th day. Heart rate and mean blood pressure were measured. Then, the experiment was terminated.

Group 3) VINPO+ISO group; (n=8): 20 mg/kg of vinpocetine was given intraperitoneally (i.p.) at first and second day. After 30 minutes, 120 mg/kg of ISO was given intraperitoneally (i.p.). No medication was given at third and fourth day. ECG was taken on the 5th day. Heart rate and mean blood pressure were measured. Then, the experiment was terminated.

Group 4) ISO+VINPO group; (n=8): 120 mg/kg of ISO was given intraperitoneally (i.p.) at first and second day. 20 mg/kg of vinpocetine was given intraperitoneally (i.p.) at third and fourth day. ECG was taken on the 5th day. Heart rate and mean blood pressure were measured. Then, the experiment was terminated.

Surgical procedure

Before the surgery, the rats were anesthetized with intraperitoneal (i.p.) injections of 10 mg/kg xylazine hydrochloride (2%) (Rompun, Bayer) and 50 mg/kg ketamine hydrochloride (Ketalar, Phizer). They were placed in the supine position on the operating table. ECG was taken. Heart rate and mean blood pressure were measured. Then, the operation area was cleaned with 10% povidone-iodine and sterile gauze. The skin, subcutaneous, fascia and thorax were opened with a 3 cm median incision made in the thorax. The mean blood pressure was measured by attaching a cannula into a carotid artery. Blood samples were obtained from all groups with intracardiac puncture for biochemical analysis. After 4-5 cc of blood samples were centrifuged, serum was obtained and was maintained at -30°C until the time of biochemical measurement. The extracts were divided vertically and horizontally. They were taken into vials containing 10% formalin and were stored at +4°C. All animals were sacrificed at the end of the procedure.

Biochemical Evaluation

5 cc of blood samples from the rats were placed in gel vacuum tubes (BD Vacutainer SST, UK) and were centrifuged at 4000 rpm for 15 min. The serum obtained were studied by an automatic analyzer system using devices in Central Laboratory, Inonu University Turgut Ozal Medical Center in order to measure the values for myoglobin (Reference Range: 14-70 ng/ml), AST (Reference Range: 5-34 U/L), ALT (Reference Range: 0-55 U/L), LDH (Reference Range: 125-243 U/L) and CK (Reference Range: 29-200 U/L) levels.

Histological Methods

At the end of the experiment, the heart tissues were kept in 10% formaldehyde. At the end of keeping, these tissues were embedded in paraffin blocks after they were passed through dehydration and polishing processes. 4-5 µm thick sections were taken from paraffin blocks. The sections, which were passed through deparaffinization and rehydration processes, underwent hematoxylin-eosin (H-E) for general histological evaluations and underwent Gomori triple staining to observe connective tissue. In the sections stained with hematoxylin-eosin (H&E) staining, organization dysfunction, myofibrillar loss, and cardiomyocytes with eosinophilic cytoplasm and pyknotic nuclei were evaluated. In the sections stained with Gomori triple staining, connective tissue density was evaluated. According to the extent of damage, it was enumerated as below:

0: Normal
1: Disorganization of cardiomyocyte structure, myofibrillar loss, cardiomyocytes with eosinophilic cytoplasm and pyknotic nuclei and an increase of less than 25% in the total amount of connective tissue
2: The ratio of cardiomyocytes with similar changes being between 25% and 50%
3: The ratio of cardiomyocytes with similar changes being between 50% and 75%
4: The ratio of cardiomyocytes with similar changes being more than 75%

10 randomly selected areas from each section (under X20 lens magnification) were examined using the Leica Q Win image analysis system.

Statistical analysis

The data are given as median (min-max). The Shapiro-Wilk test was used to determine if the data are or are not normally distributed. The Kruskal-Wallis test from the nonparametric tests was used in the analyzes. After the Kruskal-Wallis test, the Bonferroni-corrected Mann-Whitney U test was used for multiple comparisons. p-values <0.05 was considered statistically significant. IBM SPSS Statistics Base 22.0. Statistics software was used in the analyzes.

Results

The average weight of the rats prior to the experiment: It was measured as 325.63 g for group 1 (SHAM), 320.0 g for group 2 (ISO), 321.25 g for group 3 (VINPO+ISO) and 310.63 g for group 4 (ISO+VINPO)
The average weight of the rats after the experiment: It was measured as 325.38 g for group 1 (SHAM), 318.75 g for group 2 (ISO), 303.13 g for group 3 (VINPO+ISO) and 294.00 g for group 4 (ISO+VINPO).

