Protective effect of chlorogenic acid on renal ischemia/reperfusion injury in rats

Tuncay Toprak 1, Cagri Akın Sekerci 2, Hasan Riza Aydin 3, Mehmet Akif Ramazanoglu 4, Fatma Demet Arslan 3, Banu Isbilen Basok 3, Hatice Kucuk 6, Huseyin Kocakoglu 3, Hamit Zafer Aksoy 3, Seyhan Sumeyra Asci 7, Yiloren Tanidir 8

1 Fatih Sultan Mehmet Training and Research Hospital, Urology, Istanbul; 2 Marmara University Pendik Training and Research Hospital, Pediatric Urology, Istanbul; 3 Kanuni Training and Research Hospital, Urology, Trabzon; 4 Rize State Hospital, Urology, Rize; 5 Tepecik Training and Research Hospital, Biochemistry, Izmir; 6 Kanuni Training and Research Hospital, Pathology, Trabzon; 7 Kanuni Training and Research Hospital, Anesthesiology and Reanimation, Trabzon, Turkey; 8 Marmara University, School of Medicine, Urology Istanbul, Turkey.

Summary

Objectives: Ischemia/reperfusion (I/R) injury is a common cause of renal injury and to date, many pharmacological agents have been identified to decrease I/R injury. One of the potential compound that can target I/R injury is chlorogenic acid (CGA). It has potent anti-inflammatory, antibacterial, anti-oxidant, analgesic and antipyretic activities in in vitro experiments and in vivo animal models. The aim of the study was to investigate the protective characteristic of CGA on renal I/R injury.

Material and Methods: 24 rats were randomly allocated to three groups (n = 8): Sham, I/R+CGA and I/R groups. CGA was administered intraperitoneally at a dose of 20 mg/kg, 10 min before reperfusion. I/R injury was achieved by clamping the left renal artery for 45 minutes, followed by reperfusion for 4 hours. The left kidneys of the rats were examined for tissue damage by histopathological and biochemical examination. For histological evaluation, EG11 scoring system was used. For biochemical examination total oxidant status, total antioxidant status and oxidative stress index were used. The power analysis indicated that 8 subjects per group would be required to produce 80% chance of achieving statistical significance at p < 0.05 level. The results are expressed as mean ± SD. Mann-Whitney U was performed for statistical analysis.

Results: Histopathological examination of the tissue damage revealed that all kidneys in the sham group were normal. I-R group had significantly higher histopathological scores than other groups. Histopathological improvement was seen after CGA treatment. TAS, TOS and OSI values of I-R group were significantly higher than sham group (0.88 vs 0.76 (p: 0.004), 13.8 vs 7.04 (p: 0.021) and 0.15 vs 0.09 (p: 0.034), respectively). In CGA treated group TAS, TOS and OSI levels were 0.84, 6.47 and 0.07, respectively. CGA treatment resulted in significant improvement in TOS and OSI parameters.

Conclusions: CGA treatment provided marked improvement in renal histology and suppressed oxidative stress. Thus, CGA may have a protective effect in renal tissue against I/R injury.

Key words: Renal ischemia; Oxidative stress; Chlorogenic acid; Rat.

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Introduction

Ischemia/reperfusion (I/R) injury is a common cause of renal injury arising from a variety of clinical circumstances, including partial nephrectomy, renal transplan-
total oxidant (TOS) status, total antioxidant (TAS) assays. In our knowledge, there have been no studies concerning the protective effect of CGA against renal I/R injury.

**MATERIALS AND METHODS**

The experimental and surgical procedures were conducted according to routine animal care guidelines, and the Guide for the Care and Use of Laboratory Animals (19).

The approval was obtained from Institutional Animal Care and Use Committee of Karadeniz Technical University (Trabzon, Turkey) (Approval Number/ID: 2019/5). 24 male Sprague-Dawley rats (8 weeks old, weight 230-300 g) were purchased from the Karadeniz Technical University Laboratory Animals Research Centre (Trabzon, Turkey).

