

Green Tea Polyphenol Prevents Diabetic Rats From Acute Kidney Injury After Cardiopulmonary Bypass

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Background. Acute kidney injury (AKI) is a common complication accompanying cardiopulmonary bypass (CPB) and is independently associated with increased morbidity and death. Diabetes mellitus increases the risk for AKI after CPB. Epigallocatechin-3-gallate (EGCG) is a major component of the polyphenolic fraction of green tea, which possesses cardioprotective activities, as previously reported. We hypothesized that EGCG also possesses a renoprotective effect through its diverse biochemical properties and assessed the effect on renal function after CPB for diabetic rats.

Methods. Goto-Kakizaki rats developing type 2 diabetes mellitus were randomly assigned to one of the following groups: sham ($n = 10$), CPB (CPB alone, $n = 9$), or EGCG (CPB + EGCG, $n = 10$). CPB was conducted for 30 minutes at a flow rate of 100 mL/kg/min in the CPB and EGCG groups. Rats assigned to the EGCG group were administrated EGCG solution

orally for 2 weeks before CPB. We evaluated renal biochemical or histologic changes at 24 hours after CPB.

Results. Compared with the CPB group, the EGCG group exhibited milder tubular injury histologically ($p < 0.0001$) and reduced expression of kidney injury molecule-1, a biomarker for renal tubular injury ($p < 0.0001$) and 8-hydroxy-2'-deoxyguanosine ($p < 0.01$), indicating attenuated oxidant stress.

Conclusions. Preoperative oral administration of EGCG ameliorates AKI in a CPB model of diabetic rats through antioxidative properties. This simple method could be applied in a clinical setting as a prophylactic renal protection against AKI after CPB, especially for high-risk patients with diabetes mellitus.

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Acute kidney injury (AKI) develops in up to 30% of all patients who undergo cardiac operations and is associated with a fourfold increase in postoperative death or increased morbidity [1-5]. AKI requiring hemodialysis, which occurs in approximately 1% of AKI cases, leads to a mortality rate as high as 63% [4, 6]. Minimal changes of serum creatinine may deteriorate the prognosis after cardiac operations even in patients with normal renal function [5]. Use of cardiopulmonary bypass (CPB) is considered a major triggering factor of AKI after cardiac operations [7] through multiple pathophysiologies such as ischemia/reperfusion injury, thromboembolism, hypoperfusion, hemodilution, inflammation, oxidative stress, and iron toxicity due to hemolysis [1, 8]. However, the etiology is not fully understood, and there is no effective therapy for the pathologic conditions [9, 10].

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Another compromising condition for patients undergoing cardiac operations is diabetes mellitus (DM). The diabetic population accounts for more than 38% of patients undergoing cardiac operations, especially coronary revascularization [11]. DM is an independent risk factor in patients undergoing cardiac operations [12, 13] and is also reported to be a risk factor for postoperative AKI [8].

Green tea is safe, popular, and known for its antioxidant properties, mainly through scavenging reactive oxygen species such as superoxide anion, hydrogen peroxide, or hydroxyl radical [14]. Several studies have shown that green tea consumption is potentially protective against renal disease [15] through its antioxidant properties [16]. Epigallocatechin-3-gallate (EGCG) is the most abundant polyphenolic catechin in green tea. We previously demonstrated that EGCG enhances recovery from myocardial ischemia/reperfusion injury by diminishing oxidative stress [17, 18]. However, whether preoperative oral intake of green tea polyphenols produces

Dr Hyon discloses a financial relationship with Bio-Verde Inc.

Abbreviations and Acronyms

8-OHdG	= 8-hydroxy-2'-deoxyguanosine
AKI	= acute kidney injury
BG	= blood glucose
CPB	= cardiopulmonary bypass
DM	= diabetes mellitus
EGCG	= epigallocatechin-3-gallate
HO-1	= hemoxygenase-1
HR	= heart rate
Kim-1	= kidney injury molecule-1
MAP	= mean arterial pressure
mRNA	= messenger RNA
NGAL	= neutrophil gelatinase-associated lipocalin
Pao ₂	= partial pressure of arterial oxygen
PAS	= periodic acid-Schiff
RT	= rectal temperature
T-24h	= 24 hours after termination of CPB
T-cpb	= 15 minutes after CPB initiation
T-pre	= time before CPB
T-post	= 1 hour after CPB

protective effects against AKI evoked by CPB has not been reported. In this study, we used a CPB model in diabetic rats to investigate the protective effects of pre-operative oral intake of EGCG against AKI associated with CPB.

