Schisandra Chinensis Protects against Adriamycin-Induced Cardiotoxicity in Rats

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Background: Adriamycin (ADR) is an effective chemotherapeutic agent against cancers but its clinical use is limited due to its cardiotoxicity. It has been suggested that the pathogenesis involves inhibition of nucleic acid and protein synthesis, free radical formation and lipid peroxidation. *Schisandra chinensis (SC)* has strong antioxidant activity. We investigate the protective effects of SC on adriamycin-induced cardiotoxicity.

Methods: Wistar rats were divided into four groups: CONT (control), ADR, SC and SC + ADR. After treatment, the hearts of the rats were surgically removed and studied for synthesis rates of nucleic acid and protein, myocardial antioxidants and lipid peroxidation.

Results: Cardiotoxicity was characterized by ascites, congested liver and depressed cardiac function. Nucleic acid and protein synthesis were inhibited, malondialdehyde (MDA) was increased, while myocardial glutathione peroxidase (GSHPx) activity and superoxide dismutase (SOD) were decreased by ADR. In contrast, administration of SC before and concurrent with ADR significantly reduced mortality and the amount of ascites. Indexes in myocardial GSHPx, macromolecular biosynthesis and SOD activities increased with a concomitant decrease in lipid peroxidation.

Conclusion: These results suggest that ADR cardiotoxicity is associated with antioxidant deficit and SC treatment changes the antioxidant status of the heart and improves cardiac function.

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Key words: Chinese herbal medicine, adriamycin, *Schisandra chinensis*, cardiotoxicity, antioxidant.

The classical treatise on Chinese herbal medicine, the Shen Nung Pen Tsao Ching, described *Schisandra chinensis (SC)* or Wu Wei Zi, as a high-grade herbal drug useful for a wide variety of medical conditions, particularly as a kidney tonic and lung astringent. SC contains a number of compounds, including essential oils, numerous acids and lignans. Lignans are found in the seeds of the fruit and have a number of medicinal actions. Chinese herbalists use SC for coughs, night sweats, insomnia, thirst and physical exhaustion.¹ Extracts from SC fruit are used for the treatment of cardiovascular diseases, liver diseases, diseases of the central nervous system related to old age, as well as being used as a supplement in the treatment of neoplasms, diabetes, etc.² SC extracts are also used for the production of nutraceuticals (soft drinks and health foods), oral hygiene preparations and skin and hair care.

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products. SC protects against ADR cardiotoxicity

products. (3) It enhances the hepatic glutathione antioxidant system in mice, as evidenced by the hepatoprotection against carbon tetrachloride (CCl₄) toxicity. (4) SC is one of the herbal components of Sheng Mai San (SMS). SMS is routinely used to treat coronary heart disease and is known to prevent cerebral oxidative damage and adriamycin-induced cardiomyopathy in rats. (7) Our preliminary results also suggest that SMS may improve exercise tolerance in patients with congestive heart failure without causing side effects. (8) It has been reported that SC may play a major role in antioxidant activity of SMS formulations, namely in inhibiting lipid peroxidation. (9) Animal studies also suggest that water- and alcohol-based extracts of SC promote myocardial contractility without having an adverse effect on blood pressure, stimulate the respiratory center, induce uterine smooth muscle contractions, and increase hepatic glutathione levels and the activities of glucose-6-phosphate and glutathione reductase. (10)

The anthracycline antibiotic adriamycin (ADR), also known as doxorubicin, is one of the most effective chemotherapeutic agents for a wide variety of cancers. However, its clinical use is limited due to the development of life-threatening cardiomyopathy (11) and congestive heart failure. (12) It has been suggested that ADR-induced myocardial dysfunction involves the inhibition of nucleic acid as well as protein synthesis, (13) release of vasoactive amines, (14) changes in adrenergic function, (15) abnormalities in mitochondria, (16) lysosomal alterations, (17) modifications in sarclemmal Ca²⁺ transport, (18) membrane-bound enzymes, (19) imbalance in myocardial electrolytes, (20) free radical formation (21) and lipid peroxidation. (22) Although ADR-induced injury appears to be multifactorial and complex, (23) most of the studies support the view that an increase in oxidative stress, evidenced by an increase in free radicals and lipid peroxidation as well as a decrease in antioxidants, plays an important role in the pathogenesis of ADR-induced cardiotoxicity. (24)

