Evaluation of Cardioprotective Role of Vinpocetine in Isoproterenol-induced Myocardial Infarction in Rats

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ABSTRACT

Purpose: Vinpocetine is a phosphodiesterase type-1 inhibitor having strong vasodilating effect through cAMP and cGMP elevation. Vasodilators may play positive role in myocardial infarction (MI). As vinpocetine has not been explored for its cardioprotective role so far, we proposed to investigate the same in experimental model. Wistar albino rats were randomly divided into control, isoproterenol (85 mg/kg, s.c. on 6th & 7th day), vinpocetine (10 mg/kg/day, p.o.), milrinone (4 mg/kg/day, p.o.), vinpocetine + isoproterenol and milrinone + isoproterenol groups. On 8th day lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), total antioxidant capacity (TAC) in serum and reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS), Na+/K+ ATPase (NKA) in myocardium were estimated. Results: Isoproterenol-induced MI manifested a significant (P < 0.001) increase in the LDH, CK-MB, NKA, TBARS and a substantial decrease in GSH & TAC levels (P < 0.001 & P < 0.05, respectively). These levels were significantly reverted towards normal on treatment with vinpocetine and milrinone. Per se groups of these drugs however, did not show any significant changes when compared with the control. Histopathological evaluation also revealed the same trend. Conclusion: Biochemical and histopathological parameters clearly indicate the cardioprotective potential of vinpocetine due to its antioxidative and myocardial membrane stabilizing effects.

KEYWORDS: Cardiac toxicity, antioxidant, membrane-stabilization, phosphodiesterase inhibitors.

1.1 INTRODUCTION

Globally the main cause of today’s mortality is the cardiovascular diseases (CVD) whose number is rapidly increasing, expected to affect around 25 million people by 2030 [1]. CVD is a common problem which is manifested by myocardial infarction due to deficient oxygen supply to cardiac muscles [2]. In spite of having considerable amount of drugs available, a search for better, safer, efficient and cost effective drug is always there. This study is a move in this direction. We have chosen vinpocetine, a PDE1 inhibitor, to assess its cardioprotective role in this study.

1.1.1 Vinpocetine is a synthetic drug which is derived from vincamine, an alkaloid [3] and has been reported to possess antioxidative and neuroprotective property [4, 5]. It is largely used in the indications like dementia, memory loss, stroke, etc [6]. It is a PDE1 inhibitor which facilitates vasodilatation, thereby improves cerebral blood flow [7]. Activation of Phosphodiesterase-1 increases vasoconstriction due to decrease in the concentration of cGMP in arteries [8] and is appre- ciably reversed on treatment with PDE1 inhibitors [9]. Physiological effect of PDE1 inhibitor in myocardium under in vivo condition is not very clear, however IC86340, a selective PDE1 inhibitor, was found to diminish myocytes hypertrophy in an isoproterenol-intoxicated experimental model of mouse [10]. Both cAMP and cGMP plays a pivotal role in the inotropic regulation of human myocardium. Thus, the PDE-inhibitors have a therapeutic action on the heart, lung and vasculature [11]. Vinpocetine is a potent antioxidant [12] that checks atherosclerosis [13] and lipid peroxidation [14]. It is an anti-inflammatory molecule [15] which has shown liver protection against ischemia-reperfusion injury [16]. Vinpocetine is relatively safe in long-term treatment [17] and an attractive therapeutic candidate for treating various vascular diseases [18]. However, its candidature as potent cardioprotective agent is not yet explored.

1.1.2 Milrinone, a selective PDE3 inhibitor, is a treating agent for acute heart failure [19]. It controls increased pressures on myocardium, thereby enhances its pumping action [20]. Its ventricular supportive role on heart is due to elevation of cAMP and decline in its degradation [21]. Milrinone due to its combined positive inotropic and peripheral vasoconstrictive effects is clinically used to treat congestive heart failure [22, 23], myocardial infarction prior to ischemia [24, 25] and
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shows cardiotropic effects \cite{26, 27}. Thus, we have selected this PDE3 inhibitor as a standard drug in our study where isoproterenol is used to induce cardiotoxicity.

1.1.3 Isoproterenol is a β-adrenergic agonist that causes severe myocardial stress leading to necrosis \cite{28}. The changes induced in cardiac tissue by isoproterenol in experimental models, in terms of pathophysiology or morphology, portrait the similar changes as found in human MI \cite{29}. Thus, it represents a useful experimental modal for investigating cardioprotective agents.

