in vivo *24*: 265-270 (2010)

Pretreatment with Tetrandrine Has Protective Effects against Isoproterenol-induced Myocardial Infarction in Rabbits

ARNALDO PINELLI[1], SILVIO TRIVULZIO[1], SERGIO BRENNA[2], GUSTAVO GALMOZZI[3] and GIUSEPPE ROSSONI[1]

[1]*Department of Pharmacology, Chemotherapy and Medical Toxicology, Milan, Italy;*
[2]*Laboratory of Clinical Chemistry, Ospedale Niguarda Ca' Granda, Milan, Italy;*
[3]*Department of Experimental Oncology and Laboratories, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy*

Abstract. *Tetrandrine, the active principle of Stephania tetrandra radix extracts, has broad pharmacological activity, including effects on the cardiovascular system: it has been shown to reduce the size of acute myocardial infarction in rats undergoing coronary vessel ligation and to improve heart lesions in the constriction/reperfusion model by means of mechanisms involving peroxidation, calcium antagonism and coagulation. The aim of this study was to investigate whether tetrandrine has anti-infarction, antioxidant and anticoagulant effects in rabbits treated with isoproterenol, a drug capable of causing peroxide generation, calcium overload and coagulation alterations, and inducing myocardial infarction. The results showed that pretreatment with tetrandrine protects against the myocardial injuries caused by isoproterenol. It counteracted the appearance of myocardial necrotic lesions and ischemic electrocardiographic modifications, such as ST segment alterations, prevented the appearance of the plasma cardiac necrosis markers c-troponin I and myoglobin, lowered malondialdehyde levels, and prolonged partial thromboplastin time. The protective effects of tetrandrine can be attributed to its antioxidant action in lowering peroxide levels and its ability to counteract coagulating activity. Tetrandrine seems to offer full protection against myocardial infarction experimentally induced by the non-invasive treatment of rabbits with isoprotenerol.*

Tetrandrine is a pharmacologically active alkaloid isolated from the plant *Stephania tetrandra* (1), which is used in traditional Chinese medicine as an antipyretic or analgesic compound (1), and is now extensively studied as a tumour cell cytotoxic agent and angiogenesis inhibitor (2). Some studies have also shown that it has antihypertensive activity (3-6) and antiarrhythmic effects (7). Its cardiovascular activity seems to be particularly promising and deserves great attention.

It has been more recently shown that extracts of *S. tetrandra* and its active principle tetrandrine have anti-infarction activity: they decrease the incidence of arrhythmias and infarct size in isolated rat hearts following coronary vessel obstruction (8), have protective effects in rats subjected to acute coronary occlusion and reduced the infarcted area (9), reduce the area of myocardial infarction in rats undergoing myocardial ischemia and reperfusion (10), and myocardial reperfusion injuries in rats undergoing coronary ligation followed by reperfusion (11).

The above studies indicate that various mechanisms underlie the cardiac anti-ischemic effects of tetrandrine. According to Shen *et al*., tetrandrine improves ischemia/reperfusion injury in rats by inhibiting neutrophil priming and activation, and thus abolishing the subsequent infiltration and reactive oxygen species production that causes myocardial reperfusion injury (11); according to Yu *et al*. (10), the extract containing tetrandrine reduces infarct size by acting through a mechanism involving channel antagonism (10). Other authors have shown that tetrandrine also has anti-thrombotic activity by inhibiting platelet aggregation (12). In other words, tetrandrine may counteract the damage induced by coronary ischemia by means of antioxidant (11) or calcium antagonist (10) or anti-coagulation mechanisms (12).

The aim of this study was to investigate whether tetrandrine has anti-infarction, antioxidant and anticoagulant effects in rabbits treated with isoproterenol, a drug capable of causing peroxide generation (13, 14), calcium overload (15) and coagulation alterations (14, 15), and inducing myocardial infarction (14, 15).

To this end, we evaluated ECG alterations, histological lesions and the appearance of plasma cardiac necrosis markers, and verified whether the administration of tetrandrine could

Correspondence to: Professor Arnaldo Pinelli, Department of Pharmacology, Via Vanvitelli 32, 20129 Milano, Italy. Tel. +39 0250317086, Fax +39 0250316949, e-mail: arnaldo.pinelli@unimi.it

Key Words: Myocardial infarction, isoproterenol, *tetrandrine*, rabbits, histology, ECG, cardiac necrosis markers, c-troponin I, myoglobin, malondialdehyde, partial thromboplastin time.

counteract the altered plasma malondialdehyde (MDA) levels and partial thromboplastin time (PTT) observed in isoproterenol-treated animals.

