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Studies on the Deleterious Effects of Free Radicals on Myocardial Contractility and Hemodynamics in Dogs: Protection by Felodipine, a Dihydropyridine Calcium Antagonist

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ABSTRACT

Chintala, M.S., S.M. Jacinto, and B.S. Jandhyala: Studies on the deleterious effects of free radicals on myocardial contractility and hemodynamics in dogs: Protection by felodipine, a dihydropyridine calcium antagonist. Drug Dev. Res. **27**:287–295, 1992.

There is convincing evidence that re-oxygenation of ischemic organs is associated with the formation of oxygen free radicals and increases in cell calcium that exacerbate the ischemic injury. The objective of the present studies was to determine whether felodipine, a dihydropyridine calcium antagonist, would be effective in preventing cardiovascular toxicity produced by oxygen free radicals. In pentobarbital-anesthetized dogs, intravenous (i.v.) administration of xanthine plus xanthine oxidase [X + XO] resulted in a rapid fall in arterial blood pressure (by 50–60 mmHg) and heart rate (by 30–40 beats/min). All the indices reflecting cardiac function such as contractility index (max dp/dt/p), cardiac output, stroke volume, left ventricular stroke work, and left ventricular minute work were significantly attenuated during a 120 min observation period. Pretreatment of the animals with felodipine (0.01 μmol/kg, i.v.) provided moderate but significant protection from [X + XO]-induced deterioration of cardiovascular function, whereas the higher dose (0.05 μmol/kg, i.v.) effectively prevented any adverse alterations in cardiovascular function. The results obtained in the present studies suggest that oxygen free radicals facilitate calcium overload in the

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ischemic cells and can account for the efficacy of several calcium channel antagonists in preventing ischemic injury such as ischemic renal failure in various experimental models.

Key words: myocardial dysfunction, calcium channel antagonists, xanthine-xanthine oxidase

INTRODUCTION

Ischemia due to interruption of blood flow and subsequent reperfusion of the blood would occur in various clinical and surgical situations and could lead to irreversible damage to the vital organs [Hammond et al., 1985; Farber et al., 1990; Bolli et al., 1989]. It has been proposed that marked increases in cytosolic calcium and the generation of oxygen free radicals and potential interactions between these two phenomena would precipitate impairment of cellular function in the affected organs [Hearse and Tosaki, 1988; Hammond and Hess, 1985; Cheung et al., 1986; Malis and Bonventre, 1986; Kaneko et al., 1989]. The views that free radicals may potentiate or aggravate calcium-induced cell injury are supported by our recent observations that the calcium antagonists felodipine and verapamil were effective in preventing mortality induced by oxygen free radicals in anesthetized rats [Jacinto et al., 1991]. In these studies, free radicals were generated by the exogenous administration of xanthine plus xanthine oxidase [X + XO]. The present studies are undertaken to determine whether felodipine, a dihydropyridine calcium antagonist, would prevent or attenuate deleterious effects of oxygen free radicals on hemodynamics and myocardial contractility in anesthetized dogs.

MATERIALS AND METHODS

Mongrel dogs (18–22 kg) were anesthetized with sodium pentobarbital (35 mg/kg, i.v.) and placed on positive pressure ventilation (Harvard-respirator). The animals were maintained at a stable anesthetic level by continuous infusion of the anesthetic at a rate of 4 mg/kg/hr via a catheterized femoral vein. The ipsilateral femoral artery was catheterized and the catheter was advanced into the abdominal aorta to measure mean blood pressure using a pressure transducer (Statham P23dc, Statham-Gould, Oxnard, CA). Heart rate was monitored by a cardiotach (Grass 7P44C) triggered by the pressure pulse. Thoracotomy was performed on the left side at the fourth intercostal space. The ascending aorta was separated from the surrounding tissue and an electromagnetic flow probe of suitable size (12–14 mm, i.d.) was placed around the vessel to monitor cardiac output using a Statham electromagnetic flowmeter (Model SP2202). A polyethylene catheter was inserted into the left ventricle to measure left ventricular pressure and the rate of pressure increase in the left ventricle (dp/dt) was monitored electronically with a Grass differentiator (7P20). The index of contractility was computed as the ratio of max dp/dt to p where p is the ventricular pressure at max dp/dt minus left ventricular end diastolic pressure [Jandhyala et al., 1977].

The following parameters were recorded on a Grass Model 7 polygraph: mean arterial blood pressure (mmHg), heart rate (beats/min), left ventricular systolic and diastolic pressures (mmHg), and left ventricular max dp/dt (mmHg/sec). Stroke volume, left ventricular stroke work, and total peripheral resistance were calculated and expressed in standard international units [Kappagoda and Linden, 1976].

Drugs

Xanthine (Cat. No. X-0626) and xanthine oxidase (Cat. No. X-4875) were purchased from Sigma Chemical Company (St. Louis, MD). Felodipine (mol wt: 384.26) was a gift from Hässle AB (Mölndal, Sweden).

TABLE 1. Basal Values of Cardiac Output (CO), Stroke Volume (SV), Left Ventricular Stroke Work (LVSW), Left Ventricular Minute Work (LVMW), Total Peripheral Resistance (TPR), and Contractility Index (CI) in Different Groups of Dogs Prior to Administration of [X + XO]*

Groups	CO (ml/min)	SV (ml/beat)	LVSW (J/beat)	LVMW (J/min)	TPR (KPa/liter/min)	CI (units)
Solvent controls (N = 5)	1,480 ± 72	11.2 ± 1.6	0.144 ± 0.02	20.2 ± 3.9	10.2 ± 1.1	56 ± 2.9
Felodipine (0.01 μmol/kg) (N = 5)	1,545 ± 68	11.6 ± 0.6	0.161 ± 0.02	22.5 ± 1.5	9.7 ± 1.2	53 ± 3.1
Felodipine (0.05 μmol/kg) (N = 5)	1,540 ± 44	11.2 ± 0.7	0.154 ± 0.01	21.2 ± 1.0	9.6 ± 0.7	54 ± 2.4

*The values are represented as mean ± S.E.M. There were no statistically significant differences between the basal values of various groups.

Protocol

These studies were carried out in three groups of dogs and a stabilization period of 45 min was allowed in all the experiments.

Group I. Animals received the vehicle PEG-400 (0.1 ml/kg, i.v.) 10 min before administration of xanthine (X; 0.14 mg/kg, i.v.), immediately followed by xanthine oxidase (XO; 2.0 units/kg, i.v.). N = 8.

Group II. Felodipine (0.01 μmol/kg, i.v.) was administered 10 min before [X + XO]. N = 5.

Group III. Felodipine (0.05 μmol/kg, i.v.) was administered 10 min before [X + XO]. N = 5.

Alterations in various parameters were monitored for a period of 120 min after i.v. administration of [X + XO].

Statistics

All the data were calculated as mean ± S.E.M. for each group. Analysis of variance and Newman-Keuls multiple range test were employed to evaluate the data and to assign the statistical significance of the difference from the controls. While some of the data were expressed as percent of the control values, statistical computations were made with the raw scores.

RESULTS

The basal values of several variables which are not shown in the figures are tabulated in Table 1. In a total of eight control dogs, three animals died within 30 min after administration of [X + XO]; death in these dogs was evidently due to rapid cardiovascular depression. None of the animals died in the two felodipine-treated groups. Therefore, all the figures represent data from five animals in each group (Figs. 1–4).

In the vehicle-treated group, immediately after the administration of [X + XO] there was a rapid fall in heart rate, cardiac output, and arterial blood pressure (Figs. 1, 2). Subsequently, although there was a moderate recovery in heart rate response, cardiac output remained lower and hypotension persisted throughout the 120 min observation period despite sustained increases in total peripheral resistance. Hence, pronounced reduction in the arterial blood pressure after [X + XO] administration was evidently due to acute cardiac toxicity; all the indexes reflecting cardiac function such as contractility index (max dp/dt/p), cardiac output, stroke volume, left ventricular stroke work, and left ventricular minute work were significantly

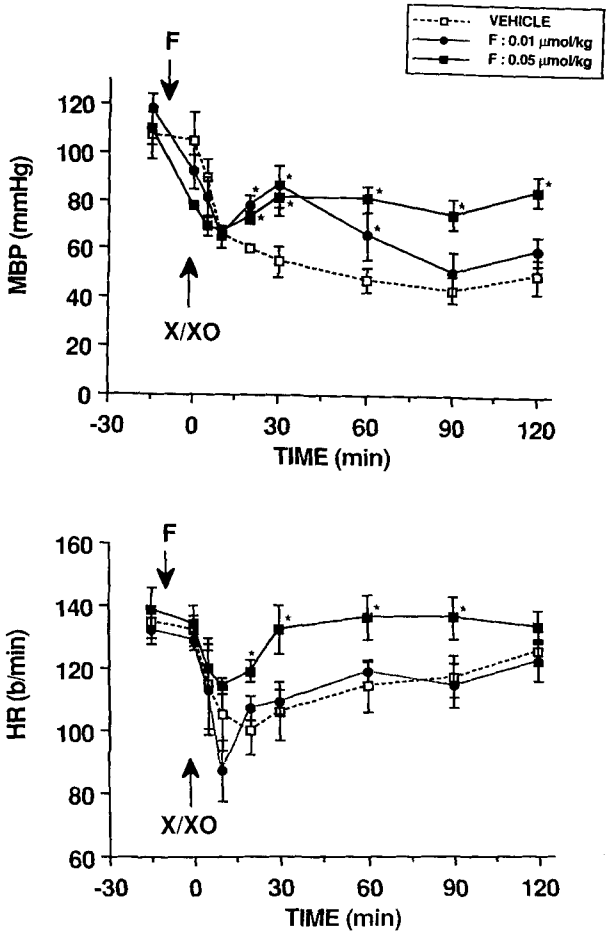

Fig. 1. Effects of [X + XO] administration on blood pressure (MBP; mean ± S.E.M.) and heart rate (HR; mean ± S.E.M.) in different groups of anesthetized dogs. The groups are as follows: vehicle controls (□, N = 5); pretreatment with a low dose of felodipine (F), 0.01 μmol/kg (●, N = 5); and pretreatment with a high dose of felodipine (F), 0.05 μmol/kg (■, N = 5). Stars indicate that the values are significantly different from that of the control group ($P < 0.05$).

attenuated during the course of the experiment (Figs. 2–4). It is also likely that the sustained increases in the peripheral resistance may have further compromised left ventricular function.

Both doses of felodipine produced anticipated decreases in the arterial blood pressure (25–30 mmHg) and provided protection against [X + XO]-induced toxicity in a dose-dependent manner. In group II, pretreatment of the animals with the lower dose of felodipine (0.01 μmol/kg) provided moderate but significant protection in that it attenuated the rate of decline in various variables and prevented deterioration of cardiovascular function during the first 30 min after [X + XO] administration. This dose was also effective in preserving myocardial contractility. The higher dose of felodipine (0.05 μmol/kg) was most effective in preventing any adverse alterations in heart rate, myocardial contractility, cardiac output, and stroke volume after [X + XO] administration; reductions in the left ventricular stroke work and minute work are evidently related to felodipine-induced decreases in the afterload (Figs. 1–4).

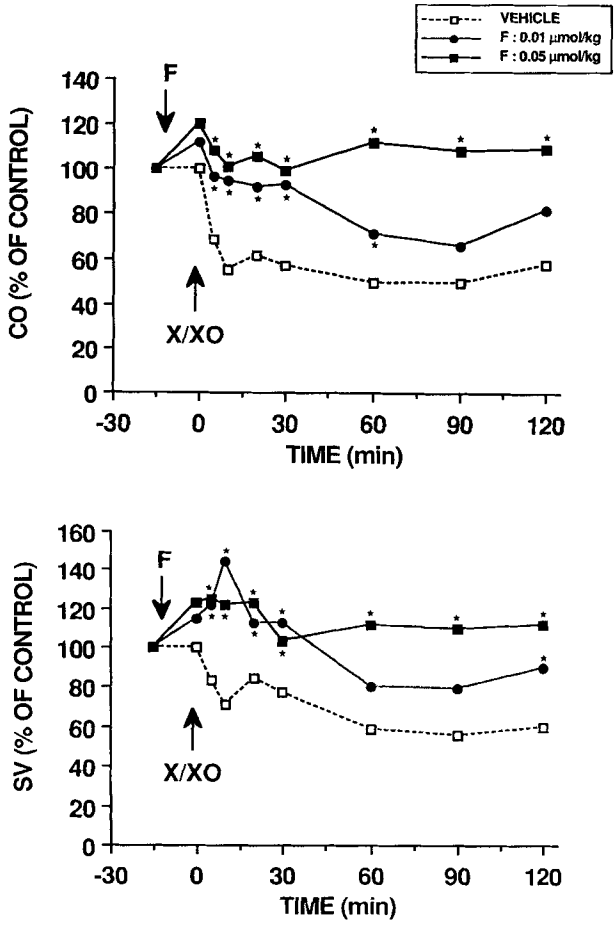

Fig. 2. Effects of [X + XO] administration on cardiac output (CO) and stroke volume (SV) in different groups of anesthetized dogs; the data are shown as percent of basal values. Basal values for CO and SV are shown in Table 1. See Figure 1 legend for explanation of groups.

Such an effect which prevented sustained increases in the peripheral resistance after [X + XO] administration may also have contributed to the salutary effects of felodipine.

DISCUSSION

Several investigators have employed [hypoxanthine + XO] and/or [X + XO] to generate free radicals and to study their toxicity on tissues in various in vitro and in vivo experimental situations [Tiede et al., 1990; Galat et al., 1989; Bratell et al., 1988; Lawson et al., 1990]. In a dog model similar to that employed in the present studies, Prasad et al. [1989] have demonstrated that i.v. administration of [X + XO] produced rapid cardiovascular depression; they also showed that pretreatment of the animals with the natural scavenger superoxide dismutase administered either alone or together with catalase provided moderate protection. These studies as well as our previous observations suggest that the cardiovascular toxicity of [X + XO] can be related to the generation of oxygen free radicals [Jacinto et al., 1991]. These observations are consistent with the evidence that oxygen free radicals can depress myocardial

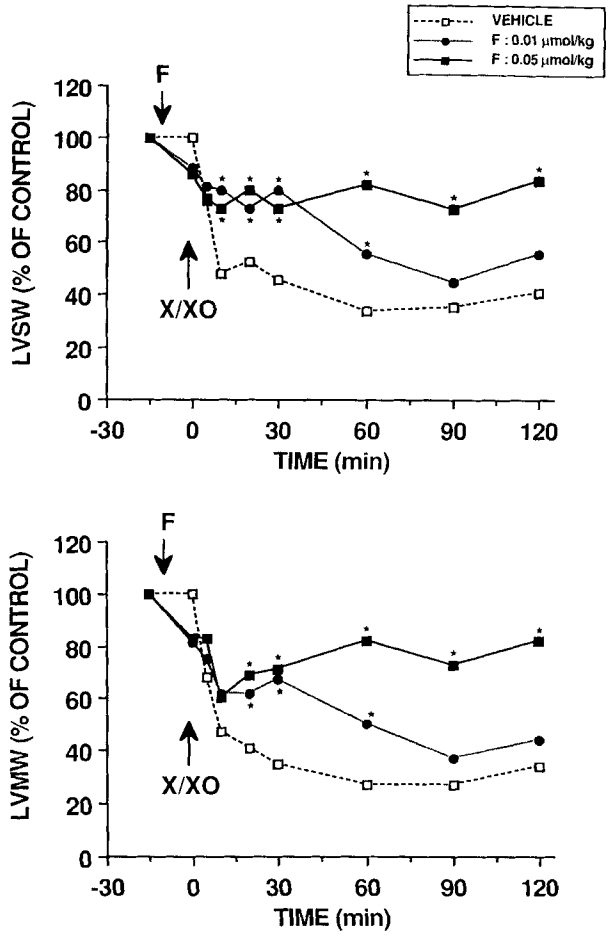

Fig. 3. Effects of [X + XO] administration on left ventricular stroke work (LVSW) and left ventricular minute work (LVMW) in different groups of anesthetized dogs; the data are shown as percent of basal values. Basal values for LVSW and LVMW are shown in Table 1. See Figure 1 legend for explanation of groups.

contractility by their inhibitory effects on the excitation-contraction coupling mechanisms and by altering electrophysiological characteristics of cardiac tissue [Goldhaber et al., 1989; Tsushima and Moffat, 1990]. Other investigators employing a variety of experimental models have also documented the beneficial effects of free radical scavengers in preventing ischemia and reperfusion-induced damage in the heart, kidneys, as well as mesenteric bed [Nejima et al., 1989; Hansson et al., 1983; Linas et al., 1990; Wang et al., 1990]. While these studies demonstrate that free radicals are cytotoxic, various cellular events that are involved in mediating tissue injury are not conclusive and remain to be clarified.

It is known that the target sites for free radicals include membrane phospholipids, nucleic acids, unsaturated fatty acids, as well as several amino acids in the membrane bound proteins [Farber et al., 1990]. Investigators have demonstrated that membrane peroxidation by free radicals would lead to inhibition of Na^+, K^+-ATPase activity and Ca^{2+}, Mg^{2+}-ATPase activity resulting in accumulation of intracellular Na^+, promotion of Ca^{2+}-influx, as well as

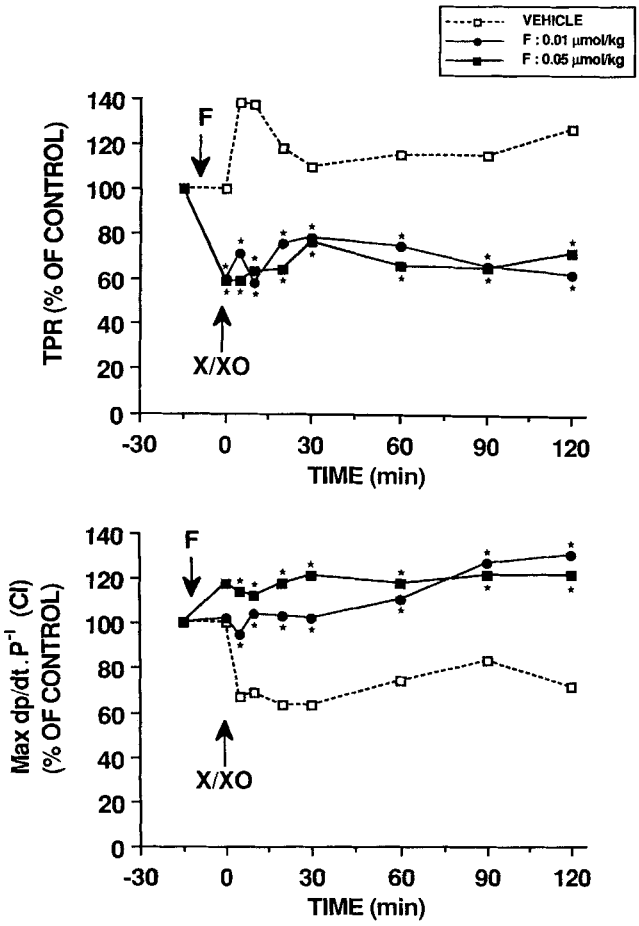

Fig. 4. Effects of [X + XO] administration on total peripheral resistance (TPR) and contractility index (CI) in different groups of anesthetized dogs; the data are shown as percent of basal values. Basal values for TPR and CI are shown in Table 1. See Figure 1 legend for explanation of groups.

inhibition of Ca^{2+}-efflux [Kaneko et al., 1989; Kim and Akera, 1987; Reeves et al., 1986]. These data suggest that the rise in cell Ca^{2+} during ischemia is further aggravated by free radicals generated during reperfusion leading to irreversible cell damage. Such a view is further supported by the studies of Malis and Bonventre [1986] which show that calcium potentiates oxygen free radical-induced injury to mitochondria.

The data obtained in the present studies support the role of an interaction between free radicals and calcium kinetics in ischemia-reperfusion injury in that free radical-induced tissue toxicity is directly related to the promotion of a rapid rise in cell calcium. Felodipine, which has the capacity to lower cell calcium by preventing influx as well as via an intracellular mechanism(s), namely enhancing calcium binding to proteins [Boström et al., 1981; Johnson et al., 1987], essentially abolished cardiac toxicity induced by free radicals in a dose-dependent manner. In the vehicle-treated group, [X + XO] administration resulted in a marked depression of myocardial contractility and function leading to hypotension and death of three of eight dogs. In contrast, in the felodipine-pretreated groups, [X + XO]-induced cardiac

toxicity was significantly attenuated by the lower dose and abolished by the higher dose of the calcium antagonist. It also should be noted that none of the ten dogs which received either dose of felodipine died as a consequence of [X + XO] administration. These data are consistent with our previous observations that both the calcium antagonists felodipine and verapamil were most effective in preventing mortality induced by [X + XO] in anesthetized rats [Jacinto et al., 1991].

It is possible to speculate whether the salutary effects of felodipine demonstrated in the present study are due at least in part to a scavenger-like action. Such a scavenging potential for dihydropyridines was originally proposed by Baarnhielm and Hansson in 1986. However, more recently Bolli [1988] and Janero and Burghardt [1989] have suggested that the antiperoxidant efficacy of dihydropyridine calcium antagonists rests with their ability to block lipid-free radical formation thereby preventing the propagation of lipid peroxidation. Therefore, the participation of mechanisms other than calcium antagonism in the ability of felodipine to provide protection against free radical-induced cardiac toxicity cannot be ruled out.

The doses of felodipine employed in the present studies yield plasma levels which are comparable to those found in patients receiving felodipine for the treatment of hypertension. Thus the present observations, which support a primary role for cell Ca^{2+} as the mediator of free radical-induced tissue toxicity, may be relevant in clinical situations, such as bypass surgery and transplantation technology, where there is a potential for reperfusion injury.

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