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Trandolapril Plus Verapamil Inhibits the Coronary Vasospasm Induced by Hypoxia Following Ischemia-Reperfusion Injury in Dogs

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ABSTRACT. 1. Dogs were exposed to hypoxemia followed by a coronary angiogram at three different times: under control conditions, after ischemia-reperfusion injury, then 30 min later.

2. In the study group, the dogs were treated with trandolapril (0.05 mg/kg) and verapamil (0.1 mg/kg) just prior to the final hypoxic challenge.

3. Under control conditions, the left anterior descending coronary artery (LAD) dilated in response to hypoxia. Following ischemia-reperfusion injury, however, it constricted significantly in response.

4. In the control group, repeat hypoxia 30 min later resulted in vasoconstriction of the LAD which was comparable to the preceding response.

5. However, in the study group, treatment with trandolapril plus verapamil inhibited the vasoconstriction in response to repeat hypoxia. GEN PHARMAC 27;6:1057–1059, 1996.

KEY WORDS. Ischemia–reperfusion injury, hypoxic vasospasm, Ca^{++} channel blocker, angiotensin converting enzyme inhibitor

INTRODUCTION

When exposed to severe hypoxia, isolated canine coronary arteries contract in an endothelium-dependent manner (Graser and Vanhoutte, 1991; Rubanyi and Vanhoutte, 1985). This response is exacerbated by previous ischemia-reperfusion injury of the coronary endothelium (Pearson *et al.*, 1987). In response to hypoxia *in vivo*, normal canine coronary arteries dilate; however, after ischemia-reperfusion injury, hypoxia induces coronary vasospasm (Pearson *et al.*, 1987). The endothelium-dependent augmentation of isolated coronary arteries induced by hypoxia can be inhibited by Ca^{2+} channel inhibition (Iqbal *et al.*, 1988). This effect is due primarily through its action on vascular smooth muscle, and not on endothelial cells. Trandolapril is an angiotensin-converting enzyme inhibitor that potentiates the endothelium-dependent relaxation to bradykinin and ADP (Vanhoutte *et al.*, 1989). Thus, it was hypothesized that the combination of trandolapril plus verapamil would be effective in inhibiting the exaggerated endothelium-dependent vasoconstriction induced by hypoxia after ischemia-reperfusion injury. The purpose of the present study was to determine if the vasospasm induced by hypoxia in reperfused coronary arteries *in vivo* can be modulated by treatment with trandolapril plus verapamil.

METHODS

Heartworm-free mongrel dogs (25 to 30 kg) of either gender were anesthetized with intravenous pentobarbital sodium (30 mg/kg bolus injection; Fort Dodge Laboratories, Fort Worth, TX), intubated with a cuffed endotracheal tube, and ventilated. The respirator was adjusted to keep blood pH between 7.35 and 7.45, P_{CO_2} between 30 and 40 mm Hg, and P_{O_2} between 100 and 120 mm Hg. The blood gas values were monitored every 30 min during the experiments (pH/Blood Gas Analyzer 813, Instrumentation Laboratory, Lexington, MA). The blood pressure, through an arterial line, and an electrocardiogram (ECG), were monitored continuously.

Experimental infarction

An 8F Judkins catheter was inserted through the left carotid artery and guided by fluoroscopy into the orifice of the left coronary artery. A 2F Fogarty embolectomy catheter was passed through the Judkins catheter and positioned in the left anterior descending (LAD) artery immediately distal to the first diagonal branch. The artery was occluded by inflation of the balloon at the tip of the Fogarty catheter with diluted radiographic contrast material. Occlusion of the coronary artery was verified by coronary angiography and electrocardiographic evidence of myocardial ischemia (typically ST segment depression in leads II and III). Heparin (2000 U) was then injected intravenously. The occlusion was terminated after 60 min by deflating the balloon slowly. Reperfusion was confirmed by coronary angiography and ECG changes. If an animal had extensive collateral blood flow and did not exhibit ischemic ECG changes 15 min after occlusion, it was excluded from the study ($n=7$).

Angiographic studies

In all animals, a control angiogram was taken under control conditions. The animals were then randomly assigned to the control group ($n=6$), or the study (treated) group ($n=6$). In both groups, a left coronary angiogram was obtained at the end of 10 min of hypoxia ($P_{O_2}=25$ to 35 mm Hg) under control conditions, again after 60 min of ischemia plus 60 min of reperfusion, and finally 30 min later. In the study group, the final hypoxic challenge was preceded

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by treatment with trandolapril plus verapamil. Systemic hypoxemia was induced by a mixture of oxygen and nitrogen into the gas reservoir of the respirator. Tidal volume and respiratory rate were adjusted so that pH and Pco_2 remained in the control range while Po_2 was lowered selectively. Verapamil 0.1 mg/kg was infused slowly (over 2 min) 10 min before the final hypoxic challenge. Trandolapril 0.05 mg/kg was infused 30 min before the final hypoxic challenge as it is a prodrug (Conen and Brunner, 1993). The dosages used were the maximum dosages which did not cause significant hypotension (less than 20 mm Hg decrease in the mean arterial pressure).

