# Alleviation of cisplatin-induced acute kidney injury using phytochemical polyphenols is accompanied by reduced accumulation of indoxyl sulfate in rats

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**Key words** Acute kidney injury, Cisplatin, Indoxyl sulfate, Uremic toxins, Polyphenols

**Abstract**

*Background* Polyphenols such as quercetin have been reported to prevent cisplatin-induced acute kidney injury (AKI). Indoxyl sulfate (IS), a uremic toxin generated in the liver, is increased in cisplatin AKI. The present study examined the effect of phytochemical polyphenols on serum and renal accumulations of IS in association with cisplatin AKI.

*Methods* Sprague-Dawley rats were treated with cisplatin (10 mg/kg body weight) by intraperitoneal injection. Polyphenols were orally administered at -24, -1, 24 and 48 hr before or after cisplatin injection. Serum levels of IS, cisplatin, serum creatinine (SCr), blood urea nitrogen (BUN) and electrolytes were measured. By using *in vitro* assay system with rat liver S9 fraction, the inhibitory potencies of several compounds on IS production were determined.

*Results* Injection of cisplatin in rats markedly elevated the SCr and BUN levles, which were accompanied by tubular injuries and the expression of kidney injury molecule-1 (Kim-1). By contrast, quercetin significantly suppressed the SCr and BUN levels in the cisplatin-treated rats and protected them against renal injury with the decreased expression of Kim-1. Quercetin had no effect on serum and renal levels of cisplatin. In addition, quercetin had no effect on cisplatin-induced renal accumulation of malondialdehyde. IS concentrations in serum, kidney, liver, intestine and lung were markedly elevated by cisplatin treatment, whereas quercetin suppressed the serum and tissue IS levels. An *in vitro* kinetic assay revealed that quercetin displayed a potent inhibitory effect on hepatic production of IS.

*Conclusion* Inhibition of IS accumulation by oral administration of quercetin alleviates cisplatin-induced AKI.

## Introduction

Acute kidney injury (AKI) is characterized by increased levels of serum creatinine (SCr) and oliguria caused by the abrupt deterioration of renal function over a period of hours to days [1]. AKI can be caused by an incident, such as a loss of renal blood flow through trauma, a bacterial infection in the blood, a prostatic hypertrophy, malignancy, or high levels of toxins or nephrotoxic drugs in the blood [2]. When the kidneys lose their filtering function, dangerous levels of metabolites accumulate in the body, including urea, nitrogenous waste products and uremic toxins. AKI is a serious condition that often requires intensive care. Nonetheless, given appropriate treatment the kidneys can restore function such that AKI can be reversed. In other circumstances, AKI subsequently progresses to chronic kidney disease (CKD), requiring dialysis or renal transplantation in severe cases [3].

Cisplatin (cis-diamminedichloroplatinum(II)) is a widely used and effective antineoplastic drug for the treatment of several solid tumors [4]. However, despite prophylactic intensive hydration and forced diuresis, AKI occurs in about a third of cisplatin-treated patients [4]. In general, cisplatin treatment impairs the S3 segment of renal proximal tubules, the highest accumulation region, by several different mechanisms, including oxidative stress, inflammation, DNA damage and apoptosis [5]. Based on the factors and/or mechanisms involved in cisplatin-induced AKI, several approaches have been used to try and alleviate and/or prevent cisplatin AKI. Quercetin, a well known bioflavonoid, appears to reduce toxicity of cisplatin in cultured tubular epithelial cells and AKI in rats [6, 7]. Resveratrol, a naturally occurring polyphenol, was reported to attenuate the structural and functional renal injuries induced by cisplatin [8]. These phytochemical polyphenols are known for their strong antioxidative activities. Quercetin was reported to attenuate alterations such as lipid peroxidation, urine volume and plasma creatinine levels as well as decrease urine osmolarity that accompany cisplatin AKI [7]. These biological effects are probably related to the antioxidant activity of quercetin. In fact, reactive oxygen species (ROS) have

been suggested as important mediators of cisplatin-induced acute renal failure. Baek S.M. et al. suggested that hydrogen peroxide is involved in the cisplatin-induced necrosis, whereas hydroxyl radical is responsible for the cisplatin-induced apoptosis [9]. Recently, Hasegawa et al. reported that cisplatin-induced AKI decreased the number and function of peroxisomes as well as mitochondria and led to increased local levels of ROS production [10]. They also suggested that Sirt1, a NAD-dependent protein deacetylase, overexpression in proximal tubules rescues cisplatin-induced AKI by maintaining peroxisomes number and function, concomitant up-regulation of catalase, and elimination of renal ROS levels [10].

We previously reported that indoxyl sulfate (IS), a typical uremic toxin generated in the liver, might be involved in the progression of cisplatin-induced AKI [11-13]. Several reports also showed that IS had potential of barrier to the kidney. For example, IS is one of the most powerful inducers of free radicals among various uremic toxins, and cellular toxicity induced by IS can be explained by induction of oxidative stress in proximal tubular cells [14]. The production of IS predominantly takes place in the liver. Dietary protein-derived tryptophan is first metabolized to indole by tryptophanase of intestinal bacteria such as *Escherichia coli*. Following intestinal absorption, indole is hydroxylated to indoxyl by cytochrome P450 (CYP) 2E1, and subsequently conjugated to IS by sulfotransferase (SULT) 1A1 in the liver [15, 16]. It is noteworthy that polyphenols, such as quercetin and resveratrol, are well-known inhibitors of CYP and SULT. Thus, we hypothesized that the protective effects of these polyphenols against cisplatin AKI might be related to the hepatic production of IS. In the present study, we examined the effect of polyphenols on serum and renal accumulations of IS in association with cisplatin-induced AKI.

## Materials and methods

### Chemicals

Cisplatin was purchased from Nippon Kayaku Co., Ltd. (Randa; Tokyo, Japan). IS, 8-methoxypsoralen, tranylcypromine, acetaminophen, irgasan, quercetin, resveratrol and thiobarbituric acid (TBA) were obtained from Sigma-Aldrich (St Louis, MO). Indole, disulfiram, N,N-diethyldithiocarbamate (DDTC), curcumin, 2,6-dichloro-4-nitrophenol (DCNP), carboxymethyl cellulose (CMC), methanol and nitric acid ($HNO_3$) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

