INIS-mf--10891

TRN: ZA8700107 -
ZA8700122

ABSTRACTS
ABSTRAKTE

CONFERENCE ON RADIONUCLIDE LABELLED CELLULAR BLOOD ELEMENTS:
APPLICATION IN ATHEROSCLEROSIS AND THROMBOSIS

3—6 FEBRUARY / FEBRUARIE 1986

BLOEMFONTEIN RSA

KONFERENSIE OOR RADIONUKLIED-GEMERKTE BLOEDSELELEMENTE:
TOEPASSING IN ATEROSKLEROSE EN TROMBOSE

THIS MEETING IS BEING PRESENTED BY THE

HIERDIE BYEENKOMS WORD AANGEBIED DEUR DIE

SOUTH AFRICAN MEDICAL RESEARCH COUNCIL
SUID-AFRIKAANSE MEDIESE NAVORSINGSRAAD

ABSTRACTS
ABSTRAKTE

CONFERENCE ON RADIONUCLIDE LABELLED CELLULAR BLOOD ELEMENTS:
APPLICATION IN ATHEROSCLEROSIS AND THROMBOSIS

3—6 FEBRUARY / FEBRUARIE 1986

BLOEMFONTEIN RSA

KONFERENSIE OOR RADIONUKLIED-GEMERKTE BLOEDSELELEMENTE:
TOEPASSING IN ATEROSKLEROSE EN TROMBOSE

THIS MEETING IS BEING PRESENTED BY THE

HIERDIE BYEENKOMS WORD AANGEBIED DEUR DIE

SOUTH AFRICAN MEDICAL RESEARCH COUNCIL
SUID-AFRIKAANSE MEDIESE NAVORSINGSRAAD

Title : PLATELET FUNCTION IN THROMBOSIS AND ATHEROSCLEROSIS: A PERSPECTIVE (1)

Authors : J F Mustard, R L Kinlough-Rathbone and M A Packham

Presented by: J F Mustard

The development of atherosclerosis is influenced by endothelial injury patterns of blood flow and the interaction of formed elements of the blood, particularly platelets and monocytes, at sites of vessel damage. Blood flow appears to be responsible for the focal nature of atherosclerotic lesions which occur primarily around vessel orifices and branches. Flow is disturbed in these regions and flow separation with low shear leads to the accumulation of metabolic products in the vessel wall and the formation of platelet aggregates, platelet accumulation at sites of endothelial cell injury, and the accumulation of leukocytes.

Platelets and monocytes play a major part in smooth muscle cell proliferation after vessel injury because when they are stimulated either as a result of their adherence to collagen or when they are exposed to thrombin generated at a site of injury, they release a factor that is mitogenic for smooth muscle cells. Platelets are one of the main constituents of mural thrombi and of occlusive thrombi in atherosclerotic vessels. Thrombin has an important role in the formation of arterial thrombi because of its ability to cause platelets to aggregate and release their granule contents and because it converts fibrinogen to fibrin which stabilizes platelet aggregates that form on injured or atherosclerotic vessels. Repeated vessel wall injury produces a more thrombogenic surface than a single injury and at sites of repeated injury thrombin may be the major factor causing thrombus formation.

Several factors cause rapid progression of atherosclerosis at sites of disturbed flow, for example hypercholesterolemia, homocystinemia, serum sickness, and possibly bacterial or viral infections. These stimuli probably exert their effects through several pathways. For example, the combined effects of hypercholesterolemia and serum sickness or mechanical injury lead to more extensive atherosclerosis than any of these conditions alone. Enhanced platelet responsiveness to aggregating and release-inducing stimuli has been reported in a number of conditions associated with atherosclerosis and thrombosis, but it is not clear whether this occurs as a result of thrombosis or whether it contributes to the development of thrombosis. The effect of cigarette smoking in enhancing platelet aggregation and predisposing towards vessel injury and thrombosis is controversial. Recent studies indicate that fish oils rich in eicosapentaenoic acid may protect against the development of atherosclerosis and thrombosis. Similarly the ingestion of some alcoholic beverages appears to inhibit platelet function and to reduce the risk of death from the complications of vascular disease.

Title : HOW LIPOPROTEIN PARTICLES RELATE TO ATHEROSCLEROSIS (2)

Author : W Gevers

Presented by: W Gevers

Interactions between different kinds of lipoprotein particles and a variety of cell types appear to be centrally important in the molecular pathology of the vascular lesions collectively known as atherosclerosis. The cellular parameters governing such events are the number, specificity and affinity of surface binding sites, while the particle parameters are local concentration and, possibly, duration of exposure. The consequences of interaction can vary greatly, ranging from selective uni- or bi-directional transfers of lipid components to the complete sequestration of particles followed either by their regurgitation in altered or unaltered form, or by their intracellular destruction and the disposal of residual constituents to intra- or extracellular destinations. In some cases, the nett result of these kinds of phenomena is the "loading" of the cell with lipoprotein-derived materials (usually lipids) while in others, lipids may be abstracted from cells, cellular lipids may end up with an altered composition or modified lipoproteins may accumulate in the cellular environment. Since most of the relevant cell types exist in organized tissues rather than in liquid suspension, extracellular matrices must also be taken into account as possible sites for lipoprotein or lipid deposition.

Many recent observations suggest that we will have a more complete understanding of atherosclerosis when we have systematically explored all the lipoprotein particles that can be differentiated in human plasma and lymph, or even in human tissues, in a great variety of circumstances. This information must be brought into experimental relation to all the cell types that have been detected in vascular lesions (notably macrophages, endothelial and smooth muscle cells) or those which make up the organs that play a quantitatively significant role in the lipid metabolism of the body (especially the liver). The breadth and sophistication of biochemical and cell-biological expertise required to carry out this task is very great indeed, but impressive progress has already been made.

In this presentation, examples will be described from our own studies and those of others, to illustrate the above conceptualizations.

Title : PATHOGENESIS OF THE ACUTE ISCHAEMIC SYNDROMES: SUDDEN DEATH, UNSTABLE ANGINA AND ACUTE MYOCARDIAL INFARCTION

Author : P J Commerford

Presented by: P J Commerford

Acute myocardial infarction and sudden death are the most important manifestations of atherosclerotic coronary artery disease. Acute myocardial infarction always results in loss of myocardium and prognosis is determined by the extent of residual, surviving myocardium. For many patients sudden death is the only manifestation of coronary artery disease. The techniques which are currently used for screening for coronary artery disease are unsatisfactory. They identify patients at risk of developing angina, but not those who will develop acute myocardial infarction or die suddenly.

Significant advances have been made in understanding the pathogenesis of the ischaemic syndromes. Coronary atherosclerotic lesions progress slowly and the progression may be accelerated by the so-called risk factors for atherosclerotic disease. Gradual stepwise progression of such lesions may ultimately lead to the development of flow-limiting stenoses and the clinical manifestations of angina pectoris - a condition with a relatively good prognosis.

More importantly, from the clinician's viewpoint, some lesions may progress very rapidly, almost certainly due to a complicating anatomic event. Plaque rupture, dissecting haemorrhage and most importantly, thrombosis have been demonstrated by means of serial coronary angiography during acute infarction and careful post-mortem studies to be important in the acute ischaemic syndromes. The understanding of the role of these processes in the important acute ischaemic syndromes of acute myocardial infarction, unstable angina pectoris and sudden death has resulted in significant changes in clinical management.

TRN: ZA8100108

(4)

Title : INHIBITORS OF VASCULAR SMOOTH MUSCLE PROLIFERATION

Authors : A Eldor, A du P Heyns, I Vlodavsky and A Panet

Presented by: A Eldor

Vascular smooth muscle (SMC) proliferation which occurs after endothelial injury is one of the initial events of atherosclerosis. Little is known about how the growth of cells in the vessel wall, induced by various growth factors is regulated. It has been shown that administration of heparin or related glycosaminoglycans inhibited SMC proliferation both in tissue culture and in animals subjected to mechanical denudation of the endothelial lining of their arteries. We have shown that endothelial injury is associated with the synthesis and release of interferon (IFN), a potent antiviral agent, and we and others have also shown that such injury is associated with the release of prostacyclin (PGI_2), a potent inhibitor of platelet activation. In the present study we investigated the effects of IFN and PGI_2 (using the stable PGI_2 analogue Iloprost, a gift from Schering, Berlin) on the proliferation of G_o G_1 arrested bovine aortic SMC.
The growth response, measured as ^{3}H-thymidine incorporation into DNA, was dependent on the concentration of the mitogen used (serum or purified platelet derived growth factor - PDGF, supplemented with plasma-derived serum-PDS).

