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].

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

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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 preoperative 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 administrated 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 (Hespander; 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