Renoprotective effect of vinpocetine and cilostazol on glycerol induced renal injury in male rats

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Abstract:

Acute kidney injury (AKI) is characterized by a sudden loss of kidney function that is established by increased serum creatinine levels and decreased urinary output. AKI is one of a group of functional kidney conditions known as acute kidney disease and disorders (AKD), which can vary in severity and self-limiting to severe and chronic. Adminstrations of glycerol generate significant elevation in serum urea and creatinine that's mean occurance of functional abnormalities in the kidney. Vinpocetine drug has many pharmacological targets with multiple action, phosphodiesterase inhibiters-1(PDE-1) inhibitor, a voltage-gated sodium channel, and Inhibitory kinase B (IKK) are 3 main molecule targets of vinpocetine. PDE1 has been implicated in the regulation of vasoconstriction, vascular and cardiac structure remodeling, and neuro-transmission. Cilostazol, a phosphodiesterase (PDE) III inhibitors, that widely used for many cases such as reduces direct vascular injury via different mechanism, such as vasodilation and antiplatelet action, anti-inflammation and platelet-leukocyte interaction minimisation, and inhibition of vascular proliferation via up-regulation of hepatocyte growth factors. In present study, we looked at the effect and mechanism of the drugs vinpocetine and cilostazol in an animal model of glycerol-induced AKI. Experiment done during the 14-day trial, rats were divided into five groups: the control group received 2ml/kg normal saline; the induction group received 10ml/kg intramuscular glycerol injection; the vinpocetine group received 5mg/kg via gavage for 14 days and on day 7 given glycerol IM, the cilostazol group received 50mg/kg for 14 days and on day 7 given glycerol IM, and the combination group received half dose vinpocetine (2.5mg/kg) and cilostazol (25mg/kg). We discovered that the induction group had higher levels of urea and creatinine, as well as increased inflammation and oxidative stress, and that their renal tissue showed morphological changes typical of AKI, whereas the combination groups reduced glycerol induce acute renal damage. This revealed that vinpocetine and cilostazol can reinforce renal rat protection by reducing serum urea and creatinine and improving histopathological changes.

Key words: AKI: acute kidney injury, PDE: Phosphodiestrase enzyme inhibitors

تأثير الفنبوسيتين وسيلوستازول على إصابة الكلى الحاد التي يسببها الجلسرين في ذكور الجرذان

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الخلاصة:
تتميز إصابة الكلى الحادة (AKI) بفقدان مفاجئ لوظائف الكلى التي تنشأ عن زيادة مستويات الكرياتينين في الدم وانخفاض إنتاج البول. AKI هي واحدة من مجموعة من أمراض الكلى الوظيفية المعروفة باسم أمراض الكلى الحادة واضطراباتها (AKD) ، والتي يمكن أن تختلف في شدتها وتقتصر على الحالات الحادة والمزمنة. تولد حقن الجلسرين ارتفاعًا كبيرًا في اليوريا والكرياتينين في الدم مما يعني حدوث تشوهات وظيفية في الكلى. يحتوي عقار Vinpocetine على العديد من الأهداف الدوائية ذات الإجراءات المتعددة ، ومثبطات phosphodiesterase-1 (PDE-1) ، وقناة الصوديوم ذات الجهد الكهربائي ، و Kinase B (IKK) هي 3 أهداف جزيئية رئيسية من vinpocetine. وقد شارك PDE1 في تنظيم تضيق الأوعية ، وإعادة تشكيل بنية الأوعية الدموية والقلب ، والانتقال العصبي. سيلوستازول ، مثبطات الفوسفوديستيراز III (PDE) ، التي تستخدم على نطاق واسع في العديد من الحالات مثل تقليل إصابة الأوعية الدموية المباشرة من خلال آلية مختلفة ، مثل توسع الأوعية والعمل المضاد للصفيحات ، ومكافحة الالتهاب وتفاعل الصفائح الدموية مع الكريات البيض ، وتثبيط تكاثر الأوعية الدموية عن طريق أعلى - تنظيم عوامل نمو خلايا الكبد. في الدراسة الحالية ، نظرنا في تأثير وآلية الأدوية vinpocetine و cilostazol في نموذج حيواني من AKI الناجم عن الجلسرين. التجربة التي أجريت خلال التجربة التي استمرت 14 يومًا ، تم تقسيم الفئران إلى خمس مجموعات: تلقت المجموعة الضابطة 2 مل / كجم من محلول ملحي ؛ تلقت مجموعة الحث 10 مل / كغ من حقن الجلسرين العضلي ؛ تلقت مجموعة vinpocetine 5 مجم / كجم عن طريق التزقيم لمدة 14 يومًا وفي اليوم السابع أعطيت الجلسرين العضلي ، تلقت مجموعة سيلوستازول 50 مجم / كجم لمدة 14 يومًا وفي اليوم السابع أعطيت الجلسرين العضلي ، وتلقت المجموعة المركبة نصف جرعة من الفينبوسيتيني (2.5 مجم / كجم) وسيلوستازول (25 مجم / كجم). اكتشفنا أن مجموعة الحث كانت تحتوي على مستويات أعلى من اليوريا والكرياتينين ، بالإضافة إلى زيادة الالتهاب والإجهاد التأكسدي ، وأن نسيجهم الكلوي أظهر تغيرات شكلية نموذجية لـ AKI ، في حين أن المجموعات المختلطة قللت من الجلسرين تسبب تلفًا كلويًا حادًا. أظهر هذا أن فينبوسيتين وسيلوستازول يمكن أن يعززوا حماية الفئران الكلوية عن طريق تقليل اليوريا والكرياتينين في الدم وتحسين التغيرات النسيجية المرضية.

