Potentiation of the Vasospastic Response to Angioplasty by Pretreatment With Fluoxetine

A Study in the Atherosclerotic Rabbit

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There is evidence that angioplasty-induced vasospasm is mediated by serotonin (5-hydroxytryptamine [5-HT]) release from platelets. We tested the hypothesis that pretreatment of the atherosclerotic rabbit with fluoxetine, a platelet-uptake inhibitor of 5-HT, would reduce vasospasm after balloon angioplasty. Short-term administration of fluoxetine reduced platelet 5-HT uptake to 4% of baseline. Daily administration of fluoxetine for 7 days reduced whole-blood 5-HT levels to 28% of baseline. Thus, fluoxetine inhibited platelet 5-HT uptake in this model as predicted. Contrary to our expectations and despite the substantial reduction in whole-blood 5-HT levels, pretreatment with fluoxetine for 1 week resulted in augmentation of angioplasty-induced vasospasm in atherosclerotic rabbits. Intrapertoneal administration of fluoxetine produced vasoconstriction in normal rabbits that was augmented by 5-HT and not reversed with LY53857, a specific serotonin receptor antagonist. We postulate that this new observation is probably a result of the inhibition of the clearance mechanism for serotonin, with resultant enhancement of the effect of serotonin released by the activated platelets that are deposited on the vessel wall surface at the time of angioplasty. A direct effect of fluoxetine on serotonergic receptors is a second possible mechanism for the observed effect. (Arteriosclerosis and Thrombosis 1993;13:907-914)

KEY WORDS • angioplasty • vasospasm • serotonin • fluoxetine

Despite the high primary success rate of percutaneous transluminal coronary angioplasty (PTCA) in the treatment of coronary artery disease, acute closure of the treated vessel within the first several hours after successful dilatation occurs in 2-10% of patients.1-5 In addition, restenosis occurs in 30-50% of successfully dilated vessels in the first 6 months after PTCA.6-9

Coronary vasospasm10-12 and mechanical obstruction by an intimal flap13-15 or thrombus16-17 have been implicated in acute vessel closure. Often these processes occur in concert, and the individual effects of each are difficult to separate.1,12 Vasospasm after angioplasty has been identified in both animals16-23 and patients.24 Its contribution to acute vessel closure is suggested by reversal of the acute occlusion in some cases with vasodilators.18,25,26

The mechanism of angioplasty-induced vasospasm is not known, but it may be caused by the release of vasoconstrictors such as serotonin from activated platelets accumulating at the site of the intimal injury that occurs during angioplasty.19,27 Other implicated mechanisms include alterations in vessel wall arachidonate metabolism,28 adrenergic nerve dysfunction,29 loss of endothelium-derived relaxation factor (EDRF),30 and release of endothelin.31-32 We have previously shown in an atherosclerotic rabbit model that pretreatment with serotonin (5-HT) receptor antagonists can prevent angioplasty-induced vasospasm both proximal and distal to the treated site.33 This suggests that serotonin released from activated platelets at the site of balloon angioplasty may be an important mediator of angioplasty-induced vasospasm.

Fluoxetine is a highly effective antidepressant agent33,34 that inhibits active uptake of serotonin into both neural cells35 and platelets.36 This latter effect reduces platelet and whole-blood serotonin levels with short- or long-term administration.37 Endothelial cells also take up serotonin.38-39 The effect of fluoxetine on the uptake of serotonin by endothelial cells is not known.

In this study, we hypothesized that pretreatment with fluoxetine would prevent angioplasty-induced vasospasm by reducing the quantity of serotonin available for release from activated platelets after angioplasty. This study will report the opposite effect, which has not previously been reported, i.e., the potentiation of vasospasm after the administration of fluoxetine.

