Effects of Verapamil on Coronary Vascular Resistance in Rabbits: Measurement with Pulsed Doppler Velocimetry

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Key Words
- coronary vasodilatation
- pulsed Doppler velocimetry
- verapamil

Background. Verapamil is an effective vasodilator. The purpose of this study was to investigate the in vivo effect of verapamil on coronary blood flow velocity and vascular resistance in anesthetized, open-chest rabbits.

Methods. Twenty-one male New Zealand white rabbits were anesthetized, and a 3-mm suction-type pulsed Doppler velocimeter probe was applied to the proximal part of the left anterior descending coronary artery after median sternotomy. The rabbits received intravenous bolus infusion of 4 different doses of verapamil (0.01 mg/kg, n = 5; 0.1 mg/kg, n = 5; 1 mg/kg, n = 5; and 10 mg/kg, n = 6). The percent changes in coronary blood flow velocity and coronary vascular resistance were examined.

Results. There was 10.0 ± 1.6% increase in coronary blood flow (CBF) and 12.5 ± 1.9% reduction in coronary vascular resistance (CVR) after infusion of 0.01 mg/kg of verapamil. The CBF increased 23.0 ± 9.5% and CVR decreased 24.2 ± 5.2% after infusion of 0.1 mg/kg of verapamil. In infusion of 1 mg/kg of verapamil in increased 34.8 ± 10.5% increase in CBF and 32.6 ± 2.5% reduction in CVR. The CBF increased 41.1 ± 14.8% and CVR decreased 45.1 ± 5.4% after infusion of 10 mg/kg of verapamil.

Conclusions. Compared with baseline condition, all doses of verapamil in increased coronary blood flow velocity and decreased coronary vascular resistance significantly in anesthetized, open-chest rabbits.

Verapamil is an effective vasodilator and plays a role in the therapy of hypertension and angina pectoris. Its effect on regional coronary resistance has been documented, but little is known regarding the dose-response effects of verapamil on total coronary vascular resistance (CVR) or systemic hemodynamic variables. Thus, there is a need for a systemic study of the effects of a wide dose range of verapamil on CVR. Pulsed ultrasound Doppler velocimetry is a convenient and accurate technique for measuring phasic changes in coronary blood flow (CBF) in small animals. The purpose of the current study is to determine the effect of verapamil on CBF and CVR in rabbits by means of pulsed Doppler velocimetry.
Methods

Animal preparation

This study was approved by the Animal Experiment Committee of the National Yang-Ming University, and the animals were cared for in accordance with the “Guide for the Care and Use of Laboratory Animals” [National Academy Press, 1996]. The methods of animal preparation and instrumentation has been used in previous studies. Twenty-one male New Zealand white rabbits (body weight ranging from 2.0 to 2.6 kg; mean ± standard deviation: 2.4 ± 0.2 kg) were anesthetized by intravenous administration of pentobarbiturate (30 mg/kg) via marginal ear veins; and maintained by 5 mg·kg⁻¹·hr⁻¹ infusion. Body temperature was maintained at 37 °C with a heating pad. The animals were intubated by means of tracheostomy and ventilated with a rodent ventilator (Harvard Apparatus, Natick, MA) using oxygen-enriched air. The right femoral artery and vein were cannulated for arterial blood pressure monitoring and fluid administration. Arterial blood pressure was measured with a Statham P23 transducer (Statham Instruments, Oxnard, CA) coupled to a pressure sensor and amplifier (Gould Instruments, Cleveland, OH), which computed the systolic, mean and diastolic arterial pressure, and heart rate. Electrocardiograph (ECG) leads were placed on four limbs of each rabbit. A median sternotomy was performed, the pericardium was incised, and the left atrium was retracted with 6-O suture. The heart was then exposed and the left anterior descending coronary artery was identified. A 3-mm suction-type pulsed Doppler velocimeter probe (20-MHz) (Triton Technology, San Diego, CA) was applied to the proximal part of the left anterior descending coronary artery, and connected to a pulsed Doppler velocimeter module (Crytal-Biotech, Northboro, MA). The signal of coronary artery blood flow was confirmed by a preamplifier and a stethoscope. The base line arterial blood pressure, heart rate, and CBF were recorded and ECG performed after a 30-minute stabilization.