The average heart weight of the rats: It was measured as 1.12 g for group 1 (SHAM), 1.25 g for group 2 (ISO), 1.24 g for group 3 (VINPO+ISO) and 1.09 g for group 4 (ISO+VINPO).

Biochemical Analyses

Serum Myoglobin, AST, ALT, LDH and CK Values:

When the myoglobin values measured in the rats were compared between the groups (Table 1), it was 452.4 ng/L in SHAM group, 194.9 ng/L in ISO group, 127.6 ng/L in VINPO+ISO group and 170.5 ng/L in ISO+VINPO group, respectively. There was no significant difference between the groups in terms of the myoglobin values (p>0.05).

When the AST values measured in the rats were compared between the groups, it was 157 U/L in SHAM group, 118 U/L in ISO group, 154 U/L in VINPO+ISO group and 114 U/L in ISO+VINPO group, respectively. There was no significant difference between the groups in terms of the AST values (p>0.05).

When the ALT values measured in the rats were compared between the groups, it was 919 U/L in SHAM group, 1039 U/L in ISO group, 827 U/L in VINPO+ISO group and 1425 U/L in ISO+VINPO group, respectively. There was no significant difference between the groups in terms of the ALT values (p>0.05).

When the LDH values measured in the rats were compared between the groups, it was 919 U/L in SHAM group, 1039 U/L in ISO group, 827 U/L in VINPO+ISO group and 1425 U/L in ISO+VINPO group, respectively. There was no significant difference between the groups in terms of the LDH values (p>0.05).

When the CK values measured in the rats were compared between the groups, it was 1813 U/L in SHAM group, 884 U/L in ISO group, 1324 U/L in VINPO+ISO group and 1463 U/L in ISO+VINPO group, respectively. There was no significant difference between the groups in terms of the CK values (p>0.05).

Tissue Variables

Tissue MDA Values: Tissue MDA levels were determined spectrophotometrically. In tissue MDA assay, the tissue homogenate was incubated in a boiling water bath for 1 hour under aerobic conditions and at pH 3.5. As a result of that, MDA, an end-product of lipid peroxidation, was reacted with thiobarbituric acid (TBA) in hot and acid medium. The resulting color was measured spectrophotometrically. When the MDA values measured in the study were compared between the groups (Table 2), it was measured as 3.31 (nmol/g tissue) in SHAM group. The MDA value showed a significant difference between SHAM group and ISO group (p<0.05). It was also measured as 14.24 (µmol/g tissue) in ISO+VINPO group and VINPO+ISO group (p<0.05).

Tissue SOD Values: SOD activity is based on the reduction of nitroblue tetrazolium (NBT) with superoxide, which is produced by the xanthine/xanthine oxidase system. As a result of the reduction of NBT with the forming superoxide radicals, the resulting formazan is measured spectrophotometrically. When the SOD values measured in the study were compared between the groups, it was measured as 1.75 (U/mg protein) in SHAM group. The SOD value showed a significant difference between SHAM group and ISO group (p<0.05). It was also measured as 0.82 (U/mg protein) in ISO group. The SOD value showed a significant difference between ISO group and ISO+VINPO group and VINPO+ISO group (p<0.05).

Tissue CAT Values: Hydrogen peroxide shows a maximum absorbance at 240 nm. Degradation of H2O2 added to the experiment medium by CAT enzyme is followed by a reduction of the absorption in the UV. When the CAT values measured in the study were compared between the groups, it was measured as 27.08 (k/mg protein) in SHAM group. The CAT value showed a significant difference between SHAM group and ISO group (p<0.05). It was also measured as 15.56 (k/mg protein) in ISO group. The CAT value showed a significant difference between ISO group and ISO+VINPO group and VINPO+ISO group (p<0.05).

Tissue GPx Values: When the GPx values measured in the study were compared between the groups, it was measured as 260.38 (µmol/g tissue) in SHAM group. The GPx value showed a significant difference between SHAM group and ISO group (p<0.05). It was also measured as 151.9 (µmol/g tissue) in ISO group. The GPx value showed a significant difference between ISO group and ISO+VINPO group and VINPO+ISO group (p<0.05).

