A double-blind, placebo-controlled study of ketanserin in patients with Prinzmetal’s angina

Evidence against a role for serotonin in the genesis of coronary vasospasm

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ABSTRACT This study was designed to test the hypothesis of a possible role of serotonin in the pathogenesis of myocardial ischemia in patients with pure vasospastic angina, since serotonin is known to cause contraction in isolated coronary arteries. This effect, as well as serotonin-induced platelet aggregation, is reversed by ketanserin, a specific S2-receptor blocker. Five male patients (49 to 68 years old) with more than six episodes/day of myocardial ischemia at rest as characterized by ST segment elevation on the electrocardiogram (ECG) were selected for the study after a 2 day run-in period of continuous ECG Holter monitoring in the absence of any therapy except that with sublingual nitrates. In a double-blind crossover protocol they received consecutive infusions of 6 hr each of ketanserin (2 mg/hr iv, preceded by a 10 mg bolus in three patients) and placebo in the following sequence: ketanserin-placebo-ketanserin-placebo in the first and placebo-ketanserin-placebo-ketanserin in the second 24 hr period. The efficacy of the infused drug was tested by exposing platelet-rich plasma, obtained from the study patients at a fixed morning time before and during ketanserin infusions, to a series of serotonin concentrations from $10^{-5}$ to $10^{-8}$M in a conventional aggregometer. A complete suppression of aggregation curves in the range of serotonin concentrations tested resulted during administration of ketanserin. The efficacy of the drug in preventing ischemic episodes was assessed by computing the ischemic episodes (recorded by Holter monitoring) and nitroglycerin consumption in each 6 hr ketanserin period and in the corresponding placebo period. A total of 171 ischemic episodes were recorded, 33 of which (19%) were symptomatic. Total numbers of ischemic episodes were 94 during ketanserin and 77 during placebo. No consistent differences in numbers of ischemic episodes and nitroglycerin consumption were observed in the comparison of the corresponding ketanserin and placebo 6 hr periods. Also, severity, as assessed both by nitroglycerin consumption and ST segment elevation, was not affected by the drug; one patient required nitrate infusion after the first day of the trial owing to a worsening of symptoms. On the basis of the results of this trial it appears unlikely that serotonin can play an important role in vasospastic angina.


A PRIMARY REDUCTION in coronary flow due to spasm of a large subepicardial coronary artery is currently considered the main pathophysiologic mechanism of Prinzmetal’s variant angina. The role of coronary artery spasm in provoking myocardial ischemia is not limited to this rather small subset of angina patients, although in variant angina coronary spasm is more easily angiographically demonstrable.

Different causes for coronary spasm have been postulated, including hyperactivity of the peripheral nervous system, segmental vascular hypersensitivity to humoral factors, activation of platelets at sites of atherosclerotic plaque or endothelial lesions, and release of either newly formed substances, such as thromboxane $A_2$, or of preformed vasoconstrictor substances, such as norepinephrine and serotonin. Serotonin (5-hydroxy-tryptamine) may contract isolated coronary arteries in vitro; human coronary arteries may undergo contraction by serotonin and the supersensitivity of atherosclerotic arteries to ergonovine, a substance widely used in provoking vasospastic angina, has been demonstrated to be partially mediated by interaction with serotonin receptors. Actions of
serotonin on blood vessels in vivo also imply indirect mechanisms, most of them commonly referred to as "amplifying effects." Among other actions serotonin amplifies blood pressure increases induced by epinephrine⁶⁷ and the vasoconstriction induced by norepinephrine.¹⁴⁻¹⁶

Despite these findings, data are still lacking on the real role of serotonin, possibly from platelets, in vasospastic angina. We therefore decided to verify the working hypothesis of a role of serotonin in the vasospasm that occurs in Prinzmetal's angina using a pure antagonistic and selective serotonin vascular receptor blocker, ketanserin (K).

Methods

Patients. Five consecutive male normotensive patients from 49 to 68 years old, who were admitted to our Coronary Care Unit (CCU) because of very frequent episodes of chest pain at rest, were selected for the study after at least 2 days of continuous electrocardiographic (ECG) monitoring (visual and Holter monitoring). In all the ECG demonstrated the presence of more than six episodes/day of myocardial ischemia characterized by transient ST segment elevation of 0.1 mV or more with or without chest pain. None of the patients were on antianginal therapy with the exception of sublingual nitrates and in none was there any evidence of prior myocardial infarction. Their clinical and angiographic characteristics are outlined in table 1. All patients gave informed consent to the study.

