Enhancement of Coronary Vasodilating Action of ATP and Adenosine by Lidoflazine

By Skoda Afonso, M.D., Ph.D., George S. O'Brien, M.D., Ph.D., and Charles W. Crampton, M.D.

ABSTRACT

Lidoflazine, a newly developed coronary vasodilator, greatly enhances the coronary vasodilating action of adenosine and ATP. Experiments were designed to study the combined coronary vasodilating effects of adenosine and lidoflazine and to quantify the enhancement of coronary vasodilation of adenosine and ATP by prior administration of lidoflazine. Results of the experiments showed that the combined coronary vasodilating effects of adenosine and lidoflazine can be classified as supra-additive. For 3 hours after the administration of lidoflazine there was a great intensification (20 to 150 times) of the vasodilating action of adenosine or ATP. An adenosine-sparing effect of lidoflazine in whole blood was also demonstrated, and the enhancement of adenosine action is in part related to this effect. It is suggested that lidoflazine may also produce a change in the sensitivity of the smooth muscle of the coronary vessels to the direct or indirect action of adenosine.

ADDITIONAL KEY WORDS coronary sinus blood flow dipyridamole thermodilution flowmeter adenosine deaminase anesthetized dogs

The coronary vasodilating action of adenosine or ATP is greatly enhanced by prior or concomitant administration of the coronary vasodilator dipyridamole (1, 2). Lidoflazine, \[1\{4, 4-di (4-fluorophenyl)-butyl\}4- (2, 6-dimethylanilinocarbonyl) -methyl-piperazine,\] is a newly developed, long-acting coronary vasodilator (3, 4). Since both lidoflazine and dipyridamole have long-lasting coronary vasodilating effects, it seemed of interest to determine whether lidoflazine, like dipyridamole, possesses the property of enhancing adenosine- and ATP-induced coronary vasodilation. We designed the present experiments to study quantitatively the combined coronary hemodynamic effects of adenosine and lidoflazine and to determine whether lidoflazine has an in vitro adenosine-sparing effect in whole blood as has been reported for dipyridamole (5-7).

Methods

The experimental procedures in this study were similar to those described in detail previously (2). Seventeen adult mongrel dogs were used. Anesthesia was induced by 3 mg of morphine sulfate/kg subcutaneously followed in 1 hour by intravenous injection of 12.5 mg of allobarbital, 50 mg of urethane/kg, 50 mg of monoethylurea/kg, and 8 mg of sodium pentobarbital/kg. During the next hour, under fluoroscopic control we placed catheters in the right atrium or pulmonary artery or both for administration of drugs and a thermodilution catheter flowmeter (8) in the coronary sinus, through the external jugular vein, for measurement of coronary sinus blood flow. Femoral arterial blood pressure was obtained from a Statham strain gauge connected to a percutaneously inserted Cournand needle. Coronary sinus blood flow and arterial blood pressure were recorded on a direct writing Sanborn polyviso 150. Cardiac output

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was measured with a Gilson model DTL dye tracer and macropolygraph after injection of indocyanine green dye through the pulmonary artery catheter. All animals received 500 units of heparin per kg body weight at the beginning of experiments.

In three dogs we measured coronary vasodilation that followed combinations of adenosine and lidoflazine in doses which alone were not effective. We measured femoral arterial blood pressure and coronary sinus blood flow during 20-minute constant rate infusions of (1) adenosine, (2) adenosine and lidoflazine, and (3) when blood pressure and coronary sinus flow had returned to control values, lidoflazine alone. Doses of lidoflazine and adenosine were selected on the basis of preliminary experiments which indicated these doses to be ineffective or barely effective in augmenting coronary sinus blood flow. Since lidoflazine was expected to influence the action of adenosine, infusions of adenosine always preceded the infusions containing lidoflazine. Aqueous solutions of lidoflazine were prepared by dissolving 400 mg of the compound in 100 ml of 0.01 N HCl.

