Biochemical Pharmacology, Vol. 46, No. 12, pp. 2327–2332, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

EFFECT OF SODIUM TANSHINONE IIA SULFONATE IN THE RABBIT MYOCARDIUM AND ON HUMAN CARDIOMYOCYTES AND VASCULAR ENDOTHELIAL CELLS

TAI-WING WU,*†‡§ LING-HUA ZENG,* KWOK-PUI FUNG,* JUN WU,*
HENRIANNA PANG,‖ ARTHUR A. GREY,‖ RICHARD D. WEISEL†‡ and JIAN YAO WANG‖

*Department of Clinical Biochemistry, University of Toronto and Toronto General Hospital, Toronto, Ontario; ‖Carbohydrate Research Centre, University of Toronto, Toronto, Ontario; and †Division of Cardiovascular Surgery and ‡The Center for Cardiovascular Research, The Toronto Hospital, Toronto, Ontario, Canada

(*Received* 23 *March* 1993; *accepted* 2 *June* 1993)

Abstract—Sodium tanshinone IIA sulfonate (STS) is a derivative of tanshinone IIA. The latter is a pharmacologically active component isolated from the rhizome of the Chinese herb *Salvia miltiorrhiza*. Liquid chromatographically pure STS was found to reduce myocardial infarct size by 53.14 ± 22.79% relative to that in the saline control in a rabbit 1 hr-ischemia and 3 hr-reperfusion model. This effect was comparable to that of Trolox (a better characterized antioxidant serving as a reference cytoprotector), which salvaged the myocardium in the same infarct model by 62.13 ± 18.91%. Also, like Trolox, STS did not inhibit oxygen uptake by xanthine oxidase (XO), a key enzyme in free radical generation. However, in contrast to Trolox, STS significantly prolonged the survival of cultured human saphenous vein endothelial cells but not human ventricular myocytes *in vitro* when these cells were separately exposed to XO-generated oxyradicals. Note that the endothelium is recognized to be a key site of oxidant generation and attack. Our findings *in vitro* and *in vivo* support the interpretation that STS is a cardioprotective substance, and that it may exert a beneficial effect on the clinically important vascular endothelium.

The rhizome of *Salvia miltiorrhiza Bunge (Labiatae)*, also known as "Tanshen" or "Danshen," is an important herb in Chinese traditional medicine for use in various coronary conditions (see review in Ref. 1). The chemical composition of this herb has been actively investigated. The organic extract of Tanshen is rich in diterpene quinones [2], some of which have been isolated [2–4] or synthesized [2, 5, 6], and their pharmacological effects have been reported in preliminary studies on myocardial ischemia-reperfusion [3, 4]. Recently, an active component, designated tanshinone IIA, was isolated from Tanshen by organic extraction, and its sodium sulfonate, i.e. sodium tanshinone IIA sulfonate (STS)¶ (Fig. 1a), was prepared for injection into patients with various heart ailments [7]. However, there was little basic information on the action of STS in the myocardium.

In the present work, we studied the effect of STS on the rabbit myocardium under oxidant stress and explored its site of action *in vitro* by using human ventricular myocytes as well as human saphenous vein endothelial cells. Throughout the paper, we will refer frequently to Trolox (TX) as a reference antioxidant [8–10], which is a well-characterized free radical scavenger similar in size to STS.

§ Corresponding author: Dr.Tai-Wing Wu, Department of Clinical Biochemistry, University of Toronto and Toronto General Hospital ES 3-404B, 200 Elizabeth St., Toronto, Ontario, Canada M5G 2C4. Tel. (416) 340-3261; FAX (416) 481-2899.

¶ Abbreviations: AVCA, anterior ventricular coronary artery; PBS, phosphate-buffered saline; STS, sodium tanshinone IIA sulfonate; TX, Trolox; and XO, xanthine oxidase.

