

## THE CALCIUM ION CHANNEL BLOCKER ISRADIPINE INHIBITS PLATELET ADHESION AND PLATELET THROMBUS FORMATION ON HUMAN VENOUS SUBENDOTHELIUM

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**SUMMARY** In order to examine the effect of isradipine, a calcium antagonist of the dihydropyridine family, on the platelet vessel wall interaction, human saphenous vein subendothelium from patients (with or without regular isradipine therapy), undergoing venous surgery was exposed to citrated human blood in a Baumgartner perfusion chamber under arterial blood flow conditions. Platelet adhesion and platelets thrombus formation were morphometrically quantified using the evaluation technique of Baumgartner. The vessels were perfused either with citrated blood taken from volunteers or hypertensives (not on therapy), or from hypertensives 30 minutes after the intake of the last 2.5mg isradipine either orally or in-vitro addition of 0.5, 1, and 5µg/ml. The source of the vessel did not influence thrombogenicity. The surface induced platelet adhesion and platelets thrombus formation both were significantly lower in experiments with blood from patients having adjusted isradipine or with in-vitro isradipine addition as compared to controlled and hypertensives without medication. As the vascular segments used were no longer able to produce any prostaglandin, the results in this perfusion model indicates a platelet-derived improvement in platelet vessel wall interaction by isradipine, which is not PGI<sub>2</sub>-mediated

**Key:** *Isradipine, platelet adhesion, thrombus, subendothelium.*

### Introduction

The exposure of an inverted vascular segment mounted on a rod and exposed to citrated blood under flow conditions occurring in humans has been introduced by Baumgartners' group in the seventies (Baumgartner et al 1972, Baumgartner 1973). In this model the rheological conditions, the characteristics of blood, and the properties of the exposed vascular surface are the main determinants. In a cooperative study about 10 years ago (Baumgartner et al 1976). It was found that no substantial difference of the time course, extent of platelet adhesion and platelet thrombus formation between the subendothelium of rabbit arteries on one hand and human arterial or venous tissues on

the other. Isradipine, a calcium antagonist of the dihydropyridine family (Fetkouska et al 1991) has been shown to potentially affect platelet function, both in-vitro (Habib et al 1986, Moncada et al 1976) and in-vivo (Sinzinger et al 1993) and has been claimed to beneficially affect platelet vessel wall interaction by stimulating PGI<sub>2</sub> formation<sup>8</sup>, EDRF-synthesis (Sinzinger et al 1993) and TBA-liberation. No definite data on vascular wall thrombogenicity, however, either on in-vitro or in-vivo studies are available presently. We therefore approach the platelet vessel wall interaction on isradipine in comparison with native citrated blood using vascular tissue samples from patients with and without current isradipine medication.

### Materials And Methods

#### *Preparation of Venous Tissue*

Saphenous venous tissue was obtained during venous surgery from 13 patients (aged 37-76 years; average age 55) 6 of them hypertensive (2 males, 4 females) being on regular isradipine therapy for over 8 weeks, and 7 (3 males, 4 females) without medication. The size of the vessels was selected considering the fact that the vessel size should fit the perfusion chamber. The vessels were rinsed with open 2 molar tris HCl-buffer (pH 7.4) and stored in tris buffer after penicillin and streptomycin addition for not longer than 4 days at 4°C (8).

#### *Blood Preparation*

Venous blood was drawn from a cubital vein without venous occlusion using a 1.2mm diameter needle into monovette vials and anticoagulated 1/10 vol. with sodium citrate. Blood was either drawn 30 minutes after the last oral injection of 2.5 mg isradipine in patients (n=8; 39-57 a, mean age: 43.7; 5 males, 3 females) suffering from hypertension without having taken any other drug since more than 2 weeks at least, from hypertensives without any medication (n=8; 35-53 a, mean age: 44.8; 6 males, 2 females) or from healthy volunteers (n=8; 32-57 a, mean age: 40.9; 6 males, 2 females). Blood drawn from healthy donors was anti coagulated as described and 0.5, 1

and 5 µg/ml isradipine were added immediately prior the perfusion experiment. Neither the patient nor the volunteers had any risk factor (beside hypertension) for the development of atherosclerosis.

#### *Perfusion procedure:*

Once the everted vessel 10–14 mm in length was mounted on the rod of the perfusion chamber, 37 ml of blood was filled into perfusion system which was circulated by a perfusion roller pump at an average flow rate of 160 ml/min pulsatile flow for different time intervals from 1–30 minutes. The whole perfusion system was kept in a bath at a constant temperature of 37 °C.

#### *Processing for morphological determination:*

The exposed segments were immediately Emerson fixed with phosphate buffered (pH 7.4) glutaraldehyde after the experiments while on the rod.