Human IFNα, recombinant human IFNα_2, a crude bovine IFN preparation prepared from virus infected bovine aortic endothelial cells or Iloprost inhibited SMC growth induced by either serum or PDGF with PDS. The extent of proliferation inhibition was related to the concentrations of the inhibitors and the mitogenic stimulus. Low concentrations of IFN and Iloprost which did not affect SMC proliferation, had a synergistic anti-proliferative effect when administered together. We also investigated whether IFN inhibited the early events in G_1 phase stimulated by the competence factor PDGF or the progression of the cells into S phase induced by PDS. The results indicated that IFN inhibited these two stages of the G_1 phase independently. Hence, other endothelial products such as IFN and PGI_2 may be operative in addition to vascular glycosaminoglycans in the control of medial SMC proliferation and the arteriosclerotic process.

Title : ENRICHMENT OF THE LIPID HYPOTHESIS OF ATHEROSCLEROSIS BY MODERN DEVELOPMENTS (5)

Author : G M B Berger

Presented by: G M B Berger

Recent developments in the field of lipoprotein metabolism have greatly enriched the content of the term "lipid hypothesis", have contributed to an understanding of fundamental biology, and will improve our effectiveness in preventing atherosclerosis. In this paper I review some of the recent advances in our knowledge of lipid transport and its disorders and attempt to relate these to the traditional use of the term "lipid hypothesis". The modern era commenced in the 1940s with the separation of the plasma lipoproteins into distinct fractions by means of analytical ultracentrifugation by Gofman and colleagues. This opened the door to the chemical characterisation of the lipoprotein fractions and an increasingly refined description of lipid transport in terms of sites and mechanisms of synthesis, interconversions of lipoproteins in plasma and the interaction of lipoproteins with cells. These dynamic processes are governed by a multitude of proteins including the apolipoproteins, the cellular receptor proteins and those involved in the intracellular processing of internalised lipoproteins, the enzymes involved in lipid and apolipoprotein synthesis, and those acting on lipids within the circulation. The use of 2-dimensional protein electrophoresis in particular has revealed considerable polymorphism amongst the apolipoproteins and, together with the use of monoclonal antibodies, has also opened the door to an understanding of the LDL receptor defects underlying the disorder of familial hypercholesterolaemia.

The protein epoch, dating from the mid-1970s, has recently been complemented by recombinant DNA technology resulting in the cloning of most of the apolipoproteins, two cellular receptor proteins (the B/E and the E receptor) and a number of the enzymes involved in lipoprotein metabolism. The conceptual and technical tools available will allow an exploration of evolutionary development, of structural-functional relationships and of genetic variation or genetic defects presenting as clinical disease. It is now possible to describe some of the genetic hyperlipidaemias in molecular terms and many of the important remaining obscurities should be cleared up in the next few years. Therapeutic drug trials, currently applied to crudely defined hyperlipidaemic categories, will in future be undertaken in terms of specific disorders categorised at the molecular level. Similar refinement is increasingly possible in terms of dietary intervention and the effects of exercise. The use of recombinant DNA technology will permit earlier and more specific diagnosis of particular disorders and will enhance our understanding of polygenic effects on lipoprotein metabolism and atherogenesis. Concurrently with these developments cellular biology is also being approached in molecular terms and there is hope that in the future an integrated understanding of the interaction of the lipoprotein system with the cells participating in atherosclerosis will become possible. The thesis of this paper will be illustrated by specific examples from the literature as well as by work from our laboratory.

Title : THE EFFECTS OF HYPERLIPIDAEMIA ON VASCULAR REACTIVITY

Author : C Rosendorff

Presented by: C Rosendorff

A diet high in saturated fat sensitises resistance vessels to a variety of vasoconstrictor neurotransmitters, including noradrenaline and serotonin, independent of the atherogenic effects of the diet. This occurs in a variety of vascular beds, including the brain, myocardium and kidney. These effects are also produced by plasma from animals or patients with obstructive jaundice and type IIa hyperlipoproteinaemia, in both of which there is hypercholesterolaemia. Conversely, a polyunsaturated diet decreases coronary vascular resistance in the isolated rat heart.

Noradrenaline vasoconstrictor sensitivity depends primarily on the density and affinity of α_1 and α_2-adrenoreceptors on vascular smooth muscle membranes. In rabbits fed a diet rich in saturated fats both α_1- and α_2-receptor density (B_{max}) increased significantly, with no significant change in affinity (K_D). In rat ventricular myocardium β-adrenoreceptor affinity increased significantly in the polyunsaturated diet group.

Possible mechanisms for changes in adrenoreceptor number and affinity in response to the fat content and composition of the diet include changes in vascular smooth muscle membrane fluidity with externalisation or internalisation of adrenergic recognition and binding sites, or some conformational change of the receptor which alters the on-off kinetics of ligand.

Title : PHYSIOLOGY OF FIBRINOLYSIS AND ITS ROLE IN THROMBOLYSIS

Authors : H R Lijnen, D Collen

Presented by: H R Lijnen

The fibrinolytic system comprises a proenzyme, plasminogen, which can be activated to the active enzyme plasmin, that will degrade fibrin, by different types of plasminogen activators. Tissue-type plasminogen activator (t-PA) present or released into the blood was shown to be aphysiologically important plasminogen activator.

Fibrinolysis in the blood is regulated by specific molecular interactions between t-PA, plasmin(ogen), fibrin and α_2-antiplasmin. Plamin(ogen) contains structures called lysine-binding sites which play a key role in its interaction with fibrin and with α_2-antiplasmin. In plasma the affinity of plasminogen for t-PA is low and no systemic plasminogen activation by t-PA occurs. Plasmin, if formed, is efficiently neutralized by α_2-antiplasmin. In the presence of fibrin, however, the affinity of plasminogen for fibrin-bound t-PA increases dramatically and efficient plasminogen activation occurs. The formed plasmin, which remains transiently complexed to fibrin, both by its lysine-binding site(s) and active center, is only slowly inactivated by α_2-antiplasmin. The fibrinolytic process thus seems to be triggered by and confined to fibrin.

Impairment of fibrinolysis may occur at several levels : deficient synthesis and/or release of t-PA from the vessel wall, deficiency or functional defects of plasminogen or fibrinogen, increased levels of inhibitors. The short half-life of t-PA in vivo (5-10 min) has been explained by rapid clearance in the liver, although plasma contains a specific fast-acting inhibitor of t-PA present in very low concentration in healthy individuals but in markedly elevated levels in several pathological conditions.

Several families have been described with congenital deficiency of α_2-antiplasmin, the fast-reacting physiological plasmin inhibitor in plasma. The homozygous and some heterozygous patients show a bleeding tendency.

Excessive activation of the fibrinolytic system and/or deficiency of inhibitors cause excessive plasmin formation and a bleeding tendency, while insufficient activation and/or excess inhibitors might be related to thrombus formation.

Title : ROLE OF PROSTAGLANDINS IN ATHEROSCLEROSIS AND THROMBOSIS (8)

Authors : J Vermylen and H Deckmyn

Presented by: J Vermylen

The role of prostaglandins in atherosclerosis and thrombosis has been a major topic of research during the past decade. The biological effects of thromboxane A_2 and prostacyclin have drawn much attention, with major emphasis on their opposite actions, resulting in the concept that the interplay of these two labile arachidonic acid metabolites is delicately balanced. We wish to emphasize the role that peroxides play in the regulation of the synthesis of eicosanoids and to suggest that altered peroxide levels may greatly disturb the thromboxane/prostacyclin balance.

Formation of thromboxane and prostacyclin depends on the release of arachidonic acid from phospholipid stores and on the oxygenation of this unsaturated fatty acid by cyclo-oxygenase. This enzyme introduces two oxygen molecules : one is involved in the formation of the cyclical structure, another in the generation of a peroxide function. In this way arachidonic acid is transformed into prostaglandin G_2. Subsequently, the peroxide function is reduced to a hydroxyl function; the resulting compound is prostaglandin H_2. Prostaglandin H_2 is the substrate for the formation of thromboxane A_2 and of prostacyclin in platelets and endothelial cells respectively.

In order to work under optimal conditions, the cyclo-oxygenase needs a number of cofactors : peroxides for the oxygenation reaction, reducing cofactors for the peroxidase reaction. In platelets, the rate of thromboxane A_2 generation seems mainly to be governed by the level of peroxides. Prolonged generation of prostacyclin by vessel wall on the other hand seems largely to be dependent on the availability of reducing cofactors for the peroxidase. An increased level of peroxides results in an enhanced production of thromboxane A_2 by platelets, and in a shortened generation of prostacyclin in areas of chronic vascular irritation. Excessive lipid peroxidation may be involved in the platelet and vessel wall dysfunctions occurring in atherosclerosis, toxaemia of pregnancy, diabetes mellitus or the haemolytic uraemic syndrome, or following the reperfusion of hypoxic tissue. Plasma contains several low molecular weight reducing cofactors for the peroxidase. Certain antithrombotic drugs (dipyridamole, nafazatrom) have similar properties and prolong prostacyclin formation by perfused vascular tissue in situ or by irritated vessels in vivo.

