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Research Article

ESTIMATION EFFICACY OF ANTITHROMBOTIC ACTIVITY OF VARIOUS ANTIPLATELET DRUGS IN MURINE MODEL

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ABSTRACT

Objective: To estimate antithrombotic effect of antiplatelet drugs, in collagen epinephrine treated swiss mice. Materials and Methods: Lung damage (pulmonary thromboembolism) in swiss mice treated with collagen (1.5 mg/kg) and epinephrine (0.5 mg/kg) by tail vein was studied by assessing parameters such as percentage protection. The effect of antiplatelet drugs (30µM oral) on above parameters was investigated .This biochemical observations were supplemented by histological examination of lung sections. The data from the dose response studies were subjected to one-way ANOVA followed by post hoc Bonferroni's Multiple Comparison test. Results: Administration of antiplatelet drugs (30µM oral) significantly increased percentage protection against collagen epinephrine induced pulmonary thromboembolism. Histological examination of lung sections revealed less thrombi generation, after administration of various antiplatelet drugs. Conclusions: The study suggests preventive action of antiplatelet drugs in collagen epinephrine induced pulmonary thromboembolism.

Keywords: Collagen, Epinephrine, Antiplatelet Drugs, Thrombi, CMC

INTRODUCTION

A number of thrombosis models have been developed for evaluating novel antithrombotic agents. The thrombosis is induced in mice employing a very small dose of platelet aggregating agents. This model is a useful first step of *in vivo* antithrombotic efficacy of antiplatelet drugs in protecting mice from death or paralysis which occur in response to thrombotic challenge. The primary aim of this study was to examine the antithrombotic effect of antiplatelet drugs in venous thrombosis induced by tail vein injection of collagen and epinephrine.Epinephrine is able to potentiate the effects of other agonists so that the combination such as (collagen and ADP) is a stronger stimulus for platelet activation than agonist alone [1].Cilostazol, a phosphodiesterase type III inhibitor that shows antiplatelet and vasodilator properties, blockade of only one pathway of ADP mediated signaling (for example, by Clopidogrel), slow onset of action (for example, of Clopidogrel), interpatient response variability with poor inhibition of platelet response in some patients (for example, to Clopidogrel) is used clinically as an antithrombotic drug in the treatment of peripheral vascular disease.

Therefore, we compared the antithrombotic effect of control with that of cilostazol, clopidogrel, in this model. The platelet collagen interaction being a very dynamic field of research is considered as an attractive target for anti-platelet drug development. With the continuous unravelling of signal transduction pathways of specific platelet receptors there remains a scope for more efforts to determine the precise description of the synergistic roles of GPIb, GPVI, and α2β1, along with a more complete assessment of the role of other components of the ECM (endothelial cell membrane) and additional platelet integrins. Intravenous administration of collagen activates platelets leading to a maximal thrombocytopenia within a few minutes. The effect is increased by additional injections of epinephrine. Activation of platelets leads to intravasal aggregation and temporary sequestration of aggregates in the lungs and other organs. Depending on the dose of agonist, this experimentally induced reduction of the number of circulating platelets is reversible within 60 min after induction. The assay is used to test the inhibitory capacity of drugs against thrombocytopenia or leucocytopenia as a consequence of *in vivo* platelet or leukocyte stimulation.

MATERIALS AND METHODS

MATERIALS

Chemicals and Drugs

The drugs which were utilized in the present study were procured from the standard pharmaceutical industries like Nicholas Piramal India Limited (NPIL), Mumbai and Zydus Cadila Health Care Limited, Ahmedabad. Cilostazol (PLETAL) -75mg tab, Clopidogrel (NOKLOT) -75mg tab were procured. The chemicals which are utilized are Collagen type v, Epinephrine Hcl, Acetic acid and Sodium chloride.

Experimental Animals

Male Swiss albino mice (20-25gm) were obtained from Bhopal Nobles' college of Pharmacy, Udaipur (Rajasthan). All the animal experiments were subjected to Institutional Animal Ethical Committee (IAEC) guidelines and were conducted according to the guidelines of Experimental Animal Care issued by the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The animals were housed in polypropylene cages and maintained on standard chow diet and water *ad libitum* and on 12hr/12hr light-dark cycle at temperature: 25±2^{0}C, humidity: 45-55% and ventilation: 10-12 exchanges/hr.

METHODS

Experimental induction of pulmonary thromboembolism

Pulmonary thromboembolism [2] was induced by a method as described [3-5, 20]. Briefly, the compounds to be tested, standard drugs or the vehicle were administered by oral route 60 minutes (or at different time points) prior to the thrombotic challenge. Ten mice were used for evaluating the effect of test compound, while a group of 5 mice was used to evaluate the effect of cilostazol or vehicle. A mixture of collagen (150µg/ml) and epinephrine (50µg/ml) was injected into the tail vein to achieve final doses of collagen (1.5 mg/kg) and epinephrine (0.5 mg/kg) to induce hind limb paralysis or death.

Histology and electron microscopy of collagen epinephrine induced pulmonary thromboembolism of mouse lung

Histology of the lung determines whether the vessels of microcirculation of these thrombi consist collagen epinephrine injection. The animals which are not dying noted as protected and which are losses their righting reflex is noted as paralyzed. The animals which not die anesthetize with ether and immediately perfused with perfusion with phosphate-buffered saline (PBS) and neutral buffer formalin 10% after the end of fixative perfusion lung excised and post-fixed in 10% NBF overnight. To For histological studies, the isolated vessel segments were processed and passed through graded alcohol series and xylene, embedded in paraffin blocks and sectioned at 5µm thickness were cut using rotary microtone and then stained with haemotoxycylin and eosin, cleared in xylene and cover slipped in DPX. Histological examination under fluorescence microscopy.

