els. The precise mechanism of these effects is unknown, but may be related to the inhibition of platelet function and/or leukocyte recruitment.

## 931-111 Platelet Activation and Aggregation by Therapeutic Doses of Heparin

Zihui Xiao, Pierre Théroux, John R. Plachetka. *Montreal Heart Institute, Montreal, Quebec, Canada*

To understand better the complex interaction of heparin with platelets, this study investigated platelet function by flow cytometry and aggregation curves, with and without the drug, in 7 pts with unstable angina and 7 normal individuals. *Method and Results:* Platelet aggregation induced by low doses ADP (0.3125 $\mu$M) and thrombin receptor agonist peptide (TRAP 0.625 $\mu$M) was quantified in platelet-rich plasma (PRP) before and after the administration of intravenous heparin at doses prolonging the aPTT 2.5 × control and also after the addition in whole blood, *ex vivo* of therapeutic concentration of heparin (0.2 U/ml), argatroban (1 ng/ml) or of an equal volume of normal saline (Control). Platelet activation was evaluated by the percentage of fluorescein positive platelets (PL%) and the binding index per platelet (BI), using antibodies directed against P-selectin (CD62) and activated GP IIb/IIIa receptor (PAC-1). Following intravenous heparin, the maximal shift in platelet aggregation (Max%) increased from 6.3 ± 3.6 to 11.6 ± 8.5 with ADP and from 4.4 ± 3.0 to 11.9 ± 5.2 ($p < 0.05$) with TRAP. The results of the *ex vivo* studies (x ± SD) were:

| | PAC-1 | | CD62 | | |
|---|---|---|---|---|---|
| | PL% | BI | PL% | BI | Max% |
| *Control* | | | | | |
| Basal | 1.80 ± 0.67 | | 0.66 ± 0.114 | 0.011 ± 0.0024 | |
| ADP | 70.1 ± 15.99 | 3.5 ± 1.48 | 10.1 ± 4.13 | 0.18 ± 0.074 | 4.3 ± 2.25 |
| TRP | 18.8 ± 16.45 | 0.81 ± 0.61 | 2.77 ± 2.02 | 0.05 ± 0.039 | 2.2 ± 1.84 |
| *Heparin* | | | | | |
| Basal | 3.60 ± 1.42* | | 1.00 ± 0.292* | 0.018 ± 0.0047† | |
| ADP | 76.6 ± 12.49* | 4.2 ± 1.70* | 13.2 ± 4.43* | 0.24 ± 0.087* | 8.8 ± 3.06† |
| TRP | 32.9 ± 25.60* | 1.5 ± 1.17* | 3.32 ± 2.24* | 0.06 ± 0.039 | 6.2 ± 1.84* |
| *Argatroban* | | | | | |
| Basal | 2.30 ± 0.75 | | 0.82 ± 0.192* | 0.015 ± 0.0041* | |
| ADP | 69.1 ± 19.62 | 3.5 ± 1.75 | 11.4 ± 4.42 | 0.21 ± 0.085 | 2.3 ± 2.07* |
| TRP | 21.0 ± 14.23 | 0.88 ± 0.56 | 2.48 ± 1.12 | 0.05 ± 0.021 | 2.0 ± 2.61 |

*$p < 0.05$ and †$p < 0.01$ vs Control

Thus, heparin, but not argatroban, a direct thrombin inhibitor, induced P-selectin expression and GP IIb/IIIa activation in the basal state and following agonist stimulation at low concentrations.

## 931-112 A Low-dose Intravenous Aspirin, Bolus Injection, Effectively Inhibits Platelet Aggregation

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Prompt inhibition of platelet aggregation is important in acute coronary syndrome and before an intervention procedure. To determine whether a single low dose of iv aspirin inhibits platelet aggregation, twenty-seven healthy volunteers (7 F and 20 M), mean age 43.5 years, were randomized double-blind to a single iv low dose of aspirin DL-lysine (L-ASA) equivalent to 2 mg/kg of aspirin, high dose (H-ASA) equivalent to 10 mg/kg, or placebo (Pl). Platelet aggregation were performed before and 1 h and 24 h after drug administration, in whole blood (WB) using electrical impedance and in platelet-rich plasma (PRP) by optical light transmittance. Baseline WB platelet aggregation (Col 3 $\mu$g/ml) was the same with L-ASA, H-ASA and Pl (24 ± 5, 23 ± 3 and 24 ± 4Ω respectively) and decreased significantly more with L-ASA and H-ASA than with Pl after 1 h. (17 ± 6, 15 ± 7 and 21 ± 5Ω $p < 0.01$) and 24 h (17 ± 7, 16 ± 6 and 25 ± 4Ω $p < 0.01$). Results in PRP were similar:

| PRP optical aggregation (%) | | Before | After ASA | |
|---|---|---|---|---|
| | | | 1 h. | 24 h. |
| ADP (5 $\mu$M): | L-ASA | 73 ± 21 | 50 ± 14* | 45 ± 15* |
| | H-ASA | 65 ± 23 | 54 ± 17* | 52 ± 16* |
| | Pl | 69 ± 18 | 72 ± 22 | 69 ± 14 |
| Col (3 $\mu$g/ml): | L-ASA | 64 ± 19 | 33 ± 13* | 36 ± 18* |
| | H-ASA | 60 ± 16 | 32 ± 17* | 34 ± 14* |
| | Pl | 55 ± 13 | 56 ± 18 | 57 ± 14 |

*$p < 0.001$ (ANOVA test) respect to Pl and the baseline values

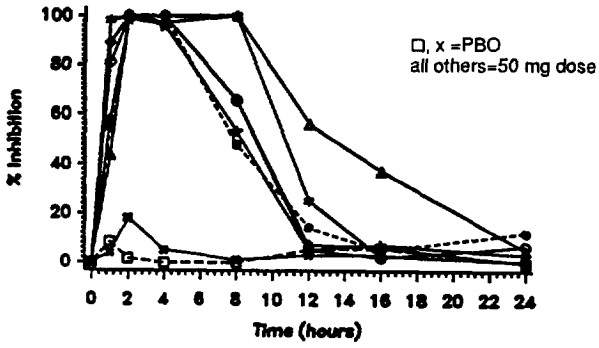
No differences in response were observed between the two doses of aspirin and no significant changes occurred between 1 and 24 hours in any group.

Effective inhibition of platelet aggregation is thus achieved within 1 hour after the administration of low-dose 2 mg/kg of iv aspirin.

## 931-113 Demonstration of Potent Inhibition of Platelet Aggregation with an Orally Active GPIIb/IIIA Receptor Antagonist

Robert J. Anders, John C. Alexander, Gary L. Hantsbarger, Dan M. Burns, Stuart D. Oliver, Graham Cole, Desmond J. Fitzgerald. *Searle Pharm., Skokie, IL; Besselaar Associates, Leeds, UK; Royal College of Surgeon, Dublin, Ireland*

This single-blind, placebo-controlled study evaluated the tolerability and pharmacodynamic (PD) response of the first dose of the oral GPIIb/IIIa receptor antagonist, SC-54684A (ethyl 3S-[[4-[[4(amino-iminomethyl)phenyl]amino}-1,4-dioxobutyl]amino]-4-pentynoate, monohydrochloride). SC-54684A (SC) is the pro-drug of the active compound, SC-54701A. Six healthy male subjects received 50 mg of SC (free base) and 2 received placebo (PBO). Results of the inhibition to ADP (20 $\mu$M) induced platelet aggregation are shown below:




Bleeding time increased a mean of 5.6 fold at 4 hrs post-dose and returned to within normal limits at 8 hrs after dosing. No significant changes in lab values or bleeding complications occurred. Peak serum concentrations coincided with peak PD effects of inhibition of platelet aggregation.

*Conclusion;* SC-54684A has a rapid onset of potent inhibition of platelet aggregation that is sustained for up to 10 hours after dosing.

## 931-114 Effects of Inhibition of Nitric Oxide Synthesis in Proximal and Distal Segments in Patients with Normal Arteries and in Patients with Coronary Artery Disease

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Inhibition of nitric oxide synthesis causes a decrease in basal diameter of distal epicardial coronary arteries in patients (pts) with normal coronary arteries (NCA). The effects of inhibition of nitric oxide synthesis with $N^G$-monomethyl-L-arginine (LNMMA) in atheromatous coronary arteries was evaluated in 13 pts with chronic stable angina (aged 57 ± 7 years, 11 males) due to angiographically documented coronary artery disease and in 8 pts (aged 50 ± 5 years, 4 males) with angiographically NCA. LNMMA was infused intracoronary at 4, 8 and 16 $\mu$mol/min each for 4 minutes. In response to low LNMMA, 4 $\mu$mol/min, there was a significant ($p < 0.05$) reduction in luminal diameter of both proximal (from 3.49 ± 0.28 to 3.35 ± 0.28 mm) and distal (from 1.33 ± 0.07 to 1.23 ± 0.06 mm) segments in patients with NCA. In patients with atheromatous arteries there was a reduction in diameter of the distal segments (from 1.44 ± 0.06 to 1.33 ± 0.07 mm) but no change occurred in the proximal segments (from 2.95 ± 0.16 to 2.89 ± 0.16 mm). In response to high LNMMA, 16 $\mu$mol/min, there was a significant ($p < 0.01$) reduction in luminal diameter of both proximal (from 2.53 ± 0.27 to 2.33 ± 0.26 mm) and distal (from 1.10 ± 0.06 to 0.99 ± 0.06 mm) segments in the pts with NCA. In the pts with atheromatous arteries the distal segments decreased in diameter (from 1.32 ± 0.07 to 1.17 ± 0.06 mm) but no change occurred in the proximal segments (from 3.16 ± 0.12 to 3.08 ± 0.14 mm). The magnitude of the distal vessel constriction was similar in both the patients with normal and in those with atheromatous arteries (−9.6 ± 2.1% and −10.9 ± 2.6% respectively, p = NS). In conclusion in pts with chronic stable angina due to coronary artery disease inhibition of basal nitric oxide synthesis causes distal coronary artery vasoconstriction, but it has no effect on proximal segments.

## 931-115 Phase I Studies on Inogatran, a New Selective Thrombin Inhibitor

Ann-Catrine Teger-Nilsson, Ulf Eriksson, David Gustafsson, Ruth Bylund, Gunnar Fager, Peter Held. *Astra Hässle AB, S-431 83 Mölndal, Sweden*

Inogatran is a new, synthetic, active site inhibitor of thrombin with a molecular weight of 439 dalton. Inogatran (pINN) selectively, rapidly and competitively binds thrombin with a Ki value of 15 nmol/l. *In vitro* it doubles the plasma

thrombin time at a concentration of 23 nmol/l and the activated partial thromboplastin time (APTT) at 1.1 μmol/l. Thrombin induced platelet aggregation is inhibited at an IC50 of 17 nmol/l. Inogatran was studied in healthy male human volunteers with regard to tolerability, pharmacokinetics and effects on haemostasis. It was given intravenously as a bolus in doses from 0.002 to 1.1 μmol/kg (n = 2–4 at each dose). The highest peak plasma concentration observed was 7 μmol/l, corresponding to an APTT prolongation of 3 times. The drug was also given as a constant infusion over 4 h at a dose of 0.73 μmol/kg per h (n = 16) which resulted in a mean plasma concentration at steady state of 1.9 μmol/l and an APTT prolongation of 2.3 times. Finally, it was given as a bolus with radiolabeled compound in a total dose of 25 μmol (n = 16). The drug was well tolerated and without side effects with the exception of slightly increased bleeding tendency at the blood sampling site. Inogatran had a volume of distribution of 0.26 ml/kg and a total plasma clearance of 6.1 ml/min per kg, resulting in a half life of about one hour. The drug was not metabolised and it was excreted unchanged with the elimination evenly distributed between urine and faeces. *Ex vivo* the thrombin time was linearly correlated to the plasma concentration while the APTT-concentration curve was non-linear. At the highest plasma concentrations a slight prolongation of the capillary bleeding time was seen in some subjects. Markers of thrombin activity (thrombin-antithrombin complex and prothrombin fragments 1–2) decreased during the constant infusion of the drug. There was no effect on fibrinolysis (PAI-1 and t-PA activities) or protein C. It is concluded that inogatran is a safe and effective anticoagulant with favourable pharmacokinetics for i.v. use.

MONDAY PM

## 932 Pathophysiology of Carditis and Cardiomyopathy

Monday, March 20, 1995, 3:00 p.m.–5:00 p.m.
Ernest N. Morial Convention Center, Hall E
Presentation Hour: 4:00 p.m.–5:00 p.m.

### 932-83 Contribution of Gi-protein Effects to β-Adrenoceptor Desensitisation in Single Ventricular Myocytes from Aged Guinea-pig Heart

Nicola Ferrara, Philip A. Poole-Wilson, Sian E. Harding. *National Heart and Lung Institute, London, UK*

We have characterised the age-related changes of contractility and β-adrenoceptor function in isolated cardiac myocytes from guinea-pigs. Pertussis toxin treatment was used to determine the contribution of Gi effects to the change in sensitivity to β-adrenoceptor stimulation. Myocytes were isolated from either adult animals from 2 weeks to 14 week of age, where body weight increases linearly with age, or senescent ones aged between 53–65 weeks. There was some indication of a decrease in contractility in maximum $Ca^{2+}$ with age, with significant differences between a young (≤4 weeks, weight < 400 g) and aged (≥8 weeks, weight > 600 g) group in contraction amplitude (% shortening) or contraction and relaxation velocities. This decline was continued into senescence, and ANOVA showed a significant difference between the three groups for percentage shortening (12.2 ± 0.9%, young, n = 31; 9.5 ± 0.6%, n = 28 aged; 6.7 ± 0.8%, n = 6, senescent; P = 0.005), and contraction or relaxation velocities (P < 0.001). There was a more pronounced decline with age for maximum contraction amplitude in isoproterenol (11.8 ± 0.7%, n = 30, young; 7.9 ± 0.5%, n = 28, aged and 5.5 ± 1.1%, n = 6, senescent; P < 0.001) and the isoproterenol/$Ca^{2+}$ ratio was reduced (P < 0.02). The EC50 value for isoproterenol was similar in young (1.7 ± 0.4 nM) and aged (1.6 ± 0.8 nM) animals, but significantly higher in senescent (16.8 ± 6 nM) (P < 0.05, ANOVA). Pertussis toxin treatment decreased the EC50 in all groups, but the effect was most pronounced for senescent animals. The final EC50 values in toxin-treated cells were 0.22 ± 0.11 nM (young), 0.19 ± 0.08 nM (aged) and 0.12 ± 0.01 nM (senescent): these were not significantly different. A general decrease in contractility of the myocyte occurs as the animal ages, but the effect is more pronounced for β-adrenoceptor stimulation than for high $Ca^{2+}$, consistent with a specific lesion in the adenylate cyclase related pathway. The ability of pertussis treatment to equalise EC50 values for isoproterenol between the groups suggests that an increase in activity of Gi contributes to the reduced β-adrenoceptor sensitivity in senescent animals.

### 932-84 Left Ventricular Collagen in Hypertension: Is Regression of LV Hypertrophy with ACE Inhibitors Really Beneficial?

H.J.F. Why, V.B. Patel [1], P.J. Richardson, V.R. Preedy [1]. *Department of Cardiology, King's College Hospital, London, U.K.;* [1] *Department of Biochemistry, King's College Hospital, London, U.K.*

Hypertensive left ventricular hypertrophy (LVH) is associated with altered left ventricular (LV) filling; this may persist despite regression of LVH with treatment. Collagen occupies about 3% of the LV mass and contributes to myocardial stiffness. Collagen content may be assessed experimentally by assay of hydroxyproline, an amino acid virtually unique to collagen. The effects of lisinopril treatment on LV hydroxyproline content were studied in 15 week old spontaneously hypertensive rats (SHR) and 15 week old normotensive Wistar Kyoto rats (WKY). Rats were treated with lisinopril (LIS) in water (5 mg/kg/day) for 8 weeks. Control rats were given plain tap water. LV hydroxyproline content was assayed by a spectrophotometric method after sacrifice. LIS lowered blood pressure (BP) in SHR significantly; BP in WKY was unaltered. LV mass was significantly greater in SHR controls than WKY controls (1062 ± 40 mg vs 896 ± 9.4 mg, p < 0.01). LV mass regressed with LIS in both SHR (1062 ± 40 vs 786 ± 18.3 mg, p < 0.001) and WKY (896 ± 9.4 vs 760 ± 33.1 mg, p < 0.01). Hydroxyproline levels were significantly higher in treated SHR than controls; no difference was seen between treated and control WKY.

| | LV hydroxyproline (mean ± SEM) | | |
|---|---|---|---|
| | μg/g tissue | μg/mg DNA | μg/mg protein |
| SHR (control) | 413.0 ± 32.0 | 143.1 ± 48.3 | 2.13 ± 0.17 |
| SHR + LIS | 525.6 ± 32.1* | 183.6 ± 10.8* | 2.72 ± 0.17* |
| WKY (control) | 452.1 ± 31.9 | 164.4 ± 7.2 | 2.40 ± 0.14 |
| WKY + LIS | 517.8 ± 26.9 | 176.2 ± 8.3 | 2.70 ± 0.13 |

*p < 0.05 vs appropriate control

Regression of LVH in SHR with lisinopril treatment was associated with significantly increased hydroxyproline per unit weight of tissue, protein and DNA. Selective regression of cardiac proteins with lisinopril may have important function implications.

### 932-85 Altered Muscle Fiber Sarcomeres in Hypertrophic Cardiomyopathy Associated with the $403^{Arg \rightarrow Gln}$ β-Myosin Heavy Chain Gene Mutation

Edward B. Lankford, Rhea Levine, Neal D. Epstein [1], Lameh Fananapazir [1], H. Lee Sweeney. *University of Pennsylvania, Philadelphia, PA;* [1] *NIH, Bethesda, MD*

We have shown that mutant cardiac β-myosin is present in slow skeletal muscle from patients with hypertrophic cardiomyopathy (HCM) caused by the $403^{Arg \rightarrow Gln}$ β-myosin heavy chain (β-MHC) gene mutation. Slow myofibers with this mutation have impaired maximal isometric tension and shortening velocity. This study examines whether ultrastructural changes account for the altered contractile properties. Single soleus muscle fibers from 4 patients (two unrelated kindreds) with the $403^{Arg \rightarrow Gln}$ mutation and 3 normal controls, were chemically permeabilized, and mounted under a light microscope at a length 10% longer than the slack length to measure sarcomeric spacing. Electronmicroscopy was used to evaluate sarcomeric structure of fixed fibers, as well as to measure the length of thick filaments obtained from elastase digestion of other fibers. Although sarcomeric structure was normal, the sarcomeric spacing was ≈20% shorter compared with normal controls (1.70 versus 2.10 μm). The isolated thick filaments were also ≈20% shorter compared to the normal controls: 1.25 ± 0.14 μm (n = 248) versus 1.55 ± 0.07 μm (n = 189), p < 0.001. This reduction of thick filament length and concomitant decrease in myosin heads, will (1) reduce the isometric force per cross-sectional area compared to fibers with normal length, and (2) increase the maximal shortening velocity as there will be more sarcomeres per unit length of muscle fiber. This observation suggests that in HCM caused by this β-MHC mutation, there is a pathway linking changes in the molecular motor to alterations in sarcomeric ultrastructure.

### 932-86 Importance of Collagen in Myocardial Ischemia and Remodeling of Abnormal Intramural Coronary Arteries ("Small Vessel Disease") in Young Patients with Hypertrophic Cardiomyopathy and Sudden Cardiac Death

Jamshid Shirani, Ruth Pick, Barry J. Maron. *Albert Einstein College of Medicine, Bronx, NY; Minneapolis Heart Institute Foundation, Minneapolis, MN*

Impaired coronary blood flow reserve and myocardial ischemia in the absence of epicardial coronary artery disease is a frequent and important pathophysiologic feature of hypertrophic cardiomyopathy (HCM). To provide the morphologic basis for such findings, we studied the hearts of 16 pts with HCM (ages 11–31 yrs; mean 20) who had died suddenly. Transmural sections of ventricular septum were studied (area 5.2 ± 2 $cm^2$) after staining with picrosirius-red; 9 ± 2 abnormal intramural coronary arteries (IMCAs) were analyzed per section. Compared to 16 structurally normal control hearts, IMCAs in HCM had substantially greater (12-fold) collagen volume fraction in media (2.5 ± 2-vs-0.2 ± 0.3%, p < 0.01), as well as larger outer diameter (120 ± 46-vs-82 ± 22 μm, p < 0.01), luminal diameter (88 ± 33-vs-62 ± 19 μm, p < 0.05), medial thickness (32 ± 20-vs-20 ± 6 μm, p < 0.05) and also perivascular