Korean J. of Pharmacology
Vol. 26, No. 1, pp. 7-12, 1990

Effects of Cyclobuxine D on the Derangement Induced by Ischemia and Reperfusion in the Isolated Rat Heart

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ABSTRACT

Cyclobuxine D is a steroidal alkaloid, which was extracted from *Buxus microphylla* var. *koreana* Nakai. In our previous studies, we clarified several pharmacological actions of cyclobuxine D: an antiinflammatory action, hypotensive and bradycardiac effects, negative inotropic effects on the several smooth muscles and cardiac muscle.

The present study was undertaken to elucidate possible mechanisms by protection of myocardial cells from ischemia and reperfusion induced derangement in cardiac function and metabolism by cyclobuxine D. For this purpose, the isolated rat heart was used. Rat hearts were perfused for 60 min under ischemia conditions in the presence and absence of cyclobuxine D and verapamil, and for 30 min under reperfusion conditions. Ischemia produced a marked decline in contractile force, an increase of resting tension, an immediate release of ATP metabolites and an accumulation of calcium in the left ventricle. Cyclobuxine D (100ng/ml) ameliorated the myocardial injury produced by ischemia.

Key Words: Cyclobuxine D, Verapamil, Isolated rat heart, Ischemia and reperfusion

INTRODUCTION

Exposure of the isolated heart to hypoxia and ischemia is accompanied by mechanical, biochemical and structural failures. The extent and severity of these abnormalities depend on many factors, such as the exogenous substrate provided, the pH, the temperature of the perfusing medium, the animal species, the concentration of certain ions normally present in the perfusion solution and the duration of hypoxia and ischemia.

Oxygen deficiency primarily induces an impairment of myocardial energy production (Jarmakani *et al.*, 1978; Joseph *et al.*, 1987), and a lack of energy in the myocardial cells may result in a deleterious disturbance of various biochemical reactions necessary for an integrity of myocardial cell function and metabolism (Hearse, 1975). Oxygen deficiency also induces alteration in membrane function, such as changes in cell membrane permeability and ion transport (Jennings, 1976) and structural defects in the membrane integrity (Willerson *et al.*, 1977). Such oxygen deficient derangements of myocardial cell function and metabolism have been demonstrated to be, more or less, protected by calcium antagonists (Cavero *et al.*, 1983; Zamanis *et al.*, 1982) and NSAIDs (Miyazaki *et al.*, 1982; Morris, 1986).

In our previous studies, we demonstrated that cyclobuxine D inhibited prostaglandin production in vivo and vitro (Lee *et al.*, 1987) and blocked potassium-activated calcium channel in the smooth muscle (Lee *et al.*, 1988). Thus, this study was carried out in order to investigate the effect of cyclobuxine D on the derangement induced by ischemia in the isolated rat heart. Verapamil (calcium channel blocking agent) was used as comparing substance.

MATERIALS AND METHODS

Perfusion of the heart

Male Sprague-Dawley rats (250-300g) were used

for this study. Five hundreds units/100g heparin was administered. After 30 min, the animals were killed by decapitation, and the heart was quickly excised and chilled in a Krebs-Hensleit solution of the following composition (mM): NaCl, 120; KCl, 4.8; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 1.25, $NaHCO_3$, 25; glucose, 11. The heart was transfered to a nonrecirculating Langendorff apparatus and perfused at 37°C with the Krebs-Hensleit solution (pH 7.4), previously equilibrated with a gas mixtrue of 95% O_2 + 5% CO_2, at a constant flow rate of 12ml/min by means of peristaltic pump (LKB 2132). The heart was preloaded with a resting tension 1.7g. Cardiac contractile force was estimated by a hook attached to the apex of the heart by means of a force development transducer (Narco T 7173). Changes in contractile force and resting tension were displayed on pen recorder.

The hearts were initially equilibrated for 30 min. Ischemia was initiated after the initial equilibration period by reducing the flow rate to 1ml/min. After 60 min, reperfusion was initiated by returning flow to the initial 12ml/min. The following drug concentrations were employed: 100ng/ml of cyclobuxine D (2.6×10^{-7}M); 100ng/ml of verapamil (2.2×10^{-7}M). For most experiment drugs were present throughout the perfusion period.

UV absorbance of the perfusate

The perfusate eluted from the perfused heart was collected at 15 min interval of ischemia and reperfusion, ranging from 0 to 90 min. The absorbance of perfusate was measured at 250nm, using a multiple wavelength detector (Hitachi 200-20). Changes in the absorbance were expressed as the absorbance $\times 10^3$ per g wet heart.