### Experimental Animals

Male Sprague-Dawley (SD) rats (Crea Japan, Inc., Tokyo, Japan) were housed in a standard animal maintenance facility at a constant temperature (20-24°C) and humidity (50-70%) and a 12/12hr light/dark cycle for about a week before the day of the experiment. All animal experiments were conducted according to the Regulations for Animal Experiments in Kumamoto University. Rats were divided into five different groups as follows: saline and CMC administered rat (saline rats), cisplatin and CMC administered rats (cisplatin rats), cisplatin and quercetin [7] administered rats (+quercetin rats), cisplatin and curcumin [17] administered rats (+curcumin rats) and cisplatin and resveratrol [18] administered rats (+resveratrol rats). Cisplatin (10 mg/kg body weight) or saline was administered by intraperitoneal injection in rats (6 weeks old). CMC (0.5 w/v%) or polyphenols (with CMC) were orally administered at -24, -1, 24 and 48 hr before or after cisplatin injection. All administrations and sacrifices were performed under surgical anesthesia using diethyl ether. Serum and tissues samples were collected 72 hr after cisplatin administration. Levels of IS, cisplatin, SCr (enzymatic method), blood urea nitrogen (BUN) (urease UV method), sodium (Na) (electrode method), chloride (Cl) (electrode method), potassium (K) (electrode method), aspartate aminotransferase (AST) (Japan Society of Clinical Chemistry (JSCC) transferable

method) and alanine aminotransferase (ALT) (JSCC transferable method) were then measured. Except for determining the levels of IS and cisplatin in serum, all other measurements were conducted at the SRL laboratory (Tokyo, Japan). For histological assessment, kidney samples were fixed in 4% phosphate buffered formaldehyde. Paraffin sections of 2 μm from excised kidney were stained with periodic acid-Schiff (PAS) base reagent. For semi-quantitative analysis of morphological changes, high-magnification fields of the outer stripe of the outer medulla in rats were randomly selected. Then, tubular injury was graded with an arbitrary score of 0-5 as follows: 0, the absence of necrosis; 1, mild necrosis; 3, moderate necrosis; 5, severe necrosis. All quantification was performed in a blinded manner.

**Western blot analysis**

Western blot analysis was performed according to a previous report [19] with some modifications. Kidneys were homogenized in an ice-cold homogenization buffer consisting of 230 mM sucrose, 5 mM Tris-HCl (pH 7.5), 2 mM ethylenediaminetetraacetic acid, 0.1 mM phenylmethanesulfonyl fluoride, 1 μg/ml leupeptin and 1 μg/ml pepstatin A. After measuring of protein content using a bicinchoninic acid (BCA) protein assay reagent (Thermo Fisher Scientific, Rockford, IL), each sample was mixed in a loading buffer (2 w/v% sodium dodecyl sulfate, 125 mM Tris-HCl, 20 v/v% glycerol and 5 v/v% 2-mercaptoethanol) and heated at 99°C for 2 min. The samples were separated by 7.5 w/v% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membrane (Immobilon-P; Millipore, Bedford, MA) by semi-dry electroblotting. The membrane was blocked overnight at 4°C with 2 v/v% ECL advance Blocking agent (GE Healthcare, Little Chalfont, UK) in 50 mM Tris-buffered saline (pH 7.6) containing 0.3 v/v% Tween 20, and then incubated for 1 hr at room temperature with a primary antibody specific for kidney injury molecule-1 (Kim-1) (gift from Dr. Inui, Kyoto University Hospital) or β-actin (Sigma-Aldrich). The blots were washed with Tris-buffered

saline containing Tween 20 and incubated with the secondary antibody (horseradish peroxidase-linked anti-rabbit immunoglobulin F (ab)2 or horseradish peroxidase-linked anti-mouse immunoglobulin F (ab)2, GE Healthcare) for 1 hr at room temperature. Immunoblots were visualized with an ECL system (ECL Advance Western Blotting Detection Kit, GE Healthcare).

**Measurement of cisplatin concentration**

Measurement of cisplatin concentration was carried out as described previously [20]. Kidneys were homogenized in 0.5 v/v% $HNO_3$. Each sample was incinerated using a super-high frequency sample degradation container (DV-7; Sanai Kagaku, Nagoya, Japan) encased with Teflon and propylene outer casing (PP-25 and PT-25, Sanai Kagaku) in a microwave oven with 0.5 v/v% $HNO_3$. Serum or the incinerated samples were diluted in $HNO_3$. The amount of platinum was determined using inductively coupled plasma-mass spectrometry (ICP-MS; Finnigan MAT ELEMENT, Bremen, Germany). Cisplatin concentration was measured as the $^{194}Pt$ concentration.

**High-performance liquid chromatography (HPLC) determination of IS concentration**

The HPLC system consisted of a Shimadzu LC-10ADVP pump and a Shimadzu RF-10AXL fluorescence spectrophotometer. A column of LiChrospher 100 RP-18 (Merck KGaA, Darmstadt, Germany) was used as the stationary phase and the mobile phase consisted of acetate buffer (0.2 M, pH 4.5). The flow rate was 1.0 ml/min at a column temperature of 40°C. The presence of IS in the eluate was monitored by means of a fluorescence detector (excitation 280 nm, emission 375 nm). Gradient condition was used for measuring samples from the *in vitro* screening assay.

**Measurement of lipid peroxidation in rat kidney**

The malondialdehyde (MDA) content, a measure of lipid peroxidation, was assayed as thiobarbituric acid-reactive substances (TBARS) in kidney. Kidneys were homogenized in an ice-cold phosphate bufferd saline, and supernatants of homogenates centrifuged at 2,000 rpm for 5 min (4°C) were mixed with 1 v/v% phosphoric acid and 0.6 w/v% TBA. The mixtures were heated at 100°C for 1 hr, and centrifuged at 10,000 *g* for 3 min after adding n-butanol. The absorbances of butanol phase at 535 and 520 nm were measured with UV/Visible spectrophotometer (Ultraspec 2000; GE healthcare) [7]. TBARS were calculated using the difference in absorption at the two wavelengths and the standard was used a 1, 1, 3, 3-tetraethoxypropane. Protein content of each sample was quantified by BCA protein assay.

**_In vitro_ inhibition assay for IS production by using rat liver S9 fraction**

Livers were harvested from male SD rats (6 weeks old). The liver homogenate in 50 mM sodium phosphate buffer (pH 7.4) containing 150 mM KCl was centrifuged at 9,000 *g* for 20 min (4°C), and the supernatant was used as the S9 fraction containing microsomal fraction with CYP and cytosol fraction with SULT. The reaction mixture (total 250 μL, dimethyl sulfoxide concentration was 1 v/v%) was 50 mM sodium phosphate buffer (pH 7.4) contained the S9 fraction (5 mg/mL), indole, reduced nicotinamide adenine dinucleotide phosphate (NADPH) (1 mM), adenosine 3'-phosphate 5'-phosphosulfate (20 μM) and uridine diphosphate glucuronic acid (1 mM) with or without several compounds that may inhibit production of IS. Incubations of the reaction mixture were carried out in a shaking water bath maintained at 37°C. The reaction was then stopped by addition of 250 μL of ice-cold methanol.

**Statistical analysis**

Data were analyzed statistically by analysis of variance and Scheffe's multiple comparison test. A P-value of less than 0.05 was considered statistically significant. All data

were represented the mean ± S.D..