#### *Morphometric evaluation of platelet adhesion and platelet thrombus formation:*

The extent to which the exposed subendothelium was covered with adherent platelets was determined morphometrically in stained cross sections (1) of 0.8 µ thickness using light microscopy as described (3). The percentage surface coverage with directly adhering platelets (C+S) was recorded as platelets in contact (c) with the surface but not spread out on it, and spread platelets (S), i.e. tightly adherent platelets. Surface coverage with a platelet aggregate of more than 5µ in height was recorded as mural thrombus (T). 100 T/S therefore expresses the extent of thrombus

formation as a percentage of the surface covered with spread platelets, i.e. adhesion-induced aggregation. One complete cross section (about 1.000 intersection points of the ocular micrometer scale with the examined surface) was evaluated per exposed vessel segment.

*Statistics:* Results were expressed as mean ± SD, level of significance was expressed with the Student's T-test

## Results

### *Morphology of the exposed veins:*

The thickness of the exposed saphenous vein segment was  $295 \pm 19 \mu$  (n =13). No significant difference among the vessels used in the different groups was seen.

### *Platelet interact with subendothelium of saphenous veins:*

Both the qualitative and quantitative platelet-vessel wall interactions were not different perfusing vessels from patients either on isradipine therapy or not (table.1). In contrast, however, if the platelets have been in contact with the drug before either in-vitro or in-vivo (table 2) there was a beneficial influence on the interaction with the reported subendothelium in the perfusion chamber.

In the presence of increasing doses of isradipine in vitro there was a dose dependent benefit visible both on platelet adhesion and adhesion-induced platelet thrombus formation (Table. 3).

Table 1: Data Showing (Mean ± SD, P<0.01; C:

Contact platelets S-spread (adhesion) platelets; T- thrombi (aggregates > 5µ in height) Group 1 (Patients on Isradipine therapy) and Group 2 (Patients not on any medication).

Group	C	C+S	T
1	9.3±2.1	71.4±4.4	4.6±1.4
2	9.5±3.1	72.3±4.1	4.7±1.3

Table 2: Showing the beneficial effects of prior contact of platelets with the Drug (Isradipine)

% of denuded Surface Covered					
Group	N	Denudation Extent	C	C+S	T
1	8	100±0	20.6±3.2	99.8±5.3	15.2±3.3
2	7	100±0	9.3±2.1	71.4±4.4	4.6±1.4
3	6	100±0	9.2±3.1	70.2±4.1	3.7±2.1

1- Control 2- Invitro Isradipine Therapy 3 - Invivo Isradipine Therapy

Table 3 : Showing the Beneficial effects of Isradipine given in Increasing Dose.

Table 3A ( 0.5 µg/ml)

Group	N	% Of Denuded Surface Covered			
		Denudation Extent	C	C+S	T
1	6	100±0	7.3±3.1	68.4±3.4	3.6±1.3
2	7	100±0	16.9±3.2	89.6±5.2	12.9±2.2
3	8	100±0	6.1±2.1	42.7±4.2	4.1±1.2

Table 3B ( 1µg/ml)

Group	N	% Of Denuded Surface Covered			
		Denudation Extent	C	C+S	T
1	6	100±0	5.2±1.2	62.3±2.2	2.6±1.2
2	7	100±0	1.6±3.1	88.6±2.1	12.5±1.2
3	8	100±0	5.1±2.1	41.2±1.2	3.3±1.3

Table 3C ( 1.5µg/ml)

Group	N	% Of Denuded Surface Covered			
		Denudation Extent	C	C+S	T
1	6	100±0	3.2±1.2	58.4±2.3	2.5±1.3
2	7	100±0	13.3±1.3	82.3±1.2	10.5±1.2
3	8	100±0	3.2±1.2	43.1±1.2	2.2±1.1

1- Patients on Isradipine Therapy 2 - Patients not on any Medication 3 - Control (Healthy Volunteers)

### Discussion

The perfusion of venous segments derived from untreated patients with citrated whole blood from hypertensives as well as volunteers without any medication does not reveal any difference concerning platelet vessel wall interaction as to the data reported earlier (Tschopp et al 1979) for volunteers' blood. After about 10 minutes the saturation with about 80% adherent platelets to the venous subendothelium was achieved (Tschopp and Baumgartner, 1981). While this figure did not change, the thrombus formation reached a plateauing maximum between 3 and 10 minutes showing a decline thereafter. Even without wrapping, no resting endothelial cells were found. Wrapping itself has been shown to increase thrombogenicity (Weiss et al, 1989) most likely due to mechanical damage and unmasking collagen fibrils being exposed there after to the surface. These findings show that the time period passing between the removal of the segments and their exposure in the perfusion chamber in this study compared to a mean of 5 weeks with human as segments earlier compared to a mean of 3 weeks with rabbit vessels. The shortest period of time between removal of vascular tissue and performing

the perfusion studies was too long in order to allow any residual PG-forming capacity. Pilot testing (Data not shown) for PGI<sub>2</sub> has also excluded this. Pilot test for the stable breakdown of PGI<sub>2</sub>; 6-oxo-PGF<sub>1α</sub>(radioimmunoassay) confirmed the former test. It thus can be concluded that the reported thromboregulatory role of vascular PGI<sub>2</sub> (Tschopp et al 1974) was not involved at all. Thus the beneficial effect must have been induced solely by a platelet-derived benefit (Akayama et al, 1999; Kerin et al, 1998; Strayser et al, 2000).

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