Effects of Persantin (RA8), A New Coronary Vasodilator, on Coronary Blood Flow and Cardiac Dynamics in the Dog

By James W. West, Ph.D., Samuel Bellet, M.D., Ugo C. Manzoli, M.D., and Otto F. Müller, M.D.

In 1951, Fischer and Roch made available an entirely new class of compounds which were basically a double-ring structure consisting of two condensed pyrimidine rings. Immediately following the introduction of this group of compounds, numerous derivatives from this pyrimido-(5, 4-d)-pyrimidine series were synthesized, and certain ones were demonstrated to possess interesting properties worthy of clinical evaluation. One of these compounds, Persantin (RA8) (fig. 1), was shown by Kadatz to have significant effects on coronary blood flow. Since this time, much interest has been stimulated by various investigators in evaluating the ability of this compound to improve coronary blood flow.

In experiments performed on normal dogs, Persantin increased the coronary blood flow as much as 123 per cent as determined by the bubble flowmeter, 90 per cent with the Thermostromuhr of Rein, and 35 per cent with the nitrous-oxide method of Kety and Schmidt. Bretschneider et al. using the Stromuhr catheter of Kanzow, reported flow increases up to 400 per cent with a 200 per cent increase persisting for 10 minutes and 100 per cent increases for several hours. In these experiments, papaverine was only half as effective and its duration of action only one-fifth that of Persantin. Theophylline was found to be the least effective agent; the coronary blood flow increased by only 25 per cent within one minute. The oxygen saturation in the coronary sinus blood increased on an average of 65 per cent during the effects of Persantin. A fall in mean arterial blood pressure was not observed when coronary optimal doses of Persantin (0.2 mg./Kg., intravenously) were administered. Higher doses were required to affect the peripheral circulation and produce a fall in blood pressure.

Various studies have also shown that Persantin does not change right atrial pressures and that myocardial oxygen consumption remains constant.

Persantin apparently has a specific metabolic action on the heart; it increases the ATP concentration of the myocardium which is especially pronounced in the presence of hypoxia. Lactic acid oxidation is slightly increased.

The above findings seemed important enough to justify further experimental investigations with this drug. Our investigation consisted of a study of the effects of Persantin on the coronary blood flow in normal dogs and in a smaller group of dogs with coronary insufficiency produced by various methods described below. The latter series comprises dogs with chronic insufficiency caused by narrowed main branches of the left coronary artery (three dogs) and myocardial infarction produced by ligation of the anterior descending branch of the left coronary artery (four dogs).

Methods

Adult mongrel dogs, weighing 20 to 30 Kg., were lightly anesthetized by intravenous injection of morphine (3 mg./Kg.), followed by intravenous injection of a combination of equal parts of pentobarbital-sodium veterinary solution (60 mg./ml.) and Dial-urethane solution (100 and
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Structural formula of Persantin (BAS) 2, 6-Bis-(diuethanolamino)-4, 8 dipiperidino-pyrimide (5, 1-d) pyrimidine.

400 mg./ml, respectively), the dosage being 0.25 ml. of the mixture per kilogram.6

The dogs with myocardial impairment were studied during various stages of the development of their altered physiological state. These consisted of two groups of dogs: (1) those at various periods following ligation of the anterior descending branch of the left coronary artery which corresponded with the acute, subacute, and healed stages of myocardial infarction; (2) those with varying degrees of coronary artery narrowing produced by placing casein rings around the circumflex and/or anterior descending branch of the left coronary artery. The casein swells and within a period of two to three weeks the lumen is considerably narrowed and in some instances almost entirely obliterated.7

Systemic blood pressure was recorded from the femoral artery, using a Statham transducer manometer and a direct-writing Sanborn polyvisos recorder. The electrocardiogram was continuously monitored on the same recorder, using a precordial lead (V2) and limb lead (III). Coronary artery blood flow was determined as outflow from the coronary sinus and quantitative measurements were obtained by timing a measured blood volume. This was also recorded on the Sanborn polyvisos recorder by the use of a Shipley-Wilson rotameter.