Experimental protocol

After the 30-minute stabilization period, the rabbits were divided into four groups to receive intraintravenous infusion of verapamil at various concentrations. Group 1 rabbits received bolus infusion of verapamil at a dose of 0.01 mg/kg (N = 5), group 2 rabbits at a dose of 0.1 mg/kg (N = 5), group 3 rabbits at a dose of 1 mg/kg (N = 5), and group 4 rabbits at a dose of 10 mg/kg (N = 6). The dose of verapamil less than 0.01 mg/kg was not administered to rabbits as its effect on CBF was minimal and could not be detected in preliminary experiments. The dose larger than 10 mg/kg was not used, either, because rabbit blood could not tolerate this high dose and would die after injection in preliminary experiments. The changes in CBF induced by the vasodilators are expressed as percentages of the baseline values. The changes in hemodynamic changes and changes in CBF among different doses of verapamil were subject to analysis of variance (ANOVA) followed by post hoc tests. The statistics analysis

Drug administration

Verapamil (Isoptin®) was purchased from Knoll AG (Ludwigshafen, Germany). It was diluted to the desired concentration with normal saline, and was administered in a volume of 0.3 mL via the marginal ear vein infusion. The concentration of verapamil that could produce detectable effect on CBF velocity (the lowest concentration) and was still tolerated by the animals (the highest concentration) were determined in a preliminary study.

Statistics analysis

All values were expressed as means ± SD. The paired data were analyzed with paired t tests. The differences in hemodynamic changes and changes in CBF among different doses of verapamil were subject to analysis of variance (ANOVA), followed by post hoc tests.
post-hoc Bonferroni tests. A $p$ value of less than 0.05 was considered statistically significant.

## Results

### Hemodynamic variables

There was no significant difference in baseline mean arterial pressure among the four groups of rabbits. Intravenous administration of verapamil significantly decreased the mean arterial pressure at all doses tested, as compared with the baseline values (Table 1). The reduction of mean arterial pressure induced by infusion of verapamil was significantly larger at the dose of 10 mg/kg than at other doses ($p < 0.01$).

The baseline heart rate was not significantly different among the four groups of rabbits. Intravenous administration of verapamil significantly decreased the heart rate at all doses tested, as compared with the baseline values (Table 1). The reduction of heart rate induced by infusion of verapamil was significantly larger at the dose of 10 mg/kg than at other doses ($p < 0.01$).

### Peak CBF velocity

Intravenous administration of verapamil at all doses tested significantly increased the peak CBF velocity, as compared with baseline values ($p < 0.01$). To account for variations in individual baseline values, the changes in CBF velocity after drug administration were expressed as per cent ages of the baseline velocities (Fig. 1). Intravenous administration of verapamil produced greater increase in peak CBF velocity at doses of 1 mg/kg (134.8 ± 0.5%) and 10 mg/kg (141.1 ± 14.8%) than 0.01 mg/kg (110.0 ± 1.6%) ($p < 0.01$).

### CVR

The changes in CVR after verapamil administration were expressed as per cent ages of the baseline values. All doses of verapamil tested decreased CVR in a dose-dependent manner (Fig. 2). Verapamil decreased CVR more at doses of 0.1 mg/kg, 1 mg/kg and 10 mg/kg than at the dose of 0.01 mg/kg (24 ± 5%, 33 ± 3%, and 45 ± 5% respectively, vs 13 ± 2%; $p < 0.001$). Verapamil decreased CVR more at doses of 1 mg/kg and 10 mg/kg than at the dose of 0.1 mg/kg (33 ± 3% and 45 ± 5%, respectively, vs 13 ± 2%; $p < 0.001$; the

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**Table 1. Hemodynamic values before and after infusion of verapamil in open-chest, anesthetized rabbits**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Mean arterial pressure (mmHg)</th>
<th>Heart rate (beat/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After infusion</td>
</tr>
<tr>
<td>0.01 (N=5)</td>
<td>71.8 ± 5.7</td>
<td>67.0 ± 5.1$^a$</td>
</tr>
<tr>
<td>0.1 (N=5)</td>
<td>69.8 ± 6.8</td>
<td>63.0 ± 5.8$^a$</td>
</tr>
<tr>
<td>1 (N=5)</td>
<td>68.8 ± 10.1</td>
<td>61.4 ± 9.3$^a$</td>
</tr>
<tr>
<td>10 (N=6)</td>
<td>66.5 ± 7.8</td>
<td>47.3 ± 6.5$^a$</td>
</tr>
</tbody>
</table>

All data are presented as mean ± SD.