All animals were kept in captivity under the same nutritional and environmental conditions. Rats were entrained under a 12:12 h dark: light cycle (lights on 6 am-6 pm) with stable temperature (21 ± 2°C) and humidity (60 ± 5%). The rats had sterile water and food available ad libitum.

**Experimental design**

Rats were randomly and equally divided into 3 groups;

1. **Vehicle- treated ischemic (I/R):** After sterile conditions were obtained, a midline laparotomy was performed. Isotonic saline (1 mg/kg) was applied intraperitoneally 10 min before the beginning of reperfusion. The left kidney pedicle was clamped with an artery clamp for 45 minutes. After 45 minutes of left renal ischemia, the occlusion clamp was removed for reperfusion for 4 hours and the incision was closed.

2. **Vehicle- treated sham (Sham):**
   - Rats underwent the same surgical procedures except unilateral renal occlusion. During the experiment, they were kept under anesthesia with gauze, soaked in saline in the abdominal cavities.

3. **CGA-treated ischemic (I/R+CGA):** After sterile conditions were obtained, a midline laparotomy was performed. CGA (Sigma-Aldrich) (20 mg/kg) was applied intraperitoneally 10 min before the beginning of reperfusion. The left kidney pedicle was clamped with an artery clamp for 45 minutes. After 45 minutes of left renal ischemia, the occlusion clamp was removed and the incision was closed.

**Administration of CGA**

CGA was dissolved in saline (vehicle) and administered intraperitoneally at a total dose of 20 mg/kg 10 minutes before reperfusion.

**Surgical procedure**

For anesthetic ketamine hydrochloride (100 mg/kg, Ketalar, Eczacibasi, Turkey) and xylazine (10 mg/kg) were used intraperitoneally. Following fluid replacement with 3 mL/kg-1 h-1 lactated Ringer’s solution, the surgical area was prepared for sterilization. Then a midline laparotomy incision was performed and the left kidney pedicle was dissected. Left renal ischemia was induced by clamping the left renal artery for 45 min for the I/R and I/R-CGA groups. For reperfusion the clamp was removed and the pulsation of renal artery was verified visually. After controlling the bleeding, the skin layers were sutured. The rats were sacrificed 4 h after completion of the reperfusion and the left kidneys were removed and stored for biochemical and histopathological examination under favorable conditions.

**Histological analysis**

Removed kidney was fixed with 10% formalin and embedded in paraffin. 5 µm tissue sections obtained for Hematoxylin and Eosin staining. An experienced, independent pathologist, who was blinded to the groups, analyzed three different tissue sections in each group, using a Zeiss Axios Imager A2 microscope (Carl Zeiss AG, Germany). The histological evaluations of the renal tissue were graded as described in the study of Medeiros et al. (20) (Table 1). The scores were applied to microscopic changes consistent with tubular necrosis: vacuolization of tubular cells, tubular lumen dilation, intra-tubular cylinders, interstitial fibrosis and tubular cell necrosis. For histological evaluation, EG11 scoring system, which was developed especially for animal studies in kidney tissues in the context of injury, was also used (21), (Table 2). This system consists of histological damage in 4 separate components: Endothelial, Glomerular, Tubular, and Interstitial.

**TAS and TOS assays**

The serum TAS and TOS levels were determined with a

**Table 1.**

| Score | Histopathological pattern |
|-------|---------------------------|
| 0     | Normal                    |
| 0.5   | Small focal damaged areas |
| 1     | ≤ 10% Cortical damaged zone |
| 2     | 10-25% Cortical damaged zone |
| 3     | 25-75% Cortical damaged zone |
| 4     | > 75% Cortical damaged zone |

**Table 2.**

The EG11 histology scoring system.

