THE EFFECTS OF VINPOCETINE ON THE PREVENTION AND TREATMENT OF THE ISCHEMIA / REPERFUSION INJURY: AN EXPERIMENTAL STUDY

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ABSTRACT

Object: Histopathological and biochemical effects of vinpocetine, which is an anti-inflammatory and antioxidant agent, on ischemia / reperfusion (I/R) injury were studied in an animal model.

Material and methods: Forty Sprague Dawley rats were divided into five groups. Group 1 underwent only a right nephrectomy. Group 2 was administrated vinpocetine after right nephrectomy. Group 3 initially underwent a right nephrectomy. Then, left kidney of this group was applied ischemia for 60 minutes, which was followed by reperfusion for 24 hours. Group 4 underwent same steps as group 3, but was administrated vinpocetine 10 mg/kg i.p. before I/R to the left kidney. Similarly, group 5 underwent same steps as group 3 but was administrated vinpocetine 10 mg/kg i.p. after I/R to the left kidney.

Results: Plasma BUN and creatinine showed no significant differences between control (Sham) group and groups that were administrated vinpocetine. Decrease in plasma urea and creatinine was detected group 5, but this finding was not present in group 4. Superoxide dismutase and glutathione reductase levels in groups 4 and 5 were significantly higher than the group 3. Parallel to this, oxidative stress index was higher in the group 3 compared to groups 2, 4, and 5 (p<0.05). No significant difference was found between levels of malondialdehyde in sham and vinpocetine administrated only group (p<0.005). All groups showed no differences in catalase levels and Total Antioxidant Capacity. Decreased tubular injury was present in the groups administrated vinpocetine before and after I/R. In control and vinpocetine given groups, there were some apoptosis in tubules after tested with caspase 3. Caspase 3 positivity was significantly higher in I/R group compared to control group. Vinpocetine if given after I/R was more effective to prevent apoptosis then to be administrated before I/R.

Conclusion: This experimental study showed that vinpocetine promotes achieving better renal functions. The results of this study suggest that vinpocetine may be used in the treatment of renal I/R.

Keywords: Animal study, kidney, rat, reperfusion injury, vinpocetine.

DOI: 10.19193/0393-6384_2017_5_127

Introduction

Ischemia defined as temporary or permanent cell and tissue damage is caused by restricted blood supply of tissue or organ. Renal ischemia may be present after renal transplantation, partial nephrectomy, cardiopulmonary bypass operations, septicemia, renal artery revascularization, trauma, and hydronephrosis¹.². Tissue reperfusion should be maintained in order to prevent permanent cell injury.

Renal ischemia / reperfusion injury (IRI) is a major cause of acute renal failure (ARF)³. Free radicals increased during re-oxygenation of the tissue in reperfusion period cause cell injury through lipid peroxidation⁴. Ischemic ARF, whether due to prerenal or renal causes, may occur due to irregularities in renal blood supply and, in inappropriately treated cases, may advance to chronic renal failure⁵,⁶. Mammalian cells harbor enzymatic and non-enzymatic mechanisms which metabolize free radicals and injury⁷. Reperfusion after ischemia promotes a paradoxical increase in tissue damage and may even cause more damage than the primary ischemia.
All these detrimental effects of ischemic and reperfusion processes collectively referred to as IRI.[8]

Two mechanisms for reperfusion injury were described: first, hydrolysis of fat acids in cell membrane by phospholipase A2 after activated by Ca++ in ischemic period; and second, emergence of free oxygen radicals (FOR)[9, 10]. The study of Ertorun in 2015 investigated the effect of Salvia L. extracts on renal tissue in renal IRI and suggested a protective effect on renal tissue and functions.[11]

Vinpocetine, an extract from lesser periwinkle plant, is a semi-synthetic alkaloid and a phosphodiesterase type 1 inhibitor. Vinpocetine has evident anti-oxidant, anti-inflammatory, neuroprotective, vasodilator, and anticonvulsant effects and increases cerebral blood flow[12]. Benefits of vinpocetine were studied in a multicenter trial with 4865 patients with chronic cerebrovascular disease and arterial hypertension[13]. This study suggested a significant decrease in severity of neurological symptoms.