Material and Methods

The animals in this study received humane care in compliance with the *Principles of Laboratory Animal Care* formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health, (www.nap.edu/catalog/5140.html).

Animals

The study used 32 male Goto-Kakizaki rats, a nonobese Wister substrain that develops type 2 DM early in life [19], aged 13 to 14 weeks and at 300 to 360 g body weight at the operation. The Goto-Kakizaki rats were purchased from Japan SLC Inc (Shizuoka, Japan).

Green Tea Polyphenol Solution

Pure form of EGCG extracted from green tea was provided from BioVerde Inc (Kyoto, Japan). An EGCG solution, at a concentration of 1 mmol/L, was made as drinking water.

Study Groups

The rats were randomly divided into the three experimental open-label groups: a sham group (n = 10), a CPB group (CPB alone, n = 11), and an EGCG group (CPB + EGCG, n = 11). Eleven rats were used in the CPB and EGCG groups because animal loss was expected. Although all rats in the sham group survived, 2 rats in the

CPB group and 1 rat in the EGCG group died of blood loss during the cannulation procedure. Therefore, the number of the sample is 10 in the sham group, 9 in the CPB group, and 10 in the EGCG group, unless otherwise stated. Rats of the EGCG group were administered the EGCG solution of 1 mmol/L as a daily fluid intake for 2 weeks before CPB, according to our protocol described previously [17]. Rats in the sham or CPB groups drank regular water during the same period (Fig 1).

Surgical Procedure

The animals were anesthetized with 3.0% isoflurane-mixed air inhalation with a vaporizer. The rats were intubated with a 16-gauge intravenous catheter (SURFLO ETFE I.V. Catheter; Terumo Corp, Tokyo, Japan) and mechanically ventilated under 30% of oxygen. Anesthesia was maintained with 1.5% to 2.0% isoflurane with an additional intraperitoneal administration of pentobarbital sodium (30 mg/kg) at CPB initiation.

CPB was performed using a modified surgical technique as described by Dong and colleagues [20]. The right femoral artery was cannulated with a 24-gauge SURFLO ETFE I.V. catheter to monitor systemic arterial pressure and to analyze arterial blood gas (epoc Blood Analysis System; Alere Medical Co, Tokyo, Japan). After systemic administration of heparin sodium (500 IU/kg), the left femoral artery was cannulated with a 24-gauge intravenous catheter as an arterial infusion line for the CPB circuit. A 17-gauge multiorificed angiocatheter (Happy Cath, 19-gauge inner diameter; Medikit Co, Tokyo, Japan) was introduced into the right internal jugular vein and advanced into the right atrium and inferior vena cava. The CPB circuit was primed with approximately 11 mL hydroxyethyl starches (Hespan; Fresenius Kabi Japan, Tokyo, Japan) solution with 0.2 mL heparin and 0.5 mL sodium bicarbonate 7% solution. Blood was pumped from a venous reservoir through a modified neonatal membrane oxygenator (MERA, Tokyo, Japan) using a tubing roller pump (model RP-VT; Furue Science Co, Tokyo, Japan).

Normothermic CPB with a flow of 100 mL/kg/min was performed for 30 minutes. During CPB, 100% oxygen gas was delivered to the oxygenator at 0.8 L/min. The target CPB flow rates (100 mL/kg/min) were based on other rat CPB studies and reported normal cardiac output of the rat [20, 21]. CPB was terminated after 30 minutes without using any vasoactive agents, and the animals were ventilated for another 60 minutes. The remaining priming solution was gradually infused and the mean arterial pressure was maintained at more than 60 mm Hg. In the sham group, the animals were cannulated and gradually infused with 11 mL of CPB priming solution without induction of CPB.