Tissue reduced glutathione (GSH) Values: 5,5'-dithio-bis-[2-nitrobenzoic acid] (DTNB) is reduced by sulfhydryl compounds to produce a yellow complex (a disulfide compound). The optical density of this yellow compound is measured at 412 nm to determine GSH activity. When the GSH values measured in the study were compared between the groups, it was measured as 28.73 (µmol/g tissue) in SHAM group. The GSH value showed a significant difference between SHAM group and ISO group (p<0.05). It was also measured as 14.24 (µmol/g tissue) in ISO group. The GSH value showed a significant difference between ISO group and ISO+VINPO group and VINPO+ISO group (p<0.05).

Tissue Total Oxidant Status (TOS) Measurement: The results, which were calibrated with hydrogen peroxide, were expressed as nmol H2O2 Eq/mg protein and were measured spectrophotometrically. When the TOS values measured in the study were compared between the groups, it was measured as 4.88 (µmol H2O2 Eqv/L) in SHAM group. The TOS value showed a significant difference between SHAM group and ISO group (p<0.05). It was also measured as 8.1 (µmol H2O2 Eqv/L) in ISO group. The TOS value showed a significant difference between ISO group and ISO+VINPO group and VINPO+ISO group (p<0.05).

Tissue Total Antioxidant Status (TAS) Measurement: It was measured spectrophotometrically and was expressed as Trolox Equiv./L. There was no significant difference between all the groups in terms of the TAS values (P>0.05).

Oxidative Stress Index (OSI): It was calculated by dividing TOS by TAC and was expressed as µmol H2O2/ mmol Trolox. When the OSI values measured in the study were compared between the groups, it was measured as 14.99 (6.72-22.23) (AU) in SHAM group. The OSI value showed a significant difference between SHAM group and ISO group (p<0.05). It was also measured as 14.99 (6.72-22.23) (AU) in SHAM group. The OSI value showed a significant difference between SHAM group and ISO group (p<0.05).

Heart Rate, Mean Blood Pressure and ECG Changes: The heart rate, mean blood pressure and ECG changes of the groups are given in Table 3. According to these findings, there was a statistically significant difference between the groups in terms of the heart rate, mean blood pressure and ECG changes (p<0.05).
Table 1. Serum myoglobin, AST, ALT, LDH, CK values (median, min-max) and p-values in groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variables</th>
<th>SHAM (n=8)</th>
<th>ISO (n=8)</th>
<th>VINPO+ISO (n=8)</th>
<th>ISO+VINPO (n=8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MYOGLOBIN (ng/L)</td>
<td>452.4 (94.1 - 762.6)</td>
<td>194.9 (6.9 - 562.3)</td>
<td>127.6 (82.9 - 832.4)</td>
<td>170.5 (52 - 440.9)</td>
<td>0.267</td>
</tr>
<tr>
<td></td>
<td>AST (U/L)</td>
<td>157 (89 - 321)</td>
<td>118 (56 - 167)</td>
<td>154 (65 - 2244)</td>
<td>114 (66 - 253)</td>
<td>0.431</td>
</tr>
<tr>
<td></td>
<td>ALT (U/L)</td>
<td>53 (37 - 123)</td>
<td>38b (25 - 60)</td>
<td>55 (50 - 177)</td>
<td>54 (31 - 80)</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>LDH (U/L)</td>
<td>919 (335 - 1515)</td>
<td>1039 (300 - 1933)</td>
<td>827 (58 - 1935)</td>
<td>1425 (130 - 4295)</td>
<td>0.530</td>
</tr>
<tr>
<td></td>
<td>CK (U/L)</td>
<td>1813 (1332 - 2349)</td>
<td>884 (317 - 3509)</td>
<td>1324 (217 - 2989)</td>
<td>1463 (251 - 2726)</td>
<td>0.220</td>
</tr>
</tbody>
</table>

p<0.05 was considered significant (difference between groups); a: It is different according to ISO group; b: It is different according to VINPO+ISO group; c: It is different according to ISO+VINPO group.