This research is the first study in the literature investigating effects of vinpocetine, an anti-inflammatory and anti-oxidant PDE type-1 inhibitor, on renal ischemia and reperfusion injury. Thence, this present study aims prevention of or protection from renal IRI via administration of vinpocetine.

Materials and methods

This study was approved by Ethical Committee of Inonu University Faculty of Medicine. Forty Sprague Dawley rats were divided in 5 groups.

Group 1 (sham group): Abdominal area of rats was cleaned with povidone iodine 10% and sterile gauze. Procedural anesthesia was utilized by xylazine hydrochloride 10 mg/kg and ketamine hydrochloride 50 mg/kg intraperitoneally (i.p.). A 3-cm-long median skin incision to the upper abdominal area was performed. Opening the peritoneum and mobilizing intestines was followed by a right nephrectomy. No further steps were performed. Surgical wound was closed.

Group 2 (Vinpocetine administrated only): Vinpocetine 10 mg/kg i.p. was administrated after right nephrectomy.

Group 3 (IRI group): After completion of the right nephrectomy, blood flow through the pedicle of left kidney was interrupted with 0.4-1.0 mm long suture. After 60 minutes, suture was loosened and blood flow was restored for 24 hours.

Group 4 (Vinpocetine before IRI group):

Vinpocetine 10 mg/kg i.p. was administrated after right nephrectomy. Then blood flow of the left kidney was interrupted for 60 minutes followed by 24 hours of reperfusion.

Group 5 (Vinpocetine after IRI group): After completing the right nephrectomy, blood flow of the left kidney was interrupted for 60 minutes. Vinpocetine 10 mg/kg i.p. was administrated and left kidney was perfused for 24 hours.

Blood samples were obtained through cardiac punctures. BUN and creatinine in plasma and malondialdehide (MDA), total anti-oxidant capacity (TAC), Glutathione reductase (GSH), superoxide dismutase (SOD), catalase (CAT), and oxidative stress index (OSI) levels in renal tissue were analyzed.

Extracted renal tissues were divided transversally and fixated with formalin 10% solution before embedding in paraffin blocks.

Tissue cuts of 5 μm thickness were investigated with Hematoxylene Eosine and Periodic Acid Schiff dyes. Renal tubular injury was investigated with semi-quantitative analyze. Renal changes were scored as 0: no injury; 1: injury in less than 25% of total area; 2: injury in 25-50% of total area; and 3: injury in more than 50% of total area[14]. Immunohistochemical analysis has been performed by immersing thick tissue cuts into sytrate tamponed and washing with Phosphate Buffered Saline after holding in H2O2 3% for 7 minutes. Tissue dyes was done with chromogene + substrate for 15 minutes and tissue cuts were dyed with Mayers hemotoxy-lene for 1 minute.

Statistical Analysis

Biochemical features were compared with Kruskal Wallis H Test. Multiple comparisons were performed with Mann Whitney U Test with Banferroni corrections. Significance level was set at p<0.001 for BUN and creatinine levels, and at p<0.05 for renal tissue features. Continuity of changes between groups regarding histopathological analysis were evaluated with Shapiro Wilk test. Prominent changes in groups were analyzed by comparing Kruskal Wallis and Mann Whitney U tests. Significance level was set at p<0.05.

Results

Mean plasma BUN and creatinine levels in Sham group (control group) was 26.50 and 0.67, respectively. In the “vinpocetine before IRI” group,
plasma BUN and creatinine levels were 20.50 and 0.64, respectively. There were no significant differences (p=1).

In the IRI group, large necrosis areas were detected in some proximal tubules (Figure 1A). Moreover, there were less tubular injury in “Vinpocetine before IRI” and “Vinpocetine after IRI” groups, and necrotic tubules were not as large as in IRI only group. However there were no statistically significant differences between these groups. Tubular protective periods were similar between “vinpocetine before IRI” and “vinpocetine after IRI” groups (p>0.05) (Figure 2B). Control and vinpocetine groups showed a few apoptotic tubules on the caspase-3 examination. Administration of vinpocetine after IRI was found to show more preventive effect against apoptosis compared to administration of vinpocetine before IRI (Figure 2 B-C).

Our findings suggest that vinpocetine promotes better renal functions. There were no significant differences between BUN and creatinine levels in control (sham) group and vinpocetine administrated groups.