Arterial blood samples were collected just before CPB, 15 minutes after CPB initiation (T-cpb) and 1 hour after CPB. Hematocrit and partial pressure of arterial oxygen and carbon dioxide were measured. Systemic arterial pressure was monitored continuously during the experiments, and mean arterial pressure and heart rate were recorded. Rectal temperatures were continuously

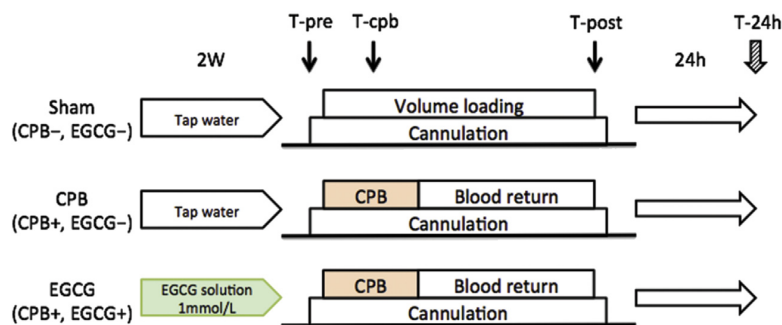


Fig 1. Study protocol. (CPB = cardiopulmonary bypass; EGCG = epigallocatechin-3-gallate; T-pre, T-cpb, and T-post = time for arterial blood sampling before, during, and 1 hour after CPB; T-24h = sacrifice and assessment at 24 hours after CPB.)

monitored and maintained at 37.0°C by an electric mattress pad and heat lamp placed for the animal and the CPB equipment.

Measurement of Serum Biochemical Parameters

At 24 hours after termination of CPB, blood samples were collected and centrifuged at 3,000 rpm for 20 minutes at 4°C, and serum was obtained. Urea nitrogen and creatinine were measured (SRL Inc, Tokyo, Japan). The concentration of serum neutrophil gelatinase-associated lipocalin (NGAL), a highly sensitive biomarker of AKI, was measured using the Rat NGAL ELISA Kit (BioPorto Diagnostics, Gentofte, Denmark) according to the manufacturer's protocol.

Histologic Examination

The kidneys were removed at 24 hours after CPB, fixed in 10% (w/v) neutral-buffered formalin, sectioned, stained with periodic acid-Schiff, and photographed using a digital microscope (BIOREVO BZ-9000; KEYENCE, Osaka, Japan). Renal injury was evaluated in a blind manner by a nephrologist. Tubular injury was defined as tubular dilatation, tubular vacuolization, cast deposition, loss of brush borders, and necrosis at the cortex. Injury was scored with a semiquantitative scale; 0 = no tubular injury; 1 = 10% or less; 2 = 10% to 25%; 3 = 26% to 50%; 4 = 51% to 75%; 5 = more than 75% of tubules injured at the cortex [22, 23].

Immunohistochemical Staining

Immunohistochemical staining of kidney injury molecule-1 (Kim-1), a highly specific and early biomarker for renal tubular injury, was performed on frozen sections using a Histofine Simple stain Rat Max PO (Nichirei Bioscience, Tokyo, Japan). The sections were blocked by Protein Block (DAKO, Tokyo, Japan) and labeled with a rat TIM-1 (also known as Kim-1) antigen affinity-purified polyclonal antibody (R&D Systems, Minneapolis, MN) at 4°C overnight. The sections were stained with horse-radish peroxidase and visualized with diaminobenzidine.