Materials and Methods

Materials, reagents and animal treatment. Isoproterenol and tetrandrine were purchased from Sigma (Milan, Italy); the reagents and instruments for evaluating c-troponin I and myoglobin came from Dade-Behring (Milan, Italy), and the reagents for measuring PTT came from Boehringer-Roche (Milan, Italy).

The study was carried out in conformity with the Guidelines for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Twenty-four male New Zealand white rabbits weighing 3000+100 g were obtained from Harlan (Correzzana, Milan, Italy), and divided into groups of six animals each. One group was treated with isoproterenol 3 mg/kg *i.p.*, the second with tetrandrine 15 mg/kg *i.m.*, and the third received a combination of tetrandrine 15 mg/kg *i.m.* and isoproterenol 3 mg/kg *i.p.*, with the tetrandrine being given ten minutes before the administration of isoproterenol. The fourth group received saline and acted as a control group.

Before treatment, the rabbits were anesthetized with urethane (700 mg/kg *i.p.*), with clonazepam (0.125 mg/kg *i.p.*) being given 30 minutes later. They were sacrificed 60 minutes after isoproterenol administration.

Electrocardiography. The electrocardiograms were recorded using an Electrocardiograph 3C (Elettronica Trentina, Trento, Italy) and ten leads: I, II, III, aVR, aVL, aVF and precordial VM (instead of V1, V2, V3), V4, V5 and V6. The recordings were made at baseline (with the animals anesthetized before starting the experiments), and then for one minute every five minutes during treatment, and the types of alterations (ST segment elevation or depression, and T wave inversion) were evaluated. In particular, the alterations observed in the last trace (recorded five minutes before sacrifice) were carefully analyzed because they would be closest to any altered plasma biochemical profiles immediately after collection.

Immediately before sacrifice, blood was collected from all of the animals using lithium-heparin (14 IU/ml) or citrate and the plasma was prepared and stored at –20˚C until assay.

Plasma cardiac markers. As it has been shown that polyclonal antibodies specifically prepared against human cardiac c-troponin I react with c-troponin I in the supernatants of heart homogenates of rabbits and other species (16), c-troponin I was evaluated in rabbit lithium-heparin plasma using a commercially available Dade-Behring reagent for human c-troponin I (17, 18), in which monoclonal antibodies are conjugated with alkaline phosphatase acting on the fluorogenic 4-methyl-umbelliferil-phosphate substrate. Myoglobin was evaluated in plasma using a Dade-Behring reagent in which the anti-myoglobin monoclonal antibody interacts with the myoglobin of the plasma sample to give a turbidimetric reaction (19).

Histology. Immediately after the sacrifice of the anesthetized animals, their hearts were removed and immediately fixed in 10% buffered formalin before being processed for histological examination. The ventricular mass was sectioned from the apex to the base of the heart in order to obtain 0.4 cm thick transverse slices, which were then embedded in paraffin after being dehydrated in alcohol (from 80% to absolute alcohol) and subsequently cleared with xylene. Five-micron thick serial histological sections were obtained from the paraffin blocks and stained with hematoxylin and eosin as previously described (20).

The histological findings are referred to in order to describe the sites of the lesions; no morphometric evaluations were made in this study. The photomicrographs were shot using a Zeiss MC 80 Axioscope photomicroscope at different magnifications (×100, ×200 and ×400).

Free plasma MDA. Free plasma MDA was extracted without hydrolysis, and assayed using the gas chromatography-mass spectrometry method described by Cighetti *et al*. (21).

Pro-coagulant activity. PTT was assayed in citrate plasma using a standard procedure (22). The plasma was diluted 1: 10 with 0.05 M phosphate buffer at pH 7.4.

Statistical analysis. The values of c-troponin I, myoglobin, MDA and PTT were analyzed using analysis of variance, and their statistical significance was evaluated using Tukey's test (23). The control and isoproterenol groups were compared with each other, and with the tetrandrine or tetrandrine plus isoproterenol groups.

Statistical significance was assumed at $p<0.05$.