| Tissue type      | Damage                        | Score |
|------------------|-------------------------------|-------|
| Tubular          | No damage                     | 0     |
|                  | Loss of Brush Border (BB) in less than 25% of tubular cells, Integrity of basal membrane | 1     |
|                  | Loss of BB in more than 25% of tubular cells, Thickened basal membrane | 2     |
|                  | (Plus) Inflammation, cast formation, necrosis up to 60% of tubular cells | 3     |
|                  | (Plus) Necrosis in more than 60% of tubular cells | 4     |
| Endothelial      | No damage                     | 0     |
|                  | Endothelial swelling          | 1     |
|                  | Endothelial disruption        | 2     |
|                  | Endothelial loss              | 3     |
| Glomerular       | No damage                     | 0     |
|                  | Thickening of Bowman capsule  | 1     |
|                  | Retraction of glomerular tuft  | 2     |
|                  | Glomerular fibrosis           | 3     |
| Tubulo/Interstitial | No damage                  | 0     |
|                  | Inflammation, haemorrhage in less than 25% of tissue | 1     |
|                  | (Plus) necrosis in less than 25% of tissue | 2     |
|                  | Necrosis up to 60%            | 3     |
|                  | Necrosis more than 60%        | 4     |
novel automatic method, developed by Erel (22, 23). The ratio of TAS to TOS is defined as oxidative stress index (OSI), expressed as percentage.

Statistical analysis
IBM SPSS 22 version (SPSS IBM, Turkey) program was used for analysis. Before starting to study, we performed power analysis. The power analysis indicated that 8 subjects per group would be required to produce 80% chance of achieving statistical significance at $p < 0.05$ level. The Kolmogorov-Smirnov test was performed to determine the normality of data. The results are expressed as mean ± SD. Mann-Whitney U was performed for statistical analysis, as appropriate. A $p$ value below 0.05 was considered statistically significant.

Table 3.
Histopathology scoring of cortical damage of the groups.

| Rats | Sham group | I/R group | I/R + CGA group |
|------|------------|-----------|-----------------|
| 1    | 0          | 0.5       | 0.5             |
| 2    | 0          | 1         | 1               |
| 3    | 0          | 1         | 1               |
| 4    | 0          | 1         | 0.5             |
| 5    | 0          | 0.5       | 0.5             |
| 6    | 0          | 0.5       | 0.5             |
| 7    | 0          | 1         | 1               |
| 8    | 0          | 0.5       | 0.5             |

Table 4.
Comparison of rats in terms of EGT1 scoring.

| Rats | Sham group | I/R group | I/R + CGA group |
|------|------------|-----------|-----------------|
| 1    | 0          | 8         | 5               |
| 2    | 0          | 7         | 3               |
| 3    | 0          | 8         | 6               |
| 4    | 0          | 8         | 3               |
| 5    | 0          | 5         | 3               |
| 6    | 0          | 5         | 4               |
| 7    | 0          | 8         | 7               |
| 8    | 0          | 4         | 6               |

Figure 1.
Histological images of rat renal cortex sections. a; Normal renal cortex (sham group), b; tubular necrosis (I/R), c; tubular injury (I/R + CGA group).

Table 5.
Comparison of groups in terms of biochemical parameters.

|               | TAS median (min-max) | P     | TOS median (min-max) | P     | OSI median (min-max) | P     |
|---------------|----------------------|-------|----------------------|-------|----------------------|-------|
| Group 1-2     | 0.84 (0.76-1.0)      | 0.021 | 6.47 (2.1-23.7)      | 0.83  | 0.07 (0.03-0.26)     | 0.52  |
| Group 2-3     | 0.76 (0.66-0.80)     | 0.0048| 7.04 (4.7-13.9)      | 0.021 | 0.09 (0.06-0.21)     | 0.034 |
| Group 1-3     | 0.84 (0.76-1.0)      | 0.49  | 6.47 (2.1-23.7)      | 0.046 | 0.07 (0.03-0.26)     | 0.040 |

(1) I/R + CGA, (2) sham, (3) I/R, Mann-Whitney U test.
ischemic damage and tubulo-interstitial damage are the hallmarks of renal IR injury which is important for complete and comprehensive documentation. For this reason, EGT I scoring system was used together with other system. Because it is reliable, simple, more informative and more detailed scoring system about the degree of tissue damage of the kidney (21). The histological study showed tubular dilation, tubular necrosis, cellular edema and inflammatory cell infiltration in the tubular interstitium. These lesions were less intense in CGA treated rats compared to untreated animals.