Experimental design

The mice were divided in four group I-III, each groups consists ten mices.

Group I: Served as control received 0.25% w/v CMC.
Group II: Serve as cilostazol 30µm
Group III: serve as clopidogrel 30µm

STATISTICAL ANALYSIS

All the results were expressed as mean ± S.E.M. The data from the dose response studies were subjected to one-way ANOVA followed by post hoc Bonferroni's Multiple Comparison test.

RESULTS

When collagen or epinephrine was tested at levels employed below in combination, no effect was observed. Epinephrine at high dose (70-80 µg) killed 70-80% of challenged mice. This was due to toxic effect of drugs which cause pressor effect and ventricular fibrillation. The combination of both the aggregating agents within 1 min after thrombotic challenge all animals become immobile, develop large protruding eyes, began grasping for breath and may be expired after 2-3min[6-7]. Results have been reported as percentage protection, which represents protection against collagen and epinephrine induced thrombosis and expressed as:

Percent Protection = [1-(P_{test} /$P_{control}$)] x 100

P_{test} - number of animals paralyzed/dead in test compound-treated group; $P_{control}$ - number of animals paralyzed/dead in vehicle treated group.

Histological examination of organs of animals that died showed massive occlusion of the microcirculation of lungs by platelet thrombi. The number of thrombi in the lungs at the time of death after the injection was reduced in clopidogrel drug treated mice, while in cilostazol treated mice, the number of thrombi were relatively more**. (Table 1)** Show that aqueous solution of cilostazol, clopidogrel at the dose employed gave significant protection against thrombotic challenge.

Table 1: Protection of mice from thrombotic challenge (tail vein injection of collagen & epinephrine) Effect of antithrombotic agents in aqueous medium

S.No.	Treatment	No. Tested	% paralyzed	% protected
1.	Control (CMC)	9/10	90%	10%
2.	Cilostazol 30 (µg/kg)	5/10	50%	50
3.	Clopidogrel 30 (µg/kg)	4/10	40	60

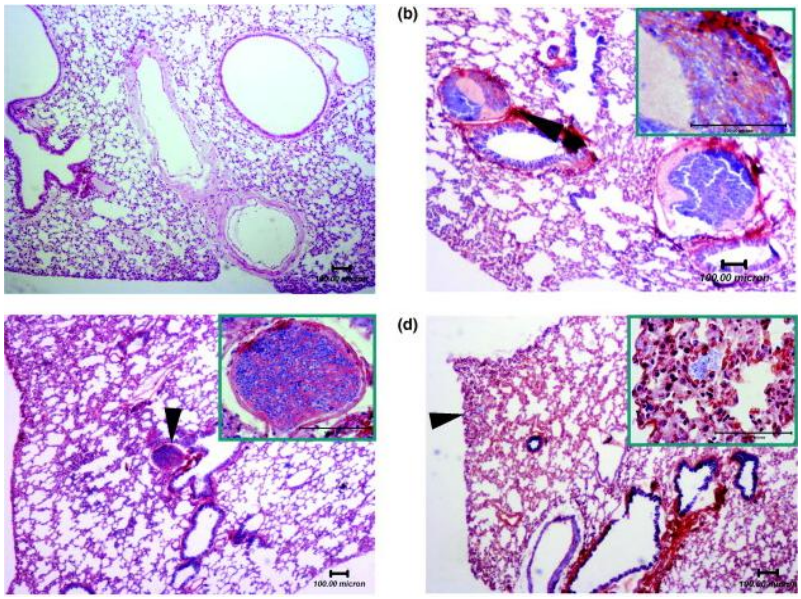

Fig 1: Lung sections stained with hematoxylin and eosin were digitally imaged using the Leica microscope(40x view inset) (a) Control, (b) control paralysed, (c) Clopidogrel, (d) Cilostazol

DISCUSSION

Availability of such a test would facilate preclinical evaluation of potentially useful agents for preventing arterial thrombosis [8-11]**.** The mouse antithrombotic test describe here fulfil this needs. It employs a small, inexpensive test animals and very small amount of aggregating agents to induce thrombosis. The test point is simple and the end point is unequivocal. Ten test animals and control can be studied in 2 hr.Obviously this method is value for studying antithrombotic agents which work primarily by inhibiting platelet thromboembolism through their effect on platelet aggregation [13] **.**In addition, Antiplatelet drugs inhibited collagen and epinephrine-induced thromboembolic death in mice in a dose-dependent manner compared with collagen treated control [12]**.**The lethal effect of aggregating agonists on mice was known to be caused by massive occlusion of the microcirculation of the lung by platelet thromboembolism or by vasoconstriction due to the increase of TXA2 and prostaglandin E in platelets. Thus, this antithrombotic activity can be mediated by inhibition of thromboxane B2 and prostaglandin E2, metabolites of arachidonic acid.

CONCLUSIONS

In summary we described a simple and inexpensive method of evaluating antithrombotic agents .This was in agreement to its antithrombotic efficacy as observed in the mice model of collagen epinephrine induced pulmonary thromboembolism. Cilostazol was also studied for comparison to better validate the experimental procedures employed. Clopidogrel exhibited better antithrombotic efficacy than cilostazol [14-19]**.** In the lung sections of mice, the number of thrombi was significantly reduced in the clopidogrel treated mice as compared to the cilostazol treated and control mice.

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