Results

General observations. Treatment with isoproterenol, and pretreatment with tetrandrine have different effects on myocardial histology, electrocardiographic profiles, plasma cardiac necrosis markers, free plasma MDA and coagulation parameters.

Electrocardiographic data. Isoproterenol administration caused ischemic lesions evaluated as ST segment alterations in DIII, aVF, VM, V4, V5 and V6. Pretreatment with tetrandrine affected the electrocardiographic profiles of the isoproterenol-treated animals insofar as no ST alterations or T wave inversions were observed in the animals receiving combined tetrandrine/isoproterenol treatment (Figure 1).

Plasma cardiac necrosis markers. The mean plasma c-troponin I level was 0.03±0.01 ng/ml in the controls, 5.98±1.29 ng/ml in the isoproterenol group, 0.11±0.04 ng/ml in the tetrandrine group, and 1.8±0.76 ng/ml in the animals receiving tetrandrine plus isoproterenol. The levels in the isoproterenol group were significantly higher ($p<0.001$) than those observed in all of the other groups, and treatment with tetrandrine very significantly lowered c-troponin I levels in the tetrandrine plus isoproterenol group ($p<0.001$).

The mean plasma myoglobin level was 2.6±0.25 ng/ml in the controls, 189.5±26.5 ng/ml in the isoproterenol group, 4.3±0.8 in the tetrandrine group, and 18.75±4.23 in the group receiving tetrandrine plus isoproterenol. The levels in the isoproterenol group were significantly higher ($p<0.001$) than those observed in all of the other groups, and treatment with tetrandrine very significantly lowered myoglobin levels in the tetrandrine plus isoproterenol group ($p<0.001$).

