

## Serotonin-mediated thrombosis and selective serotonin re-uptake inhibition

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Since the classification of platelet agonists into strong and weak began, controversy has surrounded the role of serotonin in the amplification of the generation of platelet-rich thrombi in arterial disease. Interest in serotonin-mediated platelet thrombosis stems from the detailed investigations of de Clerck and the Janssen group (1, 2). The paper by Galan et al. (3) in this issue of *Thrombosis and Haemostasis* is a systematic investigation of serotonin-mediated mechanisms of platelet aggregation and vasoconstriction reaching into fibrin-rich thrombi. The authors thoroughly assessed the influences *in vitro* of a selective serotonin reuptake inhibitor (SSRI), citalopram, on platelet aggregation and fibrin deposition, demonstrating differentiated effects of low-shear on fibrin deposition and high-shear on platelet-rich thrombus formation.

They emphasise the potency of serotonin on thrombus formation and challenge the notion that serotonin is a weak agonist of platelet-mediated thrombosis. Serotonin potentiated ADP stimulation both under acalcaemic and normocalcaemic conditions. Incubation of the cells with citalopram reduced the response to ADP as well as ADP plus serotonin. Flow cytometric studies showed that this agonist combination increased surface expression of P-selectin, binding of annexin V and factor V/Va release; the first two but not the last were inhibited by prior exposure to citalopram. Thrombin generation was promoted by serotonin but was not inhibited by pre-incubation with citalopram; this suggests a possible serotonin 2A receptor-mediated effect, rather than a serotonin transporter (SERT) effect. Perfusion studies confirmed the importance of high shear conditions upon the platelet aggregatory effects of serotonin in the presence of collagen.

That fibrin deposition is promoted by serotonin at low-shear is interesting and suggests a mechanism whereby thrombolysis is incompletely achieved. The fact that SSRI-pretreatment decreased serotonin-induced thrombus propagation supports previous studies in which dispersion of established thrombi was achieved with serotonin 2A receptor antagonism (4, 5).

Examination of clots confirmed the importance of Ca<sup>2+</sup> for serotonin-mediated fibrin deposition with shortened clotting

time and concomitant increase in clot firmness. These data chime well with clinical data that depressed patients, under SSRI treatment, have lower risk of myocardial infarction than those on other anti-depressants. Inhibition of thromboxane (TXA<sub>2</sub>) production by aspirin did not affect platelet aggregation by serotonin, ADP, or both together. This is interesting because aspirin is ineffective at *in vivo* critical stenoses where serotonin assumes dominance and may be assisted by red cell-derived ADP.

This reinforces human studies in which serotonergic inhibition has demonstrated reduced platelet aggregation when challenged by sub-maximal collagen concentrations (about one third the concentration used in the study under discussion) (6). Extracellular calcium concentration is important when relating experimental data to clinical findings, which the investigators have attended to.

The absence of bleeding time derangement seen with serotonin 2A receptor inhibition, sufficient to inhibit intracoronary thrombosis (clinically and experimentally), may have led investigators to underestimate the effectiveness of these drugs on platelet-rich thrombosis despite the large number of reports affirming this property (4, 5). SSRIs are reported to have a slightly increased risk of upper gastro-intestinal haemorrhage (7), the mechanism of which is obscure but may be due to down-regulation or shedding of glycoprotein GP-Ia (8) which suggests another mechanism whereby SSRIs reduce platelet aggregability under conditions of high shear. SERT may be related to mechanisms involved in GPIIb-IIIa activation (9) and investigators have demonstrated two-way associations between the function of SERT and GPIIb-IIIa (10).

What effects do SSRIs have on serotonin 2A receptors? Chronic SSRI administration to the rodent central nervous system reported decreased serotonin 2A receptor expression by up to 38% (11), although others found a higher number of serotonin 2A receptors in suicidal patients (12). These receptors are down-regulated by almost all anti-depressants and by almost all anti-psychotic drugs as well (13). Neither fluoxetine nor citalopram changed the number of serotonin 2A receptors (14), although their number correlated strongly with suicidal ideation. Sub-

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sequent SSRI treatment did not alter their quantity, which raises the question of whether SSRIs downregulate serotonin 2 receptors (15) or cause desensitisation (16). The only way this should matter under conditions of high shear is by effects on other receptors.