Protocol. K (R41 468, from Janssen Pharmaceuticals, Beerse, Belgium) was available in vials of 10 mg in 2 ml of saline (5 mg/ml). Always starting at 12 a.m., patients received infusions of K and placebo (P) via a continuous infusion pump set (Valleylab IV 5000 b, Boulder, CO) precalibrated at 20 ml/hr for consecutive periods of 6 hr each for a total period of 48 hr. Infusions were given according to the following sequence: K-P-K-P for the first and P-K-P-K for the second 24 hr period. This sequence was chosen to cover, once with the drug and once with P, identical periods over 2 consecutive days and to avoid the bias of daily spontaneous variability in the incidence of ischemic attacks. K was dissolved in 500 ml of saline and the bottle was protected from light with black paper. Placebo (saline) was prepared to have the same external appearance: only a code identified the bottles, and CCU personnel were instructed to change bottles in the infusion set, when required, according to the codified sequence. In addition to K or P infusions, the last three patients received a bolus (10 mg of K in 10 ml of normal saline or an identical amount of placebo) at the beginning of each infusion period. The patients' ECGs were continuously visually monitored with CCU surveillance systems and there was Holter monitoring of the two leads showing the most obvious ischemic alterations (ST segment elevation of more than 2 mm).

Blood pressure was taken every 2 hr by cuff manometer; patients were asked to report any symptoms and nitroglycerin consumption was determined from CCU reports.

Control of the efficacy of the drug. Platelet aggregation studies were performed at the same hour (9 A.M.) on 2 consecutive days in order to process platelets exposed to P and K (fourth and eighth infusion periods, respectively). From fasting patients 9 ml of venous blood was drawn in a 10 ml polypropylene syringe filled with 1 ml sodium citrate (3.8% pH 7.4). Platelet-rich plasma (PRP) was obtained by spinning blood at 150 g for 10 min. The platelet count was then adjusted to 280,000 ± 320,000 elements/ml by diluting PRP with autologous platelet-poor plasma obtained by additional spinning at 1000 g for 10 min. PRP was then exposed, in a conventional aggregometer at 37°C and with constant stirring at 1100 rpm, to a series of serotonin (5-hydroxytryptamine creatinine sulfate, Sigma Chemical, St. Louis, MO) concentrations ranging from 10⁻¹² to 10⁻⁴ M (final concentrations) and the maximum decreases in optical density during K and P infusions were compared.

Detection and analysis of ischemic episodes. The effectiveness of the drug in preventing ischemic episodes was analyzed by (1) assessment of the number of episodes of ST segment elevation as detected during Holter monitoring and (2) computation of nitroglycerin consumption, a subjective index of severity of ischemia.

Holter tapes were analyzed with the use of low-speed paper recordings of the whole period of the trial and subsequent high-speed paper recordings of all the regions showing changes from the baseline, according to a technique previously developed in our laboratory.¹⁷ Analysis of Holter tapes was performed in a blinded fashion; the investigator did not know the actual sequence of drug and P administration.

According to CCU reports ischemic episodes were also classified as symptomatic or asymptomatic. Episodes of ST segment displacement of more than 0.1 mV and lasting more than 1 min were considered. Based on the findings in the run-in period, patients had episodes of ST segment elevation only. Duration of displacement and maximum

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Angina</th>
<th>ECG during attack</th>
<th>Exercise stress test</th>
<th>Coronary arteriographic findings¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68</td>
<td>At rest + exertional</td>
<td>ST elevation in II, III, aVF</td>
<td>+</td>
<td>1 (RCA)</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>At rest</td>
<td>ST elevation in V₄–V₅</td>
<td>+</td>
<td>1 (LAD) + spasm LAD</td>
</tr>
<tr>
<td>3</td>
<td>58</td>
<td>At rest</td>
<td>ST elevation in II, III, aVF</td>
<td>–</td>
<td>2 (RCA + LCA) + spasm RCA</td>
</tr>
<tr>
<td>4</td>
<td>61</td>
<td>At rest</td>
<td>ST elevation in V₄–V₅</td>
<td>–</td>
<td>1 (LAD) + spasm LAD</td>
</tr>
<tr>
<td>5</td>
<td>67</td>
<td>At rest</td>
<td>ST elevation in V₄–V₅</td>
<td>–</td>
<td>2 (LAD + LCA)</td>
</tr>
</tbody>
</table>

LAD = left anterior descending coronary artery; RCA = right coronary artery; LCA = left circumflex coronary artery.