Methods

Overview of Experimental Design

All studies conformed to the Position of the American Heart Association on Research Animal Use and were.
approved by the Yale University Animal Use and Care Committee. Initially, atherosclerotic rabbits were treated with daily intraperitoneal injections of fluoxetine to determine the effect of the drug on platelet serotonin uptake and whole-blood serotonin levels in our experimental model. Next, the effect of fluoxetine on angioplasty-induced vasospasm was evaluated in atherosclerotic rabbits and was found to augment vasospasm after angioplasty. Finally, to determine the role of serotonin in this process, the short-term effect of fluoxetine administration on serotonin-induced vasoconstriction was determined in normal rabbits. Fluoxetine augmented serotonin-induced vasoconstriction. Ly53857, a specific serotonin receptor antagonist (5HT inhibitor), was then used to attempt to block this effect.

Induction of Focal Femoral Artery Atherosclerosis

Focal femoral artery atherosclerotic lesions were created in 4-5-kg New Zealand White rabbits by a modification of the method of LeVeen et al40 as previously described.41

Briefly, the femoral arteries of anesthetized rabbits were exposed to air dessication injury. The rabbits were then placed on a 2% cholesterol and 6% peanut oil diet (Dyets, Inc.) for 28 days. This protocol consistently resulted in the development of focal atherosclerotic lesions.41

Effect of Fluoxetine on Platelet Serotonin Uptake and Whole-Blood Serotonin Levels in the Atherosclerotic Rabbit

Ten atherosclerotic rabbits were treated daily with either fluoxetine hydrochloride (40 mg, provided by Lilly Research Laboratories, Eli Lilly Co., Indianapolis, Ind.; n=4) or sterile water (Abbott Laboratories; n=6) administered by intraperitoneal injection. Arterial blood was drawn from the central ear artery of anesthetized rabbits before and 1 hour after the first, second, and fifth doses. Blood was also drawn 24 hours after the seventh and final intraperitoneal injection. Serotonin uptake was measured by adding acid citrate dextrose to platelet-rich plasma containing 10^6 platelets to Tyrode's buffer with 0.1 μmol/L [3H]serotonin. Samples were filtered after 1 minute, and the filters were analyzed by liquid scintillation counter. Whole-blood serotonin was assayed by the liquid chromatographic-fluorometric method of Anderson et al42 with N-methylserotonin as an internal standard.

Effect of Fluoxetine on Angioplasty-Induced Spasm

After seven daily intraperitoneal injections with fluoxetine or sterile water (n=7 in each group), 14 atherosclerotic rabbits underwent unilateral femoral artery angioplasty. The angioplasty protocol has been described previously.40 Animals were anesthetized with ketamine and xylazine given intramuscularly and maintained with an 8:1 mixture of ketamine and xylazine administered intravenously. To standardize the position of the animals for subsequent angiographic studies, each rabbit was placed supine in a Perspex brace with the hind legs externally rotated and abducted and the knees fully extended. Through a midline neck incision, the right carotid artery was isolated by blunt dissection. After the vessel was ligated cranially, a 4F introducer was placed through an arteriotomy and advanced to the aortic arch under fluoroscopy. Heparin (250 units) was administered, and a 0.014 USCI Veriflex guide wire was advanced through the introducer to the distal abdominal aorta. A Med-Tech 2.5-mm angioplasty catheter was then advanced over the guide wire to the distal abdominal aorta at the level of the L4-L5 vertebral interspace. The guide wire was removed, and 1 mL of 2% lidocaine was administered into the aorta according to our standard protocol.41 A baseline angiogram was then obtained by hand injection of 8-9 mL of 50% Renograin 76 (diatrizoate meglumine and diatrizoate sodium injection USP, Squibb).

The 0.014-in. guide wire was reintroduced through the angioplasty catheter and placed across one of the femoral artery lesions. The angioplasty catheter was then advanced over the guide wire until the balloon was centered across the area of most severe angiographic stenosis. The 2.5-mm balloon was inflated to 10 atm pressure with a hand indeflator (Advanced Cardiovascular Systems, Inc.) for three 1-minute inflation periods with 1-minute intervals between each inflation. The position of the balloon and diameter of the inflated balloon were confirmed radiographically. At the completion of the dilatation protocol, the angioplasty catheter was withdrawn into the iliaca artery, and 1 mL 2% lidocaine was administered through the catheter into the angioplastied vessel according to our standard protocol. The catheter was then withdrawn to the distal abdominal aorta, and the angiogram was obtained 10 minutes after the final balloon dilatation.