MATERIALS AND METHODS

Materials. Unless otherwise stated, all chemicals used were reagent grade and supplied by the Sigma Chemical Co. (St. Louis, MO). Sodium tanshinone IIA sulfonate was obtained from Materia Medica of Academia Sinica in Shanghai, China. Xanthine oxidase (XO) was obtained from Boehringer Mannheim (Laval, Quebec), collagenase (type II and type III) from Worthington (Freehold, NJ), and fetal bovine serum from Gibco Laboratories (Grand Island, NY).

Structure analysis. The fast atom bombardment mass spectrometry (FAB-MS) spectra were acquired using a VG-Analytical ZAB-SE instrument. The samples, dissolved in methanol with thioglycerol as matrix, were bombarded by 8 keV Xenon atoms generated using the Ion-Tech Saddle field ion gun. The spectra were recorded using the VG 11-250 data system under the multi-channel analyzing mode, and the instrument was calibrated with cesium iodide. The resolution was set at 1,000 (10% valley definition) in the low resolution mode and at 10,000 in the high resolution mode. Polyethylene glycol (average molecular weight 300) was added into the matrix and used as the internal calibrant during the high resolution mass spectrometric analysis.

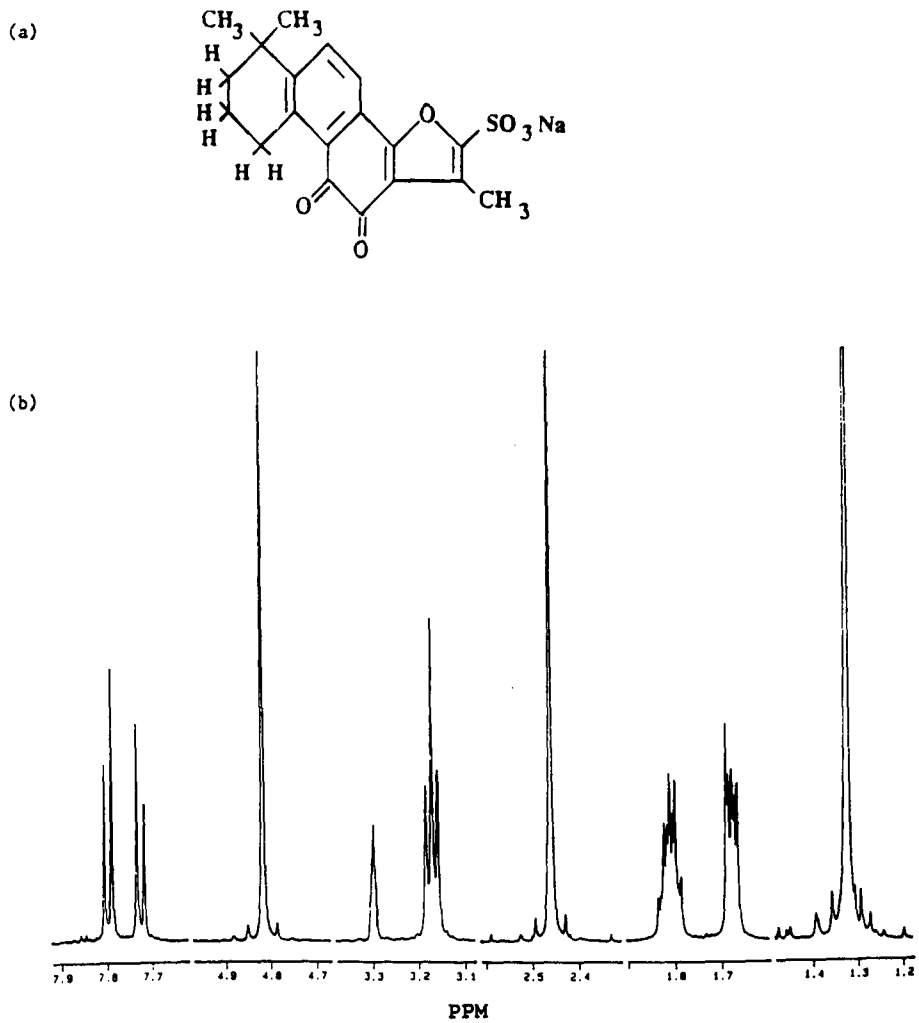

Fig. 1. (a) Chemical structure and (b) proton NMR spectrum of sodium tanshinone IIA sulfonate (STS).