الكلمات المفتاحية: AKI الفشل الكلوي الحاد, PDE فوسفودايستريز انزايم

Introduction

Acute kidney injury (AKI) is defined as a sudden loss of kidney function caused by elevated serum creatinine levels and decreased urinary output [1]. AKI is one of several functional kidney conditions known as acute kidney disease and disorders (AKD), which range in severity from mild and self-limiting to severe and chronic [2].

AKI is now known to be caused by both traumatic and non-traumatic rabidomyolysis (RM) [3]. RM is a fatal syndrome caused by skeletal muscle degeneration and muscle enzyme leakage. The development of RM has been linked to crush syndrome, strenuous exercise, prescription drugs, infectious diseases, and toxins [4]. Glycerol main mechanisim to cause AKI that was myoglobin heme causes oxidative stress and lipid peroxidation in proximal tubular cells, resulting in the release of a cascade of mediators, such as cytokines and chemokines, which leads to leukocyte activation and tubular necrosis in the renal cortical area [5]. Vinpocetine drug has many pharmacological targets with multiple action, phosphodiesterase inhibiters-1, a voltage-gated sodium channel, and IKK are 3 main molecule targets of vinpocetine. PDE1 has been implicated in the regulation of vasoconstriction, vascular and cardiac structure remodeling, and neurotrans-mission [6]. vinpocetine plays the role as a novel potent anti-inflammatory agent by inhibiting IKK activity [7]. So Vinpocetine has significant antioxidant and anti-inflammatory properties [8]. Cilostazol, a phosphodiesterase (PDE) III inhibitors, that widely used for many cases such as reduces direct vascular injury via different mechanism, such as vasodilation and antiplatelet action [9], anti-inflammation and platelet-leukocyte interaction minimisation, and inhibition of vascular proliferation via up-regulation of hepatocyte growth factors [10]. Furthermore, cilostazol may influence vascular-related growth factors and oxidative stress molecules [11]. Numerous

studies in various cells and tissues have shown that cilostazol has an inhibitory effect on reactive oxygen species and superoxide generation, along with hydroxyl radical scavenging effects [12]. It significantly reduces lipoprotein remnant particles (lipolytic degradation of triglycerides, that affect atherogenesis) and conserves endothelial health by decrease apoptosis, reducing extreme oxidative stress by minimizing mitochondrial depolarization, swelling, and reactive oxygen species production [13].

Materials and methodes

Experimental animals

The weights of thirty wistar albino male rats ranged from 120gm to 200gm. The animals were kept in cages with free access to food and water. The cages were placed in a calm, temperature-controlled environment with a 12:12 hour light/dark cycle. Before being used in this experiment, the animals were given ten days to acclimate. They were dealt with in accordance with the ethics committee in file NO: 21 approved at the Pharmacy College / Mustansiriyah University on November 29, 2021.