Table 2. Values of tissue variables (median, min-max) and p-values in groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variables</th>
<th>SHAM (n=8)</th>
<th>ISO (n=8)</th>
<th>VINPO+ISO (n=8)</th>
<th>ISO+VINPO (n=8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDA(nmol/g tissue)</td>
<td>3.31a (2.44 - 4.18)</td>
<td>7.97b,c (7.29 - 9.8)</td>
<td>3.54 (2.46 - 5.1)</td>
<td>3.04 (2.11 - 3.67)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>SOD(U/mg protein)</td>
<td>1.75a (1.13 - 2.32)</td>
<td>0.82b,c (0.52 - 1.05)</td>
<td>1.91 (1.43 - 2.44)</td>
<td>1.59 (1.24 - 2.55)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>CAT(k/mg protein)</td>
<td>27.08a (18.2 - 34.88)</td>
<td>15.56b,c (13.88 - 19.39)</td>
<td>29.49 (20.14 - 42.77)</td>
<td>26.29 (18.58 - 36.83)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>GPX(U/mg protein)</td>
<td>260.38a (228.3 - 325.65)</td>
<td>142.4b,c (120.4 - 187.1)</td>
<td>30.81 (28.06 - 32.58)</td>
<td>30.02 (27.79 - 34.39)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>GSH(µmol/g tissue)</td>
<td>28.73a (22.94 - 46.92)</td>
<td>14.24b,c (12.04 - 18.71)</td>
<td>30.81 (28.06 - 32.58)</td>
<td>30.02 (27.79 - 34.39)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>TOS(µmol H2O2Eqv / L)</td>
<td>4.88a (3.87 - 6.15)</td>
<td>8.1b,c (7.41 - 10.35)</td>
<td>5.01 (4.38 - 6.66)</td>
<td>5.55 (4.44 - 6.48)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>TAS(Trolox equivalent/L)</td>
<td>0.35 (0.24 - 0.59)</td>
<td>0.18 (0.1 - 0.48)</td>
<td>0.26 (0.11 - 0.49)</td>
<td>0.26 (0.18 - 0.48)</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td>OSI(AU)</td>
<td>14.99a (6.72 - 22.23)</td>
<td>45.12 (16.96 - 77.45)</td>
<td>19.85 (10.14 - 42)</td>
<td>23.18 (9.8 - 29.71)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

p<0.05 was considered significant (difference between groups). a: It is different according to ISO group; b: It is different according to VINPO+ISO group; c: It is different according to ISO+VINPO group.

Table 3. Heart rate, mean blood pressure and ECG changes (median, min-max) and p-values in groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variables</th>
<th>SHAM (n=8)</th>
<th>ISO (n=8)</th>
<th>VINPO+ISO (n=8)</th>
<th>ISO+VINPO (n=8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart rate</td>
<td>229.500a (220-310)</td>
<td>394b,c (350-542)</td>
<td>219.500 (189-275)</td>
<td>210 (154-305)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Mean blood pressure</td>
<td>90a (76-93)</td>
<td>111b,c (100-130)</td>
<td>84 (77-105)</td>
<td>80 (73-92)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>PR</td>
<td>44a (40-48)</td>
<td>55b,c (48-70)</td>
<td>44 (40-44)</td>
<td>44 (36-54)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>QRS</td>
<td>68a (58-88)</td>
<td>109b,c (90-132)</td>
<td>70 (52-98)</td>
<td>66 (54-98)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>QT</td>
<td>113.500a (100-120)</td>
<td>175b,c (150-194)</td>
<td>114 (100-126)</td>
<td>115 (106-120)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

p<0.05 was considered significant (difference between groups). a: It is different according to ISO group; b: It is different according to VINPO+ISO group; c: It is different according to ISO+VINPO group.

Discussion

In this study, we investigated whether VINPO (PDE I inhibitor), which has anti-inflammatory and antioxidant effects, has protective and therapeutic effects on the heart after ischemic heart injury caused by ISO administration in the rats. Studies on VINPO are being followed closely all over the world and it is a very current topic.

Serum myoglobin, total CK, LDH, AST and ALT levels were measured to investigate the effect of vinpocetine by creating experimental myocardial ischemic injury in the rats. In our study, while the ALT value was measured as 53 (37-123) in control group (SHAM), it was measured as 38 (25-60) in ISO group (group 2), 55 (50-177) in VINPO+ISO group (group 3) and 54 (31-80) in ISO+VINPO group (group 4), respectively. The ALT value was statistically significantly lower in ISO group than VINPO+ISO group (p<0.05). Collateral circulation occurs around the ischemic area in the chronic period after MI, and this is a natural defensive mechanism that limits ischemic injury [16]. Considering that the protective and therapeutic properties of vinpocetine will guide future studies, studies on ALT should also be performed.