The decrease in plasma urea and creatinine levels measured in “vinpocetine after IRI” group was not present in “vinpocetine before IRI” group. On the other hand, both groups showed less tubular injury. Signs of interstitial congestion and hemorrhage were found in all experimental groups. Tubules in sham and vinpocetine groups showed signs of apoptosis in caspase-3 testing. SOD and GSH levels were significantly higher in groups vinpocetine administrated before or after IRI compared to IRI group. OSI levels were found higher in IRI groups compared to vinpocetine administrated groups. No significant differences were found between MDA levels of control group and vinpocetine administrated group. Levels of CAT and TAC showed no statistically significant changes between groups.

Discussion

Prerenal ARF due to renal hypoperfusion forms 70% of community-based ARF and 40% of hospital-based ARF\textsuperscript{(15)}. Many agents such as low doses of dopamine infusion, diuretics, calcium channel blockers, allopurinol, N-acetylcysteine, erythropoietin, theophylline, atrial natriuretic peptide, antibodies against adhesion molecules, FOR scavengers, prostaglandins, aminoacid infusion have been evaluated in ARF prevention\textsuperscript{(16)}.

Renal IRI may be present after kidney transplant surgeries, partial nephrectomies, and suprarenal aneurysm surgeries. Recently, kidney transplantation has been widely accepted as a valid treatment option in late stage kidney failure. However, mechanism behind IRI after kidney transplantation, which is the main reason of ARF, is still unclear. Level of injury may vary by the severity and duration of ischemia. However, there is no certain treatment strategy against IRI\textsuperscript{(17)}.

Many recent studies suggest that IRI is a non-immunologic condition and is the result of many factors related to and activating each other\textsuperscript{(18, 19)}. Pathogenesis of IRI may be related to factors such as elevated intracellular Ca\textsuperscript{2+}, depletion of high energy compounds and inability of renewal of them, elevation of inflammatory cytokines and adhesion molecules, infiltration and degradation of inflammatory cells, activation and dysfunction of endothelium, and
injury and phospholipase activation of cell membrane. Most widely accepted concept is that FORs and elevated oxidative stress are main causes of renal IRI. This finding suggests that, based on creatinine levels, vinpocetine has a place in the treatment of renal failure while showing no protective effect.

Different ischemia and reperfusion time was applied in the literature to form experimental kidney IRI model. Bozkurt et al applied 45 minutes of ischemia followed by 60 minutes of reperfusion before experimented rats were sacrificed. A body of data is present in the current literature regarding the use of antioxidants in IRI of liver, heart, brain, and kidney.

Yildiz et al used Nigella sativa (black caraway) extract as exogenous anti-oxidant in their study and used, similar to our study, TAC and Total Oxidant Capacity (TOC) levels as determining factors. In their experimental renal IRI study, they found a significant decrease in TOC levels and a significant increase in TAC levels.

Table 1: Plasma BUN and creatine levels (median, min-max).

<table>
<thead>
<tr>
<th>Groups</th>
<th>BUN (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Sham)</td>
<td>26.50 (23-35)</td>
<td>0.67 (0.60-0.71)</td>
</tr>
<tr>
<td>2 (Sham+Vin)</td>
<td>20.50 (18-24)</td>
<td>0.64 (0.60-0.68)</td>
</tr>
<tr>
<td>3 (IRI)</td>
<td>125 (125-125)</td>
<td>2.59 (2.03-3.41)</td>
</tr>
<tr>
<td>4 (IRI+Vin)</td>
<td>125 (75-125)</td>
<td>2.23 (0.81-3.20)</td>
</tr>
<tr>
<td>5 (Vin+IRI)</td>
<td>125 (125-125)</td>
<td>3.20 (2.83-3.35)</td>
</tr>
</tbody>
</table>