Effect of Fluoxetine on Serotonin-Mediated Vasoconstriction

The short-term effect was tested in normal rabbits with serial angiograms. Five rabbits were anesthetized with ketamine and xylazine. A baseline angiogram was obtained through the angioplasty catheter advanced to the distal abdominal aorta. Ten micrograms of serotonin, a dose previously shown to produce significant vasoconstriction, was given as a bolus injection through the catheter.22 Angiography was repeated 5 minutes later to assess the effect of the infusion on angiographic luminal diameter. Angiography was repeated 45 minutes after administration of the serotonin to confirm that angiographic dimensions had returned to baseline. Then 40 mg fluoxetine was administered by intraperitoneal injection. One hour later (a time that correlated with significant inhibition of platelet uptake after fluoxetine treatment), angiography was repeated. After this angiogram, a second dose of 10 μg serotonin was given, and 5 minutes later a final angiogram was obtained to determine whether serotonin-induced vasoconstriction (see "Results") was affected by prior administration of fluoxetine.

Effect of Serotonin, Receptor Blockage on Fluoxetine-Induced Vasospasm

To determine whether the augmentation by fluoxetine of serotonin-induced vasoconstriction (see "Results") was mediated by 5HT receptors, six rabbits underwent the aforementioned protocol with the following modification. After an angiogram was obtained 1 hour after fluoxetine injection, 20 mg Ly53857, a serotonin (5HT) receptor-blocking agent (provided by Lilly Research Laboratories, Eli Lilly Co.), was injected as a...
bolus through the aortic catheter. Angiography was repeated 5 minutes later. Then the second dose of serotonin was given, and the final angiogram was obtained 5 minutes later to determine whether the effect of fluoxetine on serotonin-induced vasospasm could be blocked by an $S_2$ antagonist. To confirm that LY53857 did block serotonin-induced vasospasm in the absence of fluoxetine (see "Results"), four additional rabbits were injected with 10 µg serotonin after pretreatment with 1 mg LY53857. Angiography was repeated 5 minutes later. After a 45-minute delay to allow washout of the drugs, angiography was repeated to confirm return to baseline angiographic dimensions. Then, to confirm that the vessels reacted normally to serotonin, 10 µg serotonin alone was administered, and angiograms were repeated at 5 and 45 minutes. Finally, to determine whether LY53857 had an independent effect on vessels, 1 mg of the inhibitor was injected, and angiograms were obtained 5 minutes later.

**Histology**

After the postangioplasty angiogram, the animals were pressure perfused at physiological pressures and killed to obtain vessels for histology. The aorta was isolated by blunt dissection through a vertical midline abdominal incision. After the proximal abdominal aorta was ligated, a 4F cannula was inserted above the iliac bifurcation and secured in place by a silk suture. To fix the tissue at its in vivo dimensions, the distal arterial system was then perfused at a pressure of 100 mm Hg with 50 mL saline followed by 100 mL of 3% glutaraldehyde at room temperature. The animal was killed at the time of perfusion with an intravenous injection of 3 mL Nembutal (sodium pentobarbital injection, 65 mg/mL, Anthony Products Co.). A 4-6-cm section of the artery, which included the dilated segment, was removed en bloc and preserved in 3% glutaraldehyde. From this large block, the angioplasty site was identified, and 4-6-µm sections were cut from the proximal to the distal end and color coded to identify their location relative to one another. Hematoxylin and eosin and Richardson's$^{49}$ stains were used for examination by light microscopy.

**Data Analysis**

All angiograms were read by two observers blinded to the rabbits' treatment. Intraluminal diameters were measured at the distal aorta (L5-L6 intervertebral interspace), proximal femoral artery (level of the femoral head), site of the atherosclerotic lesion or angioplasty site or at a comparable level of the femoral artery in normal rabbits, and distal femoral artery (midfemur) with electronic calipers (Brown & Sharpe). The site of angioplasty was defined by clips placed in the soft tissue for groups are presented as mean±SD. Histological sections from treated arteries were examined by a pathologist (L.T.) who was blinded to the rabbits' treatment. Particular attention was directed to the presence of histological evidence of dissection and presence of thrombus.

