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Basic Science

Acute Dapagliflozin Administration Ameliorates Cardiac Surgery-Associated Acute Kidney Injury in a Rabbit Model

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Background: Several studies have shown that sodium-glucose cotransporter-2 inhibitors have a renoprotective effect on acute kidney injury (AKI), but their effect on cardiac surgery-associated AKI is unknown.

Methods and Results: AKI was induced in 25 rabbits without diabetes mellitus by cardiopulmonary bypass (CPB) for 2 h and they were divided into 5 groups: sham; dapagliflozin-treated sham; CPB; dapagliflozin-treated CPB; and furosemide-treated CPB (n=5 in each group). Dapagliflozin was administered via the femoral vein before initiating CPB. Kidney tissue and urine and blood samples were collected after the surgical procedure. There were no differences in the hemodynamic variables of each group. Dapagliflozin reduced serum creatinine and blood urea nitrogen concentrations, and increased overall urine output (all P<0.05). Hematoxylin and eosin staining showed that the tubular injury score was improved after dapagliflozin administration (P<0.01). Dapagliflozin administration mitigated reactive oxygen species and kidney injury molecule-1 as assessed by immunohistochemistry (both P<0.0001). Protein expression analysis showed improvement of inflammatory cytokines and apoptosis, and antioxidant enzyme expression was elevated (all P<0.05) through activation of the nuclear factor erythroid 2-related factor 2 pathway (P<0.01) by dapagliflozin.

Conclusions: Acute intravenous administration of dapagliflozin protects against CPB-induced AKI. Dapagliflozin may have direct renoprotective effects in renal tubular cells.

Key Words: Acute kidney injury; Cardiopulmonary bypass; Dapagliflozin; Rabbit model; Reactive oxygen species

Acute kidney injury (AKI) is a common complication after cardiac surgery (post-cardiac surgery-associated AKI: CSA-AKI) and is associated with longer stays in intensive care unit and hospital, with a high risk of death (≤30%).[1–4] A recent systematic review and meta-analysis reported a composite incidence of 22.3% for AKI in adult patients with cardiac surgery.[5] Patients who survive remain at risk of premature chronic kidney disease (CKD), even if renal function initially recovers.[6] Therefore, reducing the risk of CSA-AKI can greatly reduce mortality, morbidity, and healthcare costs, and prevent end-stage kidney disease. However, besides hemodialysis therapy, there are few effective preventive methods, so new targets or better treatment options to prevent CSA-AKI are urgently required.

CSA-AKI differs from AKI in the general population because of a unique factor, cardiopulmonary bypass (CPB). One of the most important factors related to CPB is an exaggerated inflammatory reaction through overproduction of cytokines, which causes an unregulated host response (similar to that observed during sepsis). During CPB, a sepsis-like syndrome is typically observed from the first few hours after initiation because of exposure of blood to the endogenous surface of the extracorporeal circuit,[7] which activates the complement system with release of C3a and C5a. Activated complement factors induce the production of pro-inflammatory cytokines (interleukin-1β [IL-1β] and tissue necrosis factor-α [TNFα]), and the high amount of reactive oxygen species (ROS) reduce endogenous antioxidants, which results in tubular cell death.[8]

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Sodium-glucose contransporter-2 (SGLT2) inhibitors are used to treat diabetes mellitus (DM) by inhibiting proximal tubular SGLT2, thereby reducing the reabsorption of sodium and glucose.[9] In addition to the effect of SGLT2 inhibitors on glycemic control, recent large-scale clinical studies have shown that they lower blood pressure, improve glucose toxicity, and induce hemodynamic effects in patients with type 2 DM, all of which improve cardiovascular and renal outcomes.[10–12] Moreover, a large-scale clinical trial showed that the renoprotective effect of SGLT2 inhibitors extended to patients with CKD without DM.[13,14] SGLT2 inhibitors reduce the odds of developing AKI, with and without hospitalization, in both randomized trials and real-world settings.[15] However, the mechanism of SGLT2 inhibitors in preventing AKI is not fully understood. In several animal studies, dapagliflozin administration attenuated both ischemia-reperfusion and sepsis-induced AKI.[16,17] Chi et al suggested that dapagliflozin inhibits inflammatory cytokines and ROS production, and activates nuclear factor erythroid 2-related factor 2 (Nrf2) and cytoprotective enzymes, such as NAD(P)H quinone dehydrogenase 1 (NQO1) and heme oxygenase 1.[17] In these studies, dapagliflozin appears to exert a protective effect beyond blood sugar control, possibly against oxidative stress and inflammation. However, most of the beneficial effects could be related to persistent molecular and metabolic changes because dapagliflozin treatment is given for a few days before starting experiments. To the best of our knowledge, the acute renoprotective effects of dapagliflozin administration have not been investigated, so we aimed to examine the acute renoprotective potential effects of dapagliflozin administration on CSA-AKI in non-DM rabbits.

Methods

Rabbits and Study Design

Rabbits were treated in accordance with the guiding principles for the care and use of animals during experiments. The Animals Care Committee of the Kyushu University School of Medicine approved the experimental and animal care protocols (approval number: A23-135-0, approved 15 December 2022). The 25 Japanese white rabbits (12–16 weeks, male sex, mean weight: 3.0±0.1 kg) used in this study were divided into 5 groups: (1) sham (n=5); (2) dapagliflozin-treated sham (Sham+Dapa; n=5); (3) CPB (n=5); (4) dapagliflozin-treated CPB (CPB+Dapa; n=5), and furosemide-treated CPB (CPB+F; n=5). In the CPB, CPB+Dapa, and CPB+F groups, a donor rabbit was used for the blood donation for each rabbit. The sham group did not undergo cannulation or CPB but underwent 24 h of anesthesia. The Sham+Dapa and CPB+Dapa groups were administered dapagliflozin (MedChemExpress, Monmouth Junction, NJ, USA) dissolved with 1% dimethyl sulfoxide, and 0.5 mg/kg of dapagliflozin was administered via the right femoral vein before initiating CPB. The CPB+F group was administered furosemide via the right femoral vein before initiating CPB and after weaning from CPB. The sham and CPB groups received vehicle (1% dimethyl sulfoxide). The rabbits were killed humanely at 24 h after the surgical procedure, and blood and urine samples and kidney tissue were collected (**Figure 1A**).