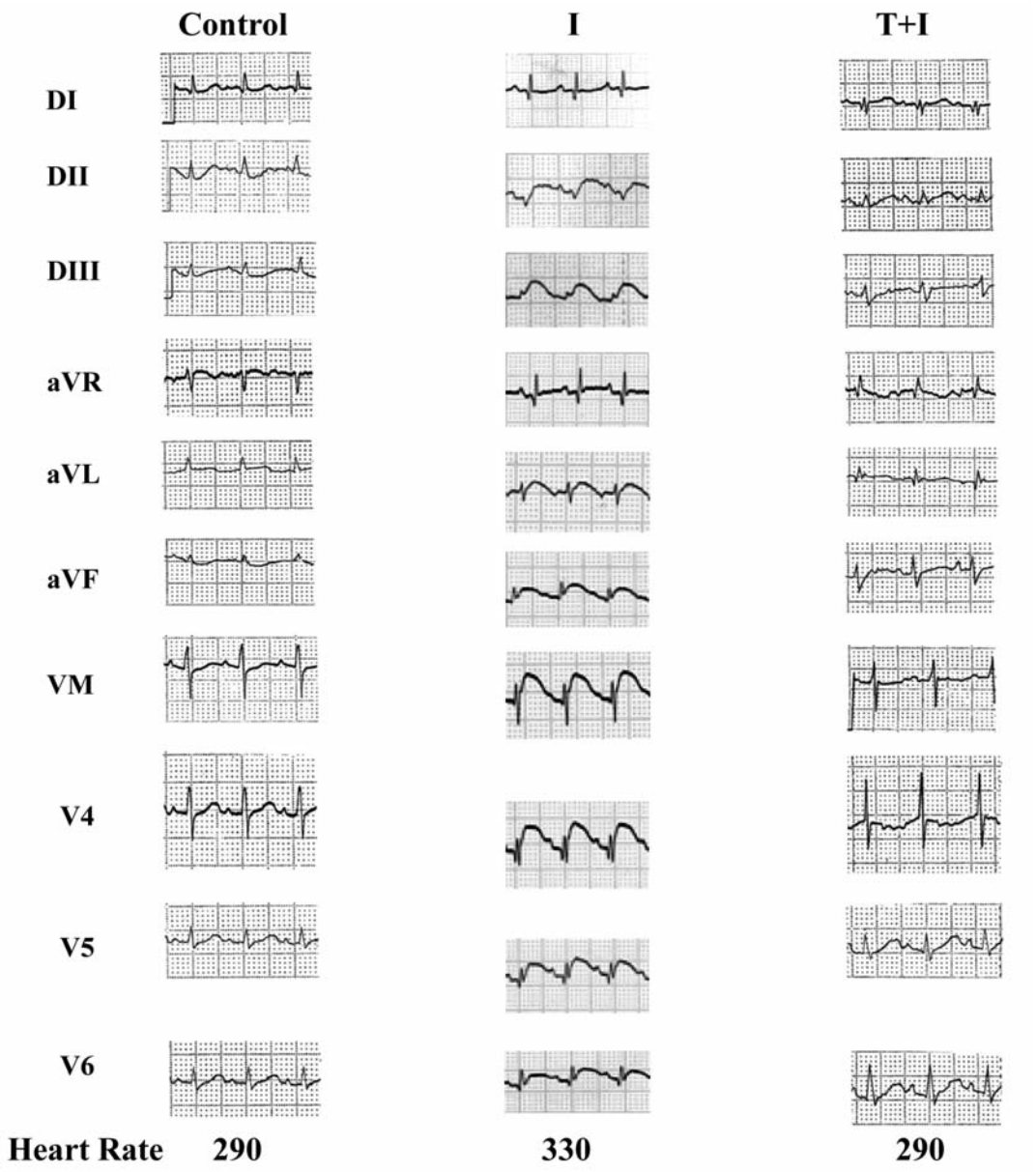

Figure 1. *Electrocardiograms of control, isoproterenol-, tetrandrine and isoproterenol-treated rabbits recorded using 3-channel electrocardiograph, with a calibration of 10 mm/mV and a chart speed of 50 mm* s^{-1}. *I: Isoproterenol-treated animals: ischemic lesions appear (ST elevation in DIII; aVF, VM, V4, V5, V6); T+I: tetrandrine and isoproterenol administration: the tracings appear similar to those of the control animals. In animals treated with tetrandrine alone the tracings appear similar to those of the control and have been omitted. The heart rate of 330 bpm shown here observed in the isoproterenol-treated rabbit was higher than that observed in all other tracings.*

Histological findings. The histological sections obtained from the hearts of the animals receiving isoproterenol alone showed various degrees of interstitial hemorrhage, presence of necrotic areas with dead myocells and vacuolar phenomena (I_1 and I_2), wavy fibres (I_3) areas with contraction band necrosis (I4) (Figure 2).

None of the histological images obtained from the animals pretreated with tetrandrine showed any remarkable alterations (T+I) (Figure 2). The histological profile of animals receiving tetrandrine alone appear similar to control (Figure not shown).

Free plasma malondialdehyde. The mean free plasma malondialdehyde level was 1.93±0.22 μmol/l in the controls, 4.15±0.38 μmol/l in the isoproterenol group, 1.97±0.21 μmol/l in the tetrandrine group, and 2.71±0.32 μmol/l in tetrandrine plus isoproterenol group. The levels in the isoproterenol group