Serial angiographic dimensions and whole-blood serotonin levels within a group of rabbits were compared by Student's $t$ test for paired observations. Angiographic dimensions and serotonin levels were compared between groups by Student's $t$ test for unpaired observations and Tukey's honestly significant difference test for comparison of multiple means. An analysis of variance with a Bonferroni correction was used when multiple comparisons were made. Comparisons of the incidence of dissection and thrombus were made by Fisher's exact test. A value of $p<0.05$ was considered statistically significant.

**Results**

**Reduction of Platelet Serotonin Uptake and Whole-Blood Serotonin Levels by Fluoxetine**

As illustrated in Figure 1, panel A, there was a 96% reduction in platelet serotonin uptake 1 hour after a single intraperitoneal dose of fluoxetine ($p<0.01$).

There was partial recovery of serotonin uptake 24 hours later, but during the 7-day treatment period, there was a cumulative dosing effect. Twenty-four hours after the seventh daily dose of fluoxetine, serotonin uptake was 0.5±0.3 pmol per 10$^7$ platelets per minute, a 93±3% reduction from pretreatment baseline of 6.8±2.1 pmol per 10$^7$ platelets per minute ($p<0.01$).

Over the 7-day treatment period, in the fluoxetine-treated rabbits, whole-blood serotonin levels declined from 4.9±1.7 to 1.4±1 µg/mL ($p<0.02$), a 72±12% reduction from baseline, as illustrated in Figure 1, panel B. In contrast, there was no reduction in whole-blood serotonin levels in rabbits treated with daily placebo injections of sterile water (4.9±1.9 to 4.7±0.9 µg/mL, $p=$NS).

Whole-blood serotonin levels at 7 days correlated well with platelet serotonin uptake ($r=0.99, p<0.02$), as shown in Figure 1, panel C. Peripheral blood platelet counts were not altered by fluoxetine administration (Figure 2).

**Enhanced Angioplasty-Induced Vasospasm After Fluoxetine Pretreatment**

In control animals treated with injections of sterile water for 1 week before angioplasty, balloon dilatation resulted in an angiographically successful angioplasty, with an increase in luminal diameter at the treated site from 1.29±0.09 to 1.77±0.08 mm ($p<0.005$, Figure 3). This was associated with mild reductions in luminal diameters at adjacent proximal (2.3±0.1 to 1.9±0.2 mm, $p<0.10$) and distal (1.70±0.09 to 1.4±0.1 mm, $p<0.06$) sites, consistent with mild vasospasm. In contrast, animals pretreated for 1 week with fluoxetine had no increase in luminal diameter at the treated site after angioplasty (1.4±0.1 to 1.4±0.2 mm). These animals exhibited severe luminal narrowing in the adjacent proximal (2.5±0.1 to 1.6±0.3 mm, $p<0.04$) and distal (1.81±0.06 to 1.2±0.3 mm, $p<0.04$) sites after angioplasty that was highly significant. Serial angiograms from a representative animal treated with fluoxetine are
Figure 1. Panel A: Bar graph reflecting platelet serotonin uptake (pmol per 10^9 platelets per minute, mean±SD) before (open bars) and after (hatched bars) dosing with fluoxetine. Panel B: Whole-blood serotonin levels (mean±SD) of animals treated with seven daily injections of water (open bars) or fluoxetine (hatched bars). Panel C: Correlation of whole-blood serotonin levels (pg/mL) after seven daily fluoxetine injections with measured platelet serotonin uptake (pmol per 10^9 platelets per minute); r=0.99; p<0.02.

Figure 2. Bar graph showing platelet count in the peripheral blood plotted against time (days) after fluoxetine administration. There was no difference compared with placebo (n=5 in each group). Open bars, placebo; hatched bars, fluoxetine.