Anesthesia and Monitoring

At the beginning of the experiments, anesthesia was induced with intramuscular ketamine (50 mg/kg) and inhalation of 3% isoflurane. The anesthetized rabbits were placed on a heating pad to maintain body temperature between 37°C and 38°C. The right femoral artery was cannulated for arterial pressure monitoring and taking blood samples, and the right femoral vein was cannulated for central venous access. A urinary catheter was surgically placed in the bladder through a midline laparotomy in the lower abdomen to measure the urine volume. After tracheostomy, the rabbits were mechanically ventilated with volume-controlled ventilation. The following ventilator settings were used: tidal volume of 8–10 mL/kg; positive end-expiratory pressure of 4 cmH_2O; respiratory rate of 40–50 breaths/min adjusted to maintain the partial pressure of carbon dioxide ([$PaCO_2$] 35–45 mmHg); and the fraction of inspired oxygen was set to maintain partial pressure of oxygen ([PaO_2] 200–300 mmHg) values of arterial blood gas samples drawn from the right femoral artery. Anesthesia was maintained with a continuous inhalation of 2% isoflurane, and infusion of fentanyl (20 μg/kg/h) and rocuronium (1 mg/kg/h). Sodium bicarbonate was administered at a rate of 10 mL/kg/h during the experiment. Arterial blood gases were monitored using the ABL80 Flex blood gas analyzer (Radiometer, CA, USA). Hemodynamic parameters, such as mean arterial pressure, heart rate, CPB flow rate, electrocardiography, and rectal temperature, were monitored continuously using a computer equipped with a data acquisition system (PowerLab and LabChart; ADInstruments, Colorado Springs, CO, USA).

CPB Circuit

The CPB circuit consisted of a mechanical heart-lung machine (HAS-P100; Senko Ikakogyo, Japan), a membrane oxygenator (Capiox Baby FX; Terumo Corporation, Japan), an air oxygen mix (SECHRIST 3500; Parker Healthcare Pty. Ltd., Australia), a heater-cooler system (HHC-51; Senko Ikakogyo), and 0.25-inch tubing. All donor rabbits were weighed and their skin was prepared. After anesthesia, median sternotomy was performed, and an 18G arterial catheter was inserted into the left ventricle. A blood sample of approximately 100−120 mL was collected 30 min before surgery. After donating blood, the rabbits were killed with an injection of 5 mL of 15% potassium chloride. The circuit was primed with 85 mL of fresh whole blood from a donor rabbit and 1,000 IU heparin.

Operative Technique and Experimental Protocol

A heparin bolus of 1,000 IU was administered, and CPB was initiated once the activated coagulation time was >480 s. Cervical cannulation for CPB was performed using a 16G arterial catheter placed in the innominate artery and an 8Fr venous cannula placed in the right external jugular vein (**Figure 1B**). During CPB, target CPB flow rates were 70–80 mL/kg/min, and sweep gas and circuit blood flow rates were refined according to $PaCO_2$ (35–45 mmHg) and PaO_2 (200–300 mmHg) values. Additional donor blood was added to the rabbits when required to achieve a target hematocrit >28%, and mean arterial pressure was maintained between 60 and 80 mmHg. The CPB was stopped after 2 h and the cannulas were removed. Ventilation was set to the pre-CPB settings, and if necessary, oxygen and breathing rates were set according to the blood gas values.

Serum Biochemistry Assessment

At 24 h after the experiment, the rabbits were killed and blood samples were collected. Serum was separated by

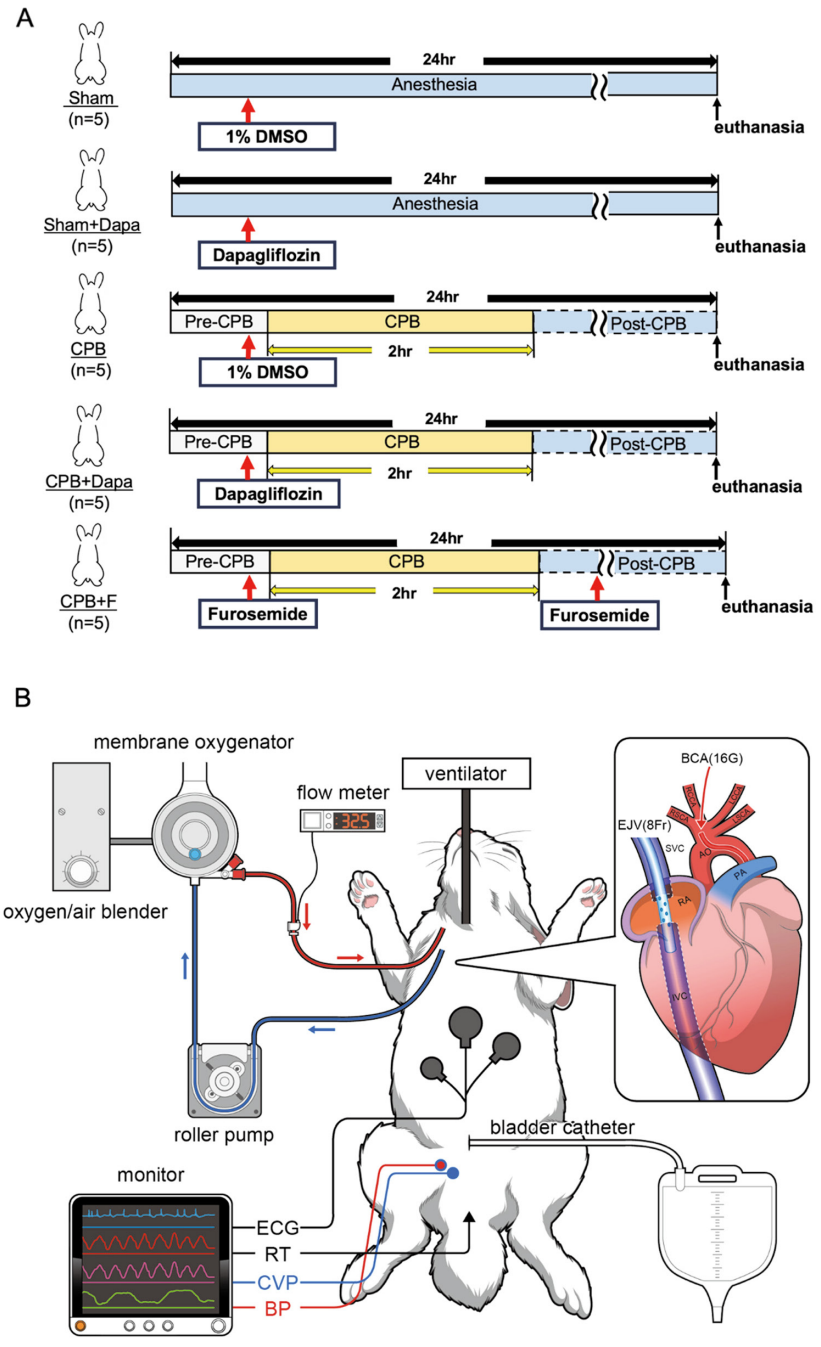

Figure 1. Schematic diagrams of the experimental protocol (**A**) and rabbit CPB model (**B**). Ao, aorta; BCA, brachiocephalic artery; BP, blood pressure; CPB, cardiopulmonary bypass; CVP, central venous pressure; Dapa, dapagliflozin; DMSO, dimethyl sulfoxide; ECG, electrocardiography; EJV, external jugular vein; F, furosemide; LCCA, left common carotid artery; LSCA, left subclavian artery; PA, pulmonary artery; RA, right atrium; RCCA, right common carotid artery; RSCA, right subclavian artery; RT, rectal temperature; SVC, superior vena cava.

centrifugation at 880 g for 10 min and stored at −80°C. A portion of the serum sample was used to measure blood urea nitrogen (BUN) and creatinine concentrations.

Histopathological Analysis

After the rabbits were killed, the kidneys were immediately removed, tissue samples were fixed in 10% formaldehyde neutral buffer solution, and then dehydrated and embedded in paraffin. Sections were stained with hematoxylin and eosin, and examined under an all-in-one fluorescence microscope (BZ-X700; Keyence, Japan); 10 random fields from each rabbit were assessed in a blinded fashion. Tubular injury was defined as intraluminal cast formation, blebbing of the apical membrane, vacuolization, and tubular dilatation. Tubular injury was evaluated and scored as follows: 0 points=0%; 1 point=1–20%; 2 points=21–40%; 3 points=41–60%; 4 points=61–80%, and 5 points=81–100%.[18] Semiquantitative measurements were performed using Image J picture analysis software, version 1.53 (National Institute of Health, Bethesda, MD, USA).

Western Blot Analysis

Western blot analysis was performed to investigate Bcl-2-associated X (Bax), B-cell lymphoma-2 (Bcl2), TNFα,

Table. Intraoperative Variables in 4 Experimental Groups (A): Sham, Sham+Dapa, CPB, and CPB+Dapa and (B): Sham, CPB, CPB+F, and CPB+Dapa

(A) Variables	Sham	Sham+Dapa	CPB	CPB+Dapa
BW (kg)	2.9±0.2	3.0±0.1	3.0±0.2	3.0±0.1
BT (°C)	38.3±0.3	38.4±0.5	38.1±0.5	38.0±0.4
HR (beats/min)	244.0±34.4	233.8±15.4	236.1±24.8	235.4±24.4
MAP (mmHg)	74.5±4.7	73.3±5.2	78.7±2.1	79.4±4.3
PaO_2 (mmHg)	267.4±26.1	251.0±23.5	289.6±13.8	284.5±21.9
$PaCO_2$ (mmHg)	37.0±1.6	42.0±5.5	40.0±2.3	39.1±2.6
Ht (%)	31.6±1.3	31.5±1.6	30.8±1.2	31.9±1.8
Hb (mg/dL)	10.2±0.5	10.2±0.5	9.9±0.4	10.3±0.6
Blood glucose (mg/dL)	114.9±11.6	122.8±16.8	180.3±11.7	140.7±43.7
CPB flow (mL/kg/min)	N/A	N/A	77.3±7.6	74.4±3.3
(B) Variables	**Sham**	**CPB**	**CPB+F**	**CPB+Dapa**
BW (kg)	2.9±0.2	3.0±0.2	3.1±0.3	3.0±0.1
BT (°C)	38.3±0.3	38.1±0.5	38.1±0.6	38.0±0.4
HR (beats/min)	244.0±34.4	236.1±24.8	232.5±15.5	235.4±24.4
MAP (mmHg)	74.5±4.7	78.7±2.1	76.3±5.6	79.4±4.3
PaO_2 (mmHg)	267.4±26.1	289.6±13.8	262.3±16.3	284.5±21.9
$PaCO_2$ (mmHg)	37.0±1.6	40.0±2.3	41.0±1.0	39.1±2.6
Ht (%)	31.6±1.3	30.8±1.2	33.6±0.6	31.9±1.8
Hb (mg/dL)	10.2±0.5	9.9±0.4	10.9±0.2	10.3±0.6
Blood glucose (mg/dL)	114.9±11.6	180.3±11.7	140.5±7.0	140.7±43.7
CPB flow (mL/kg/min)	N/A	77.3±7.6	72.5±5.3	74.4±3.3

Values are mean±standard deviation. BT, body temperature; BW, body weight; CPB, cardiopulmonary bypass; Dapa, dapagliflozin; F, furosemide; Hb, hemoglobin; HR, heart rate; Ht, hematocrit; MAP, mean arterial pressure; N/A, not applicable.

IL-1β, NQO1, and Nrf2 protein levels. Tissue samples were homogenized in lysis buffer (PRO-PREP, iNtRON, Inc., Korea) and the homogenate was centrifuged at 14,000 g for 15 min at 4°C. The supernatant was collected and then centrifuged at 14,000 g for 10 min at 4°C. Assays to determine the protein concentrations of the samples were performed by comparing the results with a known concentration of bovine serum albumin using a BCA Protein Assay Kit (Takara Bio Inc., Japan). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed on a 12% polyacrylamide gel under no-reducing conditions. Briefly, protein samples were boiled at 95°C in 5% ß-mercaptoethanol and 2.5% sodium dodecyl sulfate, and lysates equivalent to 50 μg of protein from each sample were run on the gel for 30 min at 20 mA. Proteins on the gel were transferred to a polyvinylidene fluoride membrane using the Trans-Blot Turbo Transfer System (Bio-Rad Laboratories, USA). The membrane was placed in 5% powdered milk in Tris-buffered saline containing 0.05% Tween-20 (TBST) at room temperature for 1 h. The membrane was then incubated overnight with the following primary antibodies at 4°C: Bax (bs-0127R, 1:200; Bioss, Inc., Boston, MA, USA) and Bcl2 (sc-7382, 1:1,000), NQO1 (sc-32793, 1:200), Nrf2 (sc-365949, 1:200), TNFα (sc-12744, 1:100), IL-1β (sc-12742, 1:200), and β-actin (sc-81178, 1:1,000) (Santa Cruz Biotechnology, Inc., CA, USA). After washing in TBST, the membrane was incubated with horseradish peroxidase-conjugated anti-mouse immunoglobulin (IgG) (A90-105P, 1:5,000; Bethyl Laboratories, Inc., Montgomery, TX, USA), horseradish peroxidase-conjugated anti-rabbit IgG (bs-0295G-HRP, 1:5,000; Bioss), or horseradish peroxidase-conjugated anti-Armenian hamster IgG (sc-2789, 1:5,000; Santa Cruz Biotechnology), as appropriate, for 30 min at room temperature. Blotted bands were visualized with the Clarity Western ECL Substrate (Bio-Rad Laboratories) and detected using the ImageQuant™ LAS 400 Imaging System (Fujifilm Holding Corporation, Japan). Western blot images were quantified by plotting a 2-dimensional densitogram using Image J software, version 1.53. ß-actin was used as an internal control.

Immunohistochemistry

We performed immunohistochemical analysis to investigate differences in the expression of kidney injury molecule-1 (Kim-1), 8-hydroxy-2'-deoxy-guanosine (8-OHdG), and C3a receptor (C3aR). After euthanasia of the rabbits, kidney sections were immediately fixed in 10% formaldehyde neutral buffer solution overnight at 4°C, then washed in 0.1 M phosphate-buffered saline and cryopreserved by sequentially incubating in 10%, 15%, and 20% sucrose at 4°C until the tissues sank to the bottom of the tube. Kidney pieces were then embedded in optimal cutting temperature compound (Sakura Finetek, Japan) and frozen on dry ice. Each piece was then cryosectioned into 10-μm-thick sections (CryoStar NX70 Cryostat; Thermo Fisher Scientific, Japan) and dried at room temperature for 1 h. Subsequently, the sections were incubated in 10% methanol and 0.3% H_2O_2 for 15 min to quench endogenous peroxidase activity, and then blocked in Blocking One Histo (Nacalai Tesque, Japan) for 10 min at room temperature. The blocked sections were incubated with primary antibodies for Kim-1 (sc-518008, 1:200), 8-OHdG (sc-393871, 1:200), and C3aR (sc-133172, 1:200) (Santa Cruz Biotechnology) in TBST at 4°C overnight. After washing, the sections were incubated for 1 h with biotinylated anti-mouse IgG (PK-6102; Vector Laboratories) at a 1:200 dilution in 5%

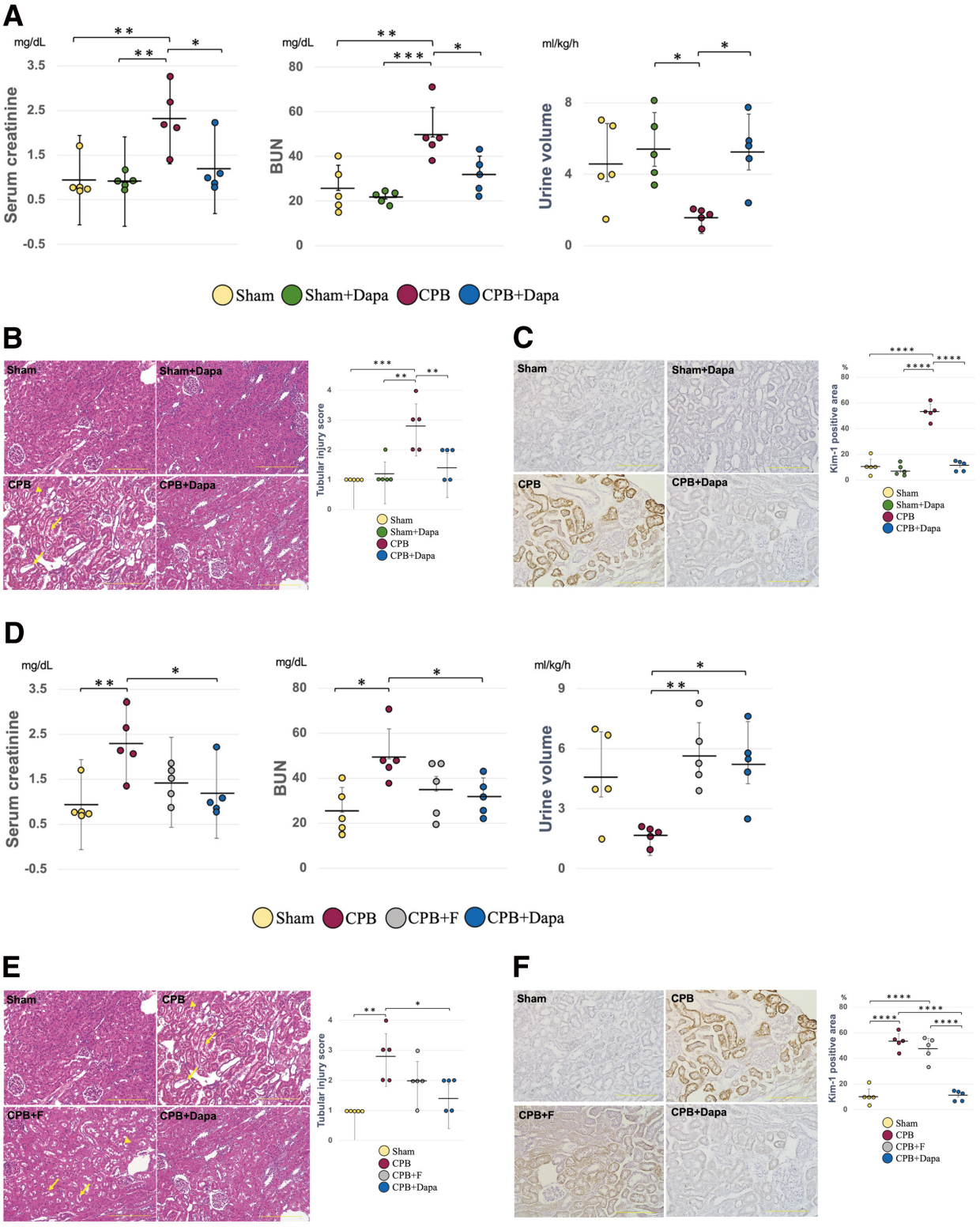

Figure 2. Effects of dapagliflozin on renal function. (**A**) Dapagliflozin pretreatment reduced BUN and serum creatinine concentrations and increased the time of diuresis in rabbits with CPB. (**B**,**E**) Images of kidney sections of the 4 groups (H&E) and semiquantitative analysis of the renal tubular injury score. The yellow arrows indicate blebbing and tubular dilation, and the yellow arrowhead indicates cast formation. (**C**,**F**) Kim-1 immunohistochemical staining in the 4 groups and semiquantitative evaluation of Kim-1-positive areas. (**D**) Furosemide administration showed no significant improvement in BUN and serum creatinine concentrations in rabbits with CPB. Data are shown as the mean and standard deviation (error bars). Scale bars=200 μm. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. BUN, blood urea nitrogen; CPB, cardiopulmonary bypass; Dapa, dapagliflozin; F, furosemide; Kim-1, kidney injury molecule-1.

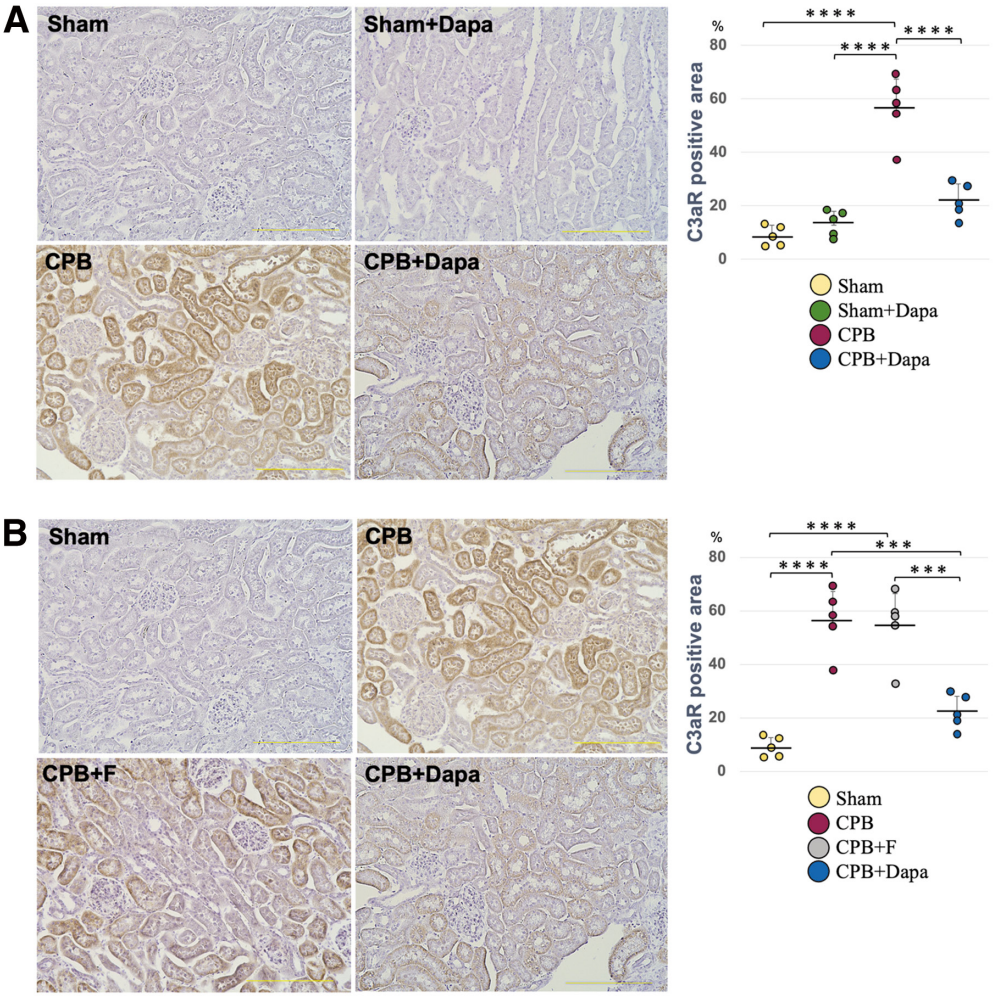

Figure 3. C3aR immunohistochemical staining and semiquantitative evaluation of C3aR-positive areas. Data are shown as the mean and standard deviation (error bars). Scale bars=200 μm. ***P<0.001; ****P<0.0001. CPB, cardiopulmonary bypass; C3aR, C3a receptor; Dapa, dapagliflozin; F, furosemide.

Blocking One Histo. The samples were then incubated with a Streptavidin Biotin Complex Peroxidase Kit (Nacalai Tesque) and colorized with diaminobenzidine/H_2O_2 solution. After staining, the slides were immersed in distilled water and then counterstained with hematoxylin to stain the nuclei blue for better visualization. The areas stained for Kim-1 and 8-OHdG, as percentages of the total area in 10 different fields of each section, were determined automatically using BZ-X Analyzer software (BZ-H3A; Keyence).

Statistical Analysis

Data are reported as the mean±standard deviation. Multiple comparisons between groups were performed using one-way analysis of variance with posthoc Tukey's multiple comparison tests. P values <0.05 were considered statistically significant. Statistical analysis was carried out using JMP version 16 (SAS institute Inc., Cary, NC, USA).

Results

Intraoperative Variables

Intraoperative variables are listed in **Table**. There were no differences in the hemodynamic variables PaO_2, $PaCO_2$, hematocrit, hemoglobin, or CPB flow among the groups.

Effects of Dapagliflozin on Renal Function and Histology

BUN and serum creatinine concentrations in the CPB group were significantly higher 24 h after CPB than in the sham group (both P<0.01). Dapagliflozin reduced the BUN and serum creatinine concentrations relative to the CPB group (both P<0.05). The urine volume/h in the CPB group was significantly lower than that in the sham group (P<0.05), and dapagliflozin increased total urine output in the CPB group (**Figure 2A**). Histological evaluation showed intraluminal cast formation, blebbing of the apical membrane, and tubular dilatation in the kidneys from the CPB group. Pretreatment with dapagliflozin attenuated renal tubular injury compared with the CPB group (P<0.01)

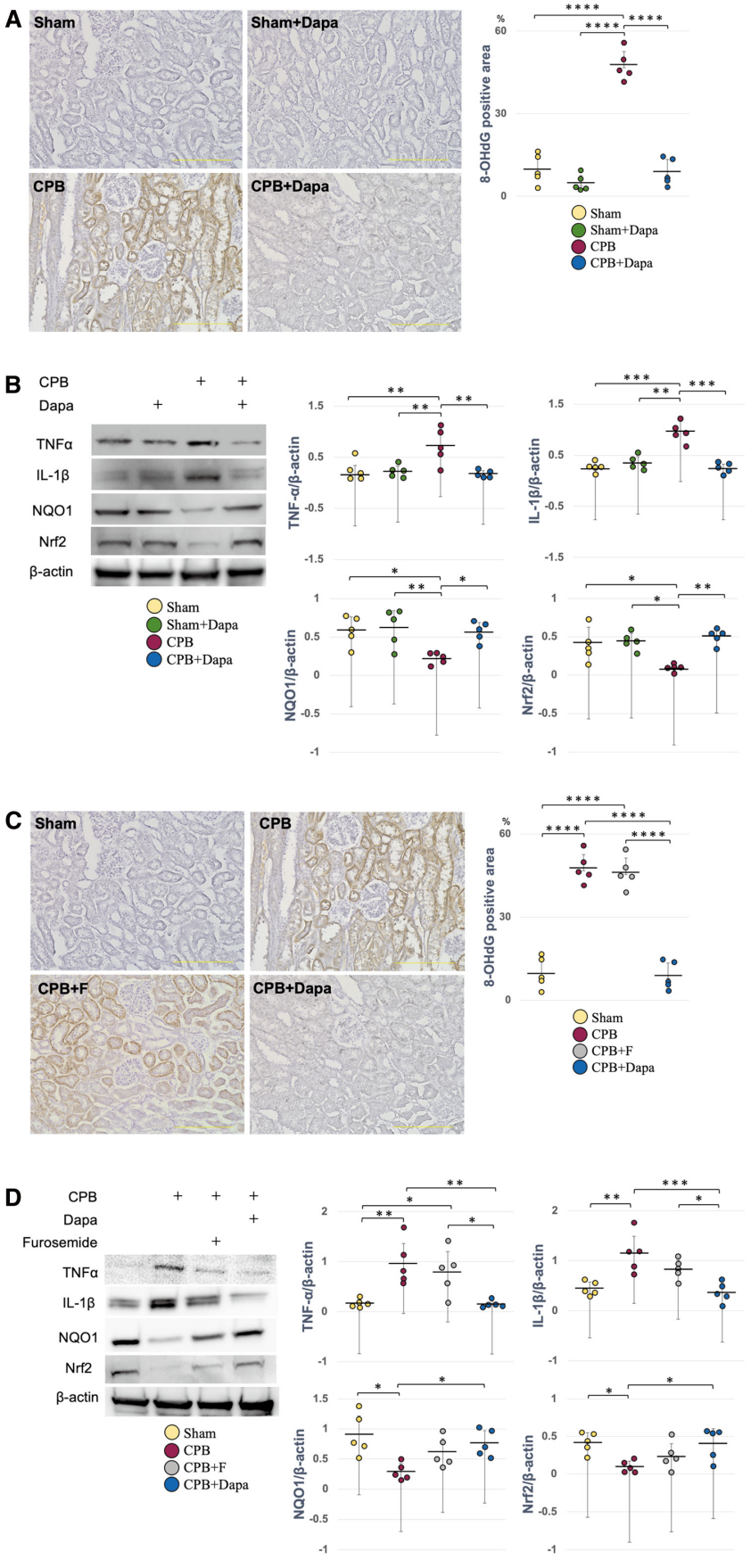

Figure 4. Effects of dapagliflozin on oxidative stress and inflammatory cytokines. (**A**,**C**) Immunohistochemical staining of 8-OHdG and semiquantitative evaluation of 8-OHdG-positive areas in 4 groups. (**B**,**D**) Representative western blot results and analysis of TNFα, IL-1β, NQO1, and Nrf2 protein levels. Data are shown as the mean and standard deviation (error bars). Scale bars=200μm. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. CPB, cardiopulmonary bypass; Dapa, dapagliflozin; F, furosemide; IL-1β, interleukin 1β; NQO1, NAD(P)H quinone dehydrogenase 1; Nrf2, nuclear factor erythroid 2-related factor 2; TNFα, tumor necrosis factor-α; 8-OHdG, 8-hydroxy-2'-deoxy-guanosine.

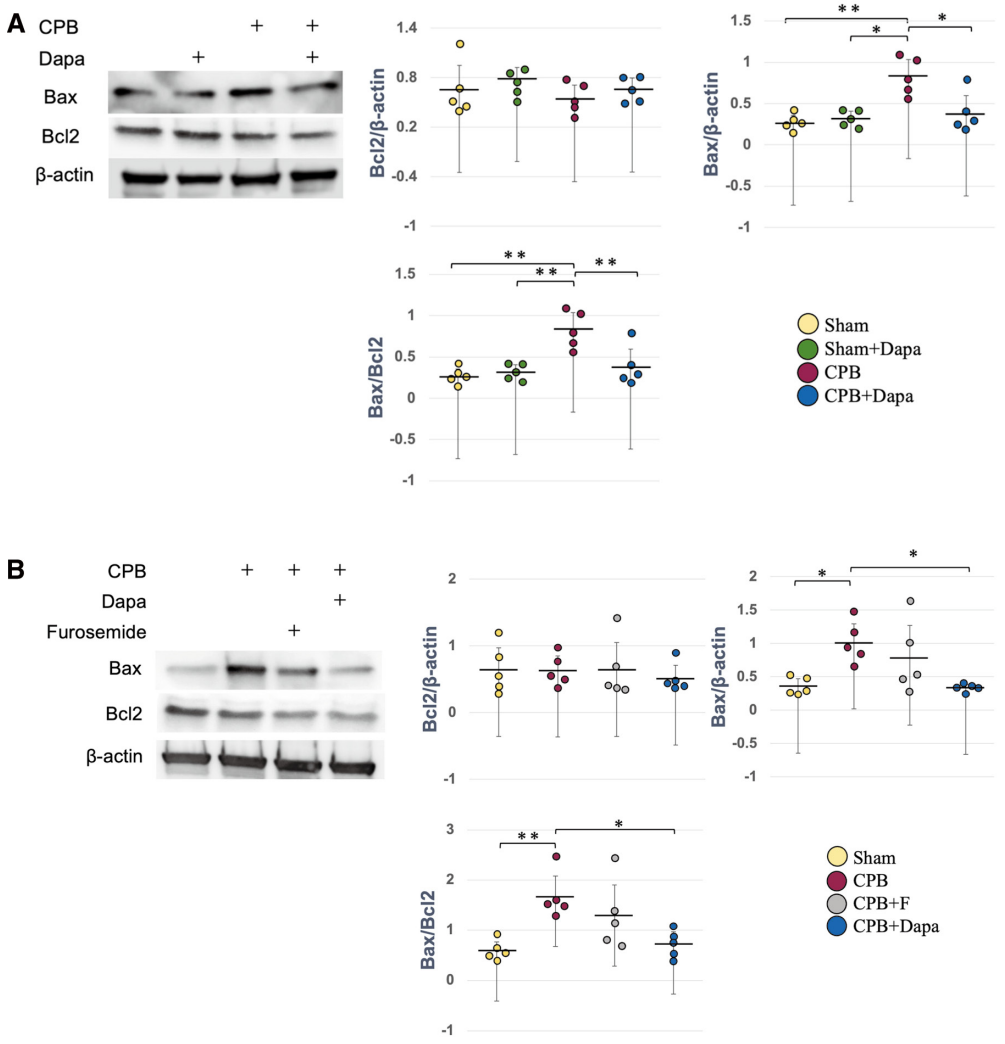

Figure 5. Effects of dapagliflozin on apoptosis. (**A**,**B**) Representative western blot results and analysis of Bax and Bcl2 protein levels, and the Bax/Bcl2 ratio. Data are shown as the mean and standard deviation (error bars). *P<0.05; **P<0.01. Bax, Bcl2-associated X protein; Bcl2, B-cell lymphoma-2; CPB, cardiopulmonary bypass; Dapa, dapagliflozin; F, furosemide.

(**Figure 2B**). The tubular injury marker Kim-1 was negative in the sham, Sham+Dapa, and CPB+Dapa groups, but a significant strong positive reaction was observed in the CPB group compared with all the other groups (P<0.0001) (**Figure 2C**). However, furosemide administration showed no significant improvement in renal function as assessed by BUN, serum creatinine, the tubular injury score, and immunohistochemical analysis of Kim-1 (**Figure 2D–F**).

Effects of Dapagliflozin on Complement Activation

Immunohistochemical analysis showed that one of the representative complements induced by CPB, C3aR, showed a significant strong positive reaction in the CPB group, but a considerable negative reaction in the sham, Sham+Dapa, and CPB+Dapa groups compared with the CPB group (P<0.0001) (**Figure 3A**). In addition, C3aR showed a significant strong positive reaction in the CPB+F group compared with the sham and CPB+Dapa groups (both P<0.001) (**Figure 3B**).

Effects of Dapagliflozin on Oxidative Stress and Inflammatory Cytokines

Immunohistochemical analysis showed that the oxidative stress marker, 8-OHdG, had a significant strong positive reaction in the CPB group compared with all the other groups (P<0.0001) (**Figure 4A**). Protein expression analysis showed that the inflammatory cytokines TNFα and IL-1β were significantly higher in the CPB group than in the sham and Sham+Dapa groups (all P<0.01), and they were significantly reduced by dapagliflozin treatment (both P<0.01, CBP group vs. CPB+Dapa group). Both NQO1 and Nrf2 protein expression was significantly lower in the CPB group than in the sham and Sham+Dapa groups (all P<0.05), and significantly increased by dapagliflozin treatment (both P<0.05, CBP group vs. CPB+Dapa group) (**Figure 4B**). With regard to the effects of a diuretic, 8-OHdG still showed a significant strong positive reaction in the CPB+F group compared with the sham and CPB+Dapa groups (P<0.0001) (**Figure 4C**). Furthermore,

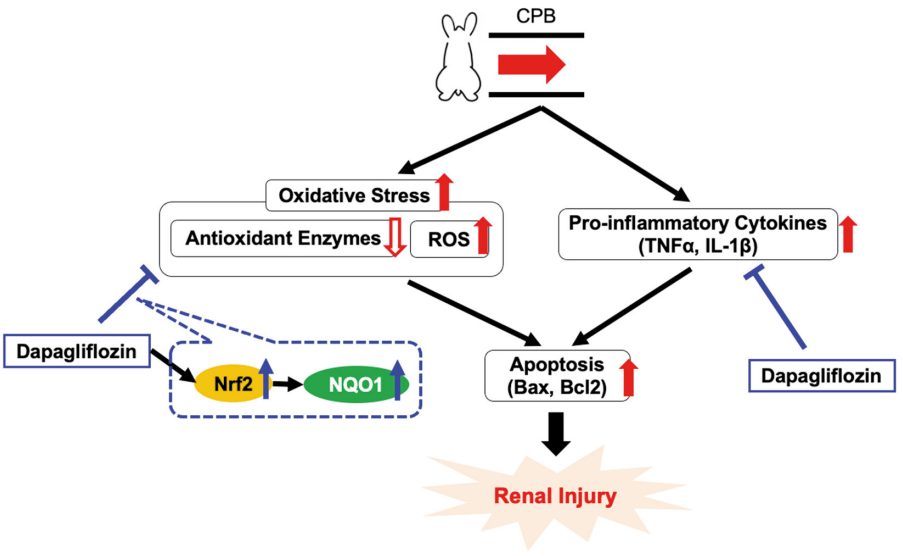

Figure 6. Schematic summary of the mechanism of the effects of dapagliflozin on CPB-induced kidney injury in rabbits. Bax, Bcl2-associated X protein; Bcl2, B-cell lymphoma-2; CPB, cardiopulmonary bypass; IL-1β, interleukin-1β; NQO1, NAD(P)H quinone dehydrogenase 1; Nrf2, nuclear factor erythroid 2-related factor 2; ROS, reactive oxygen species; TNFα, tumor necrosis factor-α.

administration of furosemide showed no beneficial effects on inflammatory cytokines or the Nrf2/NQO1 pathway (**Figure 4D**).

Anti-Apoptotic Effect of Dapagliflozin

Bax expression was significantly elevated in the CPB group compared with all the other groups ($P<0.05$). The Bax/Bcl2 ratio, which is used as an index of apoptotic signaling, also showed a significant increase in the CPB group ($P<0.01$). However, dapagliflozin pretreatment significantly decreased the Bax/Bcl2 ratio ($P<0.01$) (**Figure 5A**). Furosemide showed no significant improvement in apoptosis (**Figure 5B**).

Discussion

Our study results showed that intravenous administration of the SGLT2 inhibitor dapagliflozin before initiating CPB protected against acute in-vivo CPB-induced kidney injury in non-DM rabbits. The benefits of dapagliflozin could be related to a direct effect on the kidney because it was administered to rabbits only 30 min before initiating CPB. The direct effects of dapagliflozin relate to its ability to attenuate oxidative stress, inflammation, and apoptosis, and to activate the Nrf2 pathway in renal tubular cells, which improved renal function (improvement in serum creatinine concentrations, BUN concentrations, diuresis, Kim-1-positive immunohistochemical staining, and the tubular injury score). To the best of our knowledge, this is the first study of the acute renoprotective effect of dapagliflozin for CPB-induced AKI.

The pathophysiology of CSA-AKI remains incompletely understood. Several major injury pathways are involved, including hypoperfusion, ischemia-reperfusion injury, neurohumoral activation, inflammation, oxidative stress, nephrotoxins, and mechanical factors. During extracorporeal membrane circulation, activation of the complement system triggers inflammatory cytokines and increases ROS production, which causes cell injury and apoptosis. These conditions in turn cause further complement activation and a vicious circle of inflammation and cell damage. The kidneys account for approximately 20–25% of cardiac output and are rich in mitochondria, making them vulnerable to ROS. Oxidative stress is generally defined as an imbalance between oxidants and antioxidants in favor of oxidants, which cause an arrest of redox signaling and/or molecular damage.[19] Surgical injury to tissues and the exposure of blood to the CPB pump and circuit are associated with the activation of inflammatory pathways, and an increase in oxidative stress. Excessive oxidative stress is related to AKI. Our study showed increased protein expression of C3aR and 8-OHdG, which is one of the oxidative DNA adducts and was a marker for oxidative injury in the CPB group. The CPB group also showed increased inflammatory cytokine protein levels in renal tubules. Additionally, CPB inhibited NQO1 protein expression relative to shams.

Regarding apoptosis, Bax and Bcl2, which are members of the Bcl2 protein family, play a critical role in regulating apoptosis. Bax is an important pro-apoptotic factor that participates in the mitochondrial pathway of apoptosis, whereas Bcl2, an antiapoptotic factor, inhibits the function of Bax. Therefore, the expression level of Bax/Bcl2 is an important index of the regulation of apoptosis.[20] Previous various AKI models[16,17,21] showed an increase in the Bax/Bcl2 ratio accompanied by apoptosis of renal tubular cells. Our finding of an increase in the Bax/Bcl2 ratio associated with CPB is consistent with those previous studies.

Nrf2 is a major regulator of cytoprotective protein expression driven by antioxidant response elements, and plays a decisive role in regulating oxidative defense and redox homeostasis in cells.[22] Nrf2 upregulation vigorously attenuates oxidative stress, protects against apoptosis, and prevents renal tubular cell injury in renal ischemia-reperfusion.[23,24] Upregulation of the Nrf2-mediated signaling pathway protects against cisplatin-induced renal injury.[25]

Furthermore, Chi et al reported that dapagliflozin upregulated the Nrf2 pathway and prevented oxidative stress, an inflammatory reaction, and renal impairment in lipopolysaccharide-induced AKI in rats.[17] In the kidneys of the animals in our CPB-induced AKI rabbit model, CPB inhibited the Nrf2/NQO1 pathway.

Our findings indicated that dapagliflozin reduced oxidative stress and inflammatory cytokines. We also found that Nrf2 and NQO1 protein expression levels in the rabbits with CPB and dapagliflozin treatment were higher than those in rabbits with CPB and vehicle treatment. Moreover, dapagliflozin reduced the Bax/Bcl2 ratio in renal tissue injured by CPB. Interestingly, dapagliflozin administration had more beneficial effects on CSA-AKI than did furosemide. Furosemide directly acts on the tubules, but there is controversy on whether furosemide is a beneficial or detrimental drug for renal function. In our study, the administration of furosemide showed limited renoprotective effects on CPB-induced AKI relative to dapagliflozin, despite the same urine volume between the CPB+F and CPB+Dapa groups. These results suggested that the renoprotective effect of dapagliflozin includes not only diuresis, but also other mechanisms, such as a reduction in ROS and inflammatory cytokines, which in turn contributes to attenuating apoptosis (**Figure 6**).

Study Limitations

In this study, the AKI model was successfully established. There were obvious signs of renal tubular injury by hematoxylin and eosin staining, increased Kim-1-positive immunohistochemical staining, and an increase in Bax/Bcl2 protein expression in rabbits with CPB. However, there are some limitations. First, the relationship between oxidative stress and transfusion was not assessed. Generally, a unit of red blood cells can be stored for up to 21 days before it has to be transfused. During storage, erythrocytes undergo progressive, interrelated biochemical and morphological changes that are thought to contribute to any organ damage that might be caused by blood transfusion.[26] Although all donor blood was collected 30 min before the experiments, an effect of transfusion cannot be excluded. Second, we did not perform cardiac arrest in the current model. Although ischemia-reperfusion injury is also a main factor in developing CSA-AKI, its effect could not be completely assessed. However, using venous-arterial extracorporeal membrane oxygenation can promote tissue hypoxia through impairment of microvascular perfusion, which causes ischemic kidney damage.[27] Another potential limitation is the use of cervical cannulation. Although it is used clinically for extracorporeal membrane oxygenation in neonates and children, it is not the most commonly used cannulation strategy in adult elective cardiac surgery. Furthermore, the physiology of the rabbit is different from that of humans. Therefore, the intravenous dose of dapagliflozin (0.5 mg/kg) used in our study is not applicable to the practical clinical situation. Further studies are required to assess the optimal dose of dapagliflozin in large animals with a cardiac arrest model.

Conclusions

SGLT2 inhibitors have clinical benefits in patients, even in those without DM. This aspect has encouraged many researchers to discover other implications of SGLT2 inhibitor administration. The present study results indicated a protective effect of dapagliflozin on non-DM rabbits with CPB-induced AKI, as shown by improved serum creatinine and BUN concentrations, a decreased tubular injury score, and decreased Kim-1-positive immunohistochemical staining. Moreover, dapagliflozin reduced the production of inflammatory cytokines, and mitigated oxidative stress by decreasing ROS and activating the Nrf2/NQO1 pathway. This study provides evidence that acute administration of dapagliflozin protects against AKI caused by CPB-induced tubular injury.

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Disclosures

The authors declare that there are no conflicts of interest.

IRB Information

The Animals Care Committee of the Kyushu University School of Medicine approved the experimental and animal care protocols (approval number: A23-135-0, approved: 15 December 2022).

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