Malvadin Prevents Kidney from Renal Ischemia-Induced Oxidative Damage in Rats. Running Title: Malvidin and Acute Renal Failure

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ABSTRACT

Background: Oxidative stress is one of the suggested causes of renal ischemia reperfusion (IR) injury. Malvidin is an important anthocyanin which exhibits significant antioxidant properties. Here, we aimed to study the effect of Malvidin on oxidative stress status in vivo.

Materials and Methods: 30 Male Wistar rats were randomly assigned to three groups: (1) Sham operated, (2) renal IR (45 min ischemia followed by 24 h of reperfusion), (3) renal IR + Malvidin (100 mg/kg, P.O, 21 days). In IR group, rats were anesthetized and renal arteries occluded for 45 min followed by 24 h reperfusion. Sham-operated Rats underwent a surgical procedure identical to those of IR rats except that clamps were not applied. Then, renal functional indices (fractional excretion of sodium, plasma (blood urea nitrogen) BUN, creatinine (Cr), urine flow rate, creatinine clearance) and oxidative stress indices such as catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) were evaluated in blood and kidney tissue.

Results: Malvidin caused a fall in fractional excretion of Na, plasma BUN, Cr and rise in urine flow rate, Cr clearance in malvidin group compared with IR group. In addition malvidin administration resulted in an increase in CAT and SOD and decrease in MDA in contrast to IR group.

Conclusion: Malvidin consumption has protective effect against renal IR induced acute kidney injury, partly by inhibiting oxidative stress in renal tissues.

Keywords: Renal ischemia reperfusion, acute kidney injury, Malvidin, anthocyanin, Oxidative stress.

INTRODUCTION

Acute kidney injury (AKI) is a major clinical problem that occurs in some of the hospitalized patients especially in intensive care units. Renal ischemia reperfusion (IR) is one of the most important causative mechanisms of AKI and is associated with various clinical settings including shock, sepsis, kidney transplantation, vascular surgery, and elective urological operations [1, 2]. Although the exact mechanism of renal IR injury is not explained completely, the renal IR injury has multifactorial and interdependent causes such as inflammatory responses, leukocyte infiltration and oxidative stress. Oxidative stress has been identified to play key role in this process. The abundance of polyunsaturated fatty acids makes the kidney an organ particularly vulnerable to reactive oxygen species (ROS) attack [2, 3].

Compounds in some plants and fruits have significant antioxidant properties [4-7]. Phytochemicals are most potential antioxidant [8-11] such as Anthocyanins. Anthocyanins are a group of red, purple, violet and blue pigments that contribute to the bright colors of plant components and are broadly distributed in many fruits, vegetables and flowers that have antioxidant, Antiproliferative and anti-inflammatory properties [12-14]. Jiang et al. showed Purple potato anthocyanins could be an

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Malvidin prevents kidney from renal ischemia ...

important therapeutic agent in alcohol-induced liver injuries by inhibiting CYP2E1 expression and therefore reinforcement antioxidant defenses [12]. Cyanidin-3-O-glucoside is an anthocyanin that protects the rat heart from ischemia/reperfusion-induced apoptosis and necrosis because of the ability to reduce cytosolic cytochrome C [15]. It was demonstrated that Korean black bean anthocyanins have neuroprotective effects against kainic acid-induced excitotoxicity due to inhibiting the reactive oxygen species (ROS) accumulation [16]. Blueberry anthocyanins is effective in ameliorating radiation-induced lung injury in rats [17]. Malvidin, as an anthocyanin, is responsible for the color of red wine and blueberries, together with other anthocyanidins. Malvidin has antioxidant activity with free radical scavenging properties. It has four hydroxyls group, leading to a powerful antioxidant capacity [18, 19]. Malvidin has antihypertensive properties by inhibiting angiotensin I converting enzyme [20]. It has important role in preventing chronic inflammation by inhibiting monocyte chemotactic protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) production in the NF-κB pathway [21-27].

This study was designed to investigate the protective effects of malvidin on renal ischemia reperfusion induced acute kidney injury in rats. The present research is being carried out to highlight the possible useful effects of the usage of malvidin as a natural antioxidant in order to improve the side-effects of various clinical settings including kidney transplantation, vascular surgery, and elective urological.

Materials and Methods:

This study was approved by Shahroud University of Medical Sciences Ethics Committee by Etic CODE: IR.SHMU.REC.1396.164.

So, 30 Male Wistar rats (weight =200-250 g) were Maintained at room temperature (22 ± 2°C) in a 12:12-hour light–dark cycle and with free access to standard diet and water. Animal care was in compliance with the guidelines of the animal and human ethics committee of Shahroud University of medical sciences. The rats were randomly assigned to three groups (n=10):

1. Sham operated (2 ml/kg bw/day salin trough feeding tube for 21 days before IR),
2. renal IR (45 min bilateral ischemia),
3. renal IR + malvidin (100 mg/kg bw/day in 2 ml/kg saline for 21 days before IR).