Many experimental studies have reported that increased oxidative stress and depressed antioxidant status have deleterious effects on both cardiac structure and function. (25) Clinical studies on heart failure patients have also provided support for the role of free radicals in the pathogenesis of heart failure. (26) Since it is suggested that inhibition of nucleic acid, as well as protein synthesis, formation of free radicals and lipid peroxidation, are involved in ADR cardiotoxicity, we decided to utilize a rat model of ADR-induced cardiotoxicity to determine whether SC has cardioprotective activity.

METHODS

Preparation of Schisandra chinensis aqueous extracts

The Schisandra chinensis fruit used in the current study were purchased locally in Taipei. Identification was authenticated by experts in pharmacognosy. The authentication tests included morphological examination and identification of schisandrin B content using thin-layer chromatography according to the Chinese Pharmacopoeia. The hot-water extract was prepared by boiling the dried fruit with distilled water for five hours. The extract was filtered, freeze-dried and kept at 4°C. The yield of extraction was approximately 10.1% (w/w). The direct extract was dissolved in distilled water before use.

Animal model

Male Wistar rats, body weight 250–300 g, were maintained on a normal rat chow diet. The rats were divided into four groups: CONT (control), ADR (adriamycin treated), SC (Schisandra chinensis-treated) and SC + ADR. ADR (doxorubicin hydrochloride, Pharmacia & Upjohn SpA, Milan, Italy) was administered by intraperitoneal injection in six equal doses (each containing 3 mg/kg ADR) over a period of two weeks, with a total cumulative dose of 18 mg/kg ADR. SC was administered via a feeding tube through the mouth once a day for 30 days (5 g/kg/day; cumulative dose 150 g/kg/body weight). The SC + ADR group was treated in the same manner as SC, alternating with ADR injections. CONT rats were injected with an identical volume of normal saline. All the treated rats and the CONT rats’ body weight, general appearance, behavior, ascites, limb edema and mortality were observed for up to five weeks after the last treatment. At the end of the five-week post treatment period, the rats’ hearts were examined for synthesis rates of DNA, RNA and protein. CONT hearts were washed three times with normal
saline and once with 70% alcohol. They were then washed with phosphate buffer solution (PBS) at 4°C, fragmented and weighed. The resulting fragments were washed with PBS and then disaggregated by gentle agitation for 30 minutes with a 5 ml enzyme cocktail of trypsin (0.2%) and collagenase (0.05%). The resulting cell suspension was filtered through polyester mesh (50-µm pore size) and centrifuged at 2000 rpm for five minutes. The cell pellet was then resuspended in 5 ml Roswell Park Memorial Institute medium (RPMI) for sorting. Cell suspensions were routinely counted on a hemocytometer with trypan blue, enabling the heart cell yield to be ascertained.

In order to measure the protein, RNA and DNA synthesis rates, the aliquots of the aforementioned cell pellet suspensions (3x10^6 cells in 96 well microtiter plate) were labeled by adding 200 µl (50 µCi/ml) 3H-thymidine (20 Ci/m mole), 3H-uridine (28.9 Ci/m mole) and 3H-leucine (53 Ci/m mole). After 18 hours, the cells were collected in filter paper disks. Each disk was placed in a counting vial and 5 ml of scintillation mixture was added (0.47% PPO and 0.01% POPOP in 100% toluene). The sample was counted in a liquid scintillation counter (Nuclear-Chicago Mark 1).