Table 2: Median (min-max) and p values of tissue variables in groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>1 (Sham)</th>
<th>2 (Sham+Vin)</th>
<th>3 (IRI)</th>
<th>4 (IRI+Vin)</th>
<th>5 (Vin+IRI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/g tissue)</td>
<td>22.94 (12.21-34.96)</td>
<td>23.97 (12.23-36.14)</td>
<td>44.17 (23.28-58.09)</td>
<td>21.40 (15.40-26.55)</td>
<td>22.26 (15.00-28.59)</td>
<td>0.005</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>0.93 (0.82-1.25)</td>
<td>0.92 (0.83-1.19)</td>
<td>0.64 (0.45-0.66)</td>
<td>0.74 (0.68-0.86)</td>
<td>0.83 (0.68-1.48)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CAT (nmol/g tissue)</td>
<td>19.14 (15.74-27.24)</td>
<td>16.62 (15.39-20.65)</td>
<td>16.29 (12.77-21.71)</td>
<td>18.41 (11.11-23.56)</td>
<td>17.91 (16.81-22.30)</td>
<td>0.401</td>
</tr>
<tr>
<td>GSH (µmol/g tissue)</td>
<td>6.03 (3.87-7.85)</td>
<td>6.34 (4.02-6.93)</td>
<td>2.99 (2.53-3.96)</td>
<td>5.81 (5.15-8.06)</td>
<td>5.71 (5.31-6.98)</td>
<td>0.001</td>
</tr>
<tr>
<td>TOS (µmol H2O2/ Eq/L)</td>
<td>3.98 (2.99-4.44)</td>
<td>4.18 (3.67-4.85)</td>
<td>9.15 (6.43-13.74)</td>
<td>4.69 (2.65-5.55)</td>
<td>5.33 (3.59-6.07)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TAC (Trolox equivalent/L)</td>
<td>1.03 (0.98-1.32)</td>
<td>1.06 (0.84-1.17)</td>
<td>0.94 (0.84-1.05)</td>
<td>0.98 (0.86-1.13)</td>
<td>0.97 (0.83-1.24)</td>
<td>0.201</td>
</tr>
<tr>
<td>OSI (AU)</td>
<td>3.66 (2.25-4.65)</td>
<td>3.87 (3.40-5.72)</td>
<td>9.48 (6.54-16.30)</td>
<td>4.81 (2.51-6.02)</td>
<td>5.26 (4.09-6.01)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

This is the first study in the literature analyzing effects of vinpocetine, an anti-inflammatory and anti-oxidant PDE type-1 inhibitor, on renal ischemia and reperfusion injury. Our results showed significantly higher plasma BUN levels (125 and above) in subjects underwent IRI compared to vinpocetine administered only group, which had decreased BUN levels. This finding suggests that vinpocetine reduced the serum BUN independently from presence of I/R. Creatinine levels in control and vinpocetine administered only groups were significantly decreased compared to all three IRI groups. Furthermore, we found significantly decreased creatinine levels in “vinpocetine after the IRI” group compared to “vinpocetine before the IRI” group. Ozkan et al studied protective effects of proanthocyanidin (grape seeds) in contrast nephropathy and compared TAC and TOC levels. TOC levels were significantly decreased in drug given group compared to control group. In our study, we found no significant changes in TAC levels between groups, but a significant increase was present in TOC level of IRI group. Similarly, administration of vinpocetine after IRI decreased TOC levels while no such decrease was detected in cases vinpocetine was given before IRI.

Yun et al showed increased MDA levels in oxidative stress and lipid peroxidation in the presence of renal IRI.
Our study revealed similar results: there was no difference between control group, vinpocetine administrated only group, and vinpocetine before and after IRI groups. However, IRI only group showed a significant difference compared to other groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Histological Score</th>
<th>Caspase-3 take-up in tubules</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Sham)</td>
<td>0 (0-1)</td>
<td>2 (0-9)</td>
</tr>
<tr>
<td>2 (Sham + Vin)</td>
<td>0.5 (0-1)</td>
<td>0 (0-12)</td>
</tr>
<tr>
<td>3 (IRI)</td>
<td>1 (0-3)</td>
<td>44(30-62)</td>
</tr>
<tr>
<td>4 (IRI+Vin)</td>
<td>1 (0-3)</td>
<td>30 (8-55)</td>
</tr>
<tr>
<td>5 (Vin+IRI)</td>
<td>1 (0-3)</td>
<td>48 (15-64)</td>
</tr>
</tbody>
</table>

Table 3: Histological Evaluation of Tubuler Injury

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**References**


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