In order to block inflammatory response and oxidative stress, several drugs have been used to prevent renal I/R injury in several experimental studies (13, 14). However, the new experimental studies will help us to find the most appropriate feasible treatment. In the present study, CGA was examined for its potential effects on regulating renal I/R injury. CGA is a polyphenol, which is abundantly found in coffee, fruits and vegetables. It has been used as an antioxidant, analgesic and anti-inflammatory. It has a certain number of R-OH radicals that are capable of forming the hydrogen free radical, thereby protecting tissue cells from oxidative damage (30). It has been shown to act as a scavenger of hydroxyl radicals, peroxynitrite and superoxide radicals in a concentration-dependent manner in vitro (31). In the study of Yun et al. (32) CGA given at 10 mg/kg intraperitoneally. 10 min before ischemia and reperfusion was chosen as the most effective dose for histology evaluation for I/R induced hepatic injury.

In our study, it was administered intraperitoneally at a total dose of 20 mg/kg 10 minutes before reperfusion. Previous studies in rat models have shown that CGA is protective against hepatic and focal cerebral I/R injury (32, 33). We have observed that CGA has a protective effect against renal I/R injury in our study. We considered that CGA may serve a protective role in the rat model of renal I/R injury. To the best of our knowledge, there is no data showing the effect of CGA on I/R kidney injury and evaluating the TAS, TOS levels and histopathology together.

As a limitation of our study, since we did not perform a right nephrectomy, we did not measure plasma creatinine, the most commonly used marker as a measure of renal excretory function (34).

CONCLUSIONS

CGA treatment provided marked improvement in renal histology and suppressed oxidative stress. Thus, CGA may have a protective effect in renal tissue against I/R injury.