The count per minute (CPM) was determined by the following formula:

\[
\text{ΔCPM of control (\%) = \frac{\text{experimental groups' heart cell counts}}{\text{control group's heart cell count}} \times 100\%}
\]

**Glutathione peroxidase (GSHPx) assay**

GSHPx activity was expressed as nanomoles reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidized to nicotinamide adenine dinucleotide phosphate (NADP) per minute per milligram protein, with a molar extinction coefficient for NADPH at 340 nm of 6.22x10^6. Cytosolic GSHPx was assayed in a 3-mL cuvette containing 2.0 mL of 75 mmol/L phosphate buffer, pH 7.0. The following solutions were then added: 50 µL of 60 mmol/L glutathione, 100 µL glutathione reductase solution (30 U/mL), 50 µL of 0.12 mol/L NaN3, 0.10 of 15 mmol/L Na2EDTA, 100 µL of 3.0 mmol/L NADPH, and 100 µL of cytosolic fraction obtained after centrifugation at 20,000 g for 25 minutes. Water was added to make a total volume of 2.9 mL. The reaction was started by the addition of 100 µL of 7.5 mmol/L H2O2, and the conversion of NADPH to NADP was monitored by a continuous recording of the change of absorbance at 340 nm at 1-minute intervals for five minutes. GSHPx enzyme activity was expressed in terms of milligrams of protein.

**Superoxide dismutase assay**

The supernatant was assayed for superoxide dismutase (SOD) activity by following the inhibition of pyrogallol auto-oxidation. Pyrogallol (24 mmol/L) was prepared in 10 mmol/L HCl and kept at 4°C before use. Aliquots of supernatant (150 µg protein) were added to Tris-HCl buffer containing 25 µL pyrogallol and 10 µL catalase. The final volume of 3 mL was made up of the same buffer. Changes in absorbance at 420 nm were recorded at 1-minute intervals for five minutes. SOD activity was determined from a standard curve of percentage inhibition of pyrogallol auto-oxidation with a known SOD activity. This assay was highly reproducible, and the standard curve was linear up to 250 µg protein with a correlation coefficient of 0.998. Data are expressed as SOD units per milligram protein compared with the standard.

**Malondialdehyde assay**

Measurement of lipid peroxidation by determination of myocardial malondialdehyde (MDA) content was performed by a modified thiobarbituric acid (TBA) method. The hearts were quickly excised and washed in buffered 0.9% KCl (pH 7.4). After the atria, extraneous fat and connective tissue were removed, the ventricles were homogenized in the same buffer (10% wt/vol). The homogenate was incubated for one hour at 37°C in a water bath. A 2-mL aliquot was withdrawn from the incubation mixture and pipetted into an 8-mL Pyrex tube. One milliliter of 40% trichloroacetic acid (TCA) and one mL of 0.2% TBA were promptly added. To minimize peroxidation during the subsequent assay procedure, 2% butylated hydroxytoluene was added to the TBA reagent mixture. Tube contents were vortexed briefly, boiled for 15 minutes and then cooled in a bucket of ice for five minutes. Two milliliters of 70% TCA was then added to all tubes and the contents were again vortexed. The tubes were allowed to stand for 20 minutes. This was followed by a centrifugation of the tubes for 20 minutes at 3500 rpm. The color was read at 532 nm on a Zeiss spectrophotometer and compared with a known MDA standard.
Statistical analysis

Data were expressed as the mean ± SD. For statistical analysis of the data, group means were compared by 1-way ANOVA instead of 2-way ANOVA to explain the results.

RESULTS

General observations and mortality

The general appearance of all groups of animals was recorded during the time course of the study. After completion of ADR treatment, the animals’ fur became scruffy and developed a light yellow tinge. There were red exudates around the eyes of the ADR treated groups, which appeared to be sicker, weaker and lethargic compared with the SC + ADR group. The most predominant feature in the ADR group animals was the development of a grossly enlarged abdomen and ascites. This condition became apparent within four days after the completion of treatment with ADR. When these rats were sacrificed, all ADR group animals had a significant amount of peritoneal fluid. In addition, the liver was enlarged and congested. In the SC + ADR group, there was no peritoneal fluid. During the post-treatment period, the mortality rate was significantly higher in the ADR group, while there were no deaths in the CONT, SC and SC + ADR groups.