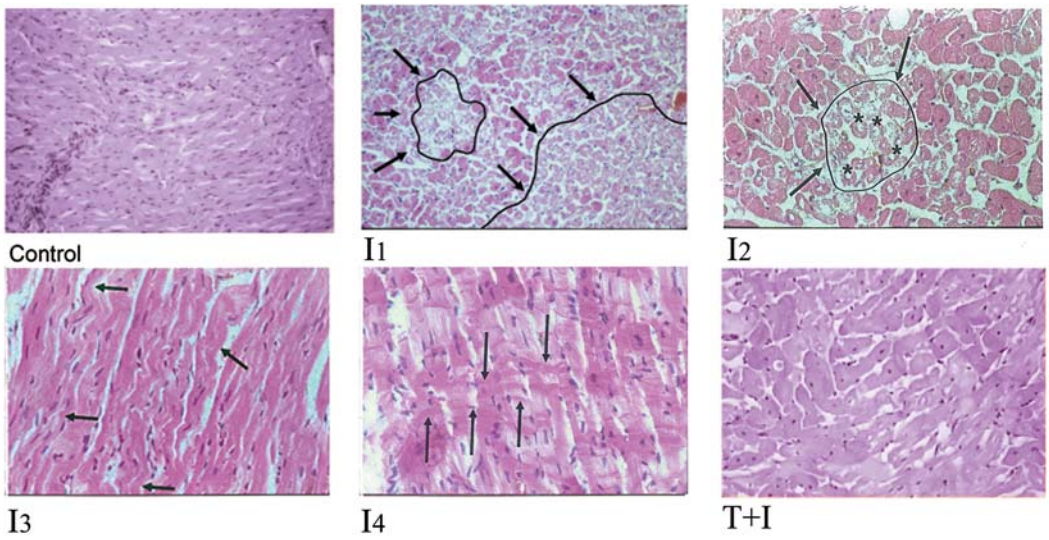

Figure 2. *Rabbit myocardium: sections taken from left ventricular anterior free wall of control; isoproterenol treated (I_1, I_2, I_3, I_4) tetradrine plus isoproterenol (T+I) treated animals. Control (×100, hematoxilin and eosin); I_1 (×200, hematoxilin and eosin) presence of necrosis areas with dead myocells and vacuolar phenomena (arrows); I_2 (×400, hematoxilin and eosin) presence of necrosis areas with dead myocells and vacuolar phenomena (arrows and asterisks); I_3 (×400, hematoxilin and eosin) section exhibiting wavy fibers (arrows); I_4 (×400, hematoxilin and eosin) presence of 'contraction bands' necrosis (arrows); T+I (×200, hematoxilin and esosin) section similar to control. Tetrandrine alone section, similar to control, has not been shown.*

were significantly higher than those observed in all of the other groups ($p<0.001$), and treatment with tetrandrine significantly lowered MDA levels in the tetrandrine plus isoproterenol group ($p<0.05$).

PTT values. The PTT values were 38.3±2.12 for controls, 30. ±1.84 with isoproterenol, 49.8±2.31 with tetrandrine and 51.5±5.12 seconds with tetrandrine plus isoproterenol.

The PTT values found in isoproterenol treated rabbits appeared to be shorter than in controls ($p<0.05$), tetrandrine-treated ($p<0.001$) and tetrandrine plus isoproterenol-treated ($p<0.001$) rabbits. The treatment with tetrandrine increased the PTT values in tetrandrine-treated and in tetrandrine plus isoproterenol-treated rabbits *vs*. controls ($p<0.01$) and *vs*. rabbits treated with isoproterenol alone ($p<0.001$).

Discussion

The administration of isoproterenol and tetrandrine, alone and in combination, has different effects on the parameters related to myocardial infarction: ECG findings, plasma c-troponin I and myoglobin levels, myocardial histology, and biochemical indicators such as plasma MDA levels and PTT. Isoproterenol treatment affected the electrocardiographic profile. The tracings of the animals receiving isoproterenol alone showed both T-wave inversions and ST-segment alterations throughout the duration of the experiment, thus suggesting the presence of ischemic myocardial damage (Figure 1). These ECG alterations are supported by the observation that the administration of isoproterenol caused the appearance of plasma c-troponin I and myoglobin, which are markers of cardiac necrosis.

The increased levels of heart injury markers after isoproterenol treatment are in line with reports by us and others concerning rats treated with isoproterenol or orciprenaline (14, 15, 24-26). Furthermore, these markers have previously been detected in rabbit plasma, which can be carried out using antibodies against human c-TnI because the peptide sequences in human and rabbit cardiac troponins are highly similar (16-18), and consequently give cross-reactivity reactions (16, 18).

The appearance of plasma cardiac necrosis markers was accompanied by histological alterations. As can be seen in Figure 2, heart histology revealed vacuoles within the cytoplasm, and the presence of edema and aspects of hydropic change and degeneration. Hydropic degeneration is due to decreased membrane ionic pump activity as a result of the lack of ATP synthesis, which also causes intracellular water accumulation and can be attributed to peroxidation-induced cell structure damage (27).

The histological examination also showed areas of coagulative necrosis in the isoproterenol-treated animals that are characteristic of the peroxide-induced death of myocardial cells (27).

Wavy fibres and contraction band necrosis are found at the periphery of an infarcted area and are attributable to systolic tugs of viable fibres adjacent to non-contractible fibres (27). We found contraction bands in our isoproterenol-treated animals and these were probably due to the peroxidation stress induced by catecholamines (28, 29). The heart alterations were

accompanied by high levels of peroxide generation, as shown by the increase in peroxide values measured by means of the MDA peroxidation marker (30). The catecholaminergic activation induced by isoproterenol favours the formation of hydroxyl radicals (31).