Title : WHOLE BLOOD THROMBOXANE A_2 AND PGI_2 PRODUCTION IN HYPERLIPIDAEMIA

Author : P N Badenhorst

Presented by: P N Badenhorst

The prevalence of familial hypercholesterolaemia among Afrikaans speaking white South Africans is 6-7 times greater than that in European and American whites. This finding is often used to explain the very high coronary heart disease death rate in this particular population group. The discovery of thromboxane and prostacyclin and the demonstration of their dramatically opposite effects on platelet aggregation and vascular tonicity resulted in the formulation of the so-called balance hypothesis. This hypothesis states that platelet aggrebility and arterial thrombosis tendency is determined by the ratio between these two substances: any shift towards thromboxane being prothrombotic and towards prostacyclin antithrombotic.

The aim of this study was to investigate the role of the balance between thromboxane and prostacyclin synthesis in the pathogenesis of the thrombotic tendency of hypercholesterolaemia.

Whole blood thromboxane A_2 and prostacyclin production in the platelets and leukocytes respectively, was measured in eight patients with familial hypercholesterolaemia and in eight age and sex matched healthy volunteers. Citrated blood samples were taken after 14h fasting and at the peak of postprandial lipaemia after a fatty meal containing 1,5g fat / 100ml plasma volume (P/S = 0.35). Prostaglandin generation in the blood samples was stimulated with collagen. Aliquots were removed at regular intervals and thromboxane B_2 and 6-keto-prostaglandin F_{1alpha}, the stable degradation products of thromboxane A_2 and prostacyclin respectively, were measured by radioimmunoassay.

Fasting thromboxane B_2 levels were significantly higher in the patients than in the controls ($p<0.001$). Postprandial thromboxane B_2 levels increased significantly in the control group ($p<0.005$), but not in the hypercholesterolaemic group ($p>1.6$). However, in the patient group the postprandial thromboxane B_2 levels were still significantly higher than those of the control group ($p<0.05$). Fasting 6-keto-prostaglandin F_{1alpha} levels did not differ significantly in the two groups ($p>0.7$). After the fatty meal the 6-keto-prostaglandin F_{1alpha} levels increased significantly in the control group ($p<0.05$), but not in the patient group ($p>0.1$). Thus in patients with familial hypercholesterolaemia, because of the increase in thromboxane A_2 production, the whole blood thromboxane/prostacyclin balance is shifted in favour of a thrombotic tendency. This finding was supported by the evidence of in vivo platelet activation as reflected by an increase in circulating platelet aggregates ($p<0.05$).

These results strengthen the view that a disturbance in the balance between thromboxane and prostacyclin may play a role in the pathogenesis of the thrombosis tendency of hypercholesterolaemia.

Title : DIETARY MODIFICATION OF PLATELET FATTY ACIDS (10)

Author : N Chetty

Presented by: N Chetty

Growing interest in n-3 fatty acids, prompted by suggestions that these could be beneficial in protecting against heart disease, has led to investigations of the response of platelets to the long chain n-3 fatty acids found mostly in marine animals. In these series of experiments 4 groups of normal volunteers participated in short term dietary experiments. Group 1 received a diet rich in eicosapentaenoic acid (EPA) (20:5 n-3) (marine source) and poor in arachidonic acid (AA) (20:4 n-6). All animal protein foods were excluded and 250-400g of fish (sardines, pilchards, herring) were ingested daily. Group 2 received a vegetarian diet poor in AA. All animal protein foods except for milk and cheese were excluded. Group 3 received a normal diet supplemented with 1.8g EPA. No dietary restrictions were imposed and all foods were allowed. Group 4 subjects received a diet rich in EPA (fresh water source - trout) and poor in AA. In all groups blood samples were tested before and after 4 weeks of the experimental diet.

In group 1 subjects the ratio of EPA to AA in whole platelets increased from 0.03 to 0.09 ($p<0.05$). Linolenic acid (18:3) increased from 1.0 $\pm$ 0.44 to 2.84 $\pm$ 1.52 ($p<0.05$) and dihomolinolenic acid (20:3) decreased from 0.89 $\pm$ 0.36 to 0.56 $\pm$ 0.29 ($p<0.05$). These changes were accompanied by a significant reduction in platelet aggregation in response to ADP, collagen and epinephrine, a reduction in thromboxane production by the platelets after stimulation and a decrease in total cholesterol. In group 2 subjects the arachidonic acid in whole platelets increased significantly and is explained by the increase in 18:2 and 18:3 intake during the vegetarian diet. Thromboxane production remained unchanged and aggregation responses to epinephrine and arachidonic acid increased. In group 3 subjects, 20:5, 22:5 and 22:6 increased significantly and 16:0 decreased significantly. Bleeding times were prolonged. There were no significant changes in platelet aggregation despite the above findings - although the height of aggregations tended to decrease. In group 4 subjects, 20:5 and 22:6 increased significantly. Bleeding times were prolonged. Platelet aggregations decreased in response to AA stimulation but were increased in response to collagen.

In conclusion these studies remain compatible with the proposition that dietary alterations in platelet membrane fatty acids, delay primary haemostasis. However, the mode of action of the fish unsaturated fatty acids requires re-evaluation in the light of the aggregation studies obtained after the trout diet.

Title : THE COMPLETE AMINO ACID SEQUENCE OF THE ERYTHRINA INHIBITOR OF TISSUE PLASMINOGEN ACTIVATOR (11)

Authors : Eugene B Dowdle, E H Merrifield and F J Joubert

Presented by : Eugene B Dowdle

The seeds of the South African legume Erythrina latissima contain a Kunitz-type trypsin inhibitor which is unique amongst similar protease inhibitors in its ability to inhibit tissue plasminogen activator. The complete amino acid sequence of the Erythrina protein has been determined. It shows extensive homology with Soybean trypsin inhibitor and Wingbean trypsin inhibitor - both proteins that have no effect on tissue plasminogen activator. The sequence of Wingbean trypsin inhibitor at the reactive site arginine is identical to that of the Erythrina inhibitor over three residues indicating that the specificity does not reside in this short sequence.

The Erythrina trypsin inhibitor may be coupled covalently to agarose to serve as an efficient affinity reagent for the one stage purification of tissue plasminogen activator.

TRN: 2A8700109

(12)

Title : FACTORS REGULATING THROMBUS FORMATION IN BABOONS

Author : S R Hanson

Presented by: S R Hanson

We have developed a baboon animal model for studying the role of physical and physiologic variables in determining the formation of platelet-fibrin thrombi in vivo. To initiate thrombus formation, both smooth and textured materials in a tubular configuration (collagen, synthetic polymers, vascular grafts) were incorporated as extension segments of a femoral arteriovenous shunt. Steady-state thromboembolic utilization of platelets was assessed by measuring the survival of platelets labelled with ^{51}Cr, ^{131}I or ^{111}In. The acute deposition of ^{111}In-platelets and ^{131}I-fibrinogen was measured quantitatively using a gamma scintillation camera. Variables of interest included the blood flow rate (arterial vs venous), the circulating platelet count, and the effects of heparin and specific platelet inhibitors.

Ten smooth-walled synthetic polymers (polyurethanes) accumulated platelets in an irregular, multifocal and changing pattern. Steady-state platelet destruction (corrected for senescent platelet removal) was directly proportional to the area of exposed surface and ranged from 2-25 x 10^8 platelets/cm^2-day. Platelet consumption was unaffected by heparin anticoagulation, and remained constant for each material of a given type despite 3-5 fold variations in blood flow rate and circulating platelet concentration. These results indicate that platelet utilization by smooth walled polymers is limited by the inherent reactivity of the surface, rather than by the availability of circulating cells.

Both knitted Dacron vascular grafts and tubings coated with Type I rat skin collagen (4 mm i.d.) were highly thrombogenic in this system, accumulating 15-30 x 10^9 platelets/cm^2 after exposure for 1 hour. The net accumulation of platelets onto both surfaces showed approximately a one-half order dependence on blood flow rate, and a dependence upon platelet count that was always greater than first-order, i.e., a doubling in platelet count between individual animals produced a greater than two-fold increase in platelet deposition. Therefore, variations in platelet count, even within the normal range, may be of considerable importance in models of experimental thrombosis and perhaps in some clinical settings.

When grafts were placed (80 cm) that removed 59 $\pm$ 5% of all circulating platelets within one hour, the platelets remaining in the circulation were normal with respect to size, density, and α-granule contents, but showed reduced levels of dense granule ADP. However, since dense granule ^{14}C-serotonin was not reduced relative to platelet ^{111}In, we conclude that reduced ADP levels may result from the selective removal of ADP-rich platelets, rather than as a consequence of platelet activation and release in vivo.