An established model of renal IR injury in rat was used [1, 28]. Briefly, rats were anesthetized with intraperitoneal pentobarbital sodium. A midline incision was made and the renal pedicles were bluntly dissected and occluded with nontraumatic vascular clips for 45 min. Then, clamps were removed gently and the kidneys were observed for a further 5 min to ensure reflow process. Then, the incision was closed in two layers with a 4-0 silk suture. The animals were then returned to their cages and allowed to recover. During the period of renal ischemia, the animals were covered with plastic wrap to prevent evaporation. In addition, animals were kept well hydrated with warm sterile saline and were maintained at a constant body temperature (~37 °C) on a heating pad. Sham-operated rats underwent surgical procedure identical to those of IR rats except that clamps were not applied. In renal IR + malvidin group, oral malvidin (Sigma) administrated 100 mg/kg intraperitoneal in 2 ml/kg saline for 21 days before IR. At the end of the reperfusion period (24 hr), the animals were anesthetized. Blood and 24-h urine samples were collected using metabolic cages for evaluation of renal functional indices: fractional excretion of sodium, plasma BUN, creatinine, urine flow rate and creatinine clearance [29]. Creatinine clearance helps to estimate the glomerular filtration rate (GFR). Kidney tissues removed for oxidative stress indices measurement. For this, we evaluated catalase (CAT), malondialdehyde (MDA) and superoxide dismutase (SOD). In order to measure sodium concentration of the plasma and urine, flame photometer apparatus was used. Plasma and urine creatinine, plasma BUN were measured by autoanalyzer. CAT activity was
determined by Aebi’s method. According to this method, activity of CAT can be measured by decomposition of H2O2. The remaining substrate concentration at a given moment of the reaction can be determined by UV spectrophotometry at 240 nm [30]. Malondialdehyde (MDA) level was evaluated in renal tissue according to the Esterbauer and Cheeseman method (spectrophotometry at 532 nm).

SOD activity was determined by the method of Paoletti and Mocali In this method superoxide anions are generated from oxygen molecules in the presence of EDTA•MnCl2 and mercapto-ethanol. NAD(P)H oxidation is linked to the availability of superoxide anions in the medium. As soon as SOD is added to the assay mixture, it inhibits nucleotide oxidation. Therefore, at high concentration of the enzyme the absorbance at 340 nm remains unchanged [32].

Statistical analysis
The statistical analysis between different groups was performed using ANOVA followed by Tukey's post-hoc test. Results with a p < 0.05 were considered as significant. The data are expressed as means ± S.E.M. All the analyses were conducted using Graphpad Prism 6 (GraphPad Software, Inc, La Jolla, Ca., USA).

Results
45 minutes renal ischemia followed by 24 hours reperfusion induced renal functional damage. Briefly, renal IR significantly increased fractional excretion of sodium, plasma BUN and creatinine levels and decreased urine flow rate and creatinine clearance in the IR group compared with sham (Table 1).

Renal oxidant - antioxidant indices showed an oxidative stress in renal tissues. In IR group, renal MDA increased and CAT and SOD decreased significantly compared with sham group (Figures 1-3).

Oral Malvidin administration partly improved renal functiona injury. Malvidin decreased plasma BUN and creatinine, fractional excretion of Na. in addition; creatinine clearance and urine flow rate were increased in malvidin group contrast to IR group (Table 1). Moreover malvidin protected renal tissues from oxidative stress damage. In this group, significant increase in SOD and GSH activity and decrease in MDA compared to IR group were observed (Figures 1-3).

Discussion
Renal ischemia reperfusion injury is one of the most common causes of AKI and occurs in some clinical situations.

In the present study, 45 min bilateral renal ischemia followed by 24 hr reperfusion resulted in increased fractional excretion of sodium, plasma BUN and creatinine levels and decreased urine flow rate and creatinine clearance in IR group demonstrated acute renal failure. Regarding the decrease in the renal tissue CAT and SOD levels and increase in MDA in IR group compared with sham group, it can be stated that renal oxidative stress was induced. Tavafi et al. have shown renal IR caused functional damage and oxidative stress induction in kidney. They have evaluated urea, creatinine and serum malondialdehyde [33]. Moreover, our previous studies have shown that renal IR resulted in renal functional damage and induced oxidative stress in kidney [34].

Oral malvidin administration improved functional and oxidative stress indices. Briefly, malvidin diminished the renal IR-induced increase in plasma BUN, creatinine, fractional excretion of sodium, creatinine clearance and urine flow rate in IR + malvidin group. In addition, CAT and SOD significantly elevated and MDA significantly decreased in this group compared with renal IR group. These tissues protective effects of malvidin may due to its antioxidant properties and scavengering of reactive oxygen species. Then, protection of renal tissue resulted in improvement of renal functional indices.