In ten dogs, we quantified the enhancement of the coronary vasodilator action of adenosine by lidoflazine. One hour after giving the anesthetic agents, we measured cardiac output, coronary sinus blood flow and femoral arterial blood pressure. Next we recorded coronary sinus blood flow and arterial pressure during constant-rate infusions of ATP or adenosine in doses which would increase coronary sinus blood flow to the level obtained before lidoflazine. Since the experiments lasted more than 3 hours, anesthesia was maintained by giving one-fifth of the initial dose of the anesthetic mixture when needed.

An in vitro adenosine-sparing effect of lidoflazine in whole blood was examined. It was observed that for 3 or more hours after 1.5 or 2.0 mg of lidoflazine/kg there was a marked increase in the coronary vasodilator response to adenosine; doses as small as 5 μg of adenosine given intravenously caused detectable increases in coronary sinus flow. Coronary vasodilator responses in these dogs made highly sensitive to adenosine by prior administration of lidoflazine were used as a bioassay for the presence of adenosine added to whole blood with or without added lidoflazine. Whole blood was collected from the animals before the injection of lidoflazine. Lidoflazine was added to the blood in vitro to approximate the calculated blood concentration of an effective dose of lidoflazine. Tests were performed in four dogs. Concentrations of adenosine and lidoflazine were respectively 10 and 40 μg/ml in two tests; 16 and 66 μg/ml in one and 20 and 40 μg/ml in the other. Control blood mixtures contained adenosine only. At various times of incubation (4 to 10 min) at room

### TABLE 1

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Adenosine alone</th>
<th>Adenosine + lidoflazine</th>
<th>Lidoflazine alone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate of Infusion (μg/ml)</td>
<td>Blood flow (ml/min)</td>
<td>Rate of Infusion (μg/ml)</td>
</tr>
<tr>
<td></td>
<td>Adenosine</td>
<td>Lidoflazine</td>
<td>Control</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
<td>------------</td>
<td>---------</td>
</tr>
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</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td></td>
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</tr>
<tr>
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<td></td>
<td>25</td>
</tr>
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<td>0.1</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>0.5</td>
<td>0.22</td>
<td>24</td>
</tr>
</tbody>
</table>

Dogs 1, 2, and 3 weighed 18.6, 20.0, and 17.7 kg, respectively.
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Results

In the first series of 3 dogs, infusions of adenosine or lidoflazaine alone produced no appreciable change in coronary sinus blood flow (Table 1); the infusion of the adenosine plus lidoflazaine produced a marked increase in coronary sinus flow in all three dogs. When the two drugs were used together, the dose of adenosine in all dogs and of lidoflazaine in one dog was half of the dose of the drugs used alone.

Figure 1 shows representative recordings from a dog (no. 6, Table 2) of the second group of ten dogs used to quantify the enhancement of coronary vasodilating action of ATP by lidoflazaine. Before administration of lidoflazaine, infusions of 1.9 and 3.8 mg of ATP/minute raised coronary flow to 129 and 378 ml/minute (Fig. 1, panels 1 and 2). Such large increases in flow cannot be ascribed wholly to the moderate accompanying increases in cardiac rate. The increase in coronary sinus flow produced by 1.5 mg of lidoflazaine/kg is shown in Figure 1 (panel 3). One hour after injection of lidoflazaine, coronary sinus flow had returned to the same level as before lidoflazaine. At this time infusions of 0.05 and 0.097 mg of ATP/minute elicited responses similar in magnitude to those produced by 1.9 and 3.8 mg of ATP/minute before lidoflazaine. Three hours after, infusions of 0.05 and 0.097 mg of ATP/minute raised coronary flow to levels of 100 and 256 ml/minute. These responses are illustrated in Figure 1 (panels 5 and 6). On the basis of the ratios of ATP doses used before and 3 hours after lidoflazaine, we estimated that the coronary vasodilating action of ATP was increased 20 to 39 fold in this dog. The data in the other nine dogs were obtained in the same manner and are shown in Table 2. In all dogs the coronary vasodilating action of ATP or adenosine was enhanced by 20 to 150 fold.

| Coronary Sinus Blood Flow (ml/min) during ATP and Adenosine Infusions | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | 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Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | Adenosine | 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In these dogs, 10 minutes after administration of lidoflazine alone there was a statistically significant increase (+120%, $P < 0.02$) in coronary sinus blood flow, associated with statistically significant increases in heart rate (+16%, $P < 0.02$) and cardiac output (+31%, $P < 0.01$). At 90 minutes after the administration, heart rate and cardiac output were at control levels and coronary sinus flow was a little elevated (+19%, $P < 0.02$).