The critical importance of fibrinogen-mediated pathways of platelet accumulation was subsequently demonstrated. When two monoclonal antibodies against the platelet glycoprotein IIb/IIIa receptor for fibrinogen were injected intravenously, greater than 80% inhibition of graft platelet deposition was observed. Similar results were achieved by injecting a synthetic peptide

Title : FACTORS REGULATING THROMBUS FORMATION IN BABOONS (cont'd)

Author : S R Hanson

Presented by: S R Hanson

representing the platelet binding sequence on the fibrinogen molecule. Conversely, no reduction in graft platelet deposition was achieved with either conventional heparin anticoagulation, or oral aspirin therapy at doses which completely blocked platelet thromboxane A_2 formation.

Title : INTERACTION OF PLATELETS WITH THE SUBENDOTHELIAL EXTRACELLULAR MATRIX

Authors : A Eldor and I Vlodavsky

Presented by: A Eldor

The extracellular matrix (ECM) produced by cultured endothelial cells has recently served as an in vitro model in the study of platelet-subendothelium interactions. We shall describe several aspects of hemostasis which can be investigated with the ECM model including:

1) The role of platelet-fibrinogen interactions - studied with platelets from thromboasthenic patients, platelets treated by a monoclonal antibody that blocks the GPIIb/IIIa complex and washed platelet suspensions

2) Aggregation-induced release reaction as compared to adhesion-induced release reaction

3) Platelet interactions with an ECM covered with a subconfluent culture of vascular endothelial cells - a model to study the effect of platelet inhibitor drugs

4) The production of a metabolically labelled ECM to study the degradation of ECM constituents by platelet enzymes. Studies on platelet hepartinase using ^{35}S-labelled ECM will be presented.

5) The ECM can serve as a model for the activation of isolated megakaryocytes (MK). MK adhesion, shape change, TXB_2 production and fragmentation induced by the ECM will be demonstrated, as well as the interaction of MK with the ECM in a flow system, under defined shear forces.

TRN: ZA8700110

Title : THE KINETICS AND SITES OF SEQUESTRATION OF PLATELETS IN ARTERIAL DISEASE (14)

Author : A du P Heyns

Presented by: A du P Heyns

The physical characteristics of In-111 make it possible to label platelets with higher specific activity and, with computer-assisted image analysis, to image the in vivo distribution of labelled platelets quantitatively. Since In-111 elutes less readily from platelets than Cr-51, measurements of In-111-platelet kinetics are also now more reliable.

The study of the kinetics of platelet deposition in aortic aneurysms has provided new insights. Platelets accumulate relatively slowly in the aneurysm and reach equilibrium and maximal activity only after 24 to 48 hours. A plateau is then maintained, suggesting that those platelets adhering to the wall of the aneurysm are in dynamic equilibrium with platelets in the circulation. The view that the deposition of the platelets on the vessel wall is temporary, was confirmed by the finding that the labelled platelets are sequestered normally in the different components of the reticulo-endothelial system.

In half of ten patients with heterozygous familial hypercholesterolaemia studied, mean In-111-platelet survival was shortened; in the others it was normal. The pattern of sequestration of platelets at the end of their life-span in the circulation, was also normal. However, there was evidence that an excess number of platelets were deposited in the microvascular circulation of the lower limbs of these patients. This could be demonstrated by comparing the rate of clearance of In-111-labelled platelets from the peripheral blood with that from the feet. The transit time of In-111-platelets was prolonged in the latter instance.

This finding suggested that the rate of clearance of labelled platelets from the peripheral tissues may reflect either the presence of intravascular platelet aggregates or in vivo platelet activation. This was further investigated in patients with atherosclerosis of the aorta or ileofemoral arteries. Survival, sites of in vivo deposition, and the sequestration pattern of In-111-labelled platelets were determined. The deposition, transit time and rate of clearance of the labelled platelets was measured in both feet with a NaI detector. The clearance of platelets from the feet was slower than normal and did not parallel the platelet survival curve constructed from blood In-111 measurements. This finding may be ascribed to at least two mechanisms: first, the transit time of activated platelets or platelet aggregates through the small vessels may be prolonged or, second, there may be sequestration of the activated or aggregated platelets in the reticuloendothelial component of the bone marrow of the tarsal bones. It would seem that this technique may provide a sensitive measurement of arterial disease, in vivo platelet activation, or for measuring the efficacy of antiplatelet drugs.

We conclude that In-111 is superior to Cr-51 as a platelet label.. It is now possible to measure the kinetics of labelled platelets accurately. The results will lead to a better understanding of the pathogenesis, and facilitate the diagnosis of arterial disease. These techniques also provide a new approach for demonstrating in vivo platelet activation.

Title : ANTITHROMBOTIC STRATEGIES FOR CARDIOVASCULAR DEVICES

Authors : L A Harker and S R Hanson

Presented by: L A Harker

Simulating human thrombotic syndromes in baboons permits objective, quantitative selection of agents and dose regimens that are truly antithrombotic in vivo. Moreover, this approach facilitates comparisons of different agents used singly or in combination.

To resolve questions of drug actions, efficacy, and interactions for platelet-modifying agents used clinically, the relative capacities and mechanisms of aspirin, dipyridamole, sulfinpyrazone, ticlopidine, suloctidil and dazoxiben were compared in the chronic steady-state arterial thromboembolism induced by arteriovenous cannulae. When given alone, dipyridamole, ticlopidine and sulfinpyrazone reduced in a dose-dependent manner the rate at which platelets were utlized by thrombus formation. In contrast, aspirin, suloctidil and dazoxiben failed to decrease cannula platelet consumption detectably. However, despite equivalent suppression of the synthesis of thromboxane B_2, aspirin, but not dazoxiben, potentiated the antithrombotic effects of dipyridamole and sulfinpyrazone in a dose-dependent fashion. Aspirin's potentiating effects are produced by mechanism(s) unrelated to its potent, irreversible inhibition of platelet cyclo-oxygenase.

To assess the effects of platelet-modifying drugs on acute thrombus formation, the deposition of ^{111}In platelets and ^{131}I-fibrinogen on knitted vascular grafts has been quantified using gamma camera imaging under high and low flow rates. Whereas ticlopidine and the combination aspirin/dipyridamole effectively reduce acute thrombus deposition, aspirin, suloctidil, sulfinpyrazone and dipyridamole fail to manifest significant effects when used singly. The antithrombotic effects of antiplatelet agents are substantially enhanced by the addition of heparin, although a conventional anticoagulating dose of heparin alone is without effect. Interestingly, the injection of murine monoclonal antibodies against the platelet GPIIb/IIIa fibrinogen receptor blocks thrombus deposition in parallel with the inhibition of platelet hemostatic function. The potency and duration of effects are dose-dependent. A synthesized peptide with high affinity binding for the same receptor, similarly produces dose-dependent interruption of thrombus deposition but of shorter duration than that produced by the monoclonal antibody.

To measure directly and in vivo the rates and time course of arterial thrombus formation and the effects of therapy, experimental iliac artery thrombosis was studied. ^{111}In-platelet activity accumulated progressively in the thrombus reaching a maximum after four days. Treatment with a combination of aspirin and dipyridamole begun immediately after graft surgery reduced ^{111}In-platelet deposition significantly, but only at time points greater than 48 hours.

Since thrombolytic therapy with tPA for acute thrombotic syndromes is associated with significant immediate recurrent thrombotic occlusion, characterization of the relative dose and duration effects of infused tPA on forming thrombus is needed in

Title : ANTITHROMBOTIC STRATEGIES FOR CARDIOVASCULAR DEVICES

Authors : L A Harker and S R Hanson

Presented by: L A Harker

developing strategies to prevent reocclusion. In these studies the deposition of both ^{111}In-platelets and ^{131}I-fibrinogen were measured under high and low flow rates with local and systemic infusion. tPA reduced the amount of thrombus formed in a dose-dependent manner that affected ^{131}I-fibrinogen deposition more profoundly than ^{111}In-platelet deposition. Although the effect dissipated within minutes after discontinuing therapy, continued infusion of tPA at reduced dosage may prevent subsequent thrombus accumulation.

These quantitative models in baboons appear to provide quantitative and objective strategies for optimizing complex antithrombotic therapies.

TRN: ZA8700112

Title : BLOOD CELL ISOLATION (16)

Author : M R Hardeman

Presented by: M R Hardeman

Since most popular techniques for blood cell labelling using ligands are not specific with respect to the kind of cell, separation or at least enrichment of the cell type under study is needed, either before or after the labelling procedure. In the literature there are many techniques described yielding more or less pure cell concentrates. The techniques currently available can be divided into 3 categories, those exploiting differences in physical properties, those in which separation is based upon differences in properties of the cell surface, and those separating cells on the basis of their functional characteristics. To the first category physical properties belong cell density, size and charge as well as optical properties. Techniques based on differences in cell surface properties mainly exploit the ability of some types of cells to adhere strongly to surfaces: however, the increasing availability of monoclonal antibodies has led to a wide application of antibody-defined surface markers. Functional properties that have been used in cell separation encompass proliferation, phagocytosis and antigen recognition.