**Results**

*Effect of phytochemical polyphenols on body weight and renal function of cisplatin-induced AKI rats*

Figure 1 shows the effect of cisplatin treatment (10 mg/kg, i.p.) on body weight and renal function with or without administration of quercetin (50 mg/kg, p.o.), curcumin (60 mg/kg, p.o.) or resveratrol (5 mg/kg, p.o.) in rats. These polyphenols were given to rats 24 hr and 1 hr before the cisplatin treatment, and 24 hr and 48 hr after the treatment. The body weights decreased approximately linearly in cisplatin-treated rats, and were not influenced by oral administration of polyphenols. SCr and BUN levels were markedly elevated in the cisplatin-treated rats compared to saline-treated rats. The elevated SCr and BUN levels were significantly suppressed by administration of quercetin. Administration of resveratrol showed a tendency to decrease SCr and BUN in rats without statistical significance. By contrast, administration of curcumin did not attenuate the SCr and BUN levels. These findings suggest that cisplatin caused partial dehydration in the rats, and that the protective effect of quercetin would surpass the effect of dehydration

Figure 2 shows the histological alterations of the kidney of rats treated with saline or cisplatin with or without administration of polyphenols. Cisplatin treatment caused severe tubular injuries, accompanied by debris accumulation, congestion and cast formation (Fig. 2b). The tubular injuries evoked by cisplatin were substantially reduced by administration of quercetin, curcumin or resveratrol (Figs. 2c, 2d &2e). Semi-quantitative scoring analysis suggested that oral administration of the polyphenols helped to protect against renal tubular injury (Fig. 2f). Changes in the expression of Kim-1 protein, a marker for AKI, during cisplatin treatment with or without quercetin were examined by Western blot analysis (Fig. 3). The expression of Kim-1 was not detected in the kidney of saline rats. By contrast, the expression of Kim-1 was clearly evident in the kidney of cisplatin-treated rats. Intriguingly, however, administration of quercetin resulted in a decreased expression of Kim-1 in the

kidney of cisplatin-treated rats.

Table 1 summarizes serum electrolytes and hepatic functions in saline- or cisplatin-treated rats with or without polyphenols. Cisplatin treatment resulted in a significant decrease in serum Cl, but not in Na and K compared to those in saline rats. Quercetin and resveratrol, but not curcumin, restored the decreased serum Cl level. The AST level was unaffected by cisplatin or polyphenols, but ALT level showed a tendency to decrease in the cisplatin-treated rats with or without administration of polyphenols.

*Effect of polyphenols on cisplatin concentrations in the serum and kidneys of cisplatin-induced AKI rats*

The findings described in the previous section suggested polyphenols reduce renal accumulation of cisplatin, thereby leading to partial protection against AKI. However, as shown in Fig. 4, administration of these polyphenols did not affect $^{194}$Pt (cisplatin) concentrations in both serum and kidney, suggesting tissue distribution and disposition of cisplatin were unaffected.

*Effect of polyphenols on IS concentrations in serum and tissues of cisplatin-induced AKI rats*

Next, we examined whether accumulations of IS in serum and tissues were affected by the polyphenols in cisplatin-treated rats. As shown in Fig. 5, IS levels in serum, kidney, liver, intestine and lung were markedly elevated in the cisplatin-treated rats compared to those in saline rats. The increases in serum and tissue IS levels were significantly suppressed by administration of quercetin or resveratrol, but not of curcumin. These results suggest that both quercetin and resveratrol inhibited production of IS, most likely in the liver. Alternatively, urinary excretion of IS might be attenuated by partial amelioration of renal function.

*Effect of quercetin on lipid peroxidation in rat kidney caused by cisplatin treatment*

Figure 6 shows lipid peroxidation in rat kidney. MDA level was markedly elevated in cisplatin-induced AKI rats. Oral administration of quercetin could not affect the increased MDA level in the cisplatin-treated rat kidney.

*In vitro inhibition assay for IS production by using the S9 fraction of rat liver.*

We developed a novel assay system for IS production from indole by using rat liver S9 fraction. As shown in Fig. 7a, the production of IS in the assay mixture was time-dependent, reaching a steady state level at 90 min. The apparent initial rate of IS production was dependent on indole concentrations, with the apparent kinetic parameters estimated by the Michaelis-Menten equation; 24.0 μM and 29.2 pmol/min/mg protein for $K_m$ and $V_{max}$, respectively (Fig. 7b). Eadie-Hofstee plots (inset in Fig. 7b) indicated a single component of IS production reaction with the comparable parameters with the Michaelis-Menten-based parameters; 27.1 μM and 30.5 pmol/min/mg protein for $K_m$ and $V_{max}$, respectively.

By using this assay system, the inhibitory potencies of several compounds (10 μM) on hepatic IS production were examined (Table 2). Known inhibitors of CYP2A6, CYP2E1 and SULT were used. Four compounds, which are known SULT inhibitors, significantly suppressed IS production. Furthermore, we compared the inhibitory potencies of five compounds by means of kinetic analysis. As shown in Fig. 8, quercetin, curcumin and resveratrol, potent inhibitors of SULT, showed a strong inhibitory effect on IS production with apparent $K_i$ values of 1.0 μM, 1.8 μM and 1.5 μM, respectively. By contrast, the apparent $K_i$ values of tranylcypromine, a CYP2A6 inhibitor, and DDTC, a CYP2E1 inhibitor, were 148 μM and 1,859 μM, respectively. These results suggested that the agents inhibiting SULT had a more potent inhibitory effect on hepatic IS production compared to those inhibiting CYP2A6 or CYP2E1.

**Discussion**

Cisplatin exerts multiple intracellular effects, causing direct cytotoxicity with ROS, activating mitogen-activated protein kinases, inducing apoptosis, and stimulating inflammation and fibrogenesis [5]. The nephrotoxicity of platinum-class drugs in animal models can be ameliorated by free radical scavenging agents, suggesting that oxidative stress-related injury is actively involved in the pathogenesis of cisplatin-induced AKI. Cisplatin increased lipid peroxidation, urine volume and plasma creatinine levels and decreased urine osmolarity. Interestingly, treatment with quercetin attenuated these alterations, highlighting the central effect of oxidative stress during cisplatin-induced AKI in rats [7]. However, the mechanisms and/or metabolites involved in the increased production of ROS and free radicals in the renal tubular cells of cisplatin-treated rats have not been identified.

It is noteworthy that IS plays a pivotal role in the generation of ROS. It was reported that IS stimulates the production of intracellular and extracellular ROS in rat mesangial cells [21]. IS also resulted in increases in the production of intracellular free radicals, such as $O^{2-}\cdot$, $HO\cdot$ and $H_2O_2$, through a pathway that most likely involves NADPH oxidase. Furthermore, Muteliefu et al. reported that IS stimulated the production of ROS by up-regulating NADPH oxidase 4, thereby inducing the expression of the osteoblast-specific proteins in human aortic smooth muscle cells [22]. AST-120, an oral charcoal adsorbent, reduced IS concentrations in the blood and inhibited oxidative stress in rats [23]. Previously, we reported that administration of AST-120 significantly decreased IS accumulation in the serum and kidney, and also ameliorated cisplatin-induced AKI [11, 12]. We suggested that IS accelerated cisplatin-induced AKI as a key mediator, and that AST-120 treatment may be useful in preventing the progression of renal dysfunction by decreasing IS accumulation in the kidney. Therefore, IS may act as a cytotoxic metabolite mediating the enhanced ROS production under cisplatin-induced AKI.

In the present study, we observed that oral administration of quercetin alleviated renal

function of cisplatin-induced AKI rats as well as significantly suppressing the accumulation of IS in the serum and kidney (Figs. 1-3 & 5). Resveratrol, but not curcumin, suppressed the IS accumulation and partially prevented AKI. Future studies regarding dosing conditions including oral administration dosage and interval and/or timing of dosing should be required to get the optimal strategy. In addition to the well-known antioxidant reaction, these polyphenols had a preventive effect on cisplatin-evoked AKI by inhibiting hepatic IS production, followed by the suppression of IS-related ROS production in the kidney. It might be speculated that oral administration of quercetin alleviated cisplatin-induced AKI, possibly by suppressing production of oxidative stress including lipid peroxidation. However, in the present study oral administration of quercetin could not affect or suppress the MDA level in the kidney (Fig. 6). Therefore, we have guessed that renal protective effect of quercetin might be a result of the decreased renal accumulation of IS, but may be independent of its antioxidant effect, indicating that decrease in IS could be related to protection of renal function. It was reported that IS had the nephrotoxic effect except ROS. Administration of IS to nephrectomized rats increased transforming growth factor-β1, tissue inhibitor of metalloproteinase-1, pro-α1 and intracellular adhesion molecule-1 and stimulated glomerular sclerosis and tubulointerstitial damage [24-26]. IS induced plasminogen activator inhibitor-1 and nuclear factor-κB in human renal tubule cells [27]. Multiple mechanisms by IS may be related to cisplatin-induced AKI.

We then performed an *in vitro* assay using rat liver S9 fraction in order to assess the inhibitory potencies of the polyphenols on hepatic IS production. It was reported that CYP2A6 is involved in oxidative metabolism of indole to give indoxyl [28]. Therefore, in addition to CYP2E1 and SULT involved in production of IS, we also focused on the role of CYP2A6 in terms of IS production in this assay. Several compounds were compared to determine their inhibitory effect on IS production in the liver. We found that CYP2A6 inhibitors (8-methoxypsoralen and tranylcypromine) [29] and CYP2E1 inhibitors (disulfiram,

DDTC and acetaminophen) [30-32] showed poor inhibitory effects on IS production, whereas SULT inhibitors (irgasan, quercetin, curcumin and resveratrol) [33-35] markedly suppressed IS production. These results suggest SULT is a rate-limiting enzyme that mediates the hepatic production of IS. In addition, these findings indicate that a couple of CYP species could be involved in the biosynthesis of IS (Fig. 8 & Table 2). CYP2A6 might play a more dominant role in the hepatic IS production pathway than CYP2E1. The present study suggests that an approach suppressing hepatic IS production could be one of the strategies for alleviation of cisplatin nephrotoxicity.

Administration of polyphenols did not affect the concentration of cisplatin in serum and kidney (Fig. 4). A previous study found that administration of AST-120 did not affect the pharmacokinetics of cisplatin in rats [36]. These findings suggest that IS accumulation is not related to cisplatin disposition and distribution. A study using ovarian cancer cells showed that quercetin and resveratrol have synergistic antiproliferative activity with cisplatin [37, 38]. Thus, a combination of these compounds may enhance the anticancer activity of cisplatin.

In addition to its toxic action in the kidney, IS is thought to affect various physiological functions of other organs. It has been reported that IS may act as a vascular toxin. In vascular smooth muscle cells, IS increases the level of osteopontin, core binding factor 1, alkaline phosphatase and ROS, thereby stimulating cell proliferation and migration [22, 39]. In vascular endothelial cells, IS appears to induce ROS, inhibit proliferation and increase endothelial microparticles release [40-42]. IS has also been shown to contribute to the remodeling of cardiac cells [43]. In hypertensive rats, administration of IS promoted aortic calcification [44]. In hemodialysis patients, IS may be involved in the pathogenesis of atherosclerosis [45]. Furthermore, serum IS concentrations are associated with the higher mortality in a cohort of CKD patients [46]. IS was demonstrated to be involved in development of renal-cardiovascular syndrome in association with AKI. These findings suggest that a strategy for suppressing IS production and/or accumulation is likely to be

beneficial for patients with kidney disease.

Inhibition of hepatic SULT activity may also decrease the level of other sulfate-conjugated uremic toxins besides IS, such as *p*-cresyl sulfate (PCS) derived from phenylalanine and tyrosine [47]. PCS may increase free radical production of leucocytes [47]. In addition, free serum PCS concentrations were associated with mortality of CKD patients [48]. Dialysis cannot remove PCS because its protein binding ratio is about 90% as well as IS [49, 50]. Decrease in sulfate-conjugated uremic toxins by administration of a SULT inhibitor may be associated with the amelioration of cisplatin-induced AKI. DCNP, a specific inhibitor of SULT, showed the strongest inhibitory effects for IS production both *in vivo* (serum and renal IS concentrations were 30.3 ± 9.8 μM and 10.6 ± 4.6 nmol/g of tissue, n = 5) and *in vitro* (percentage of IS production by inhibition was 4.0 ± 0.41, n = 4). However, in the present study, administration of DCNP (10 mg/kg) appeared to exacerbate the renal injury due to its potent toxicity in rats (SCr and BUN levels were 2.02 ± 0.47 mg/dl and 169.5 ± 37.1 mg/dl, n = 5). We suggest that prevention of cisplatin-induced AKI should require the relatively specific inhibition of SULT activity mediating hepatic generation of IS, without any toxic side effects on the kidney and liver.

In conclusion, oral administration of polyphenols, especially quercetin, showed a preventive effect on cisplatin-induced AKI in rats. Moreover, these effects were accompanied by suppression of serum and renal accumulations of IS. Agents, including phytochemical polyphenols with a potential inhibitory effect on hepatic IS production, could be useful for preventing the progression of cisplatin-induced AKI.

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**Figure legends**

**Fig. 1.** Effects of polyphenols on body weight, SCr and BUN levels in saline or cisplatin-treated rats. Relative body weights (a) were estimated as dividing the body weights at the time by body weight at 0 hr. Saline (open circle), cisplatin (closed circle), +quercetin (gray triangle), +curcumine (gray circle) and +resveratrol (gray square). SCr (b) and BUN (c) in serum were measured 72 hr after the treatment with saline or cisplatin. Each column represents the mean ± S.D. for 3-5 rats. **P<0.01 significantly different from saline rats. $^{\#}P < 0.05$, $^{\#\#}P<0.01$, significantly different from cisplatin-treated rats without polyphenols.

**Fig. 2.** Histological assessment of injury in the kidney of saline- or cisplatin-treated rats with or without oral administration of polyphenols. PAS based staining of outer stripe. a: saline, b: cisplatin treatment without polyphenols, c: cisplatin treatment with quercetin, d: cisplatin treatment with curcumin, e: cisplatin treatment with resveratrol. f: tubular injury graded with an arbitrary score of 0 to 5. $^{\#}P < 0.05$, significantly different from cisplatin treatment without polyphenols.

**Fig. 3.** Kim-1 protein expression in the kidney of saline- or cisplatin-treated rats with or without oral administration of quercetin. Tissue lysate (50 μg) from total kidney was separated by SDS-PAGE and blotted onto PVDF membrane. Specific polyclonal antibody against Kim-1 and antisera specific for β-actin were used as primary antibodies.

**Fig. 4.** Serum and renal accumulations of $^{194}Pt$ in cisplatin-treated rats with or without polyphenols. $^{194}Pt$ concentrations in serum (a) and kidney (b) were determined 72 hr after the treatment with cisplatin by ICP-MS. Each column represents the mean ± S.D. for 3-5 rats.

**Fig. 5.** Serum and tissues accumulation of IS in saline- or cisplatin-treated rats with or without oral administration of polyphenols. IS concentrations in the serum (a), kidney (b),

liver (c), intestine (d), and lung (e) were measured 72 hr after treatment with saline or cisplatin as determined by HPLC. Each column represents the mean ± S.D. for 3-5 rats. *P < 0.05, **P < 0.01, significantly different from saline rats. $^{\#}$P < 0.05, $^{\#\#}$P<0.01, significantly different from cisplatin-treated rats without polyphenols.

**Fig. 6.** Effect of quercetin on MDA levels in the kidney at 72 hr after cisplatin treatment. Each column represents the mean ± S.D. for 3-5 rats. *P < 0.05, **P < 0.01, significantly different from saline rats.

**Fig. 7.** Production of IS in rat liver S9 fractions. Time course of IS production in rat liver S9 fractions incubated at 37°C with 50 μM indole (a). Concentration dependence of IS production in S9 fractions at various concentrations of indole (b). Inset shows the apparent Eadie-Hofstee plots. Each point represents the mean ± S.D. for three independent determinations.

**Fig. 8.** Inhibition of IS production in rat liver S9 fractions in the presence of various inhibitors. Rat liver S9 fractions were incubated at 37°C with 50 μM indole in the presence of various concentrations of inhibitors. Quercetin (open triangle), curcumin (open circle), resveratrol (open square), tranylcypromine (closed circle) and DDTC (closed triangle). Each point represents the mean ± S.D. for three independent determinations.

**Table 1.** Serum electrolyte levels and hepatic indicators 72 hr after cisplatin treatment with or without oral administration of polyphenols.

| | Saline | Cisplatin | +Quercetin | +Curcumin | +Resveratrol |
|---|---|---|---|---|---|
| Na (mEq/l) | 144 ± 2 | 139 ± 3 | 139 ± 3 | 138 ± 2 | 140 ± 1 |
| Cl (mEq/l) | 103 ± 2 | 96 ± 3* | 97 ± 4 | 94 ± 4** | 100 ± 3 |
| K (mEq/l) | 4.7 ± 0.5 | 3.9 ± 0.4 | 4.1 ± 0.5 | 4.1 ± 0.6 | 4.3 ± 0.2 |
| AST (IU/l) | 126 ± 25 | 117 ± 33 | 162 ± 12 | 107 ± 36 | 116 ± 26 |
| ALT (IU/l) | 48 ± 6 | 32 ± 5** | 33 ± 4* | 29 ± 8** | 36 ± 4 |

Each value represents the mean ± S.D. for 3-5 rats. *P<0.05, **P<0.01 significantly different from control (saline) rats.

**Table 2.** Inhibition of IS production by several compounds (10 μM) in rat liver S9 fraction.

| | IS production (%) |
|---|---|
| Control | 100.0 ± 4.7 |
| 8-Methoxypsoralen | 89.2 ± 6.4 |
| Tranylcypromine | 94.2 ± 9.0 |
| Disulfirum | 100.1 ± 5.1 |
| DDTC | 102.7 ± 6.2 |
| Acetaminophen | 95.6 ± 1.6 |
| Irgasan | 76.6 ± 1.6** |
| Quercetin | 16.7 ± 4.0** |
| Curcumin | 21.8 ± 1.4** |
| Resveratrol | 15.4 ± 3.9** |

Each value represents the mean ± S.D. for three independent determinations. **P<0.01 significantly different from control.

Fig. 1.

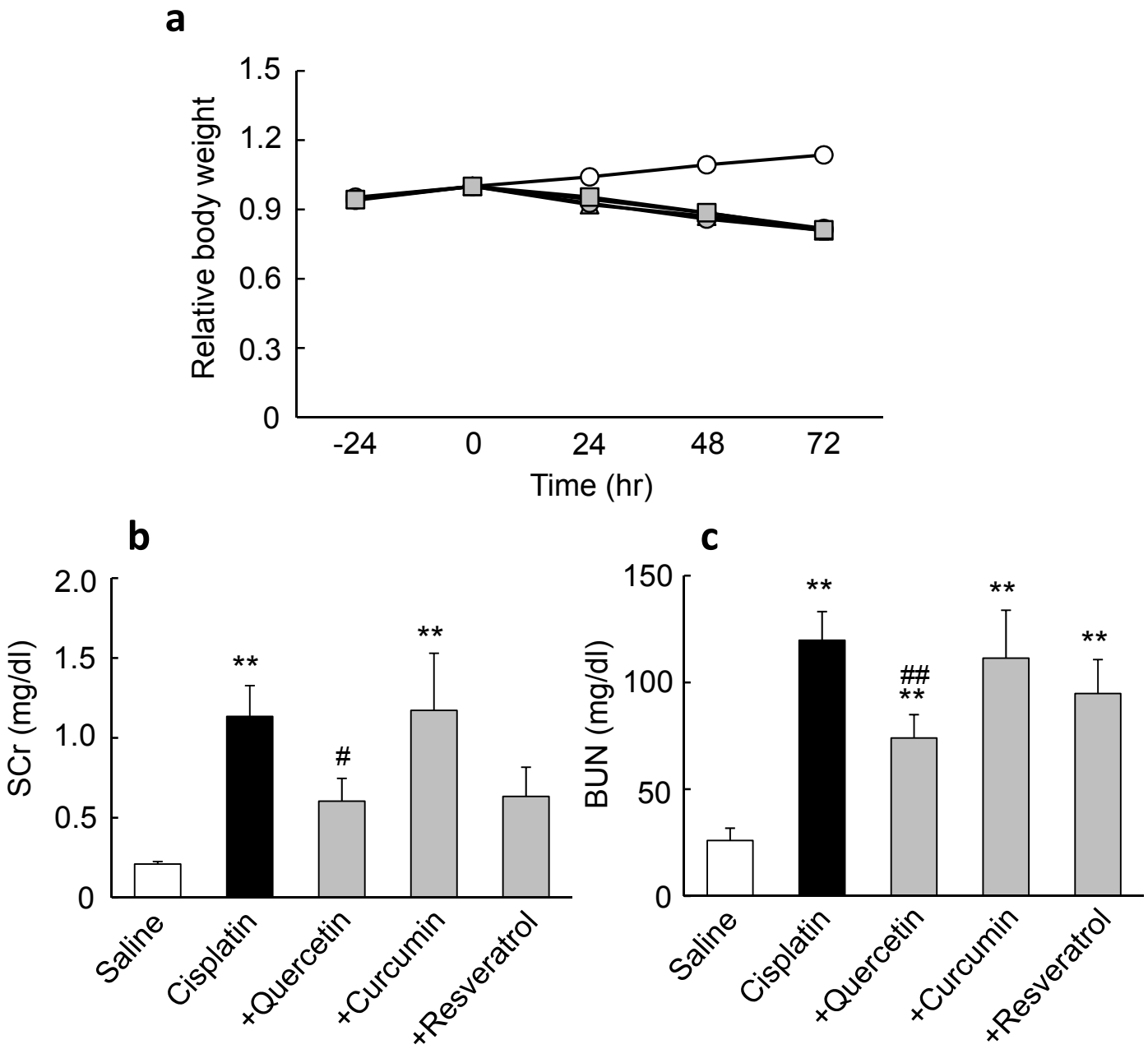

a
Relative body weight
1.5
1.2
0.9
0.6
0.3
0
-24
0
24
48
72
Time (hr)
b
SCr (mg/dl)
2.0
1.5
1.0
0.5
0
**
#
**
Saline
Cisplatin
+Quercetin
+Curcumin
+Resveratrol
c
BUN (mg/dl)
150
100
50
0
**
##
**
**
**
Saline
Cisplatin
+Quercetin
+Curcumin
+Resveratrol

Fig. 2.

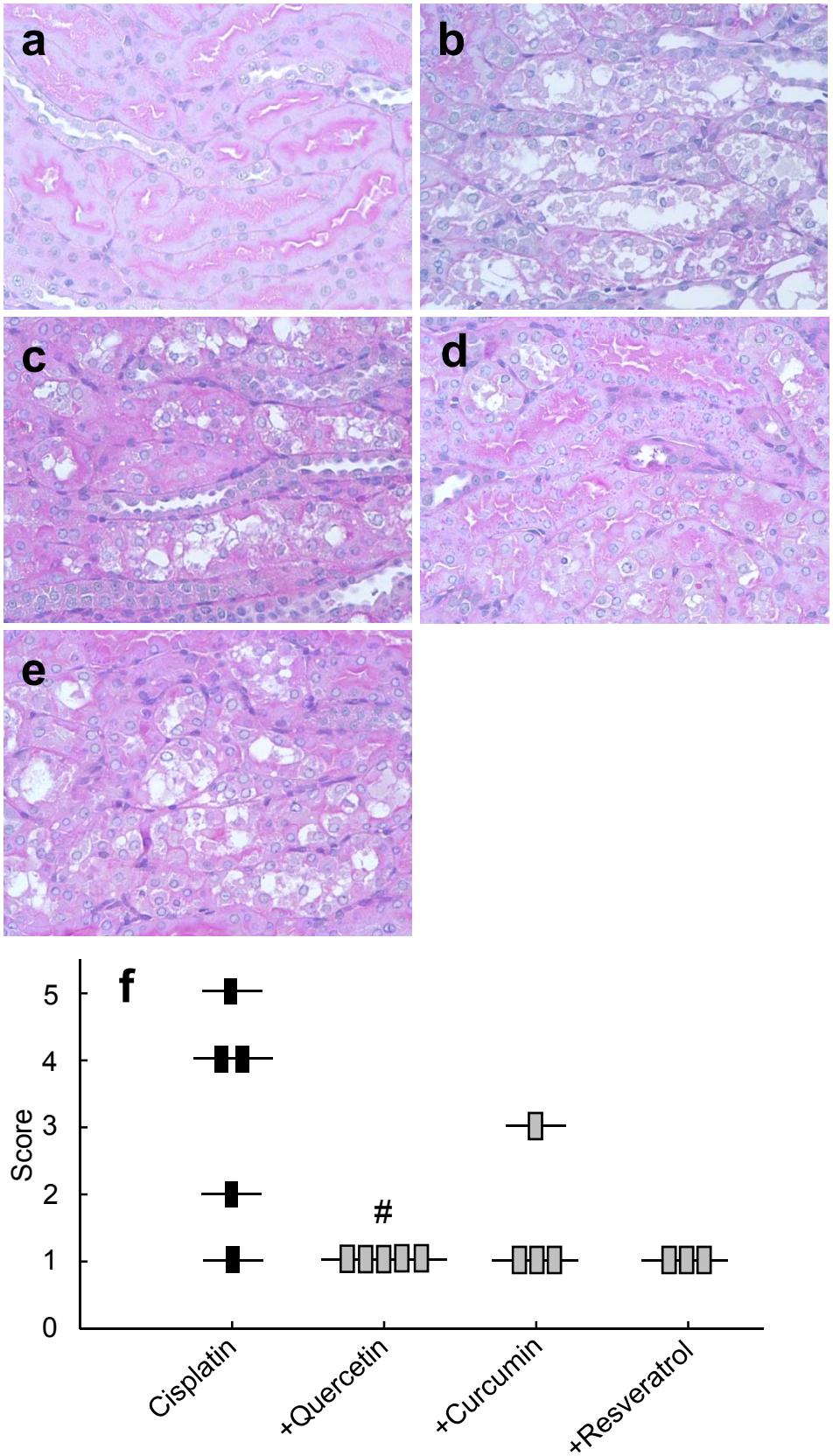

a
b
c
d
e
f
Score
0
1
2
3
4
5
#
Cisplatin
+Quercetin
+Curcumin
+Resveratrol

Fig. 3.

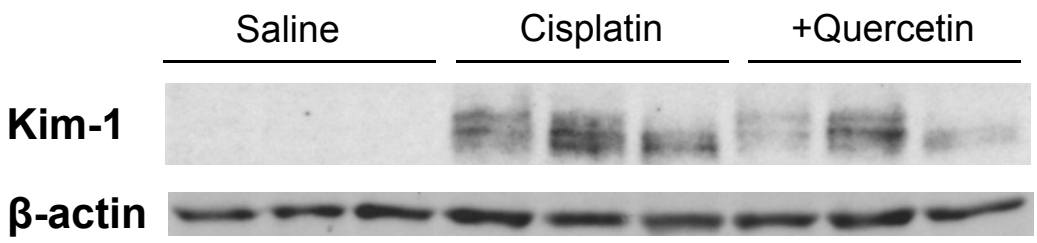


Fig. 4.

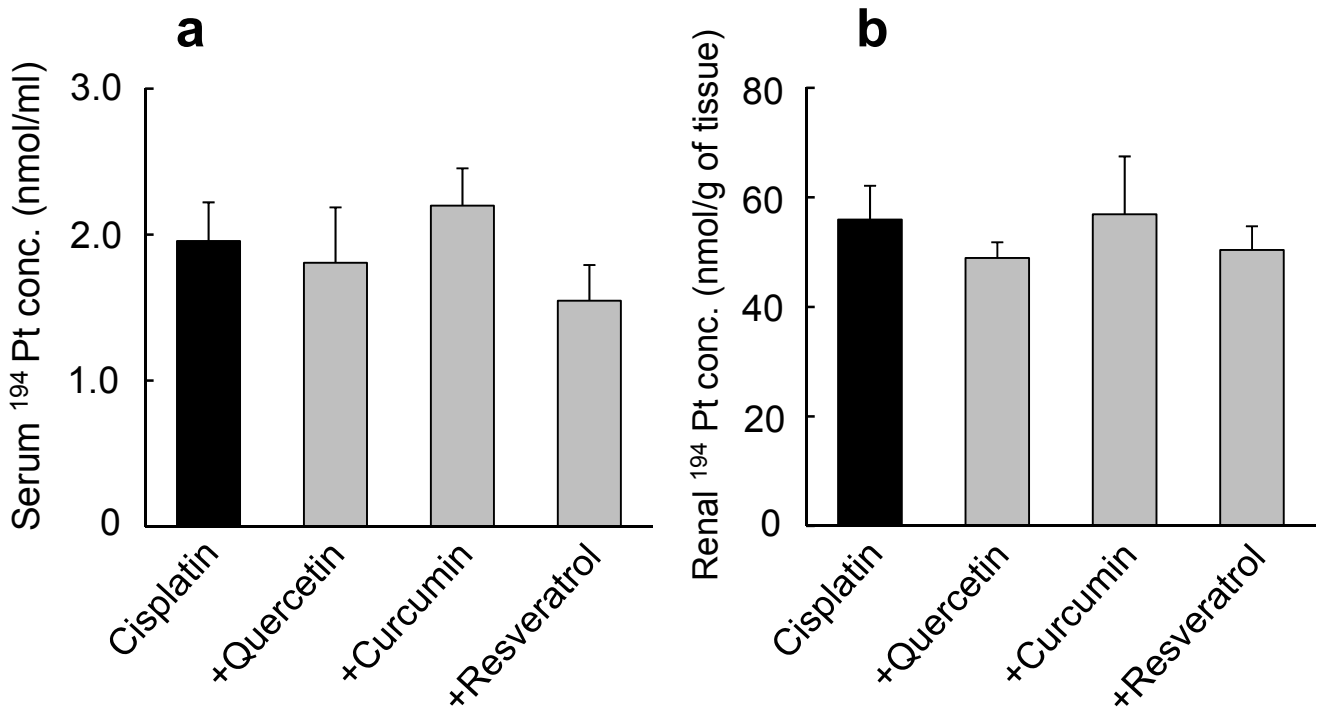

a
Serum 194 Pt conc. (nmol/ml)
3.0
2.0
1.0
0
Cisplatin
+Quercetin
+Curcumin
+Resveratrol
b
Renal 194 Pt conc. (nmol/g of tissue)
80
60
40
20
0
Cisplatin
+Quercetin
+Curcumin
+Resveratrol

Fig. 5.

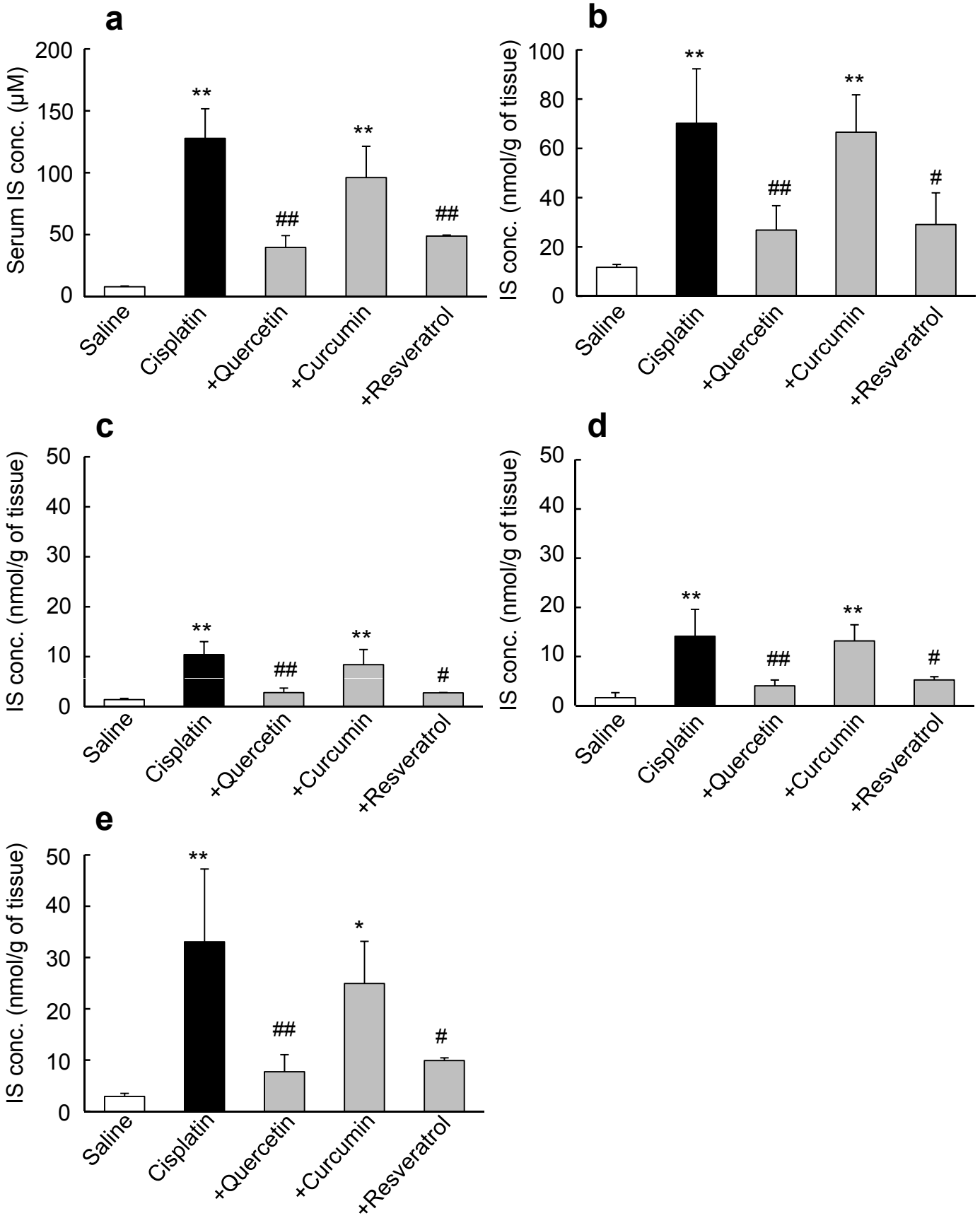

a
Serum IS conc. (μM)
200
150
100
50
0
**
**
##
##
Saline
Cisplatin
+Quercetin
+Curcumin
+Resveratrol
b
IS conc. (nmol/g of tissue)
100
80
60
40
20
0
**
**
##
#
Saline
Cisplatin
+Quercetin
+Curcumin
+Resveratrol
c
IS conc. (nmol/g of tissue)
50
40
30
20
10
0
**
**
##
#
Saline
Cisplatin
+Quercetin
+Curcumin
+Resveratrol
d
IS conc. (nmol/g of tissue)
50
40
30
20
10
0
**
**
##
#
Saline
Cisplatin
+Quercetin
+Curcumin
+Resveratrol
e
IS conc. (nmol/g of tissue)
50
40
30
20
10
0
**
*
##
#
Saline
Cisplatin
+Quercetin
+Curcumin
+Resveratrol

Fig. 6.

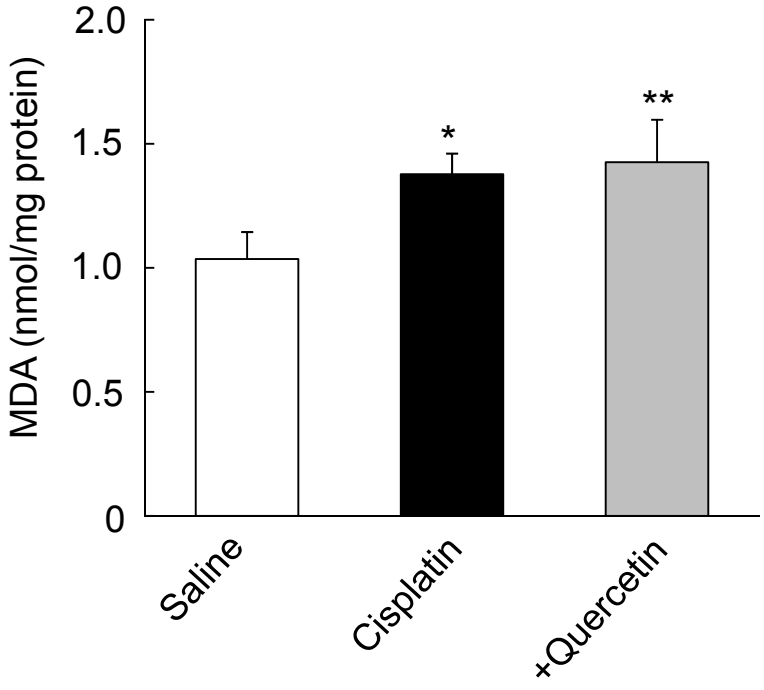


Fig. 7.

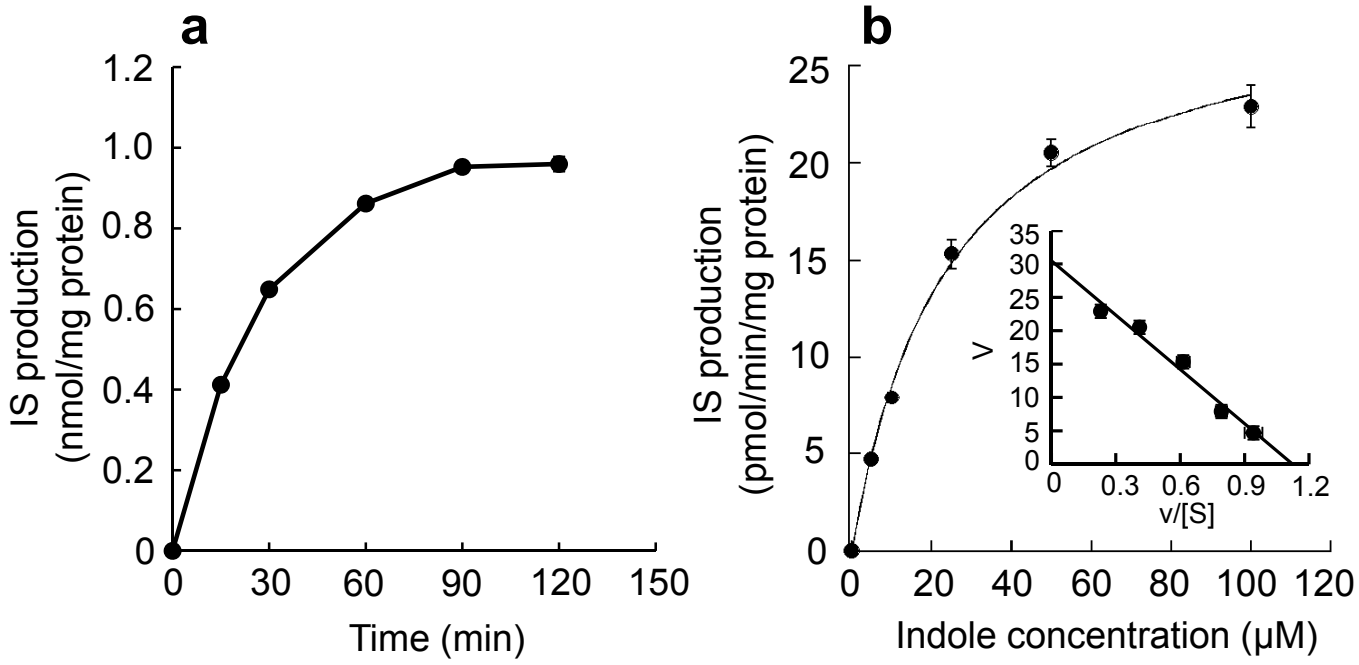
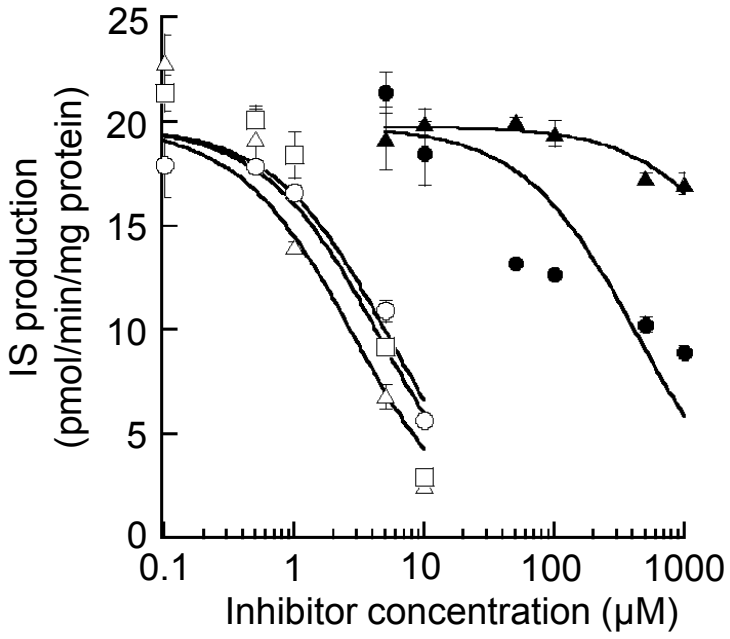

a
IS production
(nmol/mg protein)
1.2
1.0
0.8
0.6
0.4
0.2
0
0
30
60
90
120
150
Time (min)
b
IS production
(pmol/min/mg protein)
25
20
15
10
5
0
0
20
40
60
80
100
120
Indole concentration (μM)
V
35
30
25
20
15
10
5
0
0
0.3
0.6
0.9
1.2
v/[S]

Fig. 8.


IS production
(pmol/min/mg protein)
25
20
15
10
5
0
0.1
1
10
100
1000
Inhibitor concentration (μM)