Abstract
Background
Surgical operations and trauma in patients at risk of thrombosis present the problem of excessive bleeding when anti-thrombotic drugs are administered.

Purpose
(a) To analyse data from the published literature on the mechanism of occlusive arterial thrombosis and (b) to evaluate the most likely drug intervention that might circumvent this problem.

Results
(a) Activation of platelets by haemodynamic factors appeared to be more important than plaque rupture and arterial endothelial factors in the process of arterial thrombosis within arterial stenoses. (b) The process was accelerated by the well established serotonin to serotonin positive feedback thrombus growth mechanism. (c) Much animal experimental data attest to the inhibition of this process by 5HT$_{2A}$ receptor antagonism. (d) Serotonin is not involved in the haemostasis of wounds.

Conclusion
Pure serotonin 5HT$_{2A}$ antagonists will not increase traumatic bleeding and can be administered concommitantly during surgical operations and the peri-operative period.

Keywords
Wound Haemostasis; Anti-Thrombotic Therapy; Platelet Shear Stress; Serotonin; 5HT$_{2A}$; Receptor Antagonism

Introduction
All anti-thrombotic therapies cause increased bleeding which must be of great concern to surgeons, and other practitioners carrying out invasive procedures, let alone those coping with accidental trauma. In non-cardiothoracic surgical operations, one faces more complicated problems in prevention and treatment of peri-operative thrombosis than in orthopaedic operations, and that is complicated enough, with different recommendations for different types of patient [1]. This, and most of the literature on this subject are concerned with anti-coagulation for venous thrombosis and pulmonary embolism. A more general case is the recommendations for stopping anti-coagulation before planned operations [2]. In cardiovascular surgery, one may well be more concerned with dual antiplatelet therapy for arterial disease, which at least leads to less bleeding (e.g., [3]), but excess bleeding nevertheless [4]. After risk stratification analysis, various therapeutic pathways include continuing or discontinuing all antiplatelet agents or maintaining one antiplatelet agent...
Methods and Results

The Experimental Approach to a Solution

Back in 1974, Folts [6] developed an experimental model of coronary arterial thrombosis in the anesthetized dog and demonstrated inhibition of such thrombosis by aspirin [7]. There followed a number of publications from the Folts and Willerson groups confirming this and other influences of thrombus growth in the coronary artery [8]. The papers from the Willerson group included a suggestion that ketanserin was effective in this preparation; ketanserin was a crude drug with antagonistic properties to alpha-1 adrenergic receptors and serotonin 5HT₂ receptors, producing an hypothesis that 5HT₂ receptors might be a potential anti-platelet drug. Most important notable point is that a histology figure in one of these papers apparently showed no effect on the haemostatic layer of platelets bound to fibrinogen. Discussion of this with Folts, led to scepticism owing to the mixed actions of ketanserin (incidentally including a risk of arrhythmic death due to QT prolongation). Folts wanted to check the findings using a purer 5HT₂ antagonist, ritanserin. However, this confirmed powerful inhibition of coronary thrombosis growth by the drug [9].

The 5HT₂ receptor of the platelet is the 5HT₂ₐ subtype. The search was now on to find one of this class that could be used to treat arterial thrombosis without unwanted effects. There were many available and when ever tested showed anti-thrombotic activity. Most drugs with anti-serotonin properties were developed for the treatment of brain disorders such as anxiety and depression. It became possible to try out one of these, ICI 170809, in the Folts model [10]. This drug not only inhibited thrombus growth but also did so to such an extent that the thrombus eventually dispersed altogether (Figure 1). In addition it was effective when thrombus growth rate was accelerated with adrenaline [10]. In addition, if one allowed the thrombus growth to go to complete occlusion and reopened the artery with the thrombolytic tPA, re occlusion occurred which was abolished by the administration of a 5HT₂ₐ antagonist [11] (Figure 2).

Figure 1: Records of mean coronary blood flow in the stenosed circumflex coronary artery of the anesthetized dog adapted from McAuliffe et al [10] with permission. Thrombus growth is assessed by the slope of the falling blood flow as growing thrombus occludes the lumen. In the top control panel, flow is declining by 28.0 ml/min/min. In the middle panel after intravenous administration of 2µg/Kg of the 5HT₂ₐ antagonist, the rate of decline of flow is reduced to 15.9 ml/min/min. In the bottom panel after intravenous administration of 5µg/Kg, the rate of decline of flow is reduced to 3.2ml/ min/min, and after intravenous administration of 10µg/Kg, the thrombus embolises, causing increased flow.
What about the idea that $5HT_2$ antagonism leaves the haemostatic layer of blood cells and fibrinogen intact? A hint that there might be something in that idea is the result of bleeding time measurements in figure 2, which showed an increase during thrombolysis but normal values during $5HT_2$ antagonism after recovery from thrombolysis. But before going into that, it is necessary to explain why thrombosis occurs within stenoses in the first place.

**Discussion**

**Thrombosis Caused by an Arterial Stenoses**

In these animal experiments, it was only stenosis that caused coronary thrombosis, there were no plaques full of lipid, so the conventional idea that plaque rupture is the cause of occlusive thrombus must be modified. Indeed, in my practice before retirement in 2000, half the patients with acute coronary syndromes had concentric lesions, and the eccentric lesions associated with plaques had widely variable anatomy. There is, however, one thing in common with all sites of occlusive thrombus, and that is stenoses which cause very disturbed local haemodynamics [12]. Common to all these is the acceleration of blood velocity required to get the same flow through the stenoses as that through the normal sections of artery. The increased velocity, together with a variety of swirling hemodynamic abnormalities distorts the blood cells. In particular, platelets are subjected to shearing forces that activate them. Activated platelets release large amounts of serotonin from the dense granules.

One might wonder why there should be so much serotonin in platelets. A theory to explain this requires one to consider the fact that serotonin is a vital neurotransmitter in the central nervous system. It is synthetized in the gut,
secreted into the blood stream and the central nervous system has to acquire it through the serotonin re-uptake mechanism (SSRI). (Antagonists of this system are anti-depressive drugs like fluoxetine). Carcinoid syndrome is a disease in which too much serotonin is secreted by the gut and leads to pulmonary hypertension and right heart failure; therefore in normal people there needs to be a buffer to prevent plasma serotonin levels going too high. This is achieved by the same serotonin re-uptake mechanism, but now in the platelet membrane leading to storage in the dense granules. At the end of a platelet’s life the serotonin is broken down to 5-hydroxyindoleacetic acid (5HIAA), which is secreted in the urine. (A urine test for this is used to diagnose Carcinoid tumours).

This useful system evolved before humans lived long enough to develop arterial atherothrombosis. The additional difficulty is that platelets can also be activated by serotonin via their 5HT2A receptor. The serotonin released from sheared platelets then activates more platelets via this receptor so that one has a positive feedback effect. (Negative feedback causes stability; positive feedback accelerates a process). This process is stopped by antagonising the 5HT2A receptor (Figure 1).

Excess Bleeding: the problem for surgeons and trauma specialists, respectively causing and treating wounds

As indicated in the introduction, excess bleeding occurs from wounds in patients who are being treated with anti-thrombotic drugs, who may end up with “oozing coagulopathy” which can co-exist with thrombosis [13]. And trauma activates thrombosis [14] leading to worry by surgeons about the possibility of perioperative thrombosis. Essentially, these practitioners need a protection for their patients against thrombosis with no effect on operative bleeding.

Returning to the observation in the early experiments on 5HT3 antagonists inhibiting thrombosis by leaving the haemostatic layer intact, is there any other suggestion that this approach might be the answer to this problem?

The first point to make is that, if one makes or suffers a wound; what substances are there in the tissue that help to stop the bleeding? Arachidonic acid and its metabolites are part of tissue control of antigens and allergy control. The mechanism by which the active metabolites of arachidonic acid (AA), i.e., thromboxane A2 and/or prostaglandin H2 (TXA2/PGH2) induce platelet aggregation is not understood [15]. Without going into this complex subject, the point to made here is that it is an ubiquitous group of substances in tissue containing blood capillaries and wound release of these substances, and thromboxane in particular, causes platelet aggregation that helps to stop the bleeding. If one inhibits the system with aspirin, bleeding is increased. All metabolising cells in tissue have an energy system based on the phosphate compounds adenosine triphosphate (ATP), adenosine diphosphate (ADP) etc, as a group called purines; again, ubiquitous substances in tissue. These will be released by cutting into tissue and ADP in particular will cause platelet aggregation that helps to stop the bleeding, a process called haemostasis. If one inhibits the system with clopidogrel and other blockers of the P2Y12 platelet purine receptor, bleeding is increased. Serotonin also activates platelets, but there is no serotonin in tissue, only in any quantity in brain cells. Thus antagonising the serotonin receptors in platelets in wounds has no effect because of lack of agonist.