Special demands which are necessary for specific purposes, as in our case the in vivo use of a radiolabelled cell suspension, limit considerably the choice of the currently available methods. It is clear that all negative selection procedures leading to lysis or fragmentation of the cell kind under study cannot be used here, not to speak about the absolute necessity that a certain method must include the possibility of maintaining sterility. Furthermore, it is not sufficient that the physical appearance of a cellular suspension i.e. cell count, electron microscopic picture, etc. remain normal. Maintenance of viability as well as function(s), relevant to the type of study, are crucial conditions that have to be met by a suitable isolation method.

Most separation methods provide enrichment of a cell population rather than true purification. Although there are situations i.e. cell kinetic studies, where a pure suspension is desirable, a mixed cell suspension, enriched in the cell kind under study, is often adequate in most clinical studies.

Depending on the type of ligand in use, labelling efficiency is more or less impaired by the presence of plasma. Discussions are therefore still going on as to whether a lower labelling efficiency of the cells remaining in plasma, necessitating an extra wash step (centrifugation, resuspension of the pellet), outweighs the extremely high labelling efficiencies (90% or more) obtained when the cells are resuspended during the separation procedure in buffered saline, yielding a preparation ready for injection. Here a general problem i.e. the lack of suitable routine in vitro tests, reflecting the behaviour of the cells after injection, comes to the fore.

TRN: ZA8700113

Title : LABELLING AUTOLOGOUS PLATELETS OF THROMBOCYTOPENIC PATIENTS

Author : P Wessels

Presented by: P Wessels

The major advantages of In-111 is that the high labelling efficiency for platelets and the high gamma photon emission permits the labelling of platelets with a high specific activity. This permits quantitative imaging of the in vivo distribution of In-111-labelled platelets and the determination of platelet survival with platelets isolated from relatively small volumes of blood. These advantages of In-111 have been exploited to label autologous platelets of patients with severe thrombocytopenia and to study the kinetics of these platelets.

Twenty patients with immune thrombocytopenia and platelet counts ranging from 5 to 119 x 10^9/ℓ were investigated. Platelets were isolated from blood by differential centrifugation. Platelets remaining in the red cell layer were isolated by a repeated washing procedure. The platelets were labelled with In-111 in saline. A mean of 55 ± 21% were harvested from the blood and labelled with a 49 ± 24% efficiency. A total of 15,8 x 10^8 labelled platelets were reinjected. Contamination of the platelets with red cells was 1,4 ± 1,6%; 7,3 ± 5,9% In-111 was bound to plasma proteins. The labelled platelets were viable as assessed by in vitro aggregation. Recovery in the circulation, at equilibrium, was normal (55 ± 22%).

This method is simple, does not adversely affect platelet function and it is possible to harvest and label sufficient numbers of a fully representative population of platelets for kinetic studies in the presence of severe thrombocytopenia.

TRN: ZA8700114

Title : NEW TECHNIQUES AND STANDARDIZATION OF PLATELET LABELLING

Author : A du P Heyns

Presented by: A du P Heyns

The measurements of platelet survival and the in vivo distribution of labelled platelets are probably the most specific and sensitive methods of demonstrating in vivo platelet activation. Indium-111 has now firmly replaced Cr-51 as the radionuclide of choice for the labelling of platelets. The platelets may be labelled with high specific activity and, exploiting the technology of computer-assisted image analysis, the organ or regional distribution of the tagged platelets accurately quantified and imaged. Although oxine is a suitable chelate for binding In-111 to intracellular proteins, it has the disadvantage that platelets are labelled with low efficiency in the presence of plasma in the medium. In this regard, two recently described chelates, tropolone and mercaptopyridine-1-oxide (Merc), are somewhat superior.

We have experience with In-111-tropolone as platelet label. The labelling efficiency with this chelate is high; it is easy to remove contaminating proteins also labelled with the In-111; and the results of measurements of in vivo distribution and mean platelet survival time are similar to results previously obtained with In-111-oxine. There is no evidence of excessive in vivo elution of the label from platelets. More important, the severity of the "collection injury" seems to be less pronounced than with In-111-oxine. This could be demonstrated by comparing the extent and duration of the transient accumulation of labelled platelets in the liver immediately after reinjection of the labelled platelets: it was less with tropolone than with oxine. Labelling with tropolone as chelate has at least one other important advantage; platelets may also be labelled with In-113m-tropolone. In-113m is a short half-life radionuclide with physical characteristics advantageous for the study of platelet kinetics under certain specific circumstances. This label permits scintigraphy of in vivo platelet distribution; quantification of the labelled platelets in organs or regions; and even the measurement of mean platelet survival in diseases such as immune thrombocytopenic purpura, characterized by short platelet survival. It is also possible in the same patient, to simultaneously study the kinetics of different populations of platelets labelled either with In-111, In-113m or Cr-51. This may be of value for the investigation of the pathogenesis, and assessing the response to various forms of treatment in immune thrombocytopenic purpura.

Although we have no personal experience with the recently introduced Merc, a survey of the literature indicates that this chelate has some promise.

TRN: 2A8700115

Title : RADIOPHARMACEUTICAL ASPECTS OF CELL LABELLING (19)

Author : R D Neirinckx

Presented by: R D Neirinckx

Amersham International has dedicated a large effort to advance In-111 oxine to the NDA stage. With hundreds of users - and concommitant physician's Ind's - in the USA, the commercial availability of In-111 oxine will amplify the use of the product and simplify the regulatory situation.

A study of the microdosimetry of In-111 labelled leukocytes reveals that the doses delivered by the isotope after a typical cell labelling procedure are very high. A review of alternative isotopic labels will be given.

Although other isotopes will be desirable, the licensing requirements for new drug entities will of necessity delay the introduction in the main - US - market, so that for the foreseeable future, In-111 oxine will be the method of choice for all labelling.

TRN: ZA8700116

Title : PLATELET KINETICS

Author : A M Peters

Presented by: A M Peters

Following i.v. injection, radiolabelled platelets become distributed within a volume which is effectively larger than the total blood volume. The additional space can be described as the extravascular platelet pool. A considerable fraction of it is present in the spleen, with the remainder in the liver and some possibly also in the lung.

The intrasplenic platelet pool enlarges as the spleen enlarges and may increase, as a fraction of the total platelet population, from the normal of about 30% to as much as 90%. This increase is achieved largely as a result of an increase in splenic blood flow (SBF) with the mean intrasplenic platelet residence time (t) remaining more or less constant. We have measured t in a variety of conditions associated either with abnormal spleens or with abnormal platelets in an attempt to define some of the factors which may control intrasplenic platelet traffic. Our only positive finding so far is that t shows a non-linear inverse relationship with splenic perfusion (i.e., SBF/spleen volume). An interesting group of patients has been identified in whom SBF is enormously elevated in spite of only minimal splenomegaly and in these t is reduced, presumably secondary to the SBF changes. These patients have various immune complex disorders which eventually may result in splenomegaly and it may be that increased SBF is a stimulus for increase in spleen size. We have also studied t in conditions in which mean platelet lifespan (MPLS) is severely shortened. Special techniques, such as deconvolution analysis, are necessary to measure t in these circumstances, but no clear primary disturbances in t have been identified even in patients in whom the spleen extracts platelets with an efficiency of 50%.

Another probable platelet pool is the liver. Studies of the kinetics of this pool are complicated by this organ's sensitivity to platelet damage or activation during the labelling procedure. This so-called collection injury is minimized if the platelets are labelled with 111-indium in plasma using tropolone. With this technique, the kinetics appear to be consistent with uptake into a physiological pool. This results in an intrahepatic mean platelet transit time of about 1.5 times that of 99m-technetium labelled red cells. In contrast to liver and spleen we have been unable to confirm earlier suggestions that the lung is an additional site of platelet pooling. Thus we found 111-indium labelled platelets to have a mean transit time almost identical to that of 99m-technetium labelled red cells.