Oxidative damage begins when the double bonds of unsaturated fatty acids of lipids membrane are attacked by oxygen-derived free radicals, particularly OH- , thus giving rise to MDA and other oxidation products (32). Unstable and reactive peroxides can cause extensive membrane organelle and cell damage, with loss of membrane integrity, as well as mitochondrial lesions (33), and mitochondrial dysfunction leads to ATP and energy substrate depletion and massive contraction band necrosis (34).

It has been reported that isoprotenerol causes calcium channel opening (35), calcium overload, the loss of energy substrates, and myocardial necrosis (36, 37); peroxide generation seems to act synergistically with calcium overload to induce myocardial necrosis (37) in isoproterenol-treated animals.

In our experiments, tetrandrine had a protective effect against infarction and its associated signs. It protected against electrocardiographic changes as there were no signs of the ST segment or T wave alterations indicative of ischemia (Figure 1); it prevented the appearance of myocardial lesions as the histological findings were normal, and there was no observable necrosis (Figure 2); it blocked the appearance of plasma cardiac necrosis markers c-troponin I and myoglobin because, as the myocardium was not damaged even in the presence of isoproterenol, no cardiac necrosis markers were released into the plasma.

It is important to note that tetrandrine lowered the plasma MDA levels in animals receiving isoproterenol.

Tetrandrine may have protected the animals because of its antioxidant action (38). It scavenges superoxide anions (38) and blocks the conditions leading to the generation of the hydroxyl radical .OH (39). Furthermore it improves ischemia/reperfusion lesions in rat myocardium by inhibiting the neutrophil activation and infiltration and ROS production that cause myocardial reperfusion injury (11). Tetrandrine may also have protected the animals by interacting with slow calcium channels (40). Finally, isoproterenol shortens PTT as a result of adrenoreceptor stimulation (41) and peroxide production (42), and the administration of tetrandrine alone or in combination with isoproterenol significantly prolonged PTT. By means of its peroxide-scavenging activity (38), tetrandrine counteracts the increase in the levels of the peroxidation marker MDA, and this may prolong the shortened PTT caused by isoproterenol. As the conversion of prothrombin to thrombin is accelerated by the addition of calcium ions that interact with the carboxyl groups of glutamic acid of factor X and factor II (prothrombin) (43), tetrandrine may interfere with the calcium-binding sites on the coagulation factors, as it may interact with the calcium and glutamic acid residues located in calcium channels (40, 44). It therefore contributes to protection against myocardial infarction by removing the procoagulant activity induced by isoproterenol.

In conclusion, we found that tetrandrine seems to prevent experimental myocardial infarction induced in intact animals by a non-invasive technique (14, 15), and seemed to protect against myocardial necrosis by means of mechanisms possibly involving the removal of peroxides and the inhibition of coagulant activity. It may therefore be suitable for development as a protective drug in patients affected by ischemic coronary diseases associated with sympathetic oxidative stress or thrombotic events.

Acknowledgements

The Authors thank Banca Popolare di Milano (BPH) for financing this research. This study is dedicated to the memory of Professor Iraklis Galatulas (1939-1998), Professor of Pharmacology of the Medical Faculty, University of Milan from 1975 to 1998.

References

1 The Merk Index. 12 ed., New Jersey. Merk Co, p. 1577, 1996.
2 Chen YJ: Potential role of tetrandrine in cancer therapy. Acta Pharm Sinica 23: 1102-1106, 2002.
3 Chang TM, Chao KC and Lu FH: The cardiovascular effects of tetrandrine and dimethyl-tetrandrine. Acta Pharm Sinica *6*: 147-153, 1958.
4 Zeng FD, Fang DC, Leng DM and Lu FH: Influence of tetrandrine on the left ventricular function and peripheral blood vessels in canines. Acta Pharm Sinica *17*: 561-565, 1982.
5 Gao Y, Chang MY, Mao HY, Chao HY and Chen DH: The clinical observation of tetrandrine in the treatment of 270 cases of hypertension patients and hypertensive crisis. J Chin Int Med *13*: 504-507, 1965.
6 Department of Pharmacology. Wuhan Medical College and Health Department, Wuhan Textile Factory: A clinical study of antihypertensive effect of tetrandrine. Chin Med J *92*: 193-198, 1979.
7 Cha L, Qian JQ and Lu FH: Antiarrhythmic action of tetrandrine and the total alkaloids of *Sophora flavescens*. Acta Pharmacologica Sinica *2*: 26-28, 1981.
8 Yu XC, Wu S, Wang GY, Shan J, Wong TM, Chen CF and Pang KT: Cardiac effects of the extract and active components of radix *Stephaniae tetrandrae*. II. Myocardial infarct, arrhythmias, coronary arterial flow and heart rate in the isolated perfused rat heart. Life Sci *68*: 2863-2872, 2001.
9 Chen H, Chen SC, Zhang TH, Tian HC, Guan Y and Su DF: Protective effects of silybin and tetrandrine on the outcome of spontaneously hypertensive rats subjected to acute coronary artery occlusion. Int J Cardiol *41*: 103-108, 1993.
10 Yu XC, Wu S, Chen CF, Pang KT and Wong TM: Antihypertensive and anti-arrhythmic effects of an extract of radix *Stephaniae tetrandrae* in the rat. J Phar Pharmacol *56*: 115-122, 2004.
11 Shen YC, Chen CF and Sung YJ: Tetrandrine ameliorates ischaemia-reperfusion injury of rat myocardium through inhibition of neutrophil priming and activation. Br J Pharmacol *128*: 1593-1601, 1999.