A femoral vein was utilized for returning the blood to the circulation by means of a Dale-Schuster or Sigma motor pump. Catherization of the coronary sinus was accomplished in the intact animal with a special coronary sinus catheter (modified Morawitz cannula) inserted via the external jugular vein under fluoroscopic guidance. The catheter was provided with multiple openings at the tip and an inflatable balloon for securing it in the coronary sinus.8 Catherization of the main left branches of the coronary arteries (anterior descending and left circumflex) was also accomplished under fluoroscopic guidance.9 The main pulmonary artery was catheterized to obtain mixed venous blood for the estimation of cardiac output by the direct Fick method. Arterial blood samples were collected through a femoral artery catheter. Coronary sinus venous blood samples were collected by means of a small polyethylene catheter inserted in the main coronary sinus catheter. Mannuronate (10 mg./Kg) was used as an anticoagulant. The trachea was intubated for collection of the expired air by a Tissot spirometer. Expired air samples were collected from the Tissot spirometer into a tonometer for analysis of oxygen and carbon dioxide by the technique of Scholander. Oxygen uptake was calculated for periods of 5 minutes by multiplying the pulmonary ventilation (from the spirometer reading, reduced to standard conditions) by the difference between the oxygen contents of inspired and expired air. Analyses of the blood samples for oxygen and carbon dioxide were made by the manometric method of Van Slyke and Neill.10

The standard experimental procedure began with control measurements of coronary blood flow until blood flow was stabilized; this was followed by a three-minute period for the estimation of the cardiac output. Persantin was then administered intravenously or intra-arterially (coronary artery), and measurements were again obtained.

In other preparations, myocardial contractility was measured by means of a Walton strain gauge sutured directly to the exposed surface of the ventricle (left) in the area supplied by the coronary artery (left anterior descending) into which injections of Persantin were made. The amplitude of the strain-gauge record is proportional to the force of contraction.11 12 The myocardiogram was recorded on a multichannel, Sanborn polyvisos recorder.

The chest was intact in all experiments, except those involving the Walton myocardiograph. In the latter experiments, the trachea was cannulated and the lungs were inflated by the use of a Starling Ideal Pump.

CALCULATIONS

The following were calculated from the obtained data: coronary blood flow; cardiac oxygen consumption (coronary flow and coronary arteriovenous oxygen difference); cardiac output (ml./min.); cardiac work (Kg./min.) from mean blood pressure and cardiac output; total peripheral resistance (T.P.R.) (absolute units) according to Poiseuille's law by the formula T.P.R. = mean arterial pressure (mm. Hg) X 1332 / cardiac output/second; coronary vascular resistance expressed as mean arterial blood pressure + coronary flow; coronary oxygen content (volumes per cent); and myocardial efficiency estimated from the energy equivalent of the observed cardiac oxygen consumption, the actual cardiac work, and the approximate mean weight of the left ventricle.
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Following each experiment, the animal was sacrificed, the entire heart was weighed, and the left ventricle was then excised and weighed separately.13

Results

NORMAL DOGS

Since Persantin is not a water-soluble compound but is supplied for injection in a special vehicle, we embarked upon the investigation of this drug by first determining the effect of the solvent alone on the various circulatory parameters. The solvent consists of 4.0 mg. of tartaric acid (NFX), 100 mg. of polyethylene glycol 600 (highest purity), and 2 ml. of distilled water. Following this, we determined the maximal effective dose range of the drug (drug + solvent preparation). Each dog received at different times Persantin in solvent preparation, drug, solvent, and saline. In this way a comparison was made of the drug preparation (in solvent) with the drug solvent alone and with saline alone. The intravenous dosage range of the drug preparation was evaluated from 1.0 /µg./Kg. to 1.0 mg./Kg. Equivalent volumes of the drug solvent and saline were compared in each case. It was found that the optimal dose range of the drug preparation was between 200 and 500 /µg./Kg. In general, the optimal dose was approximately 200 /µg./Kg. (intravenously). However, at this dose of the drug the equivalent solvent alone produced essentially no effect. Therefore, one can attribute the effects produced on the coronary blood flow primarily to the drug itself. In general, doses of the drug under 50.0 /µg./Kg. were ineffective.

Intracoronary doses of the drug (1.0 to 200 /µg./Kg.) were also evaluated. It was found that 1.0 to 2.0 /µg./Kg. were effective in producing a definite increase in coronary blood flow and coronary venous oxygen saturation without producing changes in the systemic circulation, while equivalent volumes of solvent produced essentially no effect on these determinants. However, higher doses above 100 /µg./Kg. at times produced cardiac arrhythmias which made it difficult to evaluate the change in coronary blood flow.