TRN: ZA8700117

Title : EVALUATION OF MATHEMATICAL MODELS TO ASSESS PLATELET KINETICS

Authors : M G Lötter, A du P Heyns, P N Badenhorst, P Wessels, J M van Zyl, H F Kotze and P C Minnaar

Presented by: M G Lötter

Twelve mathematical methods used to calculate the mean platelet survival time were compared by determining the "goodness of fit" of the models to the platelet survival curves of fifteen reference subjects and 54 patients. Platelets were labelled with indium-oxine (In-111). The linear (LN), exponential, weighted mean, multiple hit (MH), Dornhorst (DH), Meuleman (ML), alpha order (AO) and polynomial (PO) mathematical models were investigated. The "goodness of fit" for the exponential model was determined by the nonlinear least squares method (EP), and also by the linear least squares method on logarithmically transformed data (EX) as is recommended. The modified weighted mean (MWM) and the usual weighted mean method (WM) obtained with these exponential models were tested. The Dornhorst (DH10) and Meuleman (ML10) models, where the potential age-dependent platelet survival times were kept constant at 10 days, were also evaluated. The "goodness of fit" results, expressed as percentage standard deviation, indicated that the LN (5.2%), EX (5.0%), EP 4.4%), WM (3.7%), DH10 (3.7%) and ML10 (3.7%) models all fitted the data significantly worse than the MWM, MH, DH, ML, AO and PO models (Range 3.2% to 3.3%). The mean platelet survival time determined with the MH model differed significantly from the results with the DH, ML and AO models. The results of mean platelet survival time calculated with different mathematical models can therefore not be compared directly. The models that fitted the platelet survival curve well varied slightly in sensitivity to noise as is indicated by the coefficient of variation of the mean platelet survival time estimates for the reference subjects (Range 7.9% to 12.0%). Fitting data to at least two mathematical models has definite advantages. If the mean platelet survival time estimated with the alpha order model is shorter than that estimated with the EP, MWM or MH models, or if the mean platelet survival time estimated with either the DH, ML, AO or PO models is longer than the LN, MWM or MH estimate of the mean platelet survival time, the data on which the calculations are based are probably invalid. We conclude that the mean platelet survival time can be reliably estimated by fitting the data to either the MWM method (if limited computing facilities are available) or the MH model. The confidence in the result will be increased if it is considered in conjunction with the finding obtained with one other model: In those cases where the platelet survival time is very short, the alpha order model is recommended. In other instances the results of the MWM method or MH model should be compared to that obtained with either the DH, ML, AO or PO models.

Title : GRANULOCYTE KINETICS

Author : A M Peters

Presented by: A M Peters

Intravascular granulocytes are thought to be distributed dynamically between two compartments, the marginating (MGP) and circulating (CGP) pools which together make up the total blood granulocyte pool (TBGP). This concept is based on (1) morphological observations through the microscope on living blood vessels, in which granulocytes can be seen to be "rolling" along the endothelial surface or moving rapidly in the axial stream, and (2) the kinetics of granulocytes radiolabelled with DF32-P. When the latter were given by i.v. injection, only 50% of the dose (the recovery) could be accounted for in the circulating blood volume. That the other 50% was physiologically marginated in blood vessels was demonstrated by showing that following exercise or adrenaline infusion the recovery could be increased to 80%.

Attempts to quantify the distribution of the MGP in man using the gamma emitting radiolabel, 111-indium, encountered difficulties because the neutrophil separation and labelling procedures resulted in artifactual early neutrophil biodistribution, the principal feature of which was intense pulmonary sequestration immediately following injection. We have developed techniques of neutrophil isolation and labelling which leave the cells fit for kinetic studies, and have quantified the distribution of the MGP in man. A number of techniques have been used: compartmental and deconvolution analysis of the time activity curves recorded by gamma camera following i.v. injection, quantification of the whole body distribution of activity and comparison of the signals emitted from 111-indium labelled neutrophils with those from previously injection 111-indium labelled red cells. We found that the MGP, which comprised 60% of the TBFP, was itself distributed between spleen (32%), liver (32%) and lung (10%), with 26% elsewhere. Neutrophil half-time in blood in normal subjects was 6.9 hr. with this labelling method, 6.6 hr. in patients with evidence of sepsis and 5.8 hr. in patients without evidence of sepsis. That the half-time in patients with sepsis was not significantly reduced, is interesting and implies that the normal routes of granulocyte migration or disposal were proportionately blocked.

111-Indium labelled neutrophils were also used to clarify the normal routes of neutrophil disposal. Neutrophils have previously been thought to end their lives in the tissues following migration from the vascular space, even in non-inflamed man. However we found that, except for losses into visible septic foci, 111-indium granulocytes were disposed of in the RES, mainly bone marrow and spleen. Extremely low recoveries of 111-indium in faeces, urine and saliva (<2%) seems to argue against migration into the peripheral tissues as an important process in the normal subject.

TRN: ZA8700119

Title : SITES OF SEQUESTRATION AND RADIATION DOSE OF IN-111-LABELLED PLATELETS (23)

Author : H F Kotzé

Presented by: H F Kotzé

The introduction of In-111 as an efficient platelet label made it possible to image and quantify the in vivo distribution of In-111-labelled platelets. Since platelets can be labelled with a high specific activity, radiation becomes important and this may curtail the clinical application of In-111 in the study of in vivo platelet kinetics.

The mean platelet lifespan in normal humans is approximately 10 days. Since a random population of platelets is labelled with In-111, the sites of sequestration of senescent platelets can only be determined at the end of their lifespan. Using the geometrical mean method of quantification in 6 normal humans, we determined that 35.6 ± 9.7% of all injected labelled platelets were sequestrated in the spleen. The liver sequestrated 28.7 ± 8.3%. Thus, about 36% of the labelled platelets were sequestrated outside the liver and the spleen. The sites of sequestration of these platelets were determined in 5 normal baboons. The baboons were sacrificed and dissected after autologous In-111-labelled platelets were cleared from the circulation. Radioactivity in the different organs was determined in a whole body counter and expressed as a percent of total radioactivity. The spleen sequestrated 33.6 ± 4.1% of the injected labelled platelets and the liver 36.7 ± 7.1%. Bone marrow sequestration was 14.4 ± 1.7%. The remaining 15.5 ± 4.0% were distributed amongst the other tissues. Hence, most senescent platelets were sequestrated in the spleen, the liver and the bone marrow. The remainder was probably used to maintain vascular integrity, or was sequestrated by the macrophages outside the three major sites of sequestration.

It is necessary to use relatively large amounts of In-111 to ensure the acquisition of good quality images, i.e. 15 - 23 MBq (0.4 - 0.6 mCi). We have estimated the radiation dose to the whole body and different organs that is received from 37 MBq (1 mCi) In-111. The calculations were done as recommended by the Medical Internal Radiation Dose Committee. The spleen received the highest radiation dose, i.e. 7.89 MGy. However, with the amounts of In-111 used in our studies the radiation dose to the spleen and other organs remained well within the safety limits laid down by the International Committee of Radiation Protection. In fact, one can use up to 86 MBq (2.3 mCi) In-111, and still be within the safety limits. It is important to note that In-114M (halflife = 49.5 days), which can contaminate the In-111-product, may increase the radiation dose. Contamination with 0.08% may increase the total weighted radiation dose by as much as 15%.

Title : DUAL ISOTOPE SCINTIGRAPHY AND QUANTIFICATION OF PLATELET DEPOSITION

Author : H Pieters

Presented by : H Pieters

Title : ANTITHROMBOTIC THERAPY IN CEREBROVASCULAR DISEASE

Authors : L A Harker and G J Del Zoppo

Presented by: L A Harker

The development of effective antithrombotic therapies for cerebrovascular disease constitutes an important public health strategy since stroke is the third leading cause of death and the commonest cause of morbidity in developed countries, and because vascular occlusion by thrombus is the precipitating cause of stroke in the majority of patients.

Aspirin has been convincingly shown to reduce stroke or death in men with transient ischemic attacks and may possibly be beneficial to women also. No additional benefit has been shown by combining aspirin with dipyridamole or sulfinpyrazone. The results with aspirin do not discriminate between platelet thrombi or vasospasm as the underlying mechanism of transient ischemic attacks since aspirin could block both thromboxane-mediated platelet aggregate formation and thromboxane-mediated vasospasm. There is a clear need to define the optimal dosage regimen since the gastrointestinal side effects of aspirin are dose-dependent. Oral anticoagulation is ineffective in reducing stroke and death in this disorder.

A number of antiplatelet agents have been evaluated regarding the secondary prevention of stroke, including aspirin, dipyridamole, sulfinpyrazone and suloctidil. No benefit has been observed with any of these platelet-modifying agents when used alone. Similarly, oral anticoagulation is ineffective in reducing stroke secondarily except for cardiogenic thromboemboli.

Recent controlled studies evaluating heparin for the early treatment of evolving stroke showed no benefit; the risk of intracranial bleeding was not increased.

Early studies in patients with acute stroke using thrombolytic therapy were interpreted to demonstrate that such therapy was not beneficial and might produce intracerebral hemorrhage. However, significant problems with study design, diagnostic specificity, and therapeutic application compromise this interpretation. For example, the frequency of hemorrhage as the cause of stroke is 15-20%, and few of these patients could be excluded in the pre-CT scan era in which these early studies were performed. However, there may still be inherent hemorrhagic risks since embolism-related hemorrhage develops in approximately 10 percent of patients with originally negative CT scans. In addition the risk of cerebral hemorrhage accompanying the use of fibrinolytic agents in patients not selected for cerebrovascular disease is low but detectable, probably 0-2 percent.