Chemicals and reagents

Because vinpocetine was water insoluble, it was dissolved in dimethylsulfoxide DMSO (5mg/ml) obtained from Sigma-Aldrich (USA). Glycerine was obtained from SDI (IRAQ), and cilostazol was obtained from Sigma-Aldrich (USA). Because cilostazol is poorly water soluble, it was dissolved in DMSO and administered orally (10 mg/ml) according to the manufacturer's instructions.

Treatments and animal group

The animals were divided into 5 groups of 6 rats each. Group 1 (Control group) rats received distilled water (2ml/kg/day) orally for 14 days and a normal saline intramuscular injection (IM) in the hind limp on day 7. Group 2 (induction group) rats were given distilled water (2ml/kg/day) and glycerol 50 percent (10ml/kg) IM in the hind limb on day 7 to induce AKI. Group 3 (vinpocetine group) rats received vinpocetine (5mg/kg) orally for 14 days and glycerol 50 percent (10ml/kg) IM in hind limp on day 7. Group 4 (cilostazol group) rats received cilostazol (50mg/kg) orally for 14 days and glycerol 50 percent (10ml/kg) IM in hind limp on day 7. Group 5 (combination group) was given half the dose. of vinpocetine and cilostazol orally for 14 days and glycerol 50 percent (10ml/kg) IM in hind limp on day 7.

Collection of blood and kidney samples

On day 14, labortary animals were euthanized under anesthesia with intraperitoneal administration of 100 mg/kg ketamine and 10 mg/kg xylazine. To collect blood from the heart, direct cardiac puncture is used. For serum separation, samples were placed in gel tubes and left at room temperature for 10 minutes before being centrifuged at 3000 rpm for 10 minutes at room temperature. Serum was collected in eppendorf tubes and stored at -20°C for estimation of renal function parameters and other biomarkers. Kidney tissue was immediately dissected out, cleaned of any adhering tissues, washed with distilled water, and weighed. Parts of the kidney were removed from each group and preserved in 10% formalin. Overnight, the kidney-fixed specimen was dehydrated, cleared, and processed.

Biochemicals analysis

Determination of Renal function test

Serum urea and Creatinine was measured as parameter of kidney function.

A) Serum Urea

In the body, protein metabolism will produce urea as primary natural result. The importance of blood urea concentration stems from its utility as an indicator of kidney function.

An enzymatic rate technique was used to determine the concentration of urea.

B) Serum Creatinine

During muscle contractions, creatinine is produced in the body at a relatively constant rate from creatine. Creatinine in the blood is then filtered from the glomeruli of the kidney and excreted in the urine. Because creatinine excretion is independent of diet and thus relatively constant in healthy individuals, the creatinine clearance test is one of most sensitive tests for identifying renal function, especially the glomerular filtration rate (GFR), with creatinine concentration in serum being almost completely dependent on its rate of excretion by the kidney. Creatinine concentration in the body frequently linked to renal diseases, as with glomerular nephritis.

Histopathological diagnosis

Is still an important part of disease prognosis and make plan for diseases treatment, several steps that include; Dehydration, Clearing, Embedding, Sectioning, Dewaxing After de-waxing, slices were treated with descending grades of ethyl alcohol (100%, 90%, 80%, and 70%, 2-3 minutes for each concentration), washed with distill water for up to 5 minutes, stained with hematoxylin for up to 5-15 minutes, and cleaned again with tap water for up to 5 minutes. Tissue sections were then stained with eosin for up to 15-30 seconds before being dehydrated in increasing grades of ethyl alcohol (70 percent ,80 percent ,90 percent and100 percent, 1 minutes for each concentration). Clarified in xylene for 10 minutes, then quickly covered with cover slip and allowed to dry overnight before being examined under a microscope.

Results

Effect of vinpocetine and cilostazol on renal function tests (serum urea and creatinine).

A. Urea level changes among tested groups

Concerning with renal function parameters, Urea levels significantly reduce ($p < 0.05$) in mean urea concentation of vinpocetine group (39.574 ± 3.4) in compared with induction group (51.574 ± 4.9), but statistical result show no significant difference ($p=0.258$) in mean concentration of vinpocetine with control group (35 ± 2.11), vinpocetine group show no signficant differences ($P=0.6$) with cilostazol and no significant differences with combination group ($P=1.00$).

The mean of serum urea in the study revealed that significant differences ($P < 0.05$) in mean concentration of induction group (51.574 ± 4.9) if we compared it wih control group (35.188 ± 2.11). cilostazol show significant difference ($P < 0.05$) in mean urea concentation (42.41 ± 3.11) with control group and induction group (51.574 ± 4.9).

Combination group significantly reduce (p-value<0.05) in mean urea concentation (39.758) in compared with induction group (51.574 ± 4.9), statistical result show no significant difference ($p=0.224$) in mean concentration of combination group if it compared with control group (35 ± 2.11), as shown in table (1), figure (1).

Table (1): Effect of vinpocetine and cilostazol on serum urea and creatinine.

Study groups	Serum Urea(mg/dl)	Serum creatinine
Control	35.188 ± 2.11 [a]	0.778 ± 0.06 [a]
Induction	51.574 ± 4.9 [b]	2.41 ± 0.25 [b]
Vinpocetine	39.574 ± 3.3 [ac]	1.34 ± 0.247 [c]
Cilostazol	42.41 ± 3.11 [c]	1.088 ± 0.18 [cd]
Combination	39.758 ± 2.1 [ac]	0.87 ± 0.091 [ad]

- *Each value represent mean ± SD*
- *Statistical analysis was done by using one-way ANOVA followed by the Tukey test*
- *Different lower-case letters indicate significant differences between groups (p<0.05)*

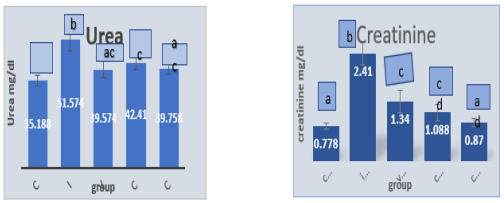

Figure (1): Effect of vinpocetine and cilostazol on serum urea and creatinine.

Figure (1): Effect of vinpocetine and cilostazol on serum urea and creatinine.

- *Each value represent mean±SD*
- *Statistical analysis was done by using one-way ANOVA followed by the post hock test*
- *Different lower-case letters indicate significant differences between groups (p<0.05)*

B. Creatinine level changes among tested groups

Concerning with renal function parameters the mean of serum creatinine in the study revealed that significant differences (*P <0.05*) in mean creatinine concentration of induction group (2.41 ± 0.25), in compaired with control group (0.778 ±0.06),

Vinpocetine group reduce serum creatinine level (1.34 ± 0.247) significantly (p<0.0.5) with induction group (*p<0.05*) in mean creatinine concentration (2.41 ± 0.25) but this reduction shows best result that significantly (p<0.0.5) reduce serum creatinine level with using combination therapy (0.87 ± 0.091) in compaired with control group (0.778 ±0.06)

Cilostazol significantly reduce *(p<0.05)* of mean creatinine concentation (1.088 ± 0.18) in compared with induction group (2.41 ± 0.25), and with control group (0.778 ±0.06), but cilostazol show no significant difference in mean creatinine concentration with combination group *(p=0.078)*.

while combination groups significantly reduce *(p<0.05)* mean creatinine concentration (0.87 ± 0.091) in compared with induction group (2.41 ± 0.25), and vinpocetine group (1.34 ± 0.247), as shown in table (1) and in figure (1)

Histopathological results

Microscopical examination of induction groups revealed the development of tubular cell swelling, tubular epithelial degeneration, interstitial cytoplasmic eosinophilic congestion, and cast formation, whereas the renal structure of the control group appeared normal. Whoever was pretreated (vinpocetine, cilostazole, combination) had slightly preserved normal renal tissue morphology, with slight swelling of renal tubules cells and less interstitial congestion as showen in fugure 2.

control group

The section of control group revealed normal appearance of renal cortex which covered by renal capsule and composed of glomeruli of nephrons and renal tubules involved proximal, distal convoluted tubules and collecting tubules, the renal medulla composed the tubular parts of loop

of henle which involve thick, thin segment and collecting tubules as in figures (2) picture (2).