Statistical analysis and calculations

End-diastolic coronary artery dimensions were measured from angiograms along a 1-cm segment of the distal LAD arteries in a blinded manner using a computer-based quantitative angiographic system (Cardiovascular Angiographic Analysis System, Pie Data Medical of Maastrict, Holland) (Abuhkalil, 1990). The changes in coronary artery dimensions are expressed as percent changes from those in the control angiograms. All results are expressed as means±SEM. Sequential data were compared by analysis of variance (ANOVA). Paired and unpaired data were analyzed by Student's *t*-test. Differences were considered to be statistically significant when P was less than 0.05.

RESULTS

The baseline LAD diameter was 1.60±0.10 mm in the control group, and 1.69±0.10 mm in the study group (P=0.52). In the control group, hypoxia under control conditions resulted in a 13.6±4.6% dilation of the LAD (P=0.017 when compared with baseline). Following reperfusion injury, hypoxia induced a 9.0±2.1% vasoconstriction of the LAD (P=0.008 when compared with baseline; P=0.01 when compared with the preceding response; Figs. 1 and 2). In the study group, hypoxia under control conditions resulted in a 17.3±6.7% vasodilation of the LAD (P=0.66 compared with the control group). Following 60 min of ischemia plus 60 min of reperfusion, the LAD constricted 11.7±3.5% in response to hypoxia (P=0.51 compared with the control group). Repeating the hypoxia 30 min later in the control group resulted in a 10.6±2.0% vasoconstriction of the LAD. This response was not significantly different when compared with the preceding response to hypoxia within the same group (P=0.55). Repeat hypoxia (30 min later) in the group treated with trandolapril plus verapamil resulted in a vasoconstriction of 1.7±7.3%. This response was significantly less when compared with the preceding response to hypoxia within the same group (P=0.049; Fig. 2).

FIGURE 1. Angiograms of the LAD taken after 10 minutes of hypoxia. Top panel: the LAD in response to hypoxia under control conditions. Bottom panel: the same artery in response to hypoxia after 60 min of ischemia followed by 60 min of reperfusion. The arrows indicate the site of occlusion.

DISCUSSION

Severe hypoxia induces endothelium-dependent augmentation of contraction of isolated canine coronary and peripheral arteries (Graser and Vanhoutte, 1991; Rubanyi and Vanhoutte, 1985: De Mey and Vanhoutte, 1983) as well as human blood vessels (Lin *et al.*, 1990). While the involvement of a diffusible endothelium-derived vasoconstrictor substance is likely (Rubanyi and Vanhoutte, 1985), the precise mechanisms underlying endothelium-dependent hypoxic contractions remain uncertain. In the present study, the coronary arteries dilated in response to hypoxia *in vivo*. This is consistent with findings reported previously (Pearson *et al.*, 1987). It is unclear why arteries contract *in vitro*, but dilate *in vivo* in response to hypoxia. It may be due to the fact that in the *in vitro* studies, the isolated coronary arteries were exposed to more severe hypoxia (Graser and Vanhoutte, 1991; Rubanyi and Vanhoutte, 1985). Moreover, it is likely that hypoxia modulates other determinants of coronary tone *in vivo*.

Following ischemia-reperfusion injury of the coronary endothelium, the isolated coronary artery demonstrates exaggerated endothelium-dependent vasoconstriction in response to hypoxia (Pearson *et al.*, 1987). In the present study, confirming previous observations, hypoxia induced vasospasm after ischemia-reperfusion injury (Pearson *et al.*, 1987). These observations imply that, following ischemia-reperfusion injury, re-exposure of the reperfused coronary artery to ischemia (hypoxia) may result in vasospasm, resulting in worsening of the myocardial ischemia. The degree of abnormal vasospasm in response to hypoxia demonstrated in this study may not by itself be sufficient to significantly alter coronary blood flow. However, ischemia-reperfusion and recurrent ischemia usually occurs in diseased arteries with already narrowed lumens. In such a setting, an abnormal vasoconstrictor response to hypoxia may be critical. Indeed, recurrent ischemia following reperfusion is not uncommon,

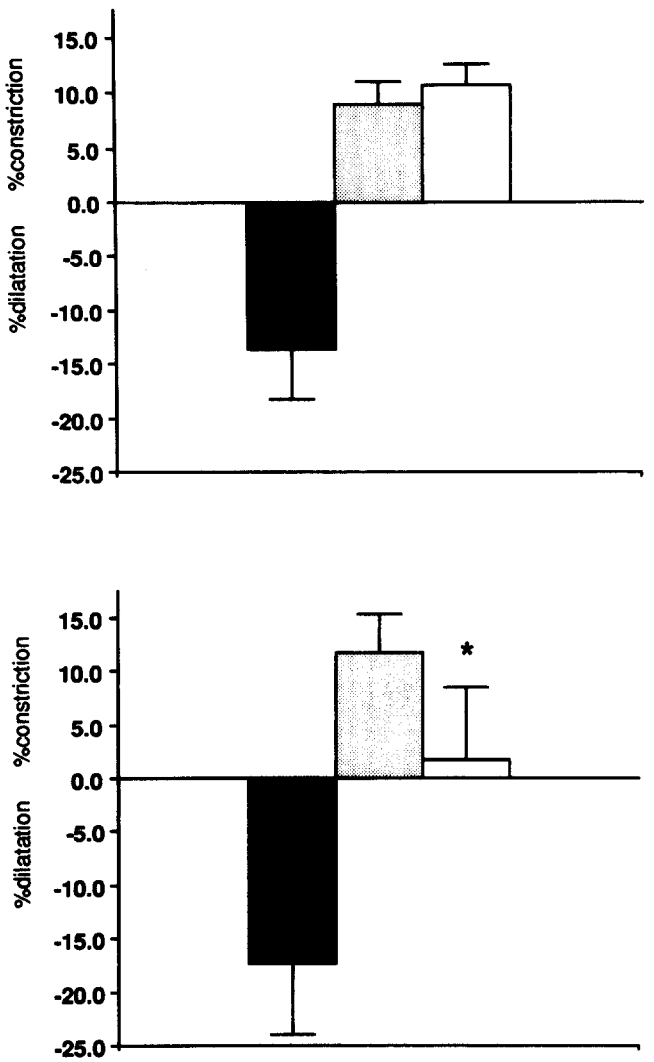

FIGURE 2. Vasoconstriction or dilation in response to hypoxia expressed as a percent change from the baseline LAD diameter. The top panel shows the response of the LAD artery in the control group. The bottom panel shows the response of the LAD in the group treated with trandolapril (0.05 mg/kg) plus verapamil (0.1 mg/kg) (study group) prior to the third hypoxic challenge. *Hypoxia-control*: response of the LAD to hypoxia under control conditions. *Hypoxia-reperfusion*: response of the LAD to hypoxia after 60 min of ischemia followed by 60 min of reperfusion. *Hypoxia-without trandolapril/verapamil*: response of the LAD to hypoxia 30 min after *hypoxia-reperfusion* in the absence of treatment with trandolapril plus verapamil. *Hypoxia-with trandolapril/verapamil*: response of the LAD to hypoxia 30 min after *hypoxia-reperfusion* in the presence of treatment with trandolapril plus verapamil. Data expressed as means±SEM ($n=6$). **Hypoxia-with trandolapril/verapamil* value is significantly different from *hypoxia-reperfusion* value within the same group ($P=0.049$). (■) Hypoxia-control; (▩) hypoxia-reperfusion; (□) hypoxia-with trandolapril/verapamil.

and can be a potentially devastating complication of thrombolytic therapy (Lincoff and Topol, 1993).

This study demonstrates that, in dogs, treatment with trandolapril plus verapamil significantly inhibits the coronary vasospasm induced by hypoxia following ischemia-reperfusion injury *in vivo*. It did not, however, restore the normal dilatory response to hypoxia. A limitation of the present study is that experiments comparing single-drug therapy with combination therapy were not performed. However, *in vitro* studies indicate that the inhibitory effect of the combination therapy in the present study can be attributed to the calcium channel blockade. Whereas Ca^{2+} channel inhibition has been shown to block the endothelium-dependent contraction induced by hypoxia (Iqbal *et al.*, 1988), recent studies demonstrate that trandolapril does not (unpublished observations).

In conclusion, in contrast to normal canine coronary arteries which dilate in response to hypoxia *in vivo*, the coronary arteries constrict in response after ischemia-reperfusion injury. While treatment with trandolapril plus verapamil did not restore the normal dilatory response to hypoxia, it did significantly inhibit the coronary vasospasm induced by hypoxia after ischemia-reperfusion injury.

SUMMARY

The purpose of the present study was to determine whether or not the hypoxic coronary vasospasm following ischemia-reperfusion injury can be modulated by treatment with trandolapril plus verapamil. Heartworm-free mongrel dogs were exposed to systemic hypoxemia followed by an angiogram at three different times: under control conditions, after ischemia-reperfusion injury, then 30 min later. In the study group, the dogs were treated with trandolapril 0.05 mg/kg and verapamil 0.1 mg/kg by intravenous injection just prior to the final hypoxic challenge. Under control conditions, the left anterior descending coronary artery (LAD) dilated significantly in response to hypoxia. Following ischemia-reperfusion injury, however, the LAD constricted significantly in response. In the control group, repeat hypoxia 30 min later resulted in vasoconstriction of the LAD which was comparable to the preceding response. However, in the study group, treatment with trandolapril plus verapamil significantly inhibited the vasoconstriction in response to repeat hypoxia. These observations demonstrate that, in dogs, treatment with trandolapril plus verapamil inhibits the coronary vasospasm induced by hypoxia following ischemia-reperfusion injury.

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