Fluoxetine Augmentation of Serotonin-Induced Vasospasm

Multiple comparisons of mean luminal diameters between groups were conducted by constructing the Scheffe's 95% simultaneous confidence intervals. The corresponding significant test consists of examining the confidence intervals for the inclusion of zero in each interval. The mean luminal diameters at baseline (45 minutes after serotonin administration) were significantly different from the mean luminal diameters after fluoxetine administration and also after a repeated bolus of serotonin. Fluoxetine-induced vasoconstriction was augmented by serotonin, but the effect was not statistically significant.

Treatment with 20 mg LY53857 did not prevent vasoconstriction after intra-arterial boluses of 10 μg serotonin in animals pretreated with fluoxetine (1.3±0.2 to 1.1±0.2 mm, p<0.001). In contrast, in four other rabbits not pretreated with fluoxetine, 1 mg LY53857 inhibited the vasoconstrictive effects of 10 μg serotonin (1.6±0.4 before versus 1.5±0.3 mm after treatment, p=NS).

Thus, in the presence of fluoxetine, serotonin-induced vasoconstriction was enhanced and could not be blocked by an S2 antagonist, even at a 20-fold higher dose than was used to block vasoconstriction in the absence of fluoxetine.

Histology

The atherosclerotic lesions produced in this model were focal, with histologically normal segments adjacent to atherosclerotic segments. The affected areas showed marked intimal hyperplasia, with a plaque consisting of fibrous tissue and a cap of myointimal cells. Lipid-laden macrophages were present at the base of the plaque and in the media and adventitia as well. No significant areas of necrosis or calcification could be identified in the
plaque. We have previously published representative examples of the histology of these lesions.41

Intimal dissections were identified after angioplasty in five of the six control vessels examined and in six of the seven vessels examined from fluoxetine-treated animals. Adherent thrombi were identified at the dissection site in five vessels in both groups. In all cases, thrombi covered <5% of the luminal circumference. No thrombi or dissections were identified in the segments proximal or distal to the angioplasty site in either group. None of the thrombi or dissections compromised the lumen of the vessel.

Discussion

The major findings of this study are that 1) short-term administration of fluoxetine inhibits platelet serotonin uptake to 4% of baseline in hypercholesterolemic, atherosclerotic rabbits; 2) daily dosing with intraperitoneal fluoxetine for 1 week reduced whole-blood serotonin levels to 28% of baseline; 3) despite reducing whole-blood serotonin levels, pretreatment with fluoxetine resulted in augmentation of angioplasty-induced vasospasm in this model; and 4) short-term administration of fluoxetine has vasoconstrictive effects that are augmented by serotonin in normal rabbits.

Fluoxetine has been shown to reduce platelet serotonin levels over time by 20–80%.37,44,45 The 72% reduction in whole-blood serotonin levels demonstrated here is consistent with published reports.37 Contrary to our expectations, however, the reduction in whole-blood serotonin levels with fluoxetine was not associated with an attenuation of angioplasty-induced vasospasm. Rather, significant vasospasm occurred both proximal and distal to the treated site. In addition, the angioplasties as a group were not successful angiographically, although there was histological evidence of dissection in most cases. In none of the animals did a thrombus or dissection compromise the vessel lumen. This finding is consistent with the presence of vasospasm at the angioplasty site in the fluoxetine-treated rabbits and would appear to be in conflict with data published earlier from our laboratory when we reported that vasospasm does not occur at the angioplasty site after angioplasty.23 In this study, a balloon matched to the lumen size was used. Histologically, there was less medial damage than reported previously, and thus, the vessel was presumably able to react to the intense vasospastic stimulus that was present in this study.

Possible mechanisms by which fluoxetine could potentiate angioplasty-induced vasospasm include 1) direct action as a vasoconstrictor, 2) potentiation of the effects of serotonin caused by upregulation of the S2 and downregulation of the S1 receptors, and 3) paralysis of the serotonin uptake mechanism with resultant enhancement of the effect of serotonin released when platelets were activated.

We found that a single intraperitoneal dose of fluoxetine caused significant femoral artery vasoconstriction that was enhanced by exogenous serotonin. Unlike many other antidepressant drugs, fluoxetine has little affinity for muscarinic, histaminic H1, or noradrenergic or noradrenergic receptors.46–49 A direct effect of fluoxetine is thus possible. It is important to note that in isolated rat vessels, fluoxetine does not potentiate the vasoconstrictive effects of either norepinephrine or serotonin.50
Angiograms from an atherosclerotic rabbit undergoing angioplasty after pretreatment for 7 days with fluoxetine. Panel A: Baseline angiogram. Top of panel is cephalad. There is focal stenosis of the right femoral artery (arrow). The left femoral artery is occluded, with the distal vessel filling via collateral vessels. Panel B: Angiogram during angioplasty demonstrating the inflated balloon filled with dye in the right femoral artery. Panel C: Angiogram 10 minutes after angioplasty, illustrating diffuse severe vasospasm of the right iliac artery (arrows). The right femoral artery is not visualized. Panel D: Angiogram 25 minutes after angioplasty, illustrating partial resolution of the right iliac artery vasospasm (I) with persistent severe vasospasm of the proximal right femoral artery (small arrows). There is successful dilatation at the site of the previous atherosclerotic lesion (large arrow). Note that moderately severe vasospasm persists in the distal vessel.

In humans, short-45 or long-term51 administration of fluoxetine does not appreciably affect α-adrenergic pressor responses. Although it is possible that vasoconstriction was, in part, a result of a lack of S1 receptor stimulation in the setting of decreased serotonin release from platelets, exogenous serotonin infusion in these rabbits caused vasoconstriction, suggesting that the S2 receptor-mediated effects predominate. Other authors have suggested that atherosclerosis may reduce or abolish serotonin-induced relaxation of vessels, either by altering the S1 receptors or by reducing the ability of the endothelium to produce EDRF.52 In this case, the latter is probably true, because the endothelium is always severely damaged during angioplasty. In addition, some experimental data suggest that the long-term administration of fluoxetine can affect the S1 and S2 receptors by downregulation of the S1 receptors and upregulation of the S2 receptors.53 The net effect, therefore, would be vasoconstriction.

Thus, two mechanisms are likely. First, the augmented serotonin-induced vasoconstriction in fluoxetine-treated rabbits may be caused by the inhibition of a major clearance pathway for serotonin, namely, reuptake into platelets. Other serotonin uptake inhibitors that, like fluoxetine, do not have significant S1 blocking effects have been shown to potentiate the serotonin pressor response in pithed rats, presumably by preventing uptake and clearance of the infused serotonin.53,54 Second, it is possible that fluoxetine exerts a direct effect through modification of both S1 and S2 receptors.55

Limitations of the Model

Hypercholesterolemic rabbits with focal atherosclerotic lesions differ from humans in many important respects. These rabbits have total cholesterol levels often exceeding 1,000 mg/dL. In addition, the atherosclerotic lesions that develop lack fibrosis, necrosis, and calcification, which are characteristic of human atherosclerosis.56 Foam cells are generally more prominent in rabbit lesions, often infiltrating the media as well as the intima.

However, this model has many similarities to human coronary atherosclerosis. Rabbit femoral arteries resemble human coronary arteries in both size and muscular composition.40 Furthermore, the short-term histological effects of angioplasty are very similar to those
was injected intraperitoneally. In patients, fluoxetine is given orally at a dose of 20 mg per day to a maximum of 80 mg per day, which is approximately 1.3 mg/kg. This describes the use of this agent widens, it is possible that some patients may exhibit an increased propensity for developing clinical entities associated with vasospasm. It is, however, important and relevant to emphasize that the dose of fluoxetine used in this study was 40 mg per rabbit, which is about 8–10 mg/kg, and that the drug was injected intraperitoneally. In patients, fluoxetine is given orally at a dose of 20 mg per day to a maximum of 80 mg per day, which is approximately 1.3 mg/kg. This study emphasizes the need for further studies of fluoxetine in humans.

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