Proton NMR was performed with a Bruker AM 500 MHz NMR spectrometer. The proton NMR spectrum of STS was recorded at 25° in a methyl-d3 alcohol solution with tetramethylsilane added as the internal reference standard. All assignments were made by first-order analysis.

Ischemia-reperfusion. Ischemia-reperfusion was performed as described previously [11]. New Zealand white male rabbits (approx. 3.0 kg) were first injected intramuscularly with ketamine HCl (Rogarsetic, 35 mg/kg) and acepromazine maleate (Atravet, 0.4 mg/kg). Anesthesia was maintained by tracheotomy and ventilating the animal with positive pressure respiration using a Harvard small animal respirator and a gas mixture of 2.5% enflurane (Ethrane, 2-chloro-1,1,2-trifluorethyl difluoromethyl ether) with oxygen (0.6 L/min). The right femoral artery was exposed and cannulated for measuring the arterial blood pressure and the right femoral vein for intravenous drip of normal saline. Following a midline sternotomy, the pericardium was opened and the heart was exposed. A dose of 50 IU/kg of heparin sulfate was given intravenously via the ear vein. The main branch of the anterior ventricular coronary artery (AVCA) was ligated temporarily with a 5–0 silk thread for 1 hr at a site between 1/3 to 1/2 the distance from apex to the atrioventricular groove. Attainment of ischemia was evidenced by the rapid change in color of the left ventricle from red to purple, accompanied by marked elevations in the S-T segment of the electrocardiogram for the saline-infused control and the STS-infused hearts (not shown). Such a change in the S-T segment was not noted in sham-operated controls, which were treated as were other rabbits, except that blood flow to the heart was only occluded for 30 sec.

Pilot experiments had shown that 1-hr ischemia here produced an optimum percentage of myocardial necrosis without reaching the "point of no return," at which most cells were no longer salvageable. After 1-hr ischemia, we observed statistically a similar percent necrosis whether the saline

reperfusion period was 3, 6 or 12 hr. Approximately 1–2 min before releasing the occlusion, a 30-mL bolus of saline alone (placebo control) or saline containing STS at 10 μmol/kg body weight was injected into the right external jugular vein, followed by a 3-hr reperfusion. Note that the saline solution containing STS was sonicated for 10 min before use. Eight animals were used in randomized order for each permutation.

After reperfusion, the rabbit was killed and the heart was harvested. To define the area previously made ischemic (the "area at risk"), we used a method similar to that described by Jolly *et al.* [12]: The AVCA was ligated in exactly the same place as before, and a bolus of 0.25% Evans Blue in saline (30 mL here) was infused into the heart via the aorta, slowly and at a pressure just sufficient to maintain closure of the aortic valve. The heart was sliced transversely into 5–6 each 0.2-cm thick slices, rinsed in saline to remove any excess Evans Blue, and then incubated in 1.25% tetrazolium red for 25 min at 25°.

The area of the left ventricle at risk of infarction (due to its anatomical dependence on the AVCA for blood flow) was identified by the lack of Evans Blue in this region. The region of infarcted myocardium within the area at risk was demarcated by the lack of staining of the tissue when immersed in tetrazolium red due to a loss of dehydrogenase enzyme(s), which converts the tetrazolium compound to a red formazan dye. After incubation with tetrazolium, the slices were weighed and the transverse ventricular sections were traced carefully onto clear plastic overlays to permit computerized planimetry of the infarct area as documented previously [10, 13]. The slices were fixed in 10% formalin, paraffin embedded, and cut to a thickness of 5–7 μm. The slices were stained with hematoxylin and eosin, as described by Jolly *et al.* [12] and by Axford-Gatley and Wilson [14]. Also, examinations by light microscopy and electron microscopy of these sections were performed to verify the reliability of the tetrazolium red stain to distinguish between necrotic and viable myocardium. In our hands, we confirmed that the percentage of necrosis estimated gravimetrically (based on weight of necrotic tissue over total weight of slice) agreed within 5% with the determination of infarct size based on planimetry. Further details concerning the protocol and validation of this histochemical determination of percent necrosis in reperfused tissue were given by Jolly *et al.* [12], Frederiks *et al.* [15] and Labbe *et al.* [16]. The myocardial salvage in the STS-treated group was calculated by the following formula:

$$\text{Myocardial salvage} = \frac{(\%\ \text{necrosis without STS} - \%\ \text{necrosis with STS})}{\%\ \text{necrosis without STS}} \times 100\%.$$