In the following data, the optimal intravenous dose employed was 200 /µg./Kg., although intracoronary injections of 2.0, 25, and 100 /µg./Kg. were given. At this intravenous dose range, Persantin produced a marked sustained effect on the coronary circulation lasting at least 20 to 30 minutes, with the peak effect being reached within 3 minutes. By intracoronary injection, the effect of the drug was only momentary, lasting less than 5 minutes unless the infused dose was similar to that which was effective by the intravenous route.

Coronary Blood Flow

Table 1, (column 1) shows the average values of coronary blood flow obtained before and after Persantin in the series of 14 normal dogs. Following intravenous injection of Persantin (200 /µg./Kg.), coronary blood flow increased on an average of 159 per cent over the control value. A graphic representation of the typical responses of this drug on coronary blood flow is illustrated in figures 2 and 3.
TABLE 1

Effect of Persantin in Normals and Dogs with Coronary Insufficiency (Mean Values)

<table>
<thead>
<tr>
<th></th>
<th>Normal dogs</th>
<th>A. Dogs with rings</th>
<th>B. Dogs with ligation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Persantin</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>Increase</td>
<td>Decrease</td>
</tr>
<tr>
<td>Coronary blood flow (ml./min.)</td>
<td>80</td>
<td>207</td>
<td>159</td>
</tr>
<tr>
<td>Cardiac O\textsubscript{2} consumption (ml./min.)</td>
<td>9.57</td>
<td>9.21</td>
<td>4</td>
</tr>
<tr>
<td>Cardiac work (Rg.-M./min.)</td>
<td>4.26</td>
<td>3.61</td>
<td>15</td>
</tr>
<tr>
<td>Cardiac output (ml./min.)</td>
<td>2489</td>
<td>2587</td>
<td>4</td>
</tr>
<tr>
<td>M.A.B.P. (mm. Hg)</td>
<td>120</td>
<td>102</td>
<td>15</td>
</tr>
<tr>
<td>Cardiac rate (beats/min.)</td>
<td>154</td>
<td>143</td>
<td>7</td>
</tr>
<tr>
<td>T.P.R. (A.U.)</td>
<td>4251</td>
<td>3199</td>
<td>25</td>
</tr>
<tr>
<td>Coronary resistance (P-F)</td>
<td>1.60</td>
<td>0.62</td>
<td>64</td>
</tr>
<tr>
<td>Coronary (A-V) O\textsubscript{2} (vol. %)</td>
<td>12.75</td>
<td>5.23</td>
<td>57</td>
</tr>
<tr>
<td>Coronary O\textsubscript{2} utilization (%)</td>
<td>78.4</td>
<td>35.7</td>
<td>55</td>
</tr>
<tr>
<td>Cardiac efficiency (%)</td>
<td>19.9</td>
<td>19.3</td>
<td>7</td>
</tr>
</tbody>
</table>

*Not determined in a sufficient number to be considered significant.

Coronary Arteriovenous Oxygen

The local vasodilating effect of Persantin is well demonstrated by its ability to markedly lower coronary arterial oxygen content, or saturation with essentially little change or saturation in the coronary venous oxygen content. This results in a narrowing of the coronary arteriovenous oxygen difference, indicating decreased oxygen extraction from the arterial system. Immediately following an intracoronary injection of Persantin, there is a marked decrease in coronary flow. In contrast, cardiac output and cardiac work are only slightly affected by the intracoronary injection of Persantin.

Coronary Resistanee

Persantin also markedly increased coronary blood flow. In doses of up to 100 mg./Kg., there was no effect on heart rate or blood pressure. However, doses of 100 mg./Kg., or higher, also produced circulatory effects, as illustrated in figure 4.

Intra coronary arterial injections of Persantin also markedly increased coronary blood flow. In doses below 100 mg./Kg., there was essentially no effect on heart rate or blood pressure. However, doses of 100 mg./Kg., or higher, also produced circulatory effects, as illustrated in figure 4.
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Effects of Persantin injected into the anterior descending branch of the left coronary artery. Note the instantaneous increase in coronary blood flow in the presence of a fall in blood pressure and a rise in heart rate. A simultaneous rise in coronary sinus venous oxygen content was also observed.