Induction group

This group showed sever nephrosis that characterized by tubular casts formation, sever vacular degeneration and necrosis of renal tubules as in figure (2) picture (3).

Vinpocetine group

The most sections of the renal cortex and medulla were similar those in control group as mention in figure (2) picture (4).

Cilostazol group

The most sections of the renal cortex showed little figures of tubular vacular degeneration and necrosis with normal renal glomeruli and the sections of renal medulla were similar those in control group as showen in figure (2) picture (5).

Combination group

The most sections of the renal cortex and medulla were similar those in control group as in figure (2) (picture 6)

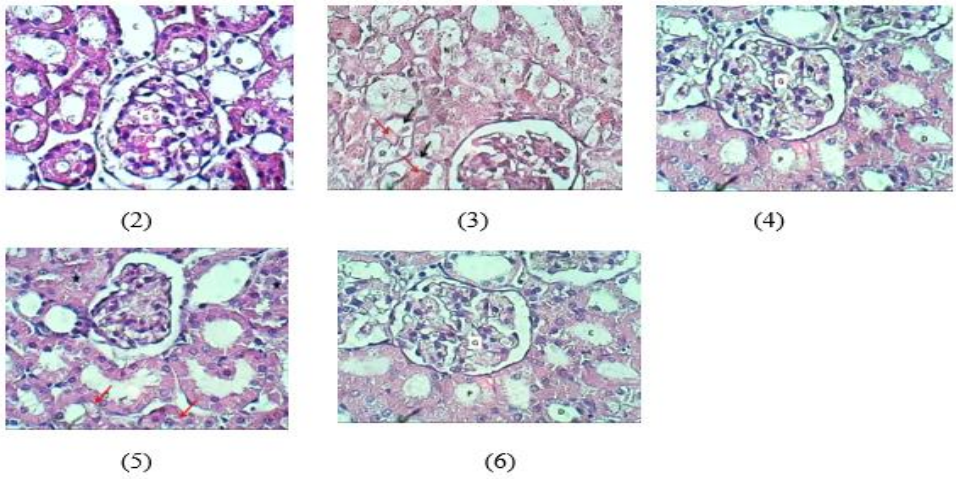

Figure (2): Effect of vinpocetine and cilostazol on histopathological changes

Effect of vinpocetine and cilostazol on renal function tests (urea and creatinine) in glycerol induced AKI.

In the current study, induction of glycerol showen significant elevation *(p<0.05)* in urea and creatinine levels in blood, thess result agree with previous studies that implies the kidney unable to excrete these products, which is an illustration of a problem with the kidney's function and these effects could be caused by changes in the tubular reabsorption threshold, renal blood flow, or glomerular infiltration rate [14],

In the current study, treatment with vinpocetine, cilostazol, and their combination (vinpocetine + cilostazol) reduce serum urea and creatinine levels significantly *(p<0.05)* when compaired with induction group and reach it near to control group these result agree with previous studies that illustated reduction in urea and creatinine due to a mechanism associated to inhibition of cytokine, oxidative stress, and apoptosis ,also these mechanisms are responsible for the enhancement in total antioxidant capacity as well as the inhibition of glycerol induced kidney NF-kB activation [15][16] .

Effects of vinpocetine and cilostazol on histopathological section in glycerol induced AKI.

In current study glycerol-treated rats had extensive proximal tubular necrosis across cortex and medulla showen area of

congestion, infiltration of inflammatory cells, and degeneration of renal cells in the kidney this agree with most previous study that's show that ischemia and toxic models frequently exhibit severe parenchymal damage [17]. marked by eosinophilic tubules with remnants of karyolytic nuclei also these results consist with some studies found that glycerol caused nephrotoxic changes [18].

In pretreatment with vinpocetine, cilostazol, combination (vinpocetine + cilostazol) significantly restored normal morphology of renal tissue, with mild swelling of renal tubule cells and less interstitial congestion. The results agree with a many previous studies, where vinpocetine treated mice showed mild tubular cell damage, a significant reduction in glomerular damage, tubular dilation, and loss of the brush border [17].

other study shows that cilostazol significantly improved the histopathological damage of renal tissue [19].

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