12 Kim HS, Zhang YH and Yun YP: Effects of tetrandrine and fangchinoline on experimental thrombosis in mice and human platelet aggregation. Planta Med *65*: 135-138, 1999.
13 Blasig IE, Blasig R and Lowe H: Myocardial lipid peroxidation during isoproterenol-induced blood flow reduction in rat myocardium. Biomed Biochim Acta *43*: S171-S174, 1984.
14 Pinelli A, Trivulzio S, Tomasoni L, Brenna S, Bonacina E and Accinni R: Isoproterenol-induced myocardial infarction in rabbits. Protection by propranolol or labetalol: a proposed non-invasive procedure. Eur J Pharmaceutical Sci *23*: 277-285, 2004.
15 Pinelli A, Trivulzio S, Tomasoni L, Bertolini B, Brenna S, Bonacina E and Vignati S: Myocardial infarction non-invasively induced in rabbits by administering isoproterenol and vasopressin: protective effects exerted by verapamil. Fund Clin Pharmacol *18*: 657-667, 2004.
16 O'Brien PJ, Dameron GW, Beck ML, Kang YJ, Erickson BK, Di Battista TH, Miller KE, Jackson KN and Mittelstadt S: Cardiac troponin T is a sensitive, specific biomarker of cardiac injury in laboratory animals. Lab Anim Sci *47*: 486-495, 1997.
17 Bodor GS, Porter S, Landt Y and Ladenson JH: Development of monoclonal antibodies for an assay of cardiac troponin-I and preliminary results in suspected cases of myocardial infarction. Clin Chem *38*: 2203-2214, 1992.
18 O'Brien PJ, Lant Y and Ladenson JH: Differential reactivity of cardiac and skeletal muscle from various species in a cardiac troponin I immunoassay. Clin Chem *43*: 2333-2338, 1997.
19 Plebani M and Zaninotto M: Diagnostic strategies using myoglobin measurement in myocardial infarction. Clin Chim Acta *272*: 69-77, 1998.
20 AFIP: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd ed. New York, McGraw-Hill book Company, 1968.
21 Cighetti G, Debiasi S, Paroni R and Allevi P: Free and total malondialdehyde assessment in biological matrices by gas chromatography-mass spectrometry: what is needed for an accurate detection. Anal Biochem *266*: 222-229, 1999.
22 Pasquinelli F: Diagnostica e Tecniche di Laboratorio. Firenze. Rosini Editrice, 1997.
23 Armitage P: Statistica Medica. 10th ed., Milano, Feltrinelli, 1991.
24 Bertinchant JP, Robert E, Polge A, Marty-Double C, Fabbro-Peray P, Poirey S, Aya G, Juan JM, Ledermann B, de la Coussaye JE and Dauzat M: Comparison of the diagnostic value of cardiac troponin I and T determination for detecting early myocardial damage and the relationship with histological findings after isoprenaline-induced cardiac injury in rats. Clin Chim Acta *298*: 13-28, 2000.
25 Bertsch T, Bleuel H, Aufenanger J and Rebel W: Comparison of cardiac troponin T and cardiac troponin I concentrations in peripheral blood during orciprenaline-induced tachycardia in rats. Exp Toxicol Pathol *49*: 467-468, 1997.
26 Bertsch T, Bleuel H, Deschl U and Rebel W: A new sensitive cardiac troponin T rapid test (TROPT) for the detection of experimental acute myocardial damage in rats. Exp Toxicol Pathol *51*: 565-569, 1999.
27 Kumar V, Abbas AK and Fausto N: Pathologic Basis of Disease. 7th ed. Philadelphia- Pennsylvania 19106, Elseviers Saunders, pp. 14-21, 575-583, 2005.