REFERENCES

1. Sagiroglu T, et al. Effects of apelin and leptin on renal functions following renal ischemia/reperfusion: An experimental study. Exp Ther Med. 2012; 3: 08-914.
2. Snoeijis MG, et al. Acute ischemic injury to the renal microvasculature in human kidney transplantation. Am J Physiol Renal Physiol. 2010; 299:F1134-40.
3. Orvieto MA, et al. Ischemia preconditioning does not confer resilience to warm ischemia in a solitary porcine kidney model. Urology. 2007; 69:984-987.
4. Eltzschig HK. Echle T Ischemia and reperfusion—from mechanism to translation. Nat Med. 2011; 17:1391.
5. Zhang J, et al. Erythropoietin pretreatment ameliorates renal ischaemia-reperfusion injury by activating PI3K/Akt signalling. Nephrology (Carlton). 2015; 20:266-72.
6. Wang L, et al. Effect of picroside II on apoptosis induced by renal ischemia/reperfusion injury in rats. Exp Ther Med. 2015; 9:817-822.
7. Fadili W, Allah MH, Laouad I. Chronic renal allograft dysfunction: risk factors, immunology and prevention. Arab J Nephrol Transplant. 2013; 6:45-50.
8. Martin GL, et al. Comparison of total, selective, and nonarterial clamping techniques during laparoscopic and robot-assisted partial nephrectomy. J Endourol. 2012; 26:152-156.
9. Conesa EL, et al. N-acetyl-L-cysteine improves renal medullary hypoperfusion in acute renal failure. Am J Physiol Renal Integr Comp Physiol. 2001; 281: R730-7.
10. Rhoden E, et al. Protective effect of allopurinol in the renal ischemia–reperfusion in uninephrectomized rats. Gen Pharmacol. 2000; 35:189-93.
11. Feitoza CQ, et al. Cyclooxygenase 1 and/or 2 blockade ameliorates the renal tissue damage triggered by ischemia and reperfusion injury. Int Immunopharmacol. 2005; 5:79-84.
12. Sahnia E, et al. The protective effects of physiological and pharmacological concentrations of melatonin on renal ischemia-reperfusion injury in rats. Urol Res. 2003; 31:188-193.
13. Hosseini F, et al. Effect of beta carotene on lipid peroxidation and antioxidant status following renal ischemia/reperfusion injury in rat. Scand J Clin Lab Invest. 2010; 70:259-263.
14. Kızılçoğlu M, et al. Beneficial effects of N-acetylcysteine and esben on renal ischemia/reperfusion injury. Ren Fail. 2011; 33:512-517.
15. Suzuki A, et al. Chorogenic acid attenuates hypertension and improves endothelial function in spontaneously hypertensive rats. J Hypertens. 2006; 24:1065-1073.
16. Dos Santos MD, et al. Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chorogenic acid. Biol Pharm Bull. 2006; 29:2216-2240.
17. Almeida AAP, et al. Antibacterial activity of coffee extracts and selected coffee chemical compounds against enterobacteria. J Agricul Food Chem. 2006; 54:8738-8743.
18. Kono Y, et al. Iron chelation by chlorogenic acid as a natural antioxidant. Biosci Biotechnol Biochem. 1998; 62:22-27.
19. Council NR. Guide for the Care and Use of Laboratory Animals. 1996, Washington, DC: The National Academies Press. 140.
20. Medeiros PJD, et al. Effect of sildenafil in renal ischemia/reperfusion injury in rats. Acta Cir Bras. 2010; 25:490-495.
21. Chavez R, et al. Kidney ischaemia reperfusion injury in the rat: the EGT I scoring system as a valid and reliable tool for histological assessment. Journal of Histology and Histopathology. 2016; 3.
22. Erel O. A novel automated method to measure total antioxidant response against potent free radicals reactions. Clin Biochem. 2004; 37:112-119.
23. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005; 38:1103-1111.
24. Ysebaert DK, et al. Identification and kinetics of leukocytes after
severe ischaemia/reperfusion renal injury. Nephrol Dial Transplant. 2000; 15:1562-74.
25. Delbridge M, et al. The effect of body temperature in a rat model of renal ischaemia-reperfusion injury. in Transplantation proceedings. 2007. Elsevier.
26. Wystrychowski W, et al. Nephroprotective Effect of Pentoxifylline in Renal Ischemia–Reperfusion in Rat Depends on the Timing of Its Administration. in Transplantation proceedings. 2014. Elsevier.
27. Tarpey MM, Wink DA, Grisham MB. Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in vivo considerations. Am J Physiol Regul Integr Comp Physiol. 2004; 286:R431-R444.
28. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem. 2004; 37:277-285.
29. Harma M, Erel O. Increased oxidative stress in patients with hydatidiform mole. Swiss Med Wkly. 2003; 133:563-6.
30. Zhang J, et al. Liquid chromatograph/tandem mass spectrometry assay for the simultaneous determination of chlorogenic acid and cinnamic acid in plasma and its application to a pharmacokinetic study. J Pharm Biomed Anal. 2010; 51:685-690.
31. Graziani G, et al. Apple polyphenol extracts prevent damage to human gastric epithelial cells in vitro and to rat gastric mucosa in vivo. Gut. 2005; 54:193-200.
32. Yun N, Kang J-W, Lee S-M. Protective effects of chlorogenic acid against ischaemia/reperfusion injury in rat liver: molecular evidence of its antioxidant and anti-inflammatory properties. J Nutr Biochem. 2012; 23:1249-1253.
33. Miao M, et al. Protective effect of chlorogenic acid on the focal cerebral ischemia reperfusion rat models. Saudi Pharm J. 2017; 25:556-563.
34. Suzuki Y, et al. Clinical validity of renal function markers including serum cystatin C on chronic kidney disease classification. Rinsho Byori. 2011; 59:345-351.