Determination of tissue calcium

Determination of tissue calcium was performed according to the method of Takeo *et al.* (1988). After perfusion, about 100mg of the left ventricle was sampled for determination of calcium. The tissue was cut into four pieces, weighed and dried at 120°C for 15hrs. After estimating the dry tissue weight, the myocardium was digested for 4hrs with 2.5ml of 0.75N HNO_3 after homogenation. After centrifugation of the digest for 10 min, at $1,000 \times g$, the supernatant fluid was diluted twice with 20mM $LaCl_3$-0.1N HCl. The calcium contents were analyzed by an atomic absorption spectrometer (Perkin-Elmer, No. 2380). The determination was performed in triplicate.

Statistics

Results were expressed as the mean ± S.E.M. Student's t-test was used for statistical evaluation of the data. Significance was taken as $p<0.05$.

RESULTS

Recovery of mechanical function

A reduction in the perfusion flow from 12 to 1 ml/min caused changes in the resting and developed tensions. The resting tension increased by 282 ± 43% at the end of the 60 min ischemia over the base-line value of 1.7 ± 0.2g (n = 7) and the developed contractile force decreased profoundly by 66%. After a 30 min reflow with 12ml/min, the mechanical abnormalities produced by ischemia were significantly decreased, although they did not entirely regress (Fig. 1 and Fig. 2). The return of flow accelerated transiently the mechanical abnormalities produced by ischemia (not expressed).

Additions of cyclobuxine D (2.6×10^{-7}M) and verapamil (2.2×10^{-7}M) to the solution perfusing the heart during ischemia did not inhibit the decline of developed contractile force while they attenuated the rise in resting tension. At the end of 30-min reperfusion with cyclobuxine D and verapamil, hearts recovered their initial active tension significantly (72% and 69% of the preischemic contractile force, respectively). Furthermore, their resting tensions were only slightly over the base-line value (Fig. 1 and Fig. 2).

Changes in UV absorbance of the perfusate

When the heart was exposed to the ischemic condition, an immediate increase in the absorbance at 250nm, of the perfusate was seen (Fig. 3). Treatment with cyclobuxine D or verapamil depressed ischemia induced increase in the absorbance. Verapamil elicited the depression of the increase more effectively than cyclobuxine D. No appreciable difference in the absorbance at 250nm of the perfusate was seen during the normal perfusion in the presence of either cyclobuxine D or verapamil, as compared with that of the control heart.

Calcium contents of the left ventricle

Calcium contents of the left ventricle were measured after ischemia and subsequent reperfusion

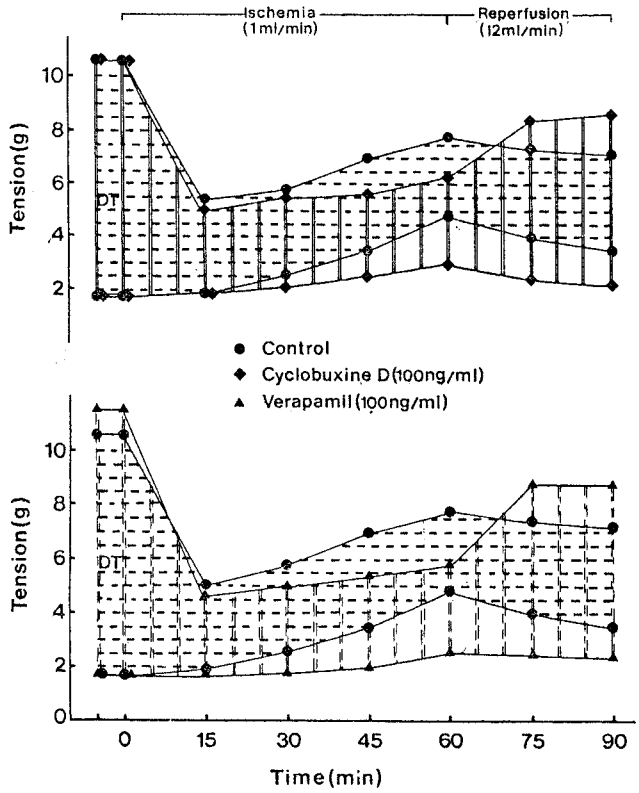

Fig. 1. Effect of 60 min ischemia and subsequent 30 min reperfusion on the developed tension in the perfused rat heart. The effects of cyclobuxine D and verapamil added to the perfusing medium are also represented. Cyclobuxine D and verapamil ameliorated the recovery of contractile force during reperfusion.