28 Todd GL, Baroldi G, Pieper GM, Clayton FC and Eliot RS: Experimental catecholamine-induced myocardial necrosis. I. Morphology, quantification and regional distribution of acute contraction band lesions. J Mol Cell Cardiol *17*: 317-338, 1985.
29 Rona G: Catecholamine cardiotoxicity. J Mol Cell Cardiol *17*: 291-306, 1985.
30 Del Rio D, Stewart AJ and Pellegrini N: A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metabol Cardiovas Dis *15*: 316-328, 2005.
31 Obata T, Hosokawa H and Yamanaka Y: *In vivo* monitoring of norepinephrine and $^{\bullet}OH$ generation on myocardial ischemic injury by dialysis technique. Am J Physiol *266*: H903-H908, 1994.
32 Valenzuela A: The biological significance of malondialdehyde determination in the assessment of tissue oxidative stress. Life Sci *48*: 301-309, 1991.
33 Long J, Wang X, Gao H, Liu Z, Liu C, Miao M and Liu J: Malonaldehyde acts as a mitochondrial toxin: Inhibitory effects on respiratory function and enzyme activities in isolated rat liver mitochondria. Life Sci *79*: 1466-1472, 2006.
34 Koster JF, Slee RG, Essed CE and Stam H: Studies on cumene hydroperoxide-induced lipid peroxidation in the isolated perfused rat heart. J Mol Cell Cardiol *17*: 701-708, 1985.
35 Byon KY and Fleckenstein A: Parallel influence of isometric tension development and O_2 consumption of isolated papillary muscles under the influence of Ca ions, adrenaline, isoproteranol and organic Ca antagonists (iproveratril, D 600, prenylamine). Pflugers Archiv – Eur J Physiol *312*: R8-R9, 1969.
36 Fleckenstein A, Janke J, Doring HJ and Leder O: Myocardial fiber necrosis due to intracellular Ca overload a new principle in cardiac pathophysiology. Rec Adv Stud Card Struct Metab *4*: 563-580, 1974.
37 Thollon C, Iliou JP, Cambarrat C, Robin F and Vilaine JP: Nature of the cardiomyocyte injury induced by lipid hydroperoxides. Cardiovas Res *30*: 648-655, 1995.
38 Cao ZF: Scavenging effect of tetrandrine of active oxygen radicals. Planta Med *62*: 413-414, 1996.
39 Castranova V: Generation of oxygen radicals and mechanisms of injury prevention. Environm Health Perspect *102 suppl*: 65-68, 1994.
40 King VF, Garcia ML, Himmel D, Reuben JP, Lam YK, Pan JX, Han GQ and Kaczorowski GJ: Interaction of tetrandrine with slowly inactivating calcium channels. Characterization of calcium channel modulation by an alkaloid of Chinese medicinal herb origin. J Biol Chem *263*: 2238-2244, 1988.
41 Ingram GI, Jones RV, Hershgold .J, Denson KW and Perkins JR: Factor-VIII activity and antigen, platelet count and biochemical changes after adrenoceptor stimulation. Br J Haematol *35*: 81-100, 1977.
42 Pratico D, Ferro D, Iuliano L, Rokach J, Conti F, Valesini G, Fitzgerald GA and Violi F: Ongoing prothrombotic state in patients with antiphospholipid antibodies: a role for increased lipid peroxidation. Blood *93*: 3401-3407, 1999.
43 Jackson CM: The biochemistry of prothrombin activation. Br J Haematol *39*: 1-8, 1978.
44 Mitterdorfer J, Sinnegger MJ, Grabner M, Striessnig J and Glossmann H: Coordination of Ca^{2+} by the pore region glutamates is essential for high-affinity dihydropyridine binding to the cardiac Ca^{2+} channel alpha 1 subunit. Biochem *34*: 9350-9355, 1995.

Received October 22, 2009
Revised March 23, 2010
Accepted March 23, 2010