In baboons thrombi form <u>in situ</u> in the microcirculation of the corpus striatum following a transient three hour period of ipsilateral middle cerebral artery occlusion, and these thrombi are prevented when antithrombotic therapy is administered prior to balloon occlusion. When urokinase therapy is given within 3 hours of experimental thrombotic occlusion, neurologic function is salvaged and cerebral infarction is reduced in the absence of detectable complicating hemorrhage.

Neurologic salvage using early thrombolytic therapy in patients with acute thrombotic stroke has also been achieved recently

Title : ANTITHROMBOTIC THERAPY IN CEREBROVASCULAR DISEASES (cont'd)

Authors : L A Harker and G J Del Zoppo

Presented by: L A Harker

using interventional neuroradiological techniques to deliver thrombolytic agents proximal to a documented vertebrobasilar or carotid artery occlusion. To date, CT-demonstratable post-perfusion hemorrhage was observed in 13 percent of patients treated by intra-arterial delivery, but none of the reported hemorrhages have resulted in functional deterioration or demise. This risk of bleeding may presumably be reduced by early short-term infusion of the thrombolytic agent, directed at the occlusion. With the advent of fibrin-specific thrombolytic agents such as tPA and scuPA, intravenous application of these agents may be safer without compromising efficacy. The specific dose-rates and evaluation require systematic controlled studies with angiographic endpoints.

TRN: ZA8700120

Title : IMAGING AND MONITORING OF HUMAN ATHEROSCLEROTIC LESIONS WITH RADIOLABELLED PLATELETS (26)

Author : H Sinzinger

Presented by: H Sinzinger

Indium-111 was introduced as a platelet label in 1976 by Thakur and has been successfully proven in experimental approaches for monitoring platelet kinetics as well as the imaging of pathological platelet accumulation such as thrombosis and experimental atherosclerotic lesions. Our interest was focused on the question of whether it would be possible to image and monitor the spontaneous course of platelet trapping over atherosclerotic lesions in man.

Platelet labelling was done in a total of more than 4 000 patients in order to calculate platelet half-life. Platelet labelling was done using a simple labelling kit developed by us with 100 Ci 111 In-oxine. Imaging of spleen and liver was done on the day after the injection of autologous labelled platelets. In parallel, we tried to detect platelet accumulation over the large arteries, especially the lower leg and the carotid artery region. Positive areas were encircled by regions of interest and the tracer uptake was quantified by dividing the actual count by the count for unaffected site. This quantitative measure, which we called platelet uptake ratio (PUR), ranged up to a value of 1,47. In total the patients suffering from clinical signs of atherosclerosis showed in less than 10% visible lesions characterized by an increased residence time of the platelets. There was a trend towards a higher PUR in younger patients, indicating a more active disease stage. The follow up of the platelet uptake for different lesion times can be differentiated pointing to a different disease activity whereas the half-life of the platelets in all these patient groups did not differ significantly. Surgical specimens obtained at different times after re-injection of the radiolabel revealed the highest platelet uptake in parietal thrombotic lesion, whereas the lowest one was found in complicated lesions.

No correlation can be found between the actual clinical stage of the disease as revealed by angiography, ultrasound and the activity as measured by the platelet uptake. However, for morphological control the gamma-counting we found a good correlation between the morphology and the uptake of the label. Intensive clinical studies have been limited so far by the availability of gamma camera units. Engymetric measurement using a portable detector system now allows continuous monitoring of the gamma-emission and a computer curve scan can be drawn for each detector separately as well as for the ratio. We present the first data from patients suffering from atherosclerosis and discuss advantages, problems and pitfalls of this new method. We demonstrated that anti-platelet therapy is able to change the PUR indicating that this new monitoring might be a very promising new tool for the diagnosis of platelet accumulation and the monitoring of the efficacy of therapeutic intervention as well.

Title : IMAGING PLATELET DEPOSITION (27)

Author : M D Ezekowitz

Presented by: M D Ezekowitz

Radionuclide labelled platelets (P) have been used to detect thrombosis (T) and P deposition in man. This paper will address the value of using indium-111 labelled P oxine method (PS) in the diagnosis of deep vein thrombosis (DVT), renal transplant rejection (RTR), left ventricular thrombi (LVT) and P deposition following balloon angioplasty (A). We will present preliminary data using radiolabelled P specific antibodies for tagging P in dogs.

In a study of 73 post-operative orthopaedic patients, PS was compared to venography (V) for the diagnosis of DVT. PS and V were read by 2 blinded readers. Only those PS and V with reader agreement were included in the analysis. Sensitivity and specificity was 13/14 (93%) and 28/29 (97%). Thus PS correlated well with V and may be used in high risk patients as a surveillance tool for DVT. To determine if PS can differentiate RTR from cyclosporine (CY) nephrotoxicity and acute tubular necrosis (ATN), we studied 5 groups of patients. Group I n=6 (3 on Imuran, 3 on Cy, Cr=1.4+/-0.64) had no clinical biochemical or histological evidence for rejection (kidney biopsy in 2). Group II n=4 (1 on Imuran, 3 on Cy Cr=2.85+/-1.07) had ATN (biopsy in 2). Group III n=3 (all on Cy CR=2.25+.-1.94) had suspected CYT; the patients improved upon withdrawal of Cy, and biopsy was negative for rejection in 2. Group IV n=11 (10 on Imuran 1 on Cy, Cr=5.61+/-4.09) had acute rejection on biopsy. In group V n=7 (3 on Imuran 4 on Cy, Cr=6.1+/-3.44) patients were under treatment for rejection for 12+/-4 days. All patients received Prednisone. P uptake index was calculated as the ratio of uptake over the transplant against a contralateral reference area. Groups I, II, III and V were significantly different from group IV, $p<0.01$ (Bonferroni). We conclude that 1) PS can be used for the diagnosis of untreated RTR. 2) Untreated PTR can be differentiated from CY nephrotoxicity and ATN by PS.

In a study comparing PS and two-dimensional echocardiography as methods of identifying LVT, the results obtained with both techniques were verified at surgery or autopsy in 34 patients. The sensitivity of PS in detecting T was 71 percent, and that of 2DE was 77 percent. The specificity of PS was 100 percent, and that of 2DE was 93 percent. We conclude that PS and 2DE have complementary roles in the detection of LVT.

Restenosis after A may be mediated through P deposition at the site of A. To determine whether P deposition at the site of A could be detected using PS, 15 patients, aged 60 $\pm$ 9 years, with iliac or femoral (n = 12), renal artery (n = 2) or distal aortic (n = 1) stenoses were studied. In 11 of 12 patients with iliac and femoral dilatations, focal uptake was demonstrated at the A site. In 4 patients (2 with renal, 1 with iliofemoral, and 1 with distal aortic stenoses), uptake was not detected. PS may be useful in predicting sites of future narrowing after A and may be used to test the efficacy of antiplatelet therapy.

Labelling P with monoclonal antibodies for IS possible in whole blood. To localize DVT and coronary T, the murine antihuman P monoclonal (7E3), was used to label P. T were induced by transcatheter placement of a copper foil followed by electrocoagu-

Title : IMAGING PLATELET DEPOSITION (Cont'd)

Author : M D Ezekowitz

Presented by: M D Ezekowitz

lation. 7E3 was labelled with 1-131 and In-111. For both isotopes 1 hr blood clearance was 54 $\pm$ 9%. In 1/3 coronary T and 4/4 with DVT clot was identified. Clot: blood ratios ranged from 7-13:1. Using the In-111 oxine method 0/3 coronary T were seen. Thus 1-131 and In-111 labelled 7E3 may be used to identify DVT. For prompt identification of coronary T more rapid clearance of P is required.

Thus PS can be used to detect T and P deposition in vivo in man and may assist in the management of patients with thromboembolic disease.

Title : ETHNIC DIFFERENCES IN VENOUS THROMBOGENESIS (28)

Authors : R C Franz, D J Jacobs, J E H Joubert, W J C Coetzee

Presented by: R C Franz

In the mid 1950's several research workers in Durban[1], Cape Town[2, 3], Johannesburg[4] and later in East Africa[5] reported higher fibrinolytic activity in blacks than in whites.

Although it has subsequently been shown that distal venous thrombosis in blacks appears to be more prevalent than previously surmised[6], it was thought that the low incidence of pulmonary embolism (P.E.) in blacks (Table I) could be at least partly attributable to ethnic differences in the plasmin system.

TABLE I: Incidence of pulmonary embolism (Necropsy Series)[7][8]

	No of Necropsies	Incidence of P.E. %
Michigan (Whites)	4 395	13,8%
Durban (Blacks)	4 527	0,7%

In view of the documented ethnic differences in the incidence of venous thromboembolism, a study was designed to evaluate several parameters of the haemostatic, fibrinolytic and lipographic profiles in three age-matched population groups with special reference to the biochemical risk factors which may be associated with the pathogenesis of thromboembolic disease i.e. 24 rural blacks, 24 urban blacks and 24 urban whites.

