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REGULAR ARTICLE

Arterial antithrombotic effects of aspirin, heparin, enoxaparin and clopidogrel alone, or in combination, in the rat

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Abstract

Introduction: Many antithrombotic drugs may have a deleterious effect on normal haemostasis leading to bleeding complications. The aim of this study was to determine if sub-therapeutic (low) doses of antithrombotic agents, when administered in combination, have enhanced efficacy without augmentation of bleeding time.
Materials and methods: The antithrombotic effects of i.v. aspirin (4–30 mg/kg), heparin (100–500 U/kg), enoxaparin (4–30 mg/kg) and clopidogrel (10–20 mg/kg) were studied in a rat Folts-like preparation of carotid arterial thrombosis. The frequency of cyclic flow reductions (CFRs; indicating occlusive thrombus formation) and bleeding time were measured. Drug doses that were singly ineffective at preventing occlusive thrombus formation were tested in the following combinations: aspirin (10 mg/kg) with heparin (250 U/kg); aspirin (4 mg/kg) with enoxaparin (4 mg/kg); and aspirin (10 mg/kg) with clopidogrel (10 mg/kg).
Results: Control period (pretreatment) CFRs were not significantly different between groups; average 7.0 ± 0.3 CFRs/30 min ($n = 64$). Tail bleeding time before drug(s) was 3.1 ± 0.1 min ($n = 86$). When administered alone, aspirin (4–30 mg/kg), heparin (250 U/kg) or enoxaparin (4 mg/kg) had no effect on CFRs or bleeding time. Heparin (500 U/kg), enoxaparin (10 and 30 mg/kg) and clopidogrel (20 mg/kg) significantly decreased CFRs. Single administration of heparin (500 U/kg) or enoxaparin (30 mg/kg) increased bleeding time by 4- or 11-fold. When co-

Abbreviations: ADP, adenosine diphosphate; CFRs, cyclic flow reductions; COX, cyclooxygenase; i.p., intraperitoneal; i.v., intravenous; LMWHs, low molecular weight heparins; TXA_2, thromboxane A_2; PRP, platelet-rich plasma.

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administered, aspirin 10 mg/kg and heparin 250 U/kg decreased CFRs, but also increased bleeding time by 11-fold. However, combination of aspirin and enoxaparin (4 mg/kg each), or aspirin and clopidogrel (10 mg/kg each), decreased CFRs with no effect on bleeding.
Conclusions: In a preparation of arterial thrombosis in the rat, combinations of sub-efficacious (low) doses of aspirin with enoxaparin or clopidogrel inhibited thrombus formation without augmenting bleeding time. However, low-dose aspirin combined with heparin, whilst inhibiting thrombus formation, exacerbated bleeding time. If these findings translate into the clinic, the use of effective low-dose combinations may have therapeutic advantages.

Introduction

Despite ongoing research and drug development, cardiovascular disease continues to be a leading cause of human mortality. Current antithrombotic agents used in the treatment of cardiovascular disease either show limited efficacy attributable to the targeting of only one key step in the antithrombotic process or profoundly affect normal haemostasis, leading to bleeding complications. In order to increase efficacy and decrease side effects, the practice of combining two or more antithrombotic agents (adjunctive therapy) is becoming more common. Clinical studies have already demonstrated the advantage of administering clopidogrel with aspirin to patients following stent implantation [1,2]. It is thought that these drugs interact in a synergistic manner, a phenomenon shown in a rabbit arteriovenous shunt model of thrombosis [3]. Adjunctive therapy may also be extended to the combination of an antiplatelet and an anticoagulant drug. To date, this has not been thoroughly investigated in humans or experimental animal preparations. In a meta-analysis, a 33% decrease in the risk of myocardial infarction or death in patients with unstable angina treated with aspirin and heparin compared with aspirin treatment alone has been demonstrated [4]. The combination of antiplatelet and anticoagulant drugs may be beneficial due to the presence of both activated platelets and thrombin in the growing thrombus [5]. Thrombin is a potent stimulus for platelet activation and plays a pivotal role in the rupture of an atherosclerotic plaque leading to thrombus formation [5].

The aim of this study was to determine if pairs of drugs which target different steps in the thrombotic process may be administered adjunctively to exert an antithrombotic effect in a rat preparation of arterial thrombosis, without extending bleeding time. Aspirin (irreversible platelet COX inhibitor) combined with either clopidogrel (irreversible inhibitor of platelet $P2Y_{12}$ receptors), heparin (anticoagulant) or enoxaparin (low molecular weight heparin) was examined.

Materials and methods

Animals

Male Sprague-Dawley rats (335—360 g) were housed at 22 °C with a 12-h light/dark cycle with free access to food and water. This study was approved by The University of Melbourne Animal Experimental Ethics Committee in accordance with the guidelines of the National Health and Medical Research Council of Australia. Rats were euthanased at the end of experiments with an intravenous (i.v.) overdose of sodium pentobarbitone (Lethabarb, Virbac, Peakhurst, NSW, Australia).

Surgical preparation

General anaesthesia was induced by intraperitoneal (i.p.) administration of pentobarbitone 60 mg/kg. A polyvinyl chloride catheter primed with heparinised saline (10 IU/ml; heparin sodium; David Bull Laboratories, Melbourne, VIC, Australia) was inserted into a femoral artery and connected to a blood pressure transducer (Cobe; Argon Medical, Athens, TX, USA) for continuous monitoring of phasic and mean arterial pressure (MAP) on a PowerLab data acquisition system (8SP; AD Instruments, Sydney, NSW, Australia). Via a tracheotomy, a respiratory pump (Model 7025, Ugo Basile, Comerio, VA, Italy) was used to ventilate the rat with room air supplemented with O_2. Arterial blood gases (pH, pCO_2 and pO_2) were assayed at regular intervals using a blood gas analyser (AVL 995 Automated Blood Gas System, Roche Australia Pty. Ltd., Sydney, NSW, Australia). Flow probes (1 mm i.d.) linked to a flow meter (T206, Transonic Systems Inc., Ithaca, NY, USA)