During the post-treatment period, the rats that died were all in the ADR group, making mortality significantly higher in this group than in any of the other groups (Table 1). All of the rats that died had a volume of ascites greater than 70 ml. Data on heart weight and ratio of heart weight to body weight are listed in Table 1. Despite the ascites, the body weight gained in the ADR group was significantly less. Mere treatment with ADR resulted in obviously lower heart weight and ratio of heart to body weight. There was no significant difference in ratio of heart to body weight for the SC + ADR, CONT and SC groups. Heart weight in the SC + ADR group was much greater than that in the ADR group.

Effects of the drugs on DNA, RNA and protein synthesis rates

Figure 1 shows that ADR inhibited synthesis rates of DNA, RNA and protein by almost 50% compared with the CONT group; while SC increased the synthesis of DNA by 45%, RNA by 60% and protein by 38%. In the SC + ADR group, DNA synthesis was increased by 20%, RNA synthesis by 60% and protein synthesis by 30%.

Antioxidant enzyme and lipid peroxidation

Different antioxidant enzyme activities were examined in all groups; these data are shown in Table 2. GSHPx activity in the ADR group was reduced by about 40% compared with the CONT group. In the SC + ADR group, GSHPx activity was almost the same as the control levels. Total SOD activity in the SC + ADR group was significantly higher than that in the ADR group. The amount of lipid peroxidation was determined by evaluating myocardial MDA content; these data are also shown in Table 2. MDA levels were lower in the SC and CONT groups, whereas the MDA content was significantly higher in the ADR group.

DISCUSSION

Repeated administration of adriamycin beyond a certain dose has been shown to cause cardiopathic changes in patients as well as in a variety of animal species. In this study, we utilized a rat model of...
ADR-induced cardiotoxicity and found that the animals had increased mortality and accumulation of ascites, as well as significantly decreased heart weight compared to the control animals. The results demonstrate that simultaneous treatment with SC mitigates ADR-induced cardiomyopathic changes as well as congestive heart failure, and indicates an improvement in cardiac function. Reduced mortality was also apparent in the SC + ADR group.

The radioactive tracer method (thymidine, uridine and leucine incorporation assay) indicated that heart cell DNA, RNA and protein synthesis rates were inhibited by ADR, which reflects its cytotoxic properties. SC, on the other hand, offered cytoprotection against ADR cardiotoxic effects by promoting heart cell macromolecular biosynthesis.

In addition to antioxidant properties, SC may also act as a stimulator for the activity of antioxidant enzymes. GSHPx is a major enzymatic mechanism for the disposal of peroxides in the heart; a prolonged depression in the level of this enzyme may lead to intracellular peroxide accumulation. Since ADR increases cardiac superoxide anion generation, the potential exists for hydrogen peroxide formation at a time when peroxide removal is impaired. It has been reported that ADR reduces the activity of GSHPx in rat hearts. SC has been shown to enhance the hepatic antioxidant system, as indicated by an increase in the hepatic GSH level as well as hepatic glutathione reductase. The enhancement in hepatic GSH status is associated with a corresponding decrease in tissue MDA levels, indicating a significant reduction in the extent of oxidative hepatocellular damage. Accordingly, it may also affect the heart, thus providing indirect and direct protection against free radical attack. These results indicate that SC can scavenge various free radicals effectively from different antioxidant system sites through enhancing the activities of the antioxidant enzymes in ADR-induced cardiotoxicity. In this regard, ADR has been shown to promote the production of free radicals and these toxic substances are known to cause myocardial dysfunction.

Data from lipid peroxidation are also in concert with this hypothesis, inasmuch as SC causes a significant attenuation in the ADR-induced increase in MDA levels.