Anthocyanins, such as malvidin, belong to the flavonoids polyphenols and they are water soluble natural
Malvadin prevents kidney from renal ischemia ... Sarvenaz Emadi, Iman Sadeghi, Hossein Khastar

pigments with red, purple and blue color from fruits, vegetables and flowers [35, 36]. Anthocyanins belong to a large group of secondary plant metabolites and occur in all forms of plant tissue. Anthocyanin pigments consist of either two or three chemical units, these being aglycon bases or flavylum rings (anthocyanidin), sugars or (potentially) acylating groups. In fact, anthocyanins and other phenols have been the focus of ever greater attention in health and medicine due to their anti-carcinogenic, anti-allergic, anti-ulceric, anti-arthritic, anti-inflammatory, and anti-oxidant properties [37].

A colored fruit-rich diet and moderate red wine consumption protect the heart against ischemia reperfusion injury, because of the several flavonoids polyphenols such as malvidin [38].

Bognar et al. demonstrated malvidin attenuated lipopolysaccharide-induced nuclear factor-kappaB, mitogenactivated protein kinase activation and reactive oxygen species production. They concluded that malvidin, the most abundant polyphenol in red wine, has beneficial effects on inflammation-mediated chronic disease such as diabetes and cardiovascular disease [18]. Huang et al in 2016 have shown blueberry malvidin significantly attenuated oxidative stress in human umbilical vein endothelial cells. They assayed xanthine oxidase-1 and superoxide dismutase as antioxidant indices [19].

Baba et al. in 2017 indicated blueberry and malvidin are potent STAT-3 inhibitors that prevent proliferation and induce apoptosis of oral cancer cells in vitro and in vivo. Blueberry and malvidin suppressed STAT-3 phosphorylation, blocked nuclear translocation of the active dimer and prevented transactivation of STAT3 target genes that play crucial roles in cell proliferation and apoptosis [39].

Tomankova et al. in 2016 demonstrated malvidin, as major wine dye, has higher antioxidant activity than Vitisin A and it was related to higher ability of malvidin to scavenge of free radicals [37].

Conclusion:

Generally, our data showed that bioactive anthocyanin malvidin in red wine, some fruits and vegetable can improve the treatment of AKI by attenuating the oxidative stress.

| Table 1. Biochemical parameters of rats in Sham, IR and IR + Malvidine groups. |
|-----------------------------------|-----------------|-----------------|
|                                  | IR + Malvidine  | IR              | Sham            |
| fractional excretion of Na, %    | 1.75 ± 0.08#    | 2.11 ± 0.1*     | 0.59 ± 0.03     |
| Plasma BUN, mg/dl                | 94.71 ± 6.31#   | 121.42 ± 8.55*  | 23.28 ± 1.55    |
| Plasma creatinine, mg/dl         | 2.9 ± 0.18#     | 3.6 ± 0.15*     | 0.28 ± 0.7      |
| Urine flow rate, μl/min.bw       | 22.42 ± 1.17#   | 16 ± 0.75*      | 31.85 ± 1.07    |
| Creatinine clearance, μl/min     | 3.62 ± 0.22#    | 2.52 ± 0.16*    | 7.9 ± 0.33      |

* Denotes p < 0.001 compared with sham and # Denotes p < 0.05 compared with IR, n = 10.
Figure 1. Renal SOD (Mean ± SEM): In IR group, renal SOD decreased significantly compared with sham group. In IR + malvidin group, significant increase in SOD compared to IR group was observed. Note: *Denotes p < 0.001 versus sham group and # Denotes p < 0.05 versus IR group.

Figure 2. Renal CAT (Mean ± SEM): In IR group, renal CAT decreased significantly compared with sham group. In IR + malvidin group, significant increase in CAT compared to IR group was observed. Note: *Denotes p < 0.001 versus sham group and # Denotes p < 0.05 versus IR group.
Malvadin prevents kidney from renal ischemia ...

Sarvenaz Emadi, Iman Sadeghi, Hossein Khastar

Figure 3. Renal MDA (mean ± SEM): In IR group, renal MDA increased significantly compared with sham group. In IR + malvidin group, significant decrease in MDA compared to IR group was observed.

Note: *Denotes p < 0.001 versus sham group and # Denotes p < 0.05 versus IR group.

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The title: Malvidine (Malvidine) is known to protect against acute renal failure in rats

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The abstract: Malvidine is an anthocyanin with strong antioxidant properties. The aim of this study was to evaluate the effect of Malvidine on the antioxidant capacity of the body. The study was divided into three groups: 1 - Sham group, 2 - Sham group + Malvidine (50 mg/kg by mouth for 45 minutes), then 24 hours reperfusion, 3 - Malvidine group (100 mg/kg by mouth for 24 hours), and 4 - Malvidine group + Malvidine (100 mg/kg by mouth for 45 minutes), then 24 hours reperfusion. The aorta, kidney, and liver tissues were processed for analysis. The results showed that Malvidine reduced BUN, creatinine, and creatinine in the sham group, and increased GSH, CAT, and SOD in the group with reperfusion. The conclusion: Malvidine has protective and therapeutic effects against acute renal failure, which may be related to the antioxidant effect.

Keywords: Reperfusion injury, acute renal failure, Malvidine, anthocyanins, antioxidant effect.