Free radical studies with cultured human ventricular myocytes and saphenous vein endothelial cells. Human ventricular myocytes were cultured in DME (Dulbecco's modified Eagle) medium supplemented with 10% fetal bovine serum, pH 7.4 [17], from 5 to 10 mg left ventricular biopsies from patients. The myocytes were identified by morphological appearance and by fluorescent antibodies to both actin and to human ventricular myosin heavy chain and light chain 1, and by electron microscopy before free radical studies [8].

Vascular endothelial cells were cultured as described earlier [8]. The segment of saphenous vein was coated with 0.1% collagenase. After 30 min of incubation at room temperature, endothelial cells were washed out and suspended in 199 medium supplemented with 20% fetal bovine serum, pH 7.4. The identity of the endothelial cells was confirmed by their morphological appearance and by fluorescent antibody to factor VIII before the free radical studies [8].

Free radical studies on cultured human ventricular myocytes and endothelial cells were done in a morphometric assay according to Wu *et al.* [18]. The cells were incubated in 0.05 M phosphate-buffered saline (PBS), pH 7.4, containing 67 IU/L XO and 2 mM hypoxanthine, at 37°. The base for comparing control versus STS was the time taken for necrosis of 95% of 10^5 cells or, conversely, the survival time of 5% of cells in each culture dish. STS was added along with XO and hypoxanthine in the cells and tested in randomized order in triplicates. Cell necrosis was observed by phase-contrast microscopy [8, 9]. The necrotic end point was corroborated by >95% loss in the ability of the cultured cells to exclude trypan blue [18], and by electron microscopy [18].

Assay of effect of STS on the rate of oxygen consumption by XO. As described previously [18], the rate of oxygen consumption as in the reaction between XO and hypoxanthine at 37° was monitored with or without STS (0.25 and 1 mM in PBS, pH 7.4) present. The percent oxygen consumed over the first 3 min was measured (N = 3) in a YSI oxygen electrode (Yellow Spring Instruments Inc.) [9, 10]. For comparison, the effects of 0.25 and 1 mM oxypurinol (a well known inhibitor of XO) in PBS, pH 7.4, were used [19].

Statistical analysis. Either Student's *t*-test or the analysis of variance (ANOVA) method was applied, as indicated. All data are expressed as means ± SD. Statistical significance was indicated by a P value < 0.05.

RESULTS

In this study, the aqueous form of STS was used. The structure of presumptive STS was deduced from its proton NMR and FAB-MS, while its purity was estimated based on, among other tests, its profile on HPLC.

The positive FAB-MS of STS exhibited pseudomolecular ions at *m/z* 397 $(M + H)^+$ and *m/z* 375 $(M - Na + 2H)^+$. High resolution data indicated *m/z* 397 and 375, consistent with an elemental composition of $C_{19}H_{18}O_6SNa$ (experimental error 3.1 mmu) and $C_{19}H_{19}O_6S$ (experimental error 0.3 mmu), respectively.