$^a$ $p < 0.05$ vs. baseline values, analyzed with paired $t$ test.

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**Fig. 1.** Changes in peak coronary blood flow (CBF) velocity, expressed as per cent ages of baseline values, after intravenous infusion of different doses of verapamil. $^*$ $p < 0.05$ vs. 0.01 mg/kg.
10 mg/kg also reduced CVR more than the 1 mg/kg (p < 0.001).

**Discussion**

We investigated the effects of verapamil on CBF and CVR in anesthetized, open-chest rabbits. All doses of verapamil tested in this study increased CBF and reduced CVR in a dose-dependent manner (Fig. 1 and 2).

With the development of the suction-type probe, CBF velocity in small animals is now measurable.\(^3,4\) We used pulsed ultrasonic Doppler velocimetry to evaluate coronary vasodilatation in rabbits in this study and in previous studies.\(^8,9\) A directional pulsed Doppler system has the advantage of easy calibration and stable zero-flow.\(^2,10\) It can measure CBF velocity in different segments of the cardiac cycle,\(^2,5\) and assess rapid changes in CBF velocity. It has proved to be suitable for studies of temporary drug intervention.\(^11\)

Pulsed Doppler velocimetry actually measures the blood flow velocity, rather than the blood flow volume itself. It is necessary to know the diameter of the vessel, and the angle between the ultrasound sound beam and the blood flow to measure the true blood flow volume.\(^7,11\) In fusion of vaso dilators, like verapamil used in this study, might the retically change the diameter of the coronary artery and affect the accuracy of the measurement of blood flow. However, there were no reports showing that changes in mean CBF velocity were measured with an electro magnetic flow meter, venous outflow collection,\(^3,13\) and microsphere-measured left ventricular perfusion.\(^3,9\) Previous reports also showed that the coronary artery diameter varies by 4% to 5% in a single cardiac cycle.\(^14,15\) In fusion of dipyridamole has been reported to decrease the coronary artery diameter by 2.6%, and ad ministration of nitrroglycerin showed to increase the internal diameter by 0.8%.\(^14,15\) Because we measured the relative change in CVR in the same artery before and after infusion of verapamil in this study, the small change in the diameter should not have affected our results.

Verapamil, the prototype of a group of drugs designated as calcium antagonists or slow channel inhibitors, has been used widely as a coronary vasodilator and antianginal agent for decades. These agents exert systemic and coronary dilator effects by means of inhibiting the inward calcium flux through the so-called slow channels in cardiac muscle and vascular smooth muscle, especially in the coronary and peripheral arteries. It was also proved with anti-ischemic effect due to non-specific anti-adrenergic effect.\(^16\) Verapamil can cause vascular smooth muscle relaxation via the mechanism of either liberation of nitric oxide or anti-spastic effect.\(^17\)

The major finding of this study was that the in fusion of verapamil re reduced the CVR and increased the CBF dose-dependently in a wide dose range (Fig. 1 and 2). Verapamil also significantly reduced mean arterial pressure and heart rate at the doses used in this study (Table 1). The effects of in fusion of verapamil on coronary hemodynamics (coronary blood flow and vascular resistance) were relatively dose-dependent, especially in low dose (0.01-1.0 mg/kg), as compared with its effects on systemic hemodynamics (heart rate and blood pressure). The effects of in fusion of verapamil seemed to be more consistent and predominant on coronary
rather than on systemic hemodynamics. Verapamil is a mod est systemic and coronary vasodilator and was noted with complex hemodynamic ef fects. In open chest anesthetized dogs, verapamil exerted no table de pres sant ef fects on car diac perfu r mance. How ever, the hypotensive ef fects of verapamil were re lated to a concomitant reduction in systemic vascular re sistance. In unanesthetized closed chest dogs, ve rapamil produced dose-dependent peripheral vasoodle with re flex in creases in myocar dial con trac til ity and heart rate. A direct myocar dial de pres sant ef fect will only be ap par ent with a dose of verapamil greater than 0.3 mg/kg.

In conclusion, we used pulsed Doppler velocimetry to in vesti gate the coronary vasodilatation ef fect of verapamil in anesthetized, open-chest rabbits. Verapamil at all doses tested in creased CBF ve loc ity sig ni fi cantly and de creased CVR dose-dependently. It also sig ni fi cantly re duced the mean arte rial pres sure and hear rate.

Acknowledgement

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