MDA, SOD, catalase (CAT), glutathione peroxidase (GPx), GSH, total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) were studied in the heart tissue as oxidative stress markers and antioxidants. Although different methods are used as a marker of lipid peroxidation in the tissue, one of the most commonly used markers is determination of MDA level. The elevation in malondialdehyde (MDA) level is a direct indication of increased lipid peroxidation. The most important marker used in the hypothesis that ischemic reperfusion injury can be reduced is MDA [10, 11].
end of the study, the MDA value was measured as 3.31 (2.44-4.18) in SHAM group. It was significantly lower in SHAM group than ISO group (p<0.05). It was significantly higher in ISO group than VINPO+ISO group and ISO+VINPO group (p<0.05). This suggested that the damage was found to be less in VINPO+ISO group than ISO group. In addition, the value in ISO+VINPO group was almost close to that in control group and vinpocetine was interpreted to be effective in terms of repairing damage.

It has been reported that the amount of glutathione decreased during ischemia due to oxidants in ischemic tissue and the inhibition of enzymes such as SOD, CAT and GPx was accelerated and thus the cells became more susceptible to the effects of oxygen radicals that formed rapidly during reperfusion [12]. In our study, similar to other studies, it was found that the levels of SOD, CAT, GPx and GSH, which were the antioxidant enzymes obtained from heart tissue samples of the rats in ISO group, decreased statistically significantly (p<0.05) [13,14]. The reduced SOD and CAT activity is consistent with other studies [15,16].

The decline in SOD and CAT activity increases superoxide and H2O2 formation and thus may lead to the formation of more toxic OH radicals [17]. There are two important enzymes involved in metabolism of H2O2, which is formed by reduction of the superoxide radical anion. One is CAT in the myocardium and the other is GPx in the myocardial cytoplasm. NADPH, which is mostly formed by oxidation of glucose-6-phosphate through the pentose phosphate pathway, provides the formation of GSH by activating glutathione reductase. Reduced GSH is converted into oxidized glutathione (GSSG) by glutathione peroxidase (GPx). This chain reduces the amount of peroxides that cause oxidative stress. GSH is an important indicator that informs about cellular oxidative events. Increased levels of GSH in the tissues or in the coronary circulation have been accepted as a sign of protecting cells from oxidative stress [17]. In this study, we found that there was a decline in the SOD, CAT, GPx and GSH activity levels in detecting ISO-induced cardiac damage. In our study, these enzymes, which decreased in ISO group, were found to be statistically significantly increased in ISO+VINPO group and VINPO+ISO group (p<0.05). Vinpocetine administration prior to ischemia has been found to significantly increase the SOD, CAT and GSH activity levels compared to control group. This shows us that vinpocetine is more effective especially before ischemia.

TAS and TOS oxidative stress markers, which reflect the total antioxidant protection in the organism against free radicals attack, were used in our study. There was no significant difference between the groups in terms of the TAS values.

Total oxidative stress (TOS) is expressed as the total value of oxidative stress. The shift in the balance between oxidants and antioxidants in favor of oxidants is termed “oxidative stress.” [18].

According the findings obtained at the end of the study, the TOS value was measured as 4.88 (3.87-6.15) in SHAM group. It was significantly lower in SHAM group than ISO group (p<0.05). The TOS value was also measured as 8.1 (7.41-10.35) in ISO group, 5.01 (4.38-6.66) in VINPO+ISO group and 5.55 (4.44-6.48) in ISO+VINPO group, respectively. It was significantly higher in ISO group than VINPO+ISO group and ISO+VINPO group (p<0.05). Based on these findings, TOS was high in group 2 (ISO group) and low in group 3 (VINPO+ISO) and group 4 (ISO+VINPO). This suggested that vinpocetine has protective and therapeutic effects. Oxidative stress index (OSI) was calculated by dividing TOS by TAS and was expressed as μmol H2O2/mmol Trolox. When the OSI values measured in the study were compared between the groups, it was measured as 14.99 (6.72-22.23) (AU) in SHAM group. The OSI value showed a significant difference between SHAM group and ISO group (p<0.05). It was also measured as 45.12 (16.96-77.45) (AU) in ISO group. The OSI value did not show significant difference between the other groups (p>0.05).

When we examined the ECG of rats before surgery in our study, 1 rat in SHAM group had ST depression, 7 rats in ISO group had ST depression, 1 rat in VINPO+ISO group had ST depression and 1 rat in ISO+VINPO group had ST depression. This suggested that the rate of occurrence of ischemia in VINPO+ISO group and ISO+VINPO group was the same as that in control group.

Consequently, vinpocetine had a positive effect on cardiac function. According to our electrophysiological, biochemical and histopathological results, it is possible to say that it exerts these beneficial effects via its powerful antioxidant, antiinflammatory and free radical scavenger effects. Despite all these, further studies are needed to get more accurate results.

References