The assignment of the NMR spectrum of STS (Fig. 1b) begins with a singlet resonance at 1.326 ppm which integrates to six protons, (6H), and is assigned to equivalent geminal methyl groups. Adjacent to

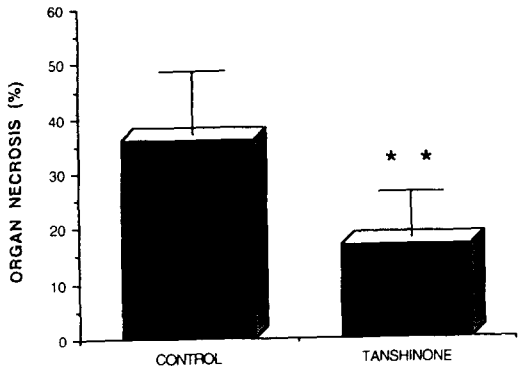

Fig. 2. Effect of STS on the salvage of the myocardium after ischemia-reperfusion in rabbits. Myocardial necrosis was obtained by infusion of saline (control) or 10 μmol/kg of STS in saline into rabbits that underwent ischemia-reperfusion. Results are the means ± SD of 8 rabbits for each group. Key: (**) $P < 0.001$.

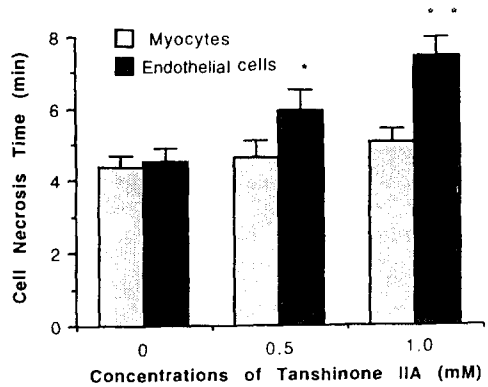

Fig. 3. Effect of STS on the time of necrosis of human myocytes and endothelial cells. Human ventricular myocytes and human saphenous vein endothelial cells were cultured as described in the text. Cells were then incubated with 67 IU/L XO and 2 mM hypoxanthine at 37° in PBS with or without 0.5 or 1 mM STS. Time for necrosis of 95% of 10^5 cells or, more accurately, the oxidant injury time for 95% of 10^5 cells was recorded. In each case, values are means ± SD, N = 4. Key: (*) $P < 0.05$, and (**) $P < 0.01$.

the latter is a methylene group responsible for a multiplet resonance centered at 1.679 ppm, (2H). The next in-line methylene group gives rise to a multiplet centered at 1.812 ppm, (2H), while the last in-line methylene group results in a triplet resonance observed at 3.172 ppm, $J = 6.4$ Hz, (2H). The latter chemical shift is typical of methylene groups covalently bonded to aromatic rings. The aromatic vicinal pair of protons on the adjacent ring system is observed as doublets at 7.798, (1H), and 7.726 ppm, (1H). Each doublet bears a coupling constant, $J = 8.2$ Hz, typical of ortho protons in aromatic ring systems. The last resonance observed in the spectrum is a singlet at 3.294 ppm, (3H), and is assigned to the methyl group on the double bond of the furan ring at the opposite end of the molecule. The chemical shifts and multiplicity of the resonances observed in the proton NMR spectrum are consistent for protons observed in the environment which makes up the structure of the diterpene quinone shown in Fig. 1a.

Both FAB-MS and ^1H NMR analyses of STS support a proposed structure as diagrammed in Fig. 1a.

The purity of STS was reflected by HPLC on a C_{18} reverse phase column (Partisil 10 ODS 3 from Phenomenex; gradient elution from 100% water to 100% methanol in 20 min, UV detection at 266 nm). Only one major peak was observed with four minor components which totally contributed less than 1.3% of the amount (data not shown). The UV chromatograms of STS before HPLC separation and of the major fraction collected after HPLC were identical.

Figure 2 shows the effect of infusing 10 μmol/kg of STS in saline on the percent salvage of the myocardium after ischemia-reperfusion in rabbits. The percent necrosis of the region at risk in the saline-treated group (N = 8) was 36.2 ± 11.3, while in the STS-treated group (N = 8) the extent of necrosis decreased significantly to 17.0 ± 8.2 ($P < 0.001$). Note that the areas at risk of both groups were statistically similar ($P > 0.05$).

The mean salvage of the rabbit myocardium after STS treatment was 53.14 ± 22.79%.