Serotonin levels, as an indicator for mood disorders and for the effect of SSRIs are not reproducible. This is probably because circulating serotonin is avidly taken up by lung capillaries, platelets and the liver; it is likely that effects are strictly local as in the synapse and the developing platelet-rich thrombus. Platelet

serotonin levels in SSRI-treated platelets *in vivo* are decreased but not abolished (17). Whether SERT inhibition would be sufficient to affect platelet aggregation would depend on local levels of liberated serotonin under conditions of high shear, which are vastly excessive even then. Galan et al. (3) demonstrate that serotonin is definitely a major agonist in platelet-rich thrombosis, although the connection between SSRIs and serotonin-2A receptors remains unclear. Further investigation of the effects of SSRIs on interactions between platelet receptors would be worthwhile.

## References

1. de Clerck FF, Janssen PA. Amplification mechanisms in platelet activation and arterial thrombosis. *J Hypertens Suppl* 1990; 8: S87–93.
2. de Clerck F. Effects of serotonin on platelets and blood vessels. *J Cardiovasc Pharmacol* 1991; 17 (Suppl 5): S1–5
3. Galan AM, Lopez-Vilchez I, Diaz-Ricart M, et al. Serotonergic mechanisms enhance platelet-mediated thrombogenicity. *Thromb Haemost* 2009; 102: 511–519.
4. Belcher P, Drake-Holland AJ, Hynd J, et al. Dispersion of coronary artery thrombi by antagonism of platelet serotonin<sub>2</sub> receptor in the dog. *Cardiovasc Res* 1992; 26: 292–296.
5. Belcher P, Drake-Holland AJ, Noble MI. Antagonism of the platelet 5HT<sub>2</sub> receptor in the presence of thrombolysis. *Int J Cardiol* 1994; 43: 11–20.
6. Menys VC. Collagen induced human platelet aggregation: serotonin receptor antagonism retards aggregate growth in vitro. *Cardiovasc Res* 1993; 27: 1916–1919.
7. Opatrny L, Delaney JA, Suissa S. Gastro-intestinal haemorrhage risks of selective serotonin receptor antagonist therapy: a new look. *Br J Clin Pharmacol* 2008; 66: 76–81.
8. Duerschmied D, Canault M, Lievens D, et al. Serotonin stimulated platelet receptor shedding by tumor necrosis factor-alpha-converting enzyme (ADAM17). *J Thromb Haemost* 2009; 7: 1163–1171.
9. Frankhauser P, Baranyai R, Ahrens T, et al. Platelet surface P-selectin expression is highly correlated with serotonin transporter density in human subjects. *Thromb Haemost* 2008; 100: 1201–1203.
10. Carneiro AMD, Cook EH, Murphy DL, et al. Interactions between integrin αIIbβ3 and the serotonin transporter regulate serotonin transport and platelet aggregation in mice and humans. *J Clin Invest* 2008; 118: 1544–1552.
11. Günther L, Liebscher S, Jähkel M, et al. Effects of chronic citalopram treatment on 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors in group and isolation-housed mice. *Eur J Pharmacol* 2008; 593: 49–61.
12. Pandey GN, Pandey SC, Dwivedi Y, et al. Platelet serotonin-2A receptors: a potential biological marker for suicidal behavior. *Am J Psychiatry* 1995; 152: 850–855.
13. Roth BL, Willins DL, Kristansen K, et al. 5-hydroxytryptamine<sub>2</sub>-family receptors (5-hydroxytryptamine<sub>2A</sub>, 5-hydroxytryptamine<sub>2B</sub>, 5-hydroxytryptamine<sub>2C</sub>): where structure meets function. *Pharmacol Ther* 1998; 79: 231–257.
14. Klimek V, Zak-Knapik J, Mackowiak M. Effects of repeated treatment with fluoxetine and citalopram, 5-HT uptake inhibitors, on 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptors in the rat brain. *J Psychiatry Neurosci* 1994; 19: 63–67.
15. Bakish D, Cavazzoni P, Chudzik J, et al. Effects of selective serotonin reuptake inhibitors on platelet serotonin parameters in major depressive disorder. *Biol Psychiatry* 1997; 41: 184–190.
16. Yamauchi M, Miyara T, Matsushima T, et al. Desensitization of 5-HT<sub>2A</sub> receptor function by chronic administration of selective serotonin reuptake inhibitors. *Brain Res* 2006; 1067: 164–169.
17. Menys VC, Smith CC, Lewins P, et al. Platelet 5-hydroxytryptamine is decreased in a preliminary group of depressed patients receiving the 5-hydroxytryptamine re-uptake inhibiting drug fluoxetine. *Clin Sci* 1996; 91: 87–92.