Review Article

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How to Treat and Prevent Arterial Thrombosis with no Increased Bleeding from Accidents, Surgical Perations and other Invasive Procedures

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Abstract

An enquiry into the lack of attention awarded to serotonin antagonism in the treatment of arterial thrombosis revealed that the mode of action of serotonin and its platelet receptor antagonists was upon thrombus growth, and not, as with anti-platelet drugs, upon the initiation of thrombosis at sites of damaged endothelium. This lack of effect could explain why this approach has been considered not to be effective. However under conditions of arterial stenosis in which there is platelet activation by increased shear stress, and during the growth phase of arterial thrombi, serotonin 5HT2A antagonism has been demonstrated to have great potentcy in dispersing thrombotic obstruction to blood flow. This mode of action, the lack of participation of serotonin in haemostasis, and the absence of serotonin in wounds accounts for the proven lack of effect of effect of pure specific 5HT2A antagonists (i.e., not ones with other actions) on operative bleeding and skin bleeding times. This lack of effect on haemostasis solves the dosing problem encountered by other anti-thrombotic drugs, with which drug concentration cannot be controlled with single fixed doses, leading to the association between increased anti-thrombotic efficacy and increased bleeding complications. Thus 5HT2A antagonism appears to be the preferred approach, from the point of view of safety and lack of bleeding risk, to thrombosis therapy in the context of traumatic accidents, surgical operations and invasive procedures such as angioplasty.

Introduction

The importance of control of intra-arterial thombosis is enhanced if this process proves crucial to the developent of atherothrombosis as well as to the terminal stages [1,2]. It is commonly thought that thrombosis and haemostasis are always linked, leading to the concept that anti-thombotic therapy is inevitably associated with excess bleeding, also called 'bleeding risk' [3]. It is true that experience with anticoagulants, which inhibit the action of thrombin, either directly or via the coagulation cascade, and with anti- platelet agents, which inhibit the thromboxane, purine or glycoprotein receptors, has confirmed the bleeding risk with these approaches [4]. At the present time, no alternative approach has been accepted, but in the case of anti-thrombotic treatment of arterial thrombosis, in which thrombin antagonism is ineffective (Belcher et al, 1996) an alternative approach is available, namely inhibition of the serotonin receptor [5-10]. This follows the recommendation of Goto (2004), that drugs which block events upstream of the final common pathway might be better antiplatelet agents than those that merely inhibit platelet aggregation [11].

The anti-serotonin approach

Serotonin (5-hydroxytryptamine, 5HT) is present in high concentration in blood platelets. Incontrast to the thromboxane, purine or glycoprotein mediators serotonin does not influence the formation of haemostatic layers, although it is implicated in shear-induced aggregation and thrombus propagation by positive feedback from the large amount of intra-platelet serotonin. It is important to distinguish between the serotonergic receptor subtypes [12]. 5HT1 receptors respond with endothelium dependent nitric oxide mediated vasodilatation, 5HT2 receptors activate plateles and mediate smooth muscle contraction. Thus in arterial lesions in which vascular smooth muscle is bared, vasoconstriction excacerbates arterial occlusion by thrombus; relief of such vasoconstriction by 5HT2A antagonists is a bonus. However, surprisingly to date, serotonin antagonism has not progressed to clinical application. Platelets are the richest source of serotonin inthe body outside the brain. Platelets acquire serotonin from the plasma by means of the cell membrane serotonin uptake mechanism, and store the serotonin in dense granules. Inhibition of this mechanism by serotonin reuptake inhibitors (SSRIs) causes depletion of platelet serotonin [13].

Serotonin dependence of acute arterial thrombosis and its inhibition by 5HT2A antagonism

Upon platelet activation (especially with high shear) high concentrations of serotonin in the platelets are released from the dense granules, and act upon platelet serotonin 5HT2A receptors to activate more platelets, thus constituting a positive feedback mechanism leading to thrombus growth [14,15]. The serotonin theory supposes that this serotonin mediation is essential for thrombotic occlusion of diseased coronary arteries, owing to the fact that such occlusions are abolished by antagonism of the platelet

5HT2A receptor even when the major stimulus of adrenaline is applied and also in the circumstances where thrombolysis has failed to clear a complete thrombotic occlusion [8,9,16,17].