¹Number and site of diseased vessels; the finding of coronary artery spasm, when it occurred, is also reported.
displacement of ST segment were also computed and heart rate was determined every 30 min. For assessment of maximum displacement of the ST segment during episodes, the following arbitrary scoring system was used: 1, episodes of from 0.1 to 0.2 mV; 2, episodes of from 0.2 to 0.3 mV; 3, episodes of 0.3 mV or more.

Statistical analysis. Comparisons of parameter values during corresponding periods of K and P administration were performed according to the following statistical methods: for number of ischemic episodes (total, symptomatic, asymptomatic) and nitroglycerin consumption — Wilcoxon's signed rank-sum test; for duration and magnitude of ST elevation, systolic and diastolic blood pressures, and heart rate — analysis of variance.

To account for the possible influence of low drug concentration during the initial hours of K infusion in patients not receiving preload with the 10 mg bolus and for the persistence of drug effect in the initial hours of P infusion, an additional statistical analysis was performed to compare the first and the second 3 hr periods of both K and P infusions.

Results

Hemodynamic and side effects of ketanserin. Blood pressure (systolic and diastolic) and heart rate, assessed as previously described and determined without knowledge of the phase of the study were not significantly affected by either K or P. No rebound phenomenon at the discontinuation of the drug was observed. Figure 1 illustrates the behavior over time of these parameters in all five patients.

No side effects of any sort were reported by any of the patients.

Effects of platelet aggregation. A virtually complete (>90%) inhibition of platelet aggregation, over the range of serotonin concentrations tested, resulted during K administration (figure 2).

Effects on ischemic episodes. Frequency of ischemic episodes (total and symptomatic, and asymptomatic by difference) and their mean duration and average maximum ST segment displacement during comparable 6 hr periods on the 2 consecutive days are reported in figure 3 together with nitroglycerin consumption. A total of 171 ischemic episodes were recorded, 33 of which (19%) were symptomatic; 94 episodes were recorded during K and 77 during P administration (NS). Subgroup (symptomatic and asymptomatic) analysis of ischemic episodes also did not reveal significant differences between P and K periods. Furthermore, no significant difference was apparent in any of the other investigated parameters (such as duration and magnitude of ST segment displacement and nitroglycerin consumption) during K and P infusions. There was also no difference found between the effects of the first and the second 3 hr periods of P and K administration, suggesting the absence of any run-in or run-off effect.

During the course of the trial one patient required nitrate infusion at the end of the first day because of a worsening of symptoms.

Discussion

On a theoretical ground platelets have multiple involvement in ischemic heart disease. Their ability to adhere to damaged endothelium or to sites of endothelial detachment has been considered one of the first pathogenic steps leading to development of atherosclerotic plaque,18,19 which is commonly considered to be
part of the usual pattern of development of ischemic heart disease. As far as its involvement in the pathogenesis of clinical manifestations of ischemic heart disease is concerned, plaque may be implicated in the origin of acute thrombotic occlusion of a coronary artery, a frequent finding in acute myocardial infarction, may produce major arrhythmic events including ventricular fibrillation, is commonly quoted as the main mechanism of sudden cardiac death, and may contribute to the pathogenesis of vasospastic angina via the release of either preformed vasoconstrictive constituents such as catecholamines or serotonin, or newly formed substances such as thromboxane A2 or leukotrienes. In this last subset, the investigation of the pathogenic role of various substances has proceeded in two directions: (1) peripheral or transcardiac assays of the substances implicated and (2) the use of specific inhibitors. Although the finding of an increase in either peripheral or coronary sinus levels of any substance raises doubts about the pathogenic mechanism (is it a primary or a secondary increase?), provided that meaningful assays for the substance are available, in our opinion results obtained with the use of specific inhibitors are more sound. For example, despite multiple reports of elevated peripheral and transcardiac levels of thromboxane B2 in patients with angina pectoris, the poor results obtained with the use of aspirin in different doses in the prevention of angina at rest points to a lack of dependence of this type of angina on thromboxane A2 levels. The results of our study with the serotonin-receptor antagonist K have similar implications.