SC not only prevented ADR-induced decreases by activating GSHPx but also increased SOD activity. It is important to note that in the ADR group, there was a significant decrease in the SOD activity. SC alone caused a 23% increase in SOD. However, in the SC + ADR group, there may have been some synergistic effect, since the increase in SOD activity was about 35%. Thus, SC improves endogenous antioxidant reserve and may improve myocardial structure and function. The mechanisms of ADR-induced decrease in GSHPx and SC-induced increase in antioxidants (GSHPx and SOD) are still not clear. This study demonstrates that SC may be providing protection by acting as an antioxidant and by pro-

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**Table 2. Effects of SC on Adriamycin-Induced Changes in GSHPx, SOD and MDA**

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>GSHPx nmoI/mg protein</th>
<th>SOD U/mg protein</th>
<th>MDA nmol/g heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONT</td>
<td>26.7 ± 2.7</td>
<td>25.8 ± 4.2</td>
<td>27.8 ± 2.3</td>
</tr>
<tr>
<td>ADR</td>
<td>19.0 ± 1.7*</td>
<td>7.6 ± 1.3*</td>
<td>44.5 ± 4.8*</td>
</tr>
<tr>
<td>SC</td>
<td>28.4 ± 1.6</td>
<td>31.8 ± 4.3</td>
<td>28.6 ± 5.1</td>
</tr>
<tr>
<td>ADR + SC</td>
<td>26.2 ± 2.4†</td>
<td>35.0 ± 3.8†</td>
<td>37.3 ± 3.5†</td>
</tr>
</tbody>
</table>

* Abbreviations: GSHPx: glutathione peroxidase; SOD: superoxide dismutase; MDA: malondialdehyde.

Data are mean ± SD from five experiments.

* p < 0.001 as compared with the CONT group
† p < 0.001 as compared with the ADR group

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**Fig. 1** Effects of SC on ADR-induced changes in protein, DNA and RNA synthesis rates of heart cells. Values are CPM of control (%).

ADR SC ADR + SC

* p < 0.001 compared with CONT group
** p < 0.001 compared with the ADR group
motivating endogenous antioxidants. However, the molecular mechanisms of ADR-induced antioxidant enzyme changes and their prevention with SC remain unclear. Since DNA strands of various enzymes can be potentially damaged directly by ADR and indirectly by ADR-produced free radicals, altered antioxidant enzyme activities could be the result of modulated gene expression and oxidative inactivation. Recent data provided evidence about both cardiomyocyte and endothelial cell apoptosis playing an important role in ADR-induced cardiotoxicity. The related mechanisms need further study.

In conclusion, it can be proposed that ADR cardiotoxicity is associated with the antioxidant deficit and SC treatment changes the antioxidant status of the heart to improve cardiac function. The mechanism may be related to the maintenance of antioxidant status and may promote heart biosynthesis. It may also be significant to elucidate the antitumor effects of an SC and ADR combination. The influence of SC on tumor response to ADR should be evaluated by further studies.

Acknowledgments

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中藥五味子對阿霉素誘導大鼠心臟毒性的拮抗作用

游智勝1,2,3 潘台龍1,2 侯毓昌2

背 景： 阿霉素是一種抗癌藥抗生素，臨床運用廣泛，不過，因其具有心肌毒性的副作用，
以致於限制了它的使用。過去的一些研究指出，阿霉素引起心臟毒性的原因包含了
核酸與蛋白質合成的抑制及自由基與過氧化脂質的產生。

方 法： 我們使用 Wistar 老鼠來建立阿霉素誘導心臟毒性的動物模型，共分成 4 組，其中一
組為對照組，其餘為治療組，分別是阿霉素、五味子、阿霉素＋五味子等 3 組。經
各組處理以後，我們觀察動物的體重、外觀、行為、腹水、四肢水腫與死亡率等情
形，並且檢測心肌細胞的 DNA、RNA 與蛋白質的合成速率、GSHPx、SOD 及 MDA
的變化，最後以 Anova 統計方法分析結果。

結 果： 阿霉素＋五味子組比阿霉素組毒性症狀出現較晚，死亡率較低。另外，阿霉素對大
鼠心肌 SOD, GSHPx 含量有顯著的抑制作用，使心肌組織內 MDA 含量增加，同時也
抑制心肌之 DNA, RNA 蛋白質的合成速率，而五味子對阿霉素所導致的心肌毒性有
拮抗作用。

結 論： 五味子對阿霉素所導致的心臟毒性有拮抗作用，其機理可能與抗自由基反應與生物
合成速率有關。
（長庚醫誌 2006;29:63-70）

關鍵字： 中草藥，阿霉素，五味子，心臟毒性，抗氧化。