1.2 MATERIALS AND METHODS

1.2.1 Drugs and chemicals
Isoproterenol, milrinone, vinpocetine, bovine serum albumin were purchased from Sigma Aldrich, (St. Louis, MO, USA). Lactate dehydrogenase (ENZOPAC™) and creatine kinase-MB kit were procured from Reckon diagnostics Pvt. Ltd, Baroda, India. Other chemicals used were of analytical grade.

1.2.2 Experimental animals
Male Wistar albino rats, weighing 255 to 295 g were used in this study. The animals were provided by the Central Animal House Facility, Jamia Hamdard, after due approval from the Institutional Animal Ethics Committee, Jamia Hamdard, New Delhi. Animals were made acclimatized by housing them in an air conditioned room under natural light and dark cycle (12 hour light/ dark) maintained at 25 ± 2°C. They were fed with standard laboratory diet (M/S Ashirwad feed industry) and water ad libitum. Ethical norms were strictly followed in this study.

1.2.3 Experimental Design and Protocol
Animals were randomly divided into six groups, each consisting of 6 rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment plan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Received vehicle (Tween 80, 0.5 ml, p.o.) for 7 days and normal saline (0.1 ml, s.c.) on 6th and 7th day.</td>
</tr>
<tr>
<td>ISO</td>
<td>Received vehicle (Tween 80, 0.5 ml, p.o.) for 7 days and administered with ISO (85 mg/kg, s.c.) on 6th and 7th day at an interval of 24 hours.</td>
</tr>
<tr>
<td>VIN</td>
<td>Received vinpocetine (10 mg/kg/day, p.o.) for 7 days and normal saline (s.c.) on 6th and 7th day.</td>
</tr>
<tr>
<td>VIN + ISO</td>
<td>Received vinpocetine (10 mg/kg/day, p.o.) for 7 days and ISO (85 mg/kg, s.c.) on 6th and 7th day at an interval of 24 hours.</td>
</tr>
<tr>
<td>MIL</td>
<td>Received milrinone (4 mg/kg/d, p.o.) for 7 days and normal saline (s.c.) on 6th and 7th day.</td>
</tr>
<tr>
<td>MIL + ISO</td>
<td>Received milrinone (4 mg/kg/day, p.o.) for 7 days and ISO (85 mg/kg, s.c.) on 6th and 7th day at an interval of 24 hours.</td>
</tr>
</tbody>
</table>

On 8th day rats were sacrificed and various biochemical parameters and histopathological analysis were performed in the serum and cardiac tissues.

1.2.4 Biochemical estimations in serum
LDH and CK-MB were determined by commercially available kit, whereas TAC was estimated by the method of Koracevic et al. \cite{30}. All estimations were carried out using UV Spectrophotometer, model no. 1601 (Shimadzu, Japan).

1.2.5 Biochemical estimations in cardiac tissue
The hearts were removed, washed in ice-cold normal saline and weighed immediately after the sacrifice. GSH was estimated by Sedlak and Lindsay method \cite{31}, TBARS by the method of Ohkawa & Ohishi \cite{32}, proteins by Lowry et al. \cite{33} and Na+/K+ ATPases by Akagawa & Tsukada; Fiske and Subbarow \cite{34, 35}.

1.2.6 Assessment of cardiac hypertrophy
The whole heart and the body weight were recorded. The ratio of heart weight to body weight (HW/BW) was then calculated.

1.2.7 Histopathological study
5-µm slices of hearts were cut out of paraffin blocks fixed with heart sample, and stained with haematoxylin and eosin (H & E) stains. The stained sections were examined and sorted out under light microscope then photomicrographs were recorded.

1.3 Statistical Analysis
This analysis was performed using Graph Pad Prism 5.1 (software). Data were compared with ANOVA (analysis of variance) followed by Tukey-Kramer multiple comparison test. All data with $P < 0.05$ were considered statistically significant.

1.4 RESULTS

1.4.1 Heart weight and heart weight/body weight ratio
ISO treated rats showed a significant ($P < 0.01$ and $P < 0.001$) increase in HW and HW/BW ratio, respectively when compared with the control. Animals when treated with vinpocetine (10 mg/kg) and milrinone (4 mg/kg) showed the significantly reduction ($P < 0.01$ and $P < 0.05$, respectively). Per se for both the drugs, however, did not show any significant changes when compared with the control, which means that they manifested almost the same status as that of control (Table 1).
1.4.2 Creatine Kinase-MB (CK-MB)
CK-MB content in myocardium on investigation showed significant (P < 0.001) rise in ISO treated rats, which was significantly (P < 0.001) reversed on treatment with vinpocetine. Milrinone also manifested a significant (P < 0.001) reduction in the level when compared with the ISO group. Drug alone treated rats showed no significant changes when compared with control (Table 2).