Analysis of the differential binding properties of various 5S-related agonists has suggested the opportunity of distinguishing between two subtypes of serotonin receptors, termed S1 and S2. The former is found in guinea pig ileum and the central nervous system and the latter in the smooth muscle of the vessel wall, bronchial tissue, and blood platelets. Previous studies on the role of serotonin under different conditions had been biased by the fact that there was no pure antagonistic and selective serotonin vascular (S2) receptor blocker. Such a compound is now available in the form of K.

K can reverse coronary contraction induced in vitro by serotonin as well as serotonin-induced (10−5M) platelet aggregation. It also appears to be a selective S2-competitive receptor blocker devoid of central nervous system effects and has been proved useful as an antihypertensive agent in elderly patients. We therefore decided to test this drug in patients with vasospastic angina in an attempt to test the hypothesis of a role for serotonin in treatment of this form of angina.

![Figure 2](image-url)

**FIGURE 2.** Effects of P (top) and K (bottom) on platelet aggregation ex vivo induced by serotonin (10−5M, final concentration). Platelet count in PRP was 270,000/mm3 in the former case, 265,000/mm3 in the latter. Note the total suppression of aggregation during K infusion.

![Figure 3](image-url)

**FIGURE 3.** Effects of P and K infusions on the number of ischemic episodes (total, symptomatic, asymptomatic), nitroglycerin (NTG) consumption, and duration and severity (scored as explained in text) of ischemic episodes in corresponding periods of the trial.
This hypothesis has been offered in the past, based mainly on the following considerations: (1) Platelets appear to be the only circulating elements containing serotonin, which they store inside the so-called dense bodies via an active process.33 (2) Platelets can release serotonin during their “release reaction,”36 which can theoretically occur in diseased coronary vessels.34 (3) Serotonin can cause, by itself, contraction in human coronary arteries30 and can amplify the vasoconstrictive response to catecholamines.13-16

Despite these facts this hypothesis has remained speculative owing to the lack of specific work aimed at addressing the point. Our results present evidence against such a role for serotonin.

Our patient population was very homogenous with respect to type of angina (only episodes of ST segment elevation at rest). All of our subjects suffered from Prinzmetal’s variant angina, which is currently considered to be mainly due to coronary vasospasm,2 and only patients in the “hot” phase of the disease were selected (more than six episodes/day). Owing to spontaneous circadian fluctuation of the episode distribution, comparisons were made between corresponding periods on 2 consecutive days. K proved to be a well-tolerated drug over the short term; patients were practically devoid of side effects and the drug did not evoke any important changes in heart rate or arterial blood pressure.

The high activity of K as an S2-receptor blocker during the infusion periods was proved by the virtually complete suppression of serotonin-induced platelet aggregation. In this context it is interesting to note that while drug concentrations at the time of sampling should be assumed, on the basis of available literature,35 to be on the order of 50 ng/ml (corresponding to 1.2 × 10−7M), the maximal serotonin concentration tested (10−5M) was still not capable of evoking any clear platelet aggregation. This finding suggests a higher receptor affinity of the drug than of the agonist itself. This was confirmed by studies in vitro of the concentration at which K inhibits platelet aggregation by serotonin 50% of the time.*

The large number of ischemic episodes observed in this study allowed reasonable statistical inference. No significant difference in the number, duration or severity of ischemic episodes or in nitroglycerin consumption was apparent between patients on drug and those on P and, more importantly, no trend toward any variation was apparent. One patient required nitrate infusion due to an apparent worsening of symptoms. Subsequently we have analyzed the differences between the first and the second halves of the drug and P infusion periods also did not demonstrate any variation from this pattern.

On the basis of our results we conclude against any practical involvement of serotonin in vasospastic angina.

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