To gain further mechanistic insights into the action of STS, we tested whether STS protects human ventricular myocytes and/or human saphenous vein endothelial cells against oxyradical damage *in vitro*. Figure 3 shows the effect of 0.5 and 1 mM STS on the time of necrosis of these cells. In the absence of XO and/or hypoxanthine, the myocytes and endothelial cells remained viable for at least 60 min. Both control cells underwent necrosis within approximately 4.5 min when exposed to XO–hypoxanthine. No significant effect over the control was observed in myocytes treated with either 0.5 or 1 mM STS. In contrast, STS prolonged survival of the endothelial cells markedly longer than in the absence of STS when the cells were exposed to XO–hypoxanthine. ANOVA confirmed statistically significant dependence of necrosis times of the endothelial cells with the concentrations of STS examined ($P < 0.05$).

Figure 4 shows the effect of STS on the rate of oxygen consumption when XO reacts with hypoxanthine, at concentrations used in our cell-based assay rather than in classical enzymological studies as exemplified by Kelley and Beardmore [19]. At a concentration of either 0.25 or 1 mM, STS had no effect on the activity of XO. In contrast, oxypurinol, a known inhibitor of XO [19], markedly reduced the initial rate of oxygen uptake by XO in a concentration-dependent manner.

DISCUSSION

The chemical composition of Tanshen has been a subject of active inquiry [1]. The alcohol extract

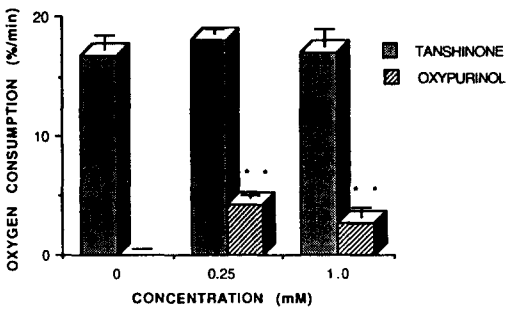

Fig. 4. Effect of STS or oxypurinol on the rate of oxygen consumption by XO. The assay was performed using an oxygen electrode as described in the text. In each case, values are means ± SD, N = 3. Note that the column at 0 concentration is the frame of reference for both STS and oxypurinol. Key: (**) $P < 0.001$.

contains diterpene quinones which are designated as tanshinones [2], whereas the aqueous extract contains, among others, lithospermate B [20] and lithospermic acid and rosemarinic acid as well as their methyl ester derivatives [21]. Some of these active components have been isolated and/or synthesized [2, 5, 6], but not all of them were pure enough for pharmacologic characterization. In the present study, STS (Fig. 1a), a tanshinone derivative, was certified to be >95% pure by HPLC, and characterized structurally by NMR (Fig. 1b) and MS before our examination of its bioactivity.

As noted at the beginning of the paper, TX was used as a frame of reference for STS in this study because it is a proven cytoprotector within the same molecular size range as STS, and its *in vitro* and *in vivo* bioactive effects have been characterized recently [8–10].

In this work, STS was found to further resemble TX in that it does not inhibit XO. In addition, both compounds appear to protect the post-ischemic reperfused rabbit myocardium to comparable extents.

Since ventricular myocytes and vascular endothelial cells are important in myocardial and other circulatory disorders including infarction [22], we had also examined whether STS protects these cells from humans [8, 17] *in vitro*. Interestingly, STS did not prolong significantly the survival of human myocytes but it did protect, in a concentration-dependent fashion, the endothelial cells against oxyradical damage generated by XO and hypoxanthine (Fig. 3). This behaviour contrasts sharply with that of Trolox, which protects ventricular myocytes but not endothelial cells [8, 18]. Although the mechanism of such contrasting cell-based behaviour between STS and TX is not immediately clear, one could speculate that there are a number of underlying causes. For example, the differential solubilities of these agents and their modes of transport into the different cells may be important determinants here. While these are pure speculations that require concrete experimental verification, we already know that the water-soluble STS is a more active oxyradical scavenger for the endothelial cells than its underivatized (less soluble) forms were. Also, in separate studies, we have observed that purpurogallin (an oak bark-derivative antioxidant) protects the vascular endothelial cells against XO-generated oxidants much more effectively than TX [18]. It is worth noting that saphenous vein endothelial cells behave similarly to aortic endothelial cells when they are exposed to oxyradicals [8, 18] and, therefore, were used in the present investigation. However, our preliminary hemodynamic studies have not revealed any substantial effect of STS on the pressure-rate index in the rabbit heart during ischemia-reperfusion. These data do not negate a possible effect (regional or global) of STS on blood circulation.

Structurally, STS does not strongly resemble any class of known antioxidants. However, it does possess unsaturated rings, which are common features in antioxidants (e.g. vitamin E and carotenoids). Although STS, like TX, does not inhibit XO, it may inhibit other sources (including enzymes involved therein) of oxyradical formation in multiple candidate pathways, such as those in leukocytes, impaired mitochondria and oxidation of catecholamines [23–25].

In summary, we have demonstrated, using highly purified and molecularly characterized STS, that this compound is a cardioprotector both in cultured human vascular endothelial cells and in the rabbit heart during ischemia-reperfusion. We have speculated that the endothelial-protective effect exhibited by STS may have clinical and perhaps systemic significance, especially since these cells are likely to be more important sites and targets of oxyradical damage than the myocytes. Such an exciting possibility warrants further investigation. *In vivo*, we have shown that STS can salvage the post-ischemic and reperfused heart almost as effectively as Trolox. Again, the precise mechanism of this salvage effect remains to be determined.

Acknowledgements—Part of this study was supported by the Medical Research Council of Canada with Grants MA-10065 and MT-12299 to T-W.W. and MT-6499 to H. Pang and A.A.G. We thank Drs. J. S. Ikonomidis and R-K. Li for providing some samples of human cells used in this work and Ms. C. Sangle for typing this manuscript.

REFERENCES

1. Chang HM and But PPH (Eds.), Danshen. In: *Pharmacology and Applications of Chinese Materia Medica*, pp. 255–268. World Scientific Publishing Co., Singapore, 1986.
2. Chang HM, Chen KP, Choang TF, Chow HF, Chui KY, Hon PM, Lau Tan FW, Yang Y, Zhong ZP, Lee CM, Sham HL, Chan CF, Cui YX and Wong HNC, Structure elucidation and total synthesis of new tanshinones isolated from *Salvia miltiorriza* Bunge (Danshen). *J Org Chem* **55**: 3537–3543, 1990.
3. Yagi A, Fujimoto K, Tanonaka K, Hirai K and Takeo S, Possible active components of tan-shen (*Salvia miltiorrhiza*) for protection of the myocardium against ischemia-induced derangements. *Planta Med* **55**: 51–54, 1989.
4. Takeo S, Tanonaka K, Hirai K, Kawaguchi K, Ogawa M, Yagi A and Fujimoto K, Beneficial effect of Tan-

Shen, an extract from the root of Salvia, on post-hypoxic recovery of cardiac contractile force. *Biochem Pharmacol* **40**: 1137–1143, 1990.
5. Danheiser RL, Casebier DS and Loebach JL, Total synthesis of dan shen diterpenoid quinones. *Tetrahedron Lett* **33**: 1149–1152, 1992.
6. Lee J, Li JH, Oya S and Snyder JK, Asymmetric synthesis of tanshindiol A. *J Org Chem* **57**: 5301–5312, 1992.
7. Shanghai Cooperative Group for the Study of Tanshinone IIA, Therapeutic effect of sodium tanshinone IIA sulfonate in patients with coronary heart disease. A double blind study. *J Tradit Chin Med* **4**: 20–24, 1984.
8. Mickle DAG, Li RK, Weisel RD, Tumiati LC and Wu TW, Water-soluble antioxidant specificity against free radical injury using cultured human ventricular myocytes and fibroblasts and saphenous vein endothelial cells. *J Mol Cell Cardiol* **22**: 1297–1304, 1990.
9. Wu TW, Hashimoto N, Wu J, Carey D, Li RK, Mickle DAG and Weisel RD, The cytoprotective effect of Trolox demonstrated with three types of human cells. *Biochem Cell Biol* **68**: 1189–1194, 1990.
10. Wu TW, Hashimoto N, Au JX, Wu J, Mickle DAG and Carey D, Trolox protects rat hepatocytes against oxyradical damage and the ischemic rat liver from reperfusion injury. *Hepatology* **13**: 575–580, 1991.
11. Wu TW, Wu J, Zeng LH, Sugiyama H, Mickle DA and Au JX, Reduction of experimental myocardial infarct size by infusion of lactosylphenyl trolox. *Cardiovasc Res* **27**: 736–739, 1993.
12. Jolly SR, Kane WJ, Bailie MN, Abrams GD and Lucchesi BR, Canine myocardial reperfusion injury: Its reduction by the combined administration of superoxide dismutase and catalase. *Circ Res* **54**: 277–285, 1984.
13. Wu T-W, Pristupa ZB, Zeng L-H, Au J-X, Wu J, Sugiyama H and Carey D, Enhancement in antioxidant-based hepatoprotective activity of Trolox by its conjugation to lactosylphenylpyranoside. *Hepatology* **15**: 454–458, 1992.
14. Axford-Gatley RA and Wilson GJ, Reduction of experimental myocardial infarct size by oral administration of α-tocopherol. *Cardiovasc Res* **25**: 89–92, 1991.
15. Frederiks WM, Frank GM and Hessling JMC, A method of quantitative analysis of the extent of necrosis in ischemic rat liver. *Exp Mol Pathol* **41**: 119–125, 1982.
16. Labbe R, Lindsay T, Gatley R, Romaschin A, Mickle D, Wilson G, Houle S and Walker P, Quantitation of postischemic skeletal muscle necrosis: Histochemical and radioisotope techniques. *J Surg Res* **44**: 45–53, 1988.
17. Mickle DAG, Li RK, Weisel RD, Birnbaum P, Wu TW, Jackowski G, Madonik MM, Bruton GW and Ingold KU, Myocardial salvage with Trolox and ascorbic acid for an acute evolving infarction. *Ann Thorac Surg* **37**: 553–557, 1989.
18. Wu TW, Wu J, Carey D and Zeng LH, Purpurogallin protects both ventricular myocytes and aortic endothelial cells of rats against oxyradical damage. *Biochem Cell Biol* **70**: 803–809, 1992.
19. Kelley WN and Beardmore TD, Allopurinol: Alteration in pyrimidine metabolism in man. *Science* **169**: 388–390, 1970.
20. Tanaka T, Movimoto S, Nonaka G-i, Nishioka I, Yokozawa T, Chung HGY and Oura H, Magnesium and ammonium-potassium lithospermates B, the active principles having a uremia-preventive effect from *Salvia miltiorrhiza*. *Chem Pharm Bull (Tokyo)* **37**: 340–344, 1989.
21. Kahda H, Takeda O, Tanaka S, Yamasaki Y, Yamashita A, Kurokawa T and Ishibashi S, Isolation of inhibitors of adenylate cyclase from Dan-shen, the root of *Salvia miltiorrhiza*. *Chem Pharm Bull (Tokyo)* **37**: 1287–1290, 1989.
22. Rutherford JD and Braunwald E, Chronic ischemic heart disease. In: *Heart Disease. A Textbook of Cardiovascular Medicine* (Ed. Braunwald E), 4th Edn, pp. 1292–1364. W. B. Saunders, Philadelphia, 1992.
23. Cross E, Halliwell B, Borish ET, Pryor WA, Ames BA, Saul RL, McCord JM and Harman D, Oxygen radicals and human disease. *Ann Intern Med* **107**: 526–545, 1987.
24. Ambrosiom G and Chiariells M, Myocardial reperfusion injury: Mechanisms and management—A review. *Am J Med* **91**: 86S–88S, 1991.
25. Downey JM, Miura T, Eddy LJ, Chambers DE, Melert T, Hearse DJ and Yellon DM, Xanthine oxidase is not a source of free radicals in the ischemic rabbit heart. *J Mol Cell Cardiol* **19**: 1053–1060, 1987.