Myocardial Contractility

Intravenous administration of Persantin in three dogs, employing four to six injections of the drug and a comparable number of solvent administrations in each dog, produced no effect on myocardial contractility (figs. 5 and 6). To evaluate better the local action of the drug on the heart's force of contraction, intracoronary arterial injections were also employed in these same three dogs. Approximately 10 to 15 injections (drug and solvent alone) were made in each of the dogs, following which there was either a slight decrease or no change in contractility. To demonstrate that the site of injection and the Walton strain gauge were not at fault, a comparison was made with local injections into the same site with a known cardiac stimulant (epinephrine) and a depressant (sodium pentobarbital). The characteristic responses of the drug are illustrated in figures 7 and 8.

Related Circulatory Dynamics

Intravenous injection of Persantin produced minimal increases in myocardial oxygen consumption and the cardiac output, while at the same time they slightly decreased the cardiac work, mean arterial blood pressure, heart rate, and cardiac efficiency (table 1). Total peripheral resistance was moderately decreased, while coronary oxygen utilization was decreased markedly. Of all of these parameters, the heart rate was the most variable; however, the overall average response was a slight decrease in rate.

To test the significance of these results, the t-test was utilized. In table 2, the significant changes were an increase in coronary blood flow and decreases in mean arterial pressure.
blood pressure, total peripheral resistance, coronary resistance, coronary arteriovenous oxygen difference, and coronary oxygen utilization. Cardiac oxygen consumption, cardiac work, cardiac output, cardiac rate, and cardiac efficiency were not changed significantly.

The electrocardiograms following intravenous (200 μg./Kg.) injections of Persantin were essentially unchanged with the exception of the alteration in heart rate.

In two experiments, the electrolyte patterns (Na, K, Mg, Cl) of the arterial and coronary venous blood were determined before and after intracoronary and intravenous injections of Persantin. The changes observed following Persantin were minimal, with no consistent change in pattern, except that sodium (in the coronary venous blood) appeared to show a consistent tendency to decrease slightly.
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DOGS WITH CHRONIC CORONARY INSUFFICIENCY: DECREASED LUMEN OF THE MAIN BRANCHES OF THE LEFT CORONARY ARTERY PRODUCED BY CASEIN RINGS

Coronary Blood Flow

In table 1, (column 2) the values for coronary blood flow on three dogs with narrowed coronary arteries (produced by casein rings) are illustrated. In this group of animals the control flow is less than the control flow observed in the normal animals, since the lumen of the coronary vessels has been markedly narrowed. Nevertheless, intravenous injections of Persantin still increased at least by 75 per cent the coronary blood flow in these animals. However, the magnitude of the flow increase was considerably less than that observed in the normal animals. A 'graphic' representation of the typical type of response of the drug on coronary blood flow is demonstrated in figure 9.

Coronary Resistance

The control resistance in these animals was considerably elevated over the resistance demonstrated in the normal animals. This resulted from the obstructive casein rings placed around the left anterior descending and circumflex branches. In spite of the reduced lumen of the main arteries, the coronary resistance was observed to decrease following Persantin (table 1).

Coronary Arteriovenous Oxygen Difference

A decrease in coronary arteriovenous oxygen difference was also observed in these animals following injections of Persantin (table 1, column 2). However, the degree of narrowing of the coronary (A-V) \( O_2 \) was less than that observed in the normal animals, because the coronary venous oxygen content and saturation could not increase as greatly as was possible in the normal dogs.

Related Circulatory Dynamics

Slight increases in cardiac oxygen consumption as well as minimal increases in cardiac output and cardiac rate were observed, while cardiac work, mean arterial blood pressure, and cardiac efficiency decreased slightly (table 1, column 2). Coronary oxygen utilization and total peripheral resistance also decreased, but to a lesser extent than in the

<table>
<thead>
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<th>Table 2</th>
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<td><strong>Effect of Persantin on Coronary Blood Flow and Cardiac Dynamics in Normal Dogs</strong></td>
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Circulation Research, Volume X, January 1965
FIGURE 9

Effects of intravenous injection of Persantin in the presence of coronary artery (left anterior descending and circumflex) narrowing. Note that coronary blood flow increases but the effect is less than in the normal dog or in the dog with coronary artery ligation. Blood pressure decreased while heart rate increased.

FIGURE 10

Effects of intravenous injection of Persantin in the presence of coronary artery (left anterior descending) ligation and healed myocardial infarction. There is an immediate sustained rise in coronary blood flow which resembles that seen in the normal dog, but somewhat less marked (see text). Blood pressure was noted to decrease slightly while heart rate increased slightly.

normal dogs. As seen in the normal dogs, the heart rate was the most variable parameter in this group of dogs.