Coronary vasodilating responses obtained in one test performed to determine if lidoflazine has an adenosine-sparing effect in whole blood are shown in Figure 2. At zero
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FIGURE 2

CF = coronary sinus blood flow in milliliters per minute. 1 = response to a single injection of 50 μg of exogenous adenosine in 5 ml of blood at zero time of incubation; 2 = at 10 minutes; 3 = at 20 minutes; 4 = response to 50 μg of adenosine + 200 μg of lidoflazine in 5 ml of blood at zero time; 5 = at 10 minutes; 6 = at 20 minutes; 7 and 8 = control responses to 50 μg of adenosine in saline at beginning and end of test. Blood temperature was verified in panels 3, 4 and 8 by turning off the heater of the flowmeter. Arrows indicate time of injections.

time of incubation, blood with adenosine only and blood with adenosine plus lidoflazine caused coronary vasodilation of the same magnitude as that produced by 50 μg of adenosine in saline. At 20 minutes of incubation, blood with adenosine only does not show any coronary vasodilating effect, whereas blood with adenosine plus lidoflazine still produces a coronary vasodilating response which is greater than that produced by blood with adenosine at 10 minutes of incubation. In all tests, blood with adenosine plus lidoflazine showed coronary vasodilating activity for longer time of incubation than blood with adenosine only; thus indicating an adenosine-sparing effect of lidoflazine in whole blood. All the blood mixtures ultimately became ineffective.

Discussion

Results of experiments described in this study show that lidoflazine has a powerful enhancing effect on the coronary vasodilatation induced by adenosine or ATP. Although chemically unrelated to dipyridamole, it shares with dipyridamole this common property (2). The enhancement obtained with lidoflazine was greater and longer lasting than that with dipyridamole, but the doses of lidoflazine used are also larger. The coronary vasodilating effects of a combination of lidoflazine and adenosine can be classified as supra-additive, as in the case of dipyridamole. Coronary and systemic hemodynamic changes after lidoflazine alone indicate that after its administration coronary sinus flow, cardiac rate, and output increased significantly. These changes are basically in accord with the findings of other investigators (4), although the doses of lidoflazine used in the current study are smaller.

The phenomenon of enhancement of adenosine action is important since adenosine compounds and adenosine deaminase are present in living tissues. It has been stated that the potentiation of adenosine-induced coronary vasodilation by dipyridamole might be explained on the basis of biochemical findings that dipyridamole has a deaminase inhibitor activity and prevents the disappearance of
exogenous adenosine in whole blood by reducing the permeability of the red blood cell membrane (5-7). From the biological assay used in the present study it is evident that lidoflazine also exhibits an adenosine-sparing effect in whole blood. It is presently not known whether this effect is related to inhibition of deaminase or a reduction of the red cell permeability to adenosine. Undoubtedly an adenosine-sparing effect would produce an enhancement of adenosine action; however, in the case of lidoflazine, the question arises whether the increase in sensitivity to adenosine observed can be solely attributed to this effect. If this is the case, then after reaching the threshold of effectiveness it would be expected that an increase in the absolute amount of adenosine infused would produce similar increases in the flow before and after lidoflazine. However, this does not seem to occur; examination of the data in Table 2 shows that after reaching the threshold of effectiveness the increases in the absolute amount of adenosine needed to produce further increases in flow are much greater before lidoflazine than those needed to produce similar increases in flow after lidoflazine.

The mechanism of enhancement of adenosine action by lidoflazine deserves further investigation. It may well be that lidoflazine also produces a change in the sensitivity of the effector cell (vascular smooth muscle) to the direct or indirect action of adenosine.

References
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