Tests of Hemostasis in the Presence of Serotonin Antagonism

There have been some experiments in which rats have had their tails cut off after giving a 5HT antagonist and there are conflicting results when compared with controls. One of the 5HT2a antagonist with effects on thrombosis shown in figure 1 (ICI 170809) was subjected to a number of tests of haemostasis. This almost happened by accident! The drug, as with most serotonin-related drugs, was synthetized and tested for serotonin antagonism with the idea that it might be good for brain disorders. All the necessary tests for toxicity, etc. were proved satisfactory for volunteer and then clinical trials which were carried out in patients with depression and anxiety. The department responsible seem not to have studied the very early basic work reports on this substance, which demonstrated that it did not pass the blood brain barrier! Obviously there could be no effect on depression or anxiety, nor was there! However, another of the laboratories in the organisation, knowing that serotonin activates platelets carried out platelet aggregation tests and the effect on that of ICI 170809 [16]. Conventional testing of platelet aggregation shows serotonin to be only a weak agonist, blocked by 5HT2 antagonists, but ICI 170809 is much more potent in inhibition of macroaggregate growth [17] because of the positive feedback effect. The results led to the carrying out of the experiment shown in figure 1. They also managed to carry out a few unpaired comparisons of bleeding time
A Potential Solution in Cardio-Thoracic Surgery for Patients at Risk of Peri-Operative Thrombosis.

on volunteers which showed no significant difference with controls. The organisation, having aimed the drug for the CNS, dropped it.

An academic group in Cardiovascular Medicine at the University of Aberdeen were able to obtain this redundant supply of the drug and carried out a trial in 2006 on stable arterial disease patients, with focus on haemostasis, particularly bleeding time. Modern haemostasis experts have reservations about the accuracy of bleeding time in assessing haemostasis, but from the surgeons’ point of view it seems to be very useful to answer the question, “Will my patient bleed more if I administer ICI 170809?”

The trial design was a double-blind, placebo controlled, paired crossover, with 99% power for showing a difference in skin bleeding time. n=48 Caucasian patients, age 69.0±6.6 years, 38 male, 10 female, with stable atherothrombotic disease and other stable chronic diseases, treated with aspirin and statin. There was no effect on bleeding time (p = 0.9729) nor on tests of haemostasis, (Ultegra point of care aggregation test and flow cytometry). The mean platelet count rose from 234.43±67.264 to 246.43±84.14 x10⁹ per litre, p = 0.024. It was concluded that ICI 170809, now renamed Th001 (Arteclere™) therapy appears to be a safe method for potential treatment and prevention of occlusive arterial thrombosis with no risk of increased bleeding [18].

Is there Any Evidence that there is No Excess Bleeding during Surgical Operations?

The most abundant evidence comes from the animal experiments described, which involved thoracotomy and dissection of a coronary artery. Normal haemostasis was secured routinely with diathermy and ligation. Subsequently, there was no further bleeding from the wounds and no oozing coagulopathy.

In humans, the only evidence is from myself (traditional self experimenter). In two knee and one hip replacements with pre-operative loading with Th001, and no postoperative routine anticoagulation, no excessive operative bleeding was reported from three different blinded orthopaedic surgeons, and there was no drop in haemoglobin.

Conclusion and Recommendation

There is every reason to try 5HT₂A antagonism before, during, and after surgery and trauma, as protection against thrombosis. The surgical community should be applying pressure to organisations with funding for research and the pharmaceutical industry to undertake clinical trials of 5HT₂A antagonism during, initially, elective surgery, in which careful and comprehensive protocols are possible.

References