Examination of patients undergoing angiography has showed that a high plasma serotonin level was significantly associated with coronary artery disease in patients younger than 70. In nearly four years of follow up high serotonin levels were also associated with cardiac events. This association persisted after adjustment for conventional risk factor [18]. Unfortunately, most of the antagonists of the platelet serotonin receptor (the 5HT2A receptor), have encountered problems, not due to the receptor blockade, that have precluded their application to human arterial thrombosis. However, Th001, ArteclereTM, formerly known as ICI170809 or AZ170809, has proved extremely potent as an inhibitor of intracoronary thrombus growth (McAuliffe et al, 1993) and appeared to be safe in a number of trials of toxicity, which are detailed in an Investigator's Brochure. Another important aspect of the McAuliffe results and those of Belcher et al. is that arterial thrombi spontaneously disperse to clear the artery for full blood flow [16,19]. The possibiity that the antagonists have a lytic effect was disproved by experiments in Aberdeen (unpublished). Indeed clearance of thrombus by thrombolytic drugs such as tPA is followed in dogs by re-occlusion, which 5HT2A antognism prevents [17]. These results are compatible with the anti-thrombolytic effect of serotonin [20]. A potent reason for pharmaceutical groups to promote 5HT2A antagonism is the involvement of 5HT2A receptors in promoting thrombosis in diabetes, diabetes now reaching epidemic proportions worldwide with cardiovascular complications the leading reason for morbidity and mortality in htis common disease [21]. Other 5HT2A recptor antagonists have been available in the past that also showed efficacy in acute arterial thrombosis, e.g., ketanserin ritanserin, LU 49938, MDL11,939, and LY53,857, MDL 28,133A, DV-7028, AT-1015, APD791 [9,17, 22-31]. The 5HT2A antagonists presently available are Th001ArteclereTM, a specific platelet receptor antagonist and nefazodone, an antidepressant now taken off the market with non-platelet effects and an unknown effect on thrombosis [32]. At present, sarpogrelate seems to be the only 5HT2A antagonist in clinical application. It is used in a wide variety of cardiovascular diseases associated with thrombosis, but appears not to have been trialed in acute coronary syndromes [33]. Th001ArteclereTM has had considerable exposure in humans, with a clean safety record. Studies of drugs with mixed receptor subtype antagonist activity we find confusing, e.g., SL65.0472 [10].

Why has the serotonin approach not been followed up?

This follows from the different mode of action of serotonin and its antagonists, namely activation by increased shear stress and blockade of positive feedback of serotonin generating thrombus growth. Increased shear stress is the very haemodynamic stimulus that occurs in a stenosed artery, and accounts for thrombus formation in both eccentric and concentric coronary artery lesions; 5HT2A recptors play a major role in highshear rate thrombus formation [34,35]. When searching for possible antiplatelet drugs, pharmaceutical groups test for platelet aggregability (often in citrated platelet rich plasma in which calcium ions have been removed) and find high aggregability to thromboxane and ADP, together with high potentcy of their antagonists, compared to little or no activity using serotonin and its antagonists. They thus incorrectly ignore the role of shear stress within stenoses and the involvement of 5HT2A receptos in that process and assume that serotonin antagonists are ineffective, even though, in McAuliffe's 1993 study, 170809 showed about ten times the potentcy of clopidogrel, the leading P2Y12 purine (ADP) receptor antagonist, in inhibition of growing thrombus *in vivo*.

A second factor that deterred pharmaceutical groups from the serotonin approach is that the first 5HT2 antagonist caused multiple problems due to its non 5HT2A properties. One of these was alpha1 adrenergic antagonism causing a drop in arterial pressure leading to trial in hypertension, a condition in which serotonin has absolutely no role. The other, more serious, was prolongation of the cardiac action potential and QT interval, causing deaths from arrhythmias. Since that time serotonin antagonists have been rigorously investigated, e.g., Th001, ArteclereTM, with the result that there is a clear absence of any cardiac electrophysiological changes attributed to serotonin or Th001ArteclereTM.

Superiority of the serotonin approach in haemostasis

Those performing research on 5HT2 antagonists since the 1980s when ketanserin became available have observed that the haemostatic layer of blood cells bound to finbrinogen is unaffected. All the early work with these compounds involved experimental surgery on animals, and it was noted that, unlike similar experiments with other anti-thrombotic drugs, there was no excess bleeding, and no oozing coagulopathy. This makes these drugs particularly attractive to surgeons wanting to protect their patients from arterial thrombosis in the peri-operative period [35]. So, is it possible that, contrary to received opinion, the anti-thrombotic activity of 5HT2A antagonists can be accompanied by a zero effect on haemostasis. That this can be the case follows from the fact that the anti-thrombotic action only comes into effect during the thrombus growth phase of the occlusive arterial thrombosis process, the lack of participation by serotonin in the haemostatic process and the absence of serotonin in wounds.