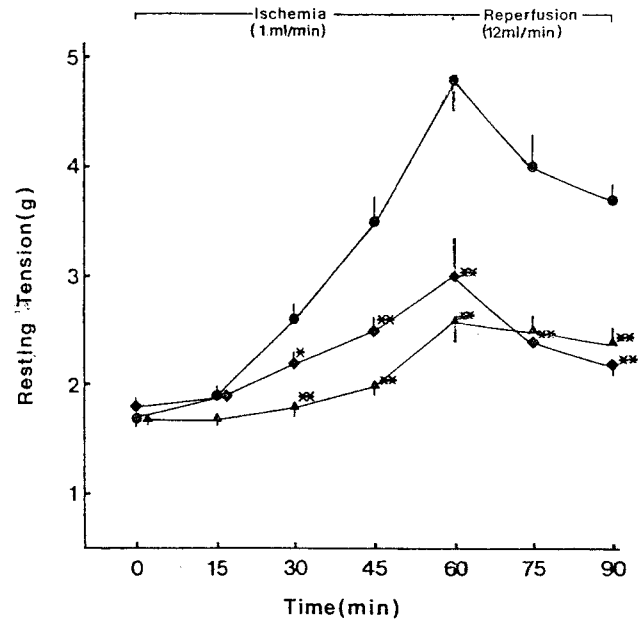

Fig. 2. Changes in resting tension of the perfused heart. Each value represents the mean and the vertical bar, the standard error of the mean. The initial resting tension was 1.7g. ●; the heart receiving 60 min ischemia and subsequent 30 min reperfusion. ◆; the heart receiving 60 min ischemia and subsequent 30 min reperfusion in the presence of cyclobuxine D (100ng/ml). ▲; the heart receiving 60 min ischemia and subsequent 30 min reperfusion in the presence of verapamil (100ng/ml). * $p<0.05$; ** $p<0.01$ from control.

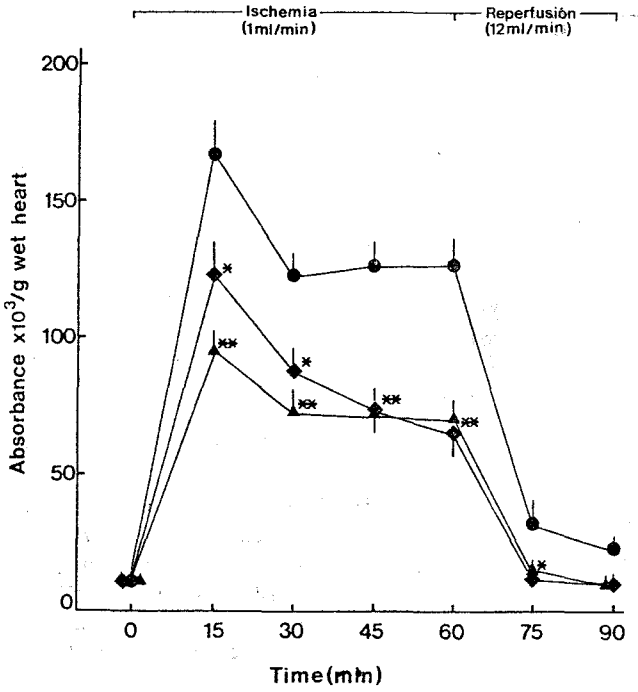

Fig. 3. Changes in the absorbance at 250nm of the perfusate eluted from hearts. ●; the perfusate from heart receiving 60 min ischemia and subsequent 30 min reperfusion. ◆; the perfusate from hearts receiving 60 min ischemia and subsequent 30 min reperfusion in the presence of cyclobuxine D (100ng/ml). ▲; the perfusate from hearts receiving 60 min ischemia and subsequent 30 min reperfusion in the presence of verapamil. * $p<0.05$; ** $p<0.01$ from control.

Table 1. Calcium contents of the left ventricle perfused under ischemia and reperfusion condition in the absence and presence of either cyclobuxine D or verapamil

	Dose (ng/ml)	N	Calcium contents
Control	—	3	8.35 ± 0.89
IS and RP	—	7	17.41 ± 1.75[a]
Cyclobuxine D	100	3	11.62 ± 2.73[b]
Verapamil	100	3	10.74 ± 1.67[b]

Calcium contents are expressed as μ moles/g dry ventricle.
N: represents the numbers of experiments.
IS and RP: represent ischemia and reperfusion, respectively.
[a]: Significantly different from the control value.
[b]: Significantly different from the value of ischemic and reperfused heart.

in the absence and presence of cyclobuxine D and verapamil. The results are shown in Table 1. Calcium contents of the myocardium perfused for 60 min ischemia and subsequent 30 min reperfusion were significantly higher than the control values. Myocardial calcium contents of hearts perfused under ischemia and reperfusion in the presence of either cyclobuxine D or verapamil were significantly lower than those of the heart not treated.

DISCUSSION

It has long been established that any type of reduced myocardial oxygen abailability results in cell damage (Hearse, 1977; Jennings *et al.*, 1975; Ganote *et al.*, 1975). Such an oxygen deficiency can be initiated either by reducing the O_2 content of the perfusing medium and maintaining flow (hypoxia) or by a reduction of flow rate to the myocardium (ischemia). In recent year it has been established that reperfusion of myocardium made sufficiently ischemic also produces a rapid tissue damage (Sakai *et al.*, 1975; Hearse, 1977). Many of the characteristics of the damage seen with the oxygen paradox parallel those of the calcium paradox, the latter involving the reintroduction of calcium after a period of calcium-free myocardial perfusion (Hearse *et al.*, 1978), possibly suggesting that calcium overload due to enhanced calcium influx mediate the damage. Many investigators demonstrated that prostaglandins contributed to cardiac injury associated with ischemia

and reperfusion. (Morris *et al.*, 1986, 1989; Margaret, 1987; Debesh *et al.*, 1987). Some studies have shown that drugs that inhibit PGs biosynthesis enhance recovery of ventricular function after reperfusion (Miyazaki *et al.*, 1982; Morris, 1986). De Deckere *et al.* (1977) demonstrated that prostacyclin is a particularly relevent cyclooxygenase-derived product since it is the predominant PG produced by the heart. The present study was undertaken to elucidate possible mechanisms by protection of myocardium for ischemia-reperfusion induced derangement in cardiac function and metabolism by cyclobuxine D, which exerted an antiinflammatory effect in rats and negative inotropic effect in smooth muscles and cardiac muscle.

A reduction of the perfusion flow from 12 to 1ml/min (ischemia) caused a decrease in developed contractile force and a steep rise in resting tension. Furthermore, poor recovery of the contractile force and the resting tension were observed upon 30 min reperfusion. Suggesting that the ischemia are sufficient to induce a severe damage in the contractile function. It should be emphasized that, as shown in Fig. 3, a significant increase in UV absorbance of the perfusate occurred ischemia and subsequent reperfusion. Although changes in UV absorbance of the perfusate do not reflect exactly the release of ATP metabolites from the heart, this may be taken as an indicator for the release of ATP metabolites. It should be mentioned that the release of ATP metabolites from the perfused heart are associated with a lack of the recovery of cardiac contractile force and the regeneration of high energy phosphate in the reperfused heart. Cyclobuxine D and verapamil effectively prevented ischemia-induced rise in resting tension and depressed ischemia induced decrease in contractile force. The recovery of the contractile force was 72 or 69% of the initial value when hearts were treated with either cyclobuxine D or verapamil during most perfusion period. The results indicate that almost complete recovery of loss of the cardiac contractile force upon reperfusion following ischemia was achieved by the treatment with either cyclobuxine D or verapamil. There is increasing evidence that recovery of cardiac contractile function after a certain period of oxygen-deficiency, is related to myocardial ATP contents and calcium accumulation (Hearse, 1979; Satoshi *et al.*, 1988; Ohhara *et al.*, 1981; Ashraf *et al.*, 1984). Cyclobuxine D prevented ischemia-induced rise in the UV absorbance of the perfusate and decreased calcium contents of the left ventricle upon reperfusion following ischemia. The results suggest that cyclobuxine D as well as verapamil is capable of protecting myocardial cells from ischemia-induced derangement in cardiac function and metabolism. We suggest the possibility that cyclobuxine D inhibits calcium entry into the myocardial cell and the release of ATP metabolites from the perfused heart. But we can not rule out the possibility that the inhibition of PGs biosynthesis by cyclobuxine D may involve in the protective mechanisms.

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=국문초록=

Ischemia에 의해 유발된 흰쥐의 적출 심장 손상에 대한 Cyclobuxine D의 보호효과

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흰쥐로 부터 심장을 적출하여 Langendorff 관류장치에 현수하여 Krebs-Hensleit 영양액으로 분당 12ml속도로 30분간 관류시킨 후 관류 속도를 분당 1ml로 줄여(ischemia)60분간 관류시키면, 적출심장의 수축력이 현저히 감소되었고, resting tension이 현저히 증가되었다. 또 적출심장으로부터 유출되는 관류액의 250nm에서의 UV흡광치는 증가되었으며, 좌심실내의 칼슘의 농도는 대조군보다 상당히 증가되었다. 본 실험에서는 흰쥐에서 항염증작용, 혈압강하 및 서맥 작용, 평활근 및 심장근에서 근이완작용을 나타내는 cyclobuxine D의 ischemia에 의해 유도된 심장손상에 대한 보호효과를 관찰하였다. Cyclobuxine D(100ng/ml)는 ischemia에 의해 유발된 적출심장의 수축력 감소와 resting tension의 증가를 유의하게 억제하였으며, 심장으로부터의 ATP metabolites의 유출과 좌심실내의 칼슘 축적을 억제시켰다. 이상의 결과는 Cyclobuxine D가 ischemia에 의해 유발된 손상으로 부터 심장을 보호할 수 있음을 나타내며, 이는 cyclobuxine D의 심장세포내의 칼슘 유입 억제작용에 기인하는 것으로 사려된다.