The level of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a major product of oxidative DNA modifications [24], which is widely used as a marker of oxidative stress against DNA [25, 26] was measured as follows: Renal tissues were

fixed overnight in Bouin's Solution (saturated picric acid-formaldehyde-acetic acid-H₂O ratio of 15:5:1:10). After deparaffinization of the specimens, 8-OHdG was stained by the avidin-biotin complex method (VECTASTAIN ABD-AP kit; Vector Laboratories Inc, Burlingame, CA). Mouse monoclonal antibody against 8-OHdG (Nikken SEIL, Shizuoka, Japan) was applied as a primary antibody. The level of 8-OHdG was quantified by the ratio of the number of 8-OHdG-staining renal tubular cells to all tubular cells under light microscopy (original magnification $\times 40$). At least three randomly selected areas were examined in each specimen, and the average of these counts was taken.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted from frozen tissues of renal cortex using an RNeasy mini kit (Qiagen, Hilden, Germany). The obtained RNA was reverse-transcribed into complementary DNA using Invitrogen SuperScript III First-Strand Synthesis SuperMix for quantitative real-time polymerase chain reaction (Life Technologies, Carlsbad, CA) according to the manufacturer's instruction. Gene-specific primers for rat Kim-1 (Taqman Gene Expression Assays, Rn00597703_m1), hemoxygenase-1 (HO-1), an ubiquitous enzyme upregulated in response to oxidant stress and inflammatory stimuli (Taqman, Rn01536932_m1), and glyceraldehyde-3-phosphate dehydrogenase (Taqman, Rn01775763_g1), a housekeeping gene used for normalization, were purchased from Applied Biosystems (Foster City, CA). Quantitative real-time polymerase chain reaction was performed by Applied Biosystems StepOnePlus Real Time PCR System, using Taqman Fast Advanced PCR Master Mix (Applied Biosystems). Glyceraldehyde-3-phosphate dehydrogenase transcript was used as an internal control.

Statistical Analysis

The values are presented as mean $\pm$ standard deviation. Statistical analyses were performed with GraphPad Prism 6.0 software (GraphPad Software Inc, La Jolla, CA). Physiologic data were assessed for any time and group effect by a two-way analysis of variance for main and interaction effects. Other intergroup comparisons were made with one-way analysis of variance. When results

Table 1. Physiologic and Blood Gas Measurements

Variable ^a	Pre-CPB			CPB			Post-CPB		
	Sham	CPB	EGCG	Sham	CPB	EGCG	Sham	CPB	EGCG
HR, beats/min	309 ± 55	293 ± 40	268 ± 39	336 ± 35	262 ± 28 ^b	272 ± 42 ^c	313 ± 40	280 ± 36	287 ± 34
MAP, mm Hg	104 ± 18	102 ± 14	92 ± 15	103 ± 21	83 ± 7 ^c	87 ± 14 ^d	90 ± 15	76 ± 10	85 ± 11
RT, °C	36.0 ± 0.7	36.2 ± 0.5	36.4 ± 0.8	36.4 ± 0.9	35.5 ± 1.0	34.8 ± 1.0	36.7 ± 0.4	36.5 ± 0.6	36.4 ± 0.3
BG, mg/dL	395 ± 58	406 ± 75	372 ± 39	551 ± 73	547 ± 76	472 ± 55 ^d	481 ± 53	520 ± 93	414 ± 86 ^e
PaO ₂ mm Hg	155 ± 23	136 ± 21	138 ± 29	156 ± 17	365 ± 126 ^f	317 ± 173 ^f	147 ± 21	127 ± 15	133 ± 18
Hematocrit, %	38.2 ± 3.4	38.9 ± 2.2	38.8 ± 2.7	34.1 ± 5.3	20.8 ± 2.0 ^f	19.7 ± 1.3 ^f	31.1 ± 2.1	26.8 ± 3.0 ^c	26.6 ± 2.3 ^c

^a All values are mean ± standard deviation. ^b $p < 0.001$ vs sham. ^c $p < 0.01$ vs sham. ^d $p < 0.05$ vs sham. ^e $p < 0.01$ vs CPB. ^f $p < 0.0001$ vs sham.

BG = blood glucose; CPB = cardiopulmonary bypass; EGCG = epigallocatechin-3-gallate; HR = heart rate; MAP = mean arterial pressure; PaO₂ = partial pressure of arterial oxygen; RT = rectal temperature.

were significant by analysis of variance, differences between individual groups were estimated with Tukey post hoc test. Statistical significance was assumed when the p value was less than 0.05.