Some drugs called 5HT2A receptor antagonists have been claimed to prolong bleeding when cutting off the tails of mice or rats, but these need to be confirmed by correct methodologically controlled skin bleeding time measurements in man as performed by Brittenden et al. [26,31,36,37].

Unpublished evidence that a 5HT2A antagonist has no effect on haemostasis

A study carried out at Aberdeen Royal Infirmary, an MHRI approved report in the public domain investigated indices of haemostasis in 48 patients with stable arterial disease [36]. This stability enabled the investigators to design a statistically paired, randomised cross-over trial of placebo versus Th001ArteclereTM.

Lack of effect on haemostasis was confirmed as follows:

Skin bleedin time and a series of ultegra and flow cytometry tests, namely
Platelet aggregation by Ultegra ASA
Platelet aggregation by Ultegra IIb/IIIa
Flow cytometry, resting fibrinogen
Flow cytometry + 10μM ADP
Flow cytometry + 1μM ADP
Flow cytometry + 1μMADP + 10μM 5HT

No significant differences were found in paired atatistical tests

Non compliance did not occur as judged by plasma Th001ArteclereTM concentration in the treated arm. Another important aspect of this

study was that the dosing used for the Th001ArteclereTM arm (10mg bd for 2 weeks) was considerably higher than the cncentration producing complete arterial clearance in the McAuliffe study. This is important consideration when investigating dosing of other anti-thrombotic drugs.

The dosing problem with anti-thrombotic therapy

The efficacy of drug action is a function of drug concentration according to the classical concentration-effect.

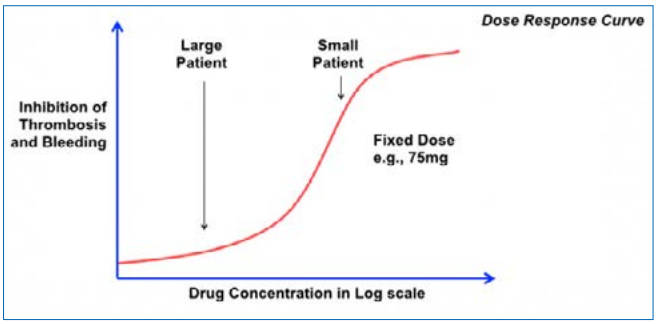
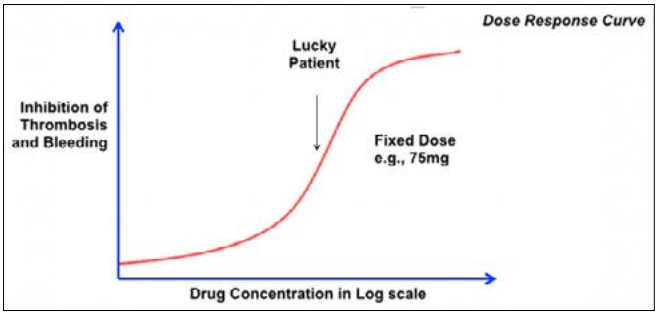
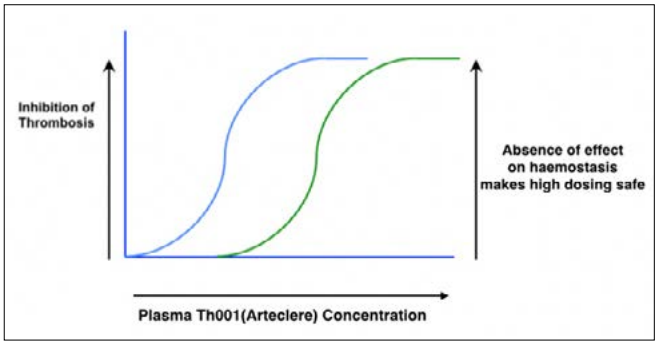


Figure 1: When drugs affect both thrombosis and haemostasis, a single fixed dose regime, e.g., 75mg clopidogrel gives different concentrations for differen body masses. A large patient may be inadequately treated whereas a small patient may have bleeding complications.



A lucky patient may receive a dose giving the correct concentration, but only by chance.

Figure 2: Patients' sensitivities to a given drug concentration varies. A patient with the blue concentration-effect curve will be inadequately treated, whereas one with the green relationsip will have high bleeding risk. A lucky patient may receive a dose giving the correct concentration, but only by chance.



The ideal solution is to use a pure selective 5HT2A antogonist such as Th001, ArteclereTM which only affects thrombosis and not haemostasis; then one can give a high dose (right hand arrow in Figure 2), that is safe for all patient weights and sensitivities.

Conflict of Interest

The author is a shareholder of Thromboserin Ltd that holds the patents on Th001 (ArteclereTM).

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