RESULTS

These results showed that the plasma fibrinolytic activity, alpha-2-macroglobulin, albumin and triglycerides of RURAL BLACKS differed significantly from those of URBAN BLACKS AND WHITES ($p<0,01$). However, the antithrombin III, gamma globulin and cholesterol values of the RURAL AND URBAN BLACKS differed significantly from those of the WHITES ($p<0,001$)[9].

Results of a similar study relating to ethnic differences in the vascular prostanoids will also be presented.

CONCLUSIONS

From these findings it would appear that the ethnic differences in the biochemical risk factors relating to thromboembolism are governed by genetic as well an environmental factors.

Title : IMAGING PLATELET DEPOSITION (Cont'd)

Author : M D Ezekowitz

Presented by: M D Ezekowitz

lation. 7E3 was labelled with 1-131 and In-111. For both isotopes 1 hr blood clearance was 54 ± 9%. In 1/3 coronary T and 4/4 with DVT clot was identified. Clot: blood ratios ranged from 7-13:1. Using the In-111 oxine method 0/3 coronary T were seen. Thus 1-131 and In-111 labelled 7E3 may be used to identify DVT. For prompt identification of coronary T more rapid clearance of P is required.

Thus PS can be used to detect T and P deposition in vivo in man and may assist in the management of patients with thromboembolic disease.

Title : TISSUE-TYPE PLASMINOGEN ACTIVATOR AS A NEW THROMBOLYTIC AGENT

Authors : H R Lijnen and D Collen

Presented by: H R Lijnen

Tissue-type plasminogen activator (t-PA) is a serine protease, different from urokinase, with a molecular weight of about 70,000 It is composed of one polypeptide chain, which is converted to a two-chain molecule by limited plasmic action.

Activation of plasminogen to plasmin occurs by cleavage of the Arg 560-Val 561 peptide bond. Kinetic analysis showed that the activation obeys Michaelis-Menten kinetics and that the presence of fibrin strikingly enhances the activation rate, by increasing the affinity of plasminogen for fibrin-bound t-PA. The directed action of plasmin towards fibrin in vivo might be explained by the low Michaelis constant in the presence of fibrin (0.16 μM) which allows efficient plasminogen activation on a fibrin clot, while its high value in the absence of fibrin (65 μM) prevents efficient activation in plasma. Plasmin formed on the fibrin surface would then be protected from rapid inactivation by α_2-antiplasmin.

Studies on the thrombolytic properties of t-PA (purified from melanoma cell cultures or obtained by recombinant DNA technology) in various animal models and in selected patients revealed that t-PA is a specific thrombolytic agent which induces thrombolysis without causing systemic activation of the fibrinolytic system. These promising initial results have encouraged the organisation of large, controlled and randomized clinical trials in patients with myocardial infarction, using t-PA obtained by recombinant DNA technology. The presently available results (TIMI trial; European multicenter trial) indicated that efficient and relatively fibrin-specific coronary thrombolysis can be obtained with t-PA.

Title : THE PRINCIPLES AND APPLICATIONS OF TREATMENT OF ATHEROSCLEROTIC LESIONS WITH PROSTAGLANDINS (30)

Author : H Sinzinger

Presented by: H Sinzinger

During the last few years the question of the value of prostaglandins in the therapy of peripheral vascular disease has not been answered satisfactorily. This is due at least in part to the fact that much too little attention has been paid to discovering the optimal infusion regimen. Assuming that the platelets are the target cell for efficacious treatment we tried to elaborate the optimal therapeutic regimen for both PGI_2 and PGE_1 by checking platelet function and the *in vivo* uptake of radiolabelled platelets. Continuous long-term infusion is limited by the occurrence of the intra-infusion and the post-infusion platelet rebound. Doing the follow-up of active human atherosclerotic lesions by platelet uptake we feel that an infusion of 4-6 hours a day at the rate of 3-5 ng/kg/min for 5 days might be optimal, at least as far as the platelet residence time is concerned. It can be shown that other prostaglandins like the stable analogue Iloprost and Prostaglandin E_1 are able to decrease the platelet uptake too.

A combination of a portable monitoring system as well as a portable infusion pump for the prostaglandin therapy allow a very simple, cheap and safe therapy on an outpatient basis. The fact that the removal of the radiolabelled platelets achieved by the therapy persists even after stopping the treatment indicates that the subendothelial layer has been rendered less thrombogenic by a still unknown mechanism which has been discovered in an experimental animal model too. We feel that therapeutic intervention at the early active atherosclerotic lesion stage at a subclinical level might be a very promising new approach for the future to prevent or at least to delay progression and the onset of human atherosclerosis. Thus, further measurements have to be performed to optimise and simplify this diagnostic aid.

TRN: ZA8700122

Title : VENOUS THROMBOSIS AND PULMONARY EMBOLISM - A PROSPECTIVE PROPHYLACTIC TRIAL IN HIGH-RISK SURGICAL PATIENTS

Authors : E J Immelman, P Jeffery and S R Benatar

Presented by: E J Immelman

Most studies of the natural and modified history of post-operative thromboembolism have relied exclusively or heavily on the ^{125}I fibrinogen uptake test and no large study has employed routine pulmonary embolus surveillance.

919 Patients over 40 years undergoing elective major abdominal surgery were randomised to receive no prophylaxis, low dose heparin (5000 units 8-hourly) or sodium pentosan polysulphate (SPP) (50 mg 12-hourly) during a 6-year prospective trial. Screening for deep venous thrombosis (DVT) and pulmonary embolism (PE) was applied to every patient on a routine basis : for DVT, ^{125}I fibrinogen, Doppler ultrasound and bilateral ascending phlebography on Day 6; for PE, pre- and 7th day post-operative chest roentgenogram, four-view perfusion lung scan and spirometry. V/Q scans were performed in selected patients and routinely from 1979. Analysis of 880 acceptable control, heparin and SPP patients revealed a ^{125}I fibrinogen incidence of DVT of 18%, 9% ($p = 0.002$) and 10% ($p = 0.005$) respectively, with a phlebographic incidence of 21%, 12% ($p = 0.01$) and 14% ($p = 0.016$). The major effect of both prophylactic methods was to reduce calf DVT and bilateral DVT. Phlebographic popliteal or more proximal involvement was present in 4.2%, 3.1% and 2.8% of patients respectively. The accuracy of the fibrinogen data was compared to venography as the reference standard in 715 patients (1430 limbs) with complete protocols. Sensitivity for thrombosis in the calf, popliteal and femoral vein was 70%, 68% and 39% respectively, with specificities of 98%, 98% and 100% respectively. New post-operative pulmonary perfusion defects were present in 21%, 19% and 18% and were regarded as diagnostic of PE in 5%, 4% and 6% respectively. A trend towards increased bleeding and transfusion requirements was evident in the two prophylactic groups. Proximal segment DVT and non-fatal PE are uncommon post-operative events and, to date, neither prophylactic regimen has effected a significant reduction. Both regimens do, however, produce a modest reduction in predominantly silent calf DVT. In the light of these results, the risk/benefit ratio of routine pharmacological prophylaxis is debatable.

In the past it has been assumed that a reduction in the incidence of venous thrombosis should proportionately reduce pulmonary embolism rates. As this study indicates, this assumption may be fallacious. Approximately 80% of post-operative thrombi are minor, restricted to the calf and, as our long-term follow-up study has indicated, are probably of no clinical significance and, therefore, not important to prevent. Fibrinogen uptake lacks sensitivity or is insensitive to thrombus in the ilio-femoral segment, which is the precursor site of the majority of fatal pulmonary emboli. Conclusions based on ^{125}I fibrinogen uptake should be viewed with caution.

Title : ANTIPLATELET THERAPY

Author : J Vermylen

Presented by: J Vermylen

Platelet function inhibitors can act at the levels of platelet receptors, of the signal-response coupling or of the amplification loops. A strategy is being developed that blunts the response of platelets to stimuli by increasing the level of intraplatelet cyclic AMP in the vicinity of vascular lesions.

Antiplatelet therapy is essentially aimed at preventing rather than curing disease. Its effectiveness has mainly been established by large-scale, long-term epidemiological studies. Alternative less expensive and time-consuming methodologies to evaluate the potential of new antiplatelet agents seem essential. Such "in vivo" screening procedures have included the bleeding time and measurement of platelet survival. Quantitation of platelet accumulation at the site of vascular lesions (e.g. following surgery or angioplasty) with the technologies discussed at this conference may result in a breakthrough in the evaluation of antiplatelet agents.

At the present time, there is evidence for a moderate but real efficacy of platelet function inhibitors in patients with unstable angina pectoris, in survivors of a myocardial infarction, in the prevention of aorto-coronary bypass closure, following heart valve surgery, in patients with transient cerebral ischaemic attacks, in peripheral vascular disease, in membranoproliferative glomerulonephritis, in the vascular complications of pregnancy and in the prevention of postoperative deep vein thrombosis. Further progress will depend not only on the development of better treatment regimens, