Schisandrin B Enhances Renal Mitochondrial Antioxidant Status, Functional and Structural Integrity, and Protects against Gentamicin-Induced Nephrotoxicity in Rats

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Schisandrin B (Sch B), a dibenzocyclooctadiene derivative isolated from the fruit of Schisandra chinensis, has been shown to protect against oxidative damage in liver, heart and brain tissues in rodents. In the present study, the effect of long-term Sch B treatment (1–10 mg/kg/d×15) on gentamicin-induced nephrotoxicity was examined in rats. Sch B treatment protected against gentamicin-induced nephrotoxicity, as evidenced by significant decreases in plasma creatinine and blood urea nitrogen levels. The nephroprotection was associated with the enhancement in renal mitochondrial antioxidant status, as assessed by the level/activity of reduced glutathione, α-tocopherol and Mn-superoxide dismutase, as well as the improvement/preservation of mitochondrial functional and structural integrity, as assessed by the extents of ATP generation capacity, malondialdehyde production, Ca\(^{2+}\) loading and cytochrome c release, as well as the sensitivity to Ca\(^{2+}\)-induced permeability transition, in control and gentamicin-intoxicated rats. In conclusion, long-term Sch B treatment could enhance renal mitochondrial antioxidant status as well as improve mitochondrial functional and structural integrity, thereby protecting against gentamicin nephrotoxicity.

Key words schisandrin B; gentamicin; mitochondria; free radical; kidney

Schisandrin B (Sch B) is the most abundant, active dibenzocyclooctadiene derivative isolated from the fruit of Schisandra chinensis (FS), a traditional Chinese herb clinically used for the treatment of viral and chemical hepatitis. \(^1\) Recent studies from our laboratory have demonstrated the protective effect of Sch B on free radical-induced damage in various organs including the heart, liver and brain in rodents. \(^2\) Investigations on biochemical mechanism(s) involved in the generalized tissue protection afforded by Sch B have revealed its capability of enhancing mitochondrial antioxidant status, which is a crucial determinant in cell survival. \(^3\) Given the anti-oxidative stress potential of Sch B, it has been proposed to be used as a universal cell protectant against tissue damage caused by endogenous and exogenous oxidants. \(^4\) While the protection of Sch B against oxidative stress-induced injury has been demonstrated in various tissues including those of the heart, liver and brain, \(^5\) it is still unclear whether Sch B treatment can produce any beneficial effect on the resistance of kidney tissue to oxidative stress-induced damage.

In the present study, we investigated the effect of long-term treatment with Sch B on gentamicin-induced nephrotoxicity in rats. Gentamicin, which is a commonly used aminoglycoside antibiotics for the treatment of severe gram negative bacterial infections, has been found to cause nephrotoxic side effects. \(^5\) To elucidate the biochemical mechanism involved in the nephroprotection, renal mitochondrial antioxidant status as well as mitochondrial functional and structural integrity were assessed in control and Sch B-treated rats, without and with gentamicin challenge.

MATERIALS AND METHODS

Herbal Material Dried FS was imported from mainland China. It was authenticated and supplied by a commercial dealer (Lee Hoong Kee Ltd.) in Hong Kong. Sch B was purified from the petroleum ether extract of FS, with the purity being higher than 95% as determined by HPLC analysis. \(^6\)

Animal Care Adult female Sprague-Dawley rats (8—10 weeks; 200—250 g) were maintained under a 12-h dark/light cycle at about 22 °C, and allowed food and water ad libitum. Experimental protocols were approved by the Research Practice Committee at the Hong Kong University of Science & Technology.

Drug Treatment Animals were randomly divided into groups, with five animals in each. In the Sch B treatment groups, rats were intragastrically administered with Sch B (dissolved/suspended in olive oil) at a daily dose of 1 or 10 mg/kg from day 1 to day 15 of the experiment. This dosage regimen was found to be effective in protecting against myocardial ischemia/reperfusion injury in rats. \(^8\) Sch B-untreated animals received the vehicle (i.e. olive oil) only. Gentamicin (Sigma Chemical Co., St. Louis, MO, U.S.A.) was administered intraperitoneally at a daily dose of 100 mg/kg (dissolved in 1% ethanol in isotonic saline) from day 10 to day 15 (i.e., 6 doses). Non-gentamicin-treated animals were given the vehicle. Twenty-four hours after the last dosing with Sch B and gentamicin (i.e. on day 16), blood samples were drawn from phenobarbital-anesthetized animals by cardiac puncture. Animals were then sacrificed by cardiac excision and both kidneys were harvested for biochemical analysis.

Preparation of Tissue Homogenate and Mitochondrial Fraction Minced kidney tissues (ca. 0.6 g) were homogenized in 6 ml of ice-cold sucrose buffer (0.25 M sucrose, 0.1 mM Na\(_2\)EDTA, 5 mM Tris/HCl, pH 7.4) with a Teflon-glass homogenizer at 2000 rpm for 8—10 complete strokes. Mitochondrial pellets were prepared from tissue homogenates by centrifugation at 800×g at 4 °C for 30 min, as described. \(^7\) Mitochondrial pellets were then resuspended in 1 ml of homogenizing buffer and constituted the mitochondrial fraction.
Mitochondrial Antioxidant Status  Levels of mitochondrial reduced glutathione (GSH) and α-tocopherol (α-TOC) as well as the activity of Mn-superoxide dismutase (SOD) were measured.7,8

Mitochondrial Functional and Structural Integrity Mitochondrial ATP generation capacity was measured as described in Leung et al. (2005).7 The value of mitochondrial ATP generation capacity was estimated by computing the area under the curve of the graph plotting ATP generated (nmol/mg protein) against time (0—20 min) and expressed in arbitrary unit. The extents of mitochondrial malondialdehyde (MDA) production, cytochrome c release and calcium loading, as well as the sensitivity of mitochondria to Ca2+-induced permeability transition were measured, as described.8

Biochemical Analysis Renal function was indirectly assessed by measurements of plasma creatinine and blood urea nitrogen (BUN) levels using assay kits (Bioassay Systems, Hayward, CA, U.S.A.). The protein concentration of mitochondrial fractions was determined using a Bio-Rad protein assay kit.

Statistical Analysis Data were analyzed by one-way Analysis of Variance (ANOVA). Post hoc multiple comparisons were done with LSD. p-Values <0.05 were regarded as statistically significant.

RESULTS

Long-term treatment with Sch B (1—10 mg/kg/d×15 d, p.o.) caused slight decreases in plasma creatinine and BUN levels in rats (Fig. 1). Gentamicin treatment (100 mg/kg/d×6 d, i.p.) significantly elevated plasma creatinine (2.3-fold) and BUN (3.7-fold) levels, indications of nephrotoxicity. Sch B treatment protected against gentamicin-induced nephrotoxicity, as evidenced by dose-dependent decreases in plasma creatinine (10—30%) and BUN (14—32%) levels, when compared to the Sch B-untreated and gentamicin-intoxicated control. Sch B treatment enhanced the renal mitochondrial antioxidant status, as indicated by increases in the level/activity of GSH (44—29%), α-TOC (7—18%) and Mn-SOD (13%) in a dose-dependent manner (Table 1). In contrast, gentamicin treatment caused the impairment in renal mitochondrial antioxidant status, as evidenced by significant decreases in the level/activity of GSH (44%), α-TOC (33%) and Mn-SOD (31%). The nephroprotection afforded by Sch B treatment against gentamicin toxicity was associated with the dose-dependent increases in the level/activity of mitochondrial GSH (11—20%), α-TOC (6—12%) and Mn-SOD (5—9%), when compared to the Sch B-untreated and gentamicin-intoxicated control.

Sch B treatment significantly and dose-dependently increased the renal mitochondrial ATP generation capacity (13—24%), when compared to the Sch B-untreated control (Fig. 2). Gentamicin intoxication caused a slight but significant decrease in the mitochondrial ATP generation capacity. Sch B treatment significantly increased the renal mitochondrial ATP generation capacity (8—13%) in gentamicin-intoxicated rats, when compared to the Sch B-untreated and gentamicin-intoxicated control.

Sch B treatment decreased the extents of renal mitochondrial MDA production (7—11%), Ca2+ loading (11—21%) and cytochrome c release (4—8%), as well as the susceptibility of mitochondria to Ca2+-induced permeability transition (6—9%) to varying extents (Figs. 3a—d), all of which are indirect measures of mitochondrial structural integrity. Gentamicin treatment caused a disruption in renal mitochondrial structural integrity, as evidenced by significant increases in

![Graph showing plasma creatinine levels](Image)

Table 1. Effect of Long-Term Sch B Treatment on Renal Mitochondrial Antioxidant Status in Control and Gentamicin-Intoxicated Rats

<table>
<thead>
<tr>
<th>Sch B Treatment</th>
<th>GSH (nmol/mg protein)</th>
<th>α-TOC (ng/mg protein)</th>
<th>Mn-SOD (mU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.92±0.02</td>
<td>95.1±0.07</td>
<td>33.4±0.82</td>
</tr>
<tr>
<td>Sch B 1 mg/kg</td>
<td>2.26±0.07(n)</td>
<td>102±1.30(b)</td>
<td>34.2±0.54(a)</td>
</tr>
<tr>
<td></td>
<td>(18)</td>
<td>(7)</td>
<td>(13)</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>2.47±0.20(n)</td>
<td>112±1.10(c)</td>
<td>37.6±0.24(c)</td>
</tr>
<tr>
<td></td>
<td>(29)</td>
<td>(18)</td>
<td>(13)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1.08±0.05(n)</td>
<td>63.5±0.06(n)</td>
<td>23.2±0.68(b)</td>
</tr>
<tr>
<td>Control</td>
<td>(−44)</td>
<td>(−33)</td>
<td>(−31)</td>
</tr>
<tr>
<td>Sch B 1 mg/kg</td>
<td>1.20±0.02</td>
<td>67.4±1.51(b)</td>
<td>24.3±0.50</td>
</tr>
<tr>
<td></td>
<td>(14)</td>
<td>(6)</td>
<td>(5)</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>1.30±0.06</td>
<td>71.4±0.64(b)</td>
<td>25.4±0.75(a)</td>
</tr>
<tr>
<td></td>
<td>(20)</td>
<td>(12)</td>
<td>(9)</td>
</tr>
</tbody>
</table>

Animals were treated with Sch B and gentamicin as described in Fig. 1. Renal mitochondrial reduced glutathione (GSH) and α-tocopherol (α-TOC) levels as well as Mn-superoxide dismutase (SOD) activity were measured. The number in parentheses represents the percent change compared to the respective 1 mg/kg Sch B group.

![Graph showing blood urea nitrogen levels](Image)

Fig. 1. Effect of Long-Term Sch B Treatment on Gentamicin-Induced Nephrotoxicity in Rats

Animals were orally treated Sch B (1—10 mg/kg/d) for 15 d. from day 10 to 15 d. animals were intraperitoneal injected with gentamicin (100 mg/kg). Twenty-four hours after the last dosing with Sch B and gentamicin, animals were sacrificed and plasma and blood samples were obtained. Plasma creatinine and blood urea nitrogen (BUN) levels were measured. Values given are mean±S.E.M., with n=5. a Significantly different from the non-gentamicin control (CON); b significantly different from the gentamicin control; c significantly different from the respective 1 mg/kg Sch B group.
the extents of MDA production (17%), Ca$^{2+}$ loading (35%), and cytochrome c release (43%), as well as the sensitivity to Ca$^{2+}$-induced permeability transition (32%), when compared to the Sch B-untreated and gentamicin-intoxicated control. Sch B treatment preserved the mitochondrial structural integrity in gentamicin-intoxicated rats, as indicated by significant decreases in the values of mitochondrial parameters to varying extents (6—17%), when compared to the Sch B-untreated and gentamicin-intoxicated control.

**DISCUSSION**

Gentamicin treatment caused the impairment in renal function, as manifested in the increases in plasma creatinine and BUN levels. Nephrotoxicity induced by gentamicin has been found to be a complex phenomenon characterized by a severe proximal renal tubular necrosis followed by renal failure.9) The reduction in glomerular filtration rate, as indicated by the increase in plasma creatinine level, would be accompanied by an increase in BUN level when a marked renal parenchymal injury occurs.10) While long-term Sch B treatment produced a slight decrease in plasma creatinine and BUN levels in rats, it suppressed the gentamicin-induced increases in plasma creatinine and BUN levels in a dose-dependent manner. The nephroprotection afforded by Sch B treatment against gentamicin toxicity was associated with the enhancement of mitochondrial antioxidant status in both control and gentamicin-intoxicated rats. A huge body of experimental evidence has suggested that reactive oxygen species (ROS) are involved in the pathogenesis of gentamicin nephrotoxicity.5,9) In this regard, gentamicin was found to enhance the production of hydrogen peroxide by rat renal cortical mitochondria in vitro.11) Our finding of impairment of renal mitochondrial antioxidant components by gentamicin intoxication supports the role of ROS in gentamicin-induced renal damage. Given the crucial role of maintenance of mitochondrial antioxidant capacity in cell survival,3) the ability of Sch B to enhance renal mitochondrial antioxidant status is likely related to the nephroprotection against gentamicin toxicity.

The finding of enhancement of renal mitochondrial antiox-
idant status is consistent with our recent study which showed that long-term treatment with Sch B at the same oral dosage enhanced the mitochondrial antioxidant capacity in various tissues of rats. The beneficial effect of Sch B treatment on kidney tissues further supports the tissue non-specific action of Sch B in enhancing the mitochondrial antioxidant system. In addition, the enhancement of mitochondrial antioxidant status was associated with the improvement and/or preservation of mitochondrial functional and structural integrity, particularly in gentamicin-intoxicated condition. The maintenance of mitochondrial functional and structural integrity is crucial for cell survival and death. As regards the Ca\(^{2+}\)-induced permeability transition, the opening of mitochondrial permeability transition pore is critically involved in the development of cellular dysfunction and cell death. The mitochondrial Ca\(^{2+}\) overload caused by gentamicin toxicity, as observed in the present study, predisposes the mitochondria to permeability transition. The release of cytochrome c from mitochondrial inner membrane, which was also shown in the present study and believed to be an event secondary to the onset of mitochondrial permeability transition, is a key step leading to apoptosis. The ability of Sch B to increase the resistance of mitochondria to Ca\(^{2+}\)-induced permeability transition may be an important determinant involved in the protection against gentamicin-induced nephrotoxicity. In conclusion, the results indicate that long-term Sch B treatment could enhance renal mitochondrial antioxidant status as well as improve mitochondrial functional and structural integrity, thereby protecting against gentamicin nephrotoxicity.

REFERENCES