DOGS WITH MYOCARDIAL INFARCTION: CORONARY ARTERY LIGATION

Coronary Blood Flow

The control coronary blood flow in this group of animals was found to be lower than the flow in the normal dogs, but not as low as in the dogs with coronary insufficiency (narrowed arteries). Following Persantin, coronary blood flow increased markedly (165 per cent). This increase in flow was greater than that observed in the dogs with coronary insufficiency and equal to that obtained in the normal dogs (figure 10). However, the total flow was considerably below that observed in the normal control animals. A typical response is illustrated in figure 10.

Coronary Resistance

In this group of dogs, the coronary resistance was found to be greater than in the normal dogs, but not as high as in the dogs with coronary narrowing. However, Persantin decreased the resistance in this group of animals more than in the dogs with coronary narrowing. The magnitude of the change was within the range of the response seen in the normal dogs (table 1, column 3).

Coronary Arteriovenous Oxygen Difference

Persantin decreased markedly the coronary arteriovenous oxygen difference in this group of animals by increasing the coronary venous oxygen content and saturation. The degree of response was greater than in the dogs with coronary narrowing and equal to that observed in the normals (table 1, column 3).

Related Circulatory Dynamics

Persantin produced minimal increases in cardiac oxygen consumption, cardiac work, and cardiac output, while at the same time it decreased slightly mean arterial blood pressure, cardiac rate, and cardiac efficiency. Total peripheral resistance was decreased moderately, while coronary oxygen utilization was markedly decreased. Heart rate was the most variable of the parameters measured.

Discussion

Coronary vasodilators may be divided into two main groups: (1) those which increase the coronary blood flow but at the same time produce a stimulating effect upon the heart muscle and increase cardiac work (epineph-
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rime and other sympathomimetic amines, nicotine, and aminophylline), and (2) those which tend to increase coronary blood flow without a significant increase in cardiac work. The latter is accomplished in the presence of a decrease in coronary vascular resistance. To this group belong the nitrites. There is considerable evidence, chiefly in the experimental animal, that the nitrites, mainly nitroglycerin, increase coronary blood flow.\textsuperscript{9} \textsuperscript{14-17} Recent studies have confirmed these observations in the normal human subject.\textsuperscript{18} However, in subjects with coronary artery disease, coronary blood flow and myocardial oxygen consumption were only slightly increased following nitroglycerin; the coronary resistance remained essentially fixed, and coronary blood flow and myocardial consumption remained either unchanged or actually decreased.\textsuperscript{19}

It would appear from the data obtained above that Persantin might also belong to the second group, namely, that in which there is an increase in coronary blood flow without an increase in cardiac contractility or cardiac work. The maximum dose to accomplish this effect was 200 $\mu$g./Kg., intravenously. It is of considerable interest that this increase in coronary flow occurred not only in the normal animal but also in the animal with coronary insufficiency, which often was of a severe grade.

The mode of action of this preparation in increasing coronary blood flow has not been entirely elucidated. There is some suggestive evidence that it manifests a direct effect in producing vascular dilatation. This effect is more pronounced in certain vascular depots, especially the coronary arteries. There is some evidence that it manifests an effect on nucleoside metabolism of the heart muscle,\textsuperscript{5} and, in addition, an increase in ATP in the myocardium has been observed in the presence of hypoxia.\textsuperscript{2}

In view of these findings in the experimental animal, the use of Persantin merits a more thorough investigation in the human subject. The dose which would result in the optimum increase in coronary blood flow and the blood level required to accomplish this end are being investigated at the present time.

Summary

The effect of a new coronary vasodilator (Persantin), which produces a marked increase in coronary blood flow (up to 159 per cent in normal animals) without increasing cardiac contractility or cardiac work, has been studied in the experimental animal. This is associated with a marked decrease in coronary resistance. There is an increase in the coronary venous oxygen content and a decrease in the coronary A-V oxygen difference; the cardiac output is only slightly affected. Increase in the coronary blood flow has also been observed in dogs in which coronary insufficiency has been produced by coronary ligation and by narrowing of the lumen of the coronary artery by the application of casein rings. This was accompanied by no significant increase in cardiac work.

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