Results

Physiologic Variables and Blood Gas Analyses During CPB

Table 1 reports the physiologic variables and blood gas analyses throughout the study period. There were no significant differences in all baseline variables among the three groups. Mean arterial pressure, heart rate, and rectal temperatures remained stable throughout the experiments in the sham group but were significantly lower in the CPB and EGCG groups at T-cpb. In every rat of the CPB and EGCG groups, partial pressure of arterial oxygen at T-cpb was significantly higher than that of the sham group. This fact indicates that CPB was successfully established in each animal. In the sham group, there was no significant change in partial pressure of arterial oxygen during the experimental period. The hematocrit during and after CPB significantly decreased in the CPB and EGCG groups compared with the sham group. The blood glucose values in the EGCG group were

significantly lower than the other two groups at T-cpb or 1 hour after CPB. The difference in blood glucose between the sham and CPB groups was not significant.

Renal Function After CPB

Compared with sham rats, serum creatinine level at 24 hours after termination of CPB was significantly elevated in CPB rats (0.20 ± 0.02 vs 0.25 ± 0.05 mg/dL, $p = 0.0016$; Fig 2A). The serum creatinine level in the EGCG group was lower than that in the CPB group (0.22 ± 0.02 vs 0.25 ± 0.05 mg/mL, $p = 0.048$). These results indicate that EGCG pretreatment ameliorated renal dysfunction associated with CPB. The difference in creatinine levels between the sham and EGCG groups was not significant.

Assessment for Extent of AKI After CPB

Serum NGAL level and messenger (m)RNA expression of Kim-1 in renal tissues are shown in Figure 2B and C, respectively. NGAL is a highly sensitive biomarker for early detection of AKI; however, the level of NGAL also increases by other causes such as inflammation or infection. On the other hand, Kim-1 is a highly specific biomarker of renal tubular injury.

The CPB group had higher serum NGAL levels than the sham group (2.35 ± 2.68 vs 0.23 ± 0.13 pg/mL,

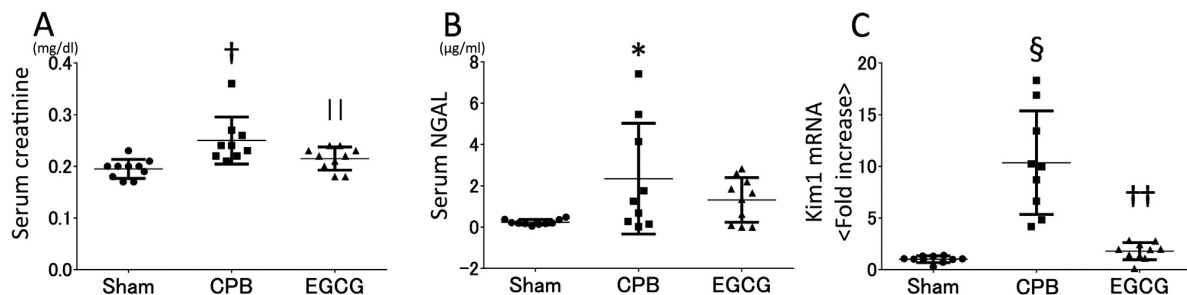


Fig 2. Biochemical measurements and markers of acute kidney injury. (A) Serum creatinine. (B) Serum neutrophil gelatinase-associated lipocalin (NGAL). (C) Messenger RNA (mRNA) expression of kidney injury molecule-1 (Kim-1) in renal tissues. All values are means ± standard deviation. * $p < 0.05$ vs sham, † $p < 0.01$ vs sham, § $p < 0.0001$ vs sham; || $p < 0.05$ vs CPB, †† $p < 0.0001$ vs CPB. (CPB = cardiopulmonary bypass; EGCG = epigallocatechin-3-gallate.)

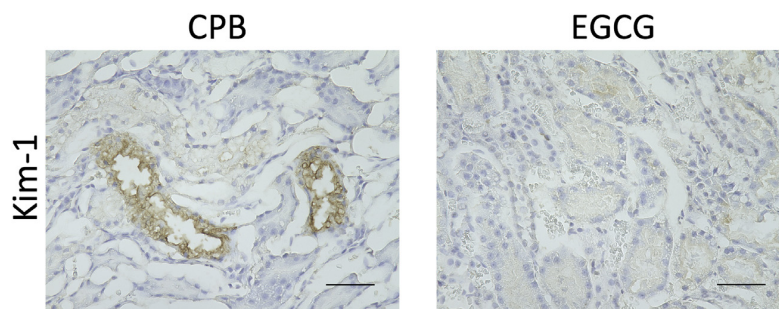


Fig 3. Immunohistologic findings of kidney injury molecule-1 (Kim-1) for cardiopulmonary bypass (CPB) and epigallocatechin-3-gallate (EGCG) groups. Scale bar, 50 μ m.

$p = 0.023$). The difference in NGAL levels between the CPB and EGCG groups was not significant.

The CPB group had significantly higher Kim-1 mRNA expression compared with the sham group (10.4 ± 5.0 -fold vs 1.0 ± 0.3 -fold increase, $p < 0.0001$), whereas EGCG treatment significantly attenuated CPB-induced renal overexpression of Kim-1 (1.8 ± 0.8 -fold vs 10.4 ± 5.0 -fold increase, $p < 0.0001$). Immunohistochemistry for Kim-1 showed diffuse staining in the proximal renal tubules of the CPB group, less staining in the EGCG group (Fig 3), and no staining in the sham group. These results indicate that pretreatment with EGCG attenuated AKI after CPB.

Histologic Assessment for Renal Tubular Damage After CPB

The extent of renal tubular damage assessed by histologic analyses is shown in Figure 4. Severe sublethal tubular injury, indicated by swelling, loss of brush border, or diffuse intracytoplasmic vacuolization, was observed in the CPB group. Semiquantitative assessment of renal tubular damage demonstrated a significant increase in tubular injury scores in CPB rats compared with sham rats (2.33 ± 0.87 vs 0.3 ± 0.48 , $p < 0.0001$; Fig 5A). Compared with CPB rats, EGCG rats exhibited statistically milder renal injury with focal tubular vacuolization (2.33 ± 0.87 vs 0.9 ± 0.74 , $p = 0.005$). These results indicate that EGCG rescued kidneys from sublethal renal tubular damage related to CPB. Obvious acute tubular necrosis was not seen in any section.

Oxidative Stress Level at Kidneys After CPB

Representative renal cortex images of 8-OHdG immunohistochemical staining are shown in Figure 6. Darkly stained 8-OHdG-positive nuclei were obviously fewer in the sham or EGCG groups compared with the CPB group. As shown in Figure 5B, the ratio of 8-OHdG-stained cells was significantly higher in the CPB group than in the sham group (0.47 ± 0.11 vs 0.15 ± 0.09 ; CPB: $n = 6$, sham: $n = 4$; $p = 0.0005$). This ratio was also significantly higher in the CPB group than the ratio in the EGCG group (0.47 ± 0.11 vs 0.28 ± 0.08 ; CPB: $n = 6$, EGCG: $n = 6$; $p = 0.0097$). The difference between the sham and EGCG groups was not significant. These results suggest that the renoprotective effects of EGCG are mediated by antioxidative properties.

Real-time polymerase chain reaction examination showed that HO-1 mRNA expression was elevated in the CPB group compared with the sham group (2.39 ± 0.87 vs 1.00 ± 0.57 , $p = 0.0002$). HO-1 mRNA expression level was lower in the EGCG group than in the CPB group (1.59 ± 0.4 vs 2.39 ± 0.87 , $p = 0.032$; Fig 5C).

Comment

A number of studies have addressed the protective effects of EGCG in variety of animal models [18, 19, 27]. Several studies have reported that EGCG supplementation significantly reduces renal damage both in subacute and chronic phases. However, no report is available on the renoprotective effect of EGCG after CPB in a rodent

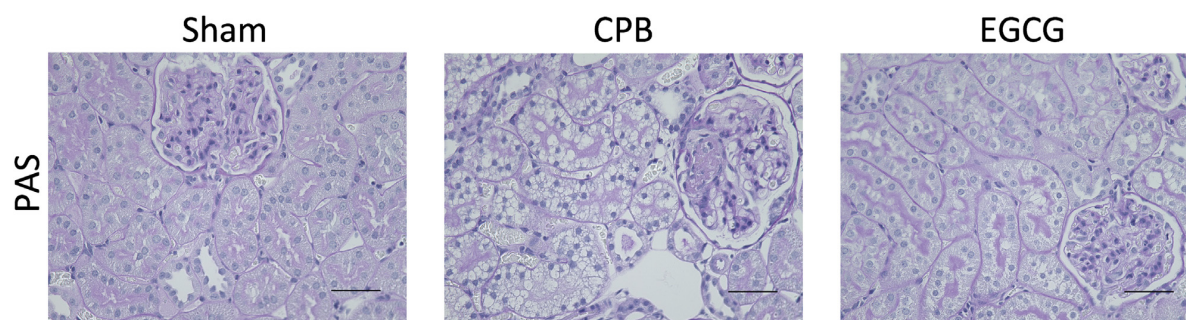


Fig 4. Periodic acid-Schiff (PAS) staining for sham, for cardiopulmonary bypass (CPB), and epigallocatechin-3-gallate (EGCG) groups. Scale bar, 50 μ m.

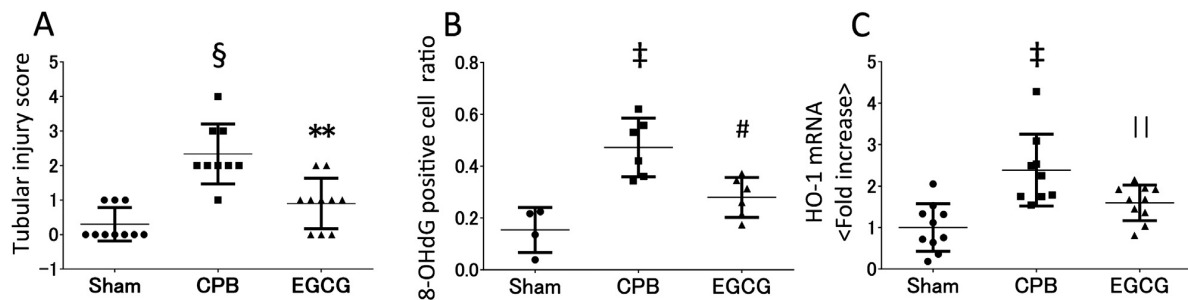


Fig 5. Histologic analysis and oxidative stress in sham, cardiopulmonary bypass (CPB), and epigallocatechin-3-gallate (EGCG) groups. (A) Tubular injury score, 0 = no tubular injury; 1 = less than 10%; 2 = 10% to 25%; 3 = 26% to 50%; 4 = 51% to 75%; 5 = more than 75% of tubules injured. (B) Staining ratio for 8-hydroxy-2'-deoxyguanosine (8-OHdG) showing the number of the 8-OHdG-stained cells/total tubular cells. (C) Messenger RNA (mRNA) expression of hemoxygenase-1 (HO-1) in renal tissues. All values are means $\pm$ standard deviation. § p < 0.0001 vs sham; || p < 0.05 vs CPB; # p < 0.01 vs CPB; ** p < 0.001 vs CPB.

model. In the present study, we demonstrated that 30 minutes of CPB induced renal damage and that oral pretreatment with EGCG attenuated the renal damage after CPB in diabetic rats. The findings of 8-OHdG immunohistochemical staining suggest that the antioxidative properties of EGCG are involved in this effect. Sahin and colleagues [27] proposed that HO-1, which is a nuclear factor erythroid 2-related factor 2-mediated antioxidative enzyme produced in response to oxidant stress or inflammatory stimuli, plays a major role in protecting the kidney. In the present study, the level of HO-1 mRNA expression in the CPB group was significantly higher than the level in the EGCG group. Our results suggest that EGCG might reduce the oxidative stress itself, which causes the renoprotective effect other than the HO-1-mediated mechanism in the acute phase of this CPB model. We need further investigation of the mechanisms of antioxidant effects by EGCG.

In our study, to assess the effect of CPB itself on renal function, we avoided blood transfusion, excessive hemodilution, or use of any vasoconstrictor. Our simple strict protocol of using short CPB circuits with a small priming volume made it difficult to maintain stable hemodynamic conditions at high flow rates for an extended duration of CPB. In the preliminary experiments of CPB using Wister rats, we found that 30 minutes of CPB was adequate

to obtain acceptable survival and reproducible results but was insufficient to induce significant renal injury in normal rats. Therefore, we hypothesized that disease model rats would be more susceptible to the stress of CPB and selected the multigenetic type 2 DM rats, Goto-Kakizaki rats, as the study subject. The 30-minute CPB on DM rats successfully induced typical and obvious renal pathologic injuries, with an excellent survival rate.

We recognize that our study has several limitations. A previous study from our group showed that oral pretreatment with 1 mmol/L EGCG had better outcomes on cardioprotective effects compared with 0.1 mmol/L or 10 mmol/L EGCG in a rat cardiac ischemia/reperfusion injury model [18]. According to these results, we selected the 2-week oral protocol with 1 mmol/L EGCG in this study. We require further investigations to determine the optimal dose for renal tissues. For a clinical application of this prophylactic therapy, we also need to establish a regimen of oral administration of EGCG considering several reported clinical studies showing the safety and pharmacokinetics of green tea polyphenols with long-term oral administration of EGCG [28].

We were able to indicate a renoprotective effect and an antioxidative effect of EGCG using this CPB model on DM rats. However, to clarify the detailed mechanisms of EGCG on CPB, we need to assess the time effect of CPB

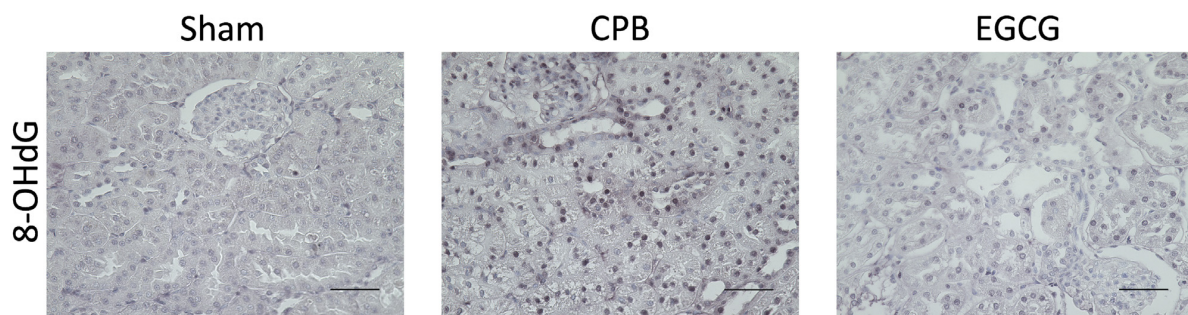


Fig 6. Immunohistological findings of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in sham, cardiopulmonary bypass (CPB) and epigallocatechin-3-gallate (EGCG) groups. Scale bar, 50 μ m.

on renal function and the recovery process from renal damage as well as the influences of EGCG on them. We also need to evaluate whether the effect of EGCG in nondiabetic rats is similar to that in diabetic rats. Along with developments of devices and technical improvements, the CPB model for more than 90 minutes with low morbidity has become possible. Preparations for further studies taking into account the influence of the CPB time and recovery process are in progress.

In summary, oral pretreatment with EGCG attenuated kidney injury after our CPB model using diabetic rats. Suppressive mechanisms for oxidative stress may be involved in the renoprotective effects of EGCG. These findings demonstrate the potential utility of EGCG as a therapeutic agent and provide a convenient and patient-friendly method for the prevention of postoperative AKI in high-risk cardiac surgical patients with DM.

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