1.4.3 Lactate Dehydrogenase (LDH)
Cardiac LDH content on estimation showed significant (P < 0.001) increase in the ISO rats, which was significantly (P < 0.001) brought down on treatment with vinpocetine. A similar trend was shown by milrinone, i.e., a significant (P < 0.001) reduction in the level of LDH when compared with the ISO group. Per se groups of these drugs however, manifested the same concentration of this parameter when compared with that of control (Table 2).

1.4.4 Total Antioxidant Capacity (TAC)
Myocardial TAC content in ISO group was found to be significantly (P < 0.01) decreased. Animals when treated with vinpocetine and milrinone significantly (P < 0.05) brought down this parameter towards normal level. Per se groups did not show any significant changes when compared with the control group (Table 2).

Table 1: Effect of vinpocetine and milrinone on heart weight and heart weight/body weight ratio in ISO administered rats

<table>
<thead>
<tr>
<th>Group</th>
<th>HW (g)</th>
<th>HW/BW (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.932 ± 0.056</td>
<td>3.42 ± 0.13</td>
</tr>
<tr>
<td>ISO</td>
<td>1.337 ± 0.066**</td>
<td>4.79 ± 0.16**</td>
</tr>
<tr>
<td>VIN</td>
<td>0.952 ± 0.067</td>
<td>3.48 ± 0.18</td>
</tr>
<tr>
<td>VIN + ISO</td>
<td>1.049 ± 0.071*</td>
<td>3.80 ± 0.18*</td>
</tr>
<tr>
<td>MIL</td>
<td>0.952 ± 0.060</td>
<td>3.78 ± 0.17</td>
</tr>
<tr>
<td>MIL + ISO</td>
<td>1.045 ± 0.072*</td>
<td>3.60 ± 0.20*</td>
</tr>
</tbody>
</table>

HW - Heart weight; HW/BW - Heart weight/Body weight; C - control; ISO - isoproterenol (85 mg/kg); VIN - vinpocetine (10 mg/kg); VIN + ISO - vinpocetine (10 mg/kg) + isoproterenol (85 mg/kg); MIL - milrinone (4 mg/kg); MIL + ISO - milrinone (4 mg/kg) + isoproterenol. Data are mean ± SEM (n = 6). *P < 0.01 vs. C; **P < 0.001 vs. C; *P < 0.05 vs. ISO; **P < 0.001 vs. ISO.

1.4.5 Na+/K+-ATPases (NKA)
NKA content in the cardiac tissue on examination showed significant (P < 0.001) decrease in the ISO treated rats, which was significantly (P < 0.001) elevated with vinpocetine. Milrinone also had shown a significant (P < 0.001) increment in the level when compared with the ISO group. Per se for both the drugs, however, did not show any significant changes when compared with control, which means that they manifested almost the same status as that of control (Figure 1).

Table 2: Effect of vinpocetine and milrinone on isoproterenol-induced changes in cardiac LDH, CK-MB and TAC.

<table>
<thead>
<tr>
<th>Group</th>
<th>LDH (IU/L)</th>
<th>CK-MB (IU/L)</th>
<th>TAC (µM/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>82.76 ± 10.04</td>
<td>110.52 ± 5.86</td>
<td>3.29 ± 0.58</td>
</tr>
<tr>
<td>ISO</td>
<td>227.88 ± 7.73**</td>
<td>263.33 ± 11.69**</td>
<td>1.26 ± 0.17**</td>
</tr>
<tr>
<td>VIN</td>
<td>86.26 ± 10.40</td>
<td>123.26 ± 10.92</td>
<td>3.31 ± 0.30</td>
</tr>
<tr>
<td>VIN + ISO</td>
<td>112.26 ± 13.59*</td>
<td>165.52 ± 13.53*</td>
<td>2.90 ± 0.15*</td>
</tr>
<tr>
<td>MIL</td>
<td>90.23 ± 9.91</td>
<td>136.81 ± 13.48</td>
<td>3.53 ± 0.59</td>
</tr>
<tr>
<td>MIL + ISO</td>
<td>99.57 ± 11.43*</td>
<td>167.11 ± 14.50*</td>
<td>3.11 ± 0.29*</td>
</tr>
</tbody>
</table>

LDH - Lactate dehydrogenase; CK-MB-creatine kinase-MB; TAC - total antioxidant capacity; C - control; ISO - isoproterenol (85 mg/kg); VIN - vinpocetine (10 mg/kg); VIN + ISO - vinpocetine (10 mg/kg) + isoproterenol (85 mg/kg); MIL - milrinone (4 mg/kg); MIL + ISO - milrinone (4 mg/kg) + isoproterenol. Data are mean ± SEM (n=6). *P < 0.01 vs. C; **P < 0.001 vs. C; *P < 0.001 vs. ISO; **P < 0.01 vs. ISO.

1.4.6 Thiobarbituric Acid Reactive Substances (TBARS)
The malondialdehyde (MDA) content in the cardiac tissue showed significant (P < 0.001) increase in ISO treated rats when compared with the control and significant (P < 0.001; P < 0.01, respectively) decrease in vinpocetine and milrinone treated rats, when compared with the ISO group. Drug alone treated groups; however, showed no significant changes when compared with the control (Figure 2).
1.4.7 Reduced Glutathione (GSH)

GSH content in the cardiac tissue on examination showed significant (P < 0.001) decrease in the ISO treated rats, which was significantly (P < 0.001) elevated with vinpocetine. Milrinone also had shown a significant (P < 0.01) increment in the level when compared with the ISO group. *Per se* for both the drugs, however, did not show any significant changes when compared with control, which means that they manifested almost the same status as that of control (Figure 3).

Lipid peroxidation increases under mild stress and causes alteration in the membrane structure, leading to its damage and enzyme inactivation [38]. In our study treatment with vinpocetine and milrinone significantly restored TBARS levels indicating maintained integrity of myocardium.

1.5 DISCUSSION

Vinpocetine is a known PDE1 inhibitor, being prescribed against cognitive disorders, has shown strong cardioprotective effect on isoproterenol-induced MI in rats. This protection is evident by the biochemical profile of various parameters, HW/BW ratio and histopathological examination. LDH and CK-MB in ISO intoxicated rats have been found to be raised in the serum showing the myocardial damage. Our drug has considerably reverted these parameters and thereby indicates prevention in myocardial damage. Serum LDH concentration serves as an indicator of myocardial injury, it oozes out of the damaged area into the blood stream and get raised [36]. CK-MB also shows the same trend, as it increases in the serum on myocardial damage, thereby reflecting the disintegrity of the membrane. So more the damage more the CK-MB leaks out into the blood. ISO causes cardiotoxicity due to generation of oxygen and other highly reactive radicals which causes severe oxidative stress in the myocardium. Our body’s natural defense system combats with this stress. GSH is an antioxidant molecule which remains in the reduced form under mild stress; however, their concentration goes down as it neutralizes the oxidative stress and gets converted into G-S-S-G. As ISO induces oxidative stress, the reduced glutathione concentration goes down. Vinpocetine and milrinone have shown significant increase in GSH when administered into rats. Similarly TAC of a cell reflects its defending capacity against the oxidant injury. Therefore, any substance which increases the TAC level will be manifesting cardiac protection. Vinpocetine and milrinone significantly decrease this level as evident in Table 2 and manifest cardiac protection which goes fine with the earlier finding [37].

Histopathological data also indicated considerable damage of the cell by ISO which is evident by myocardial edema, increased myofibril thickness, pyknotic nucleus and necrosis. These morphological changes however, got considerably reverted and normal cellular architecture was restored on treatment with vinpocetine and milrinone.
HW/BW ratio also indicated the changes in myocardial environment. These changes might arise due to infarction in the myocardium which goes fine with earlier findings \[42\]. In our study, we have noticed a significant rise in HW and HW/BW ratio in ISO challenged rats which significantly declined on treatment with our test drugs, suggesting their cardioprotective effect. Our drug alone treated groups have

**Figure 4. Light micrographs of cardiac tissue section (H & E stain):**

(a) Control heart showing no microscopic abnormality; (b) ISO group showing edema, increased myofibril thickness, pyknotic nucleus and minimal neutrophilic infiltration along with necrosis (arrows); (c) VIN + ISO group showing less pyknotic nuclei, no necrosis with less edema and infiltration (arrows); (d) MIL + ISO treated rat heart showing normal myocardial architecture and orientation of myocytes. No edema with significant reduction in infarction is seen (arrows); (e & f) VIN alone and MIL alone treated myocardium showing no structural changes, pyknotic nucleus, disarrangement of myofibrils and necrosis.
shown no significant changes in the myocardium as compared to the control group, thereby manifesting no effects on rat’s heart.

1.6 CONCLUSION
Considering all the biochemical, histopathological parameters and comparing its effect with milrinone, which is a PDE3 inhibitor and known cardioprotective agent, we have come to this conclusion that vinpocetine is a potential cardioprotective agent and its effect is quite comparable with milrinone. Thus, this study reveals the new role of PDE1 inhibitors as cardioprotective agents.

1.7 ACKNOWLEDGMENTS
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1.8 Conflict of interest: None

1.9 REFERENCES:
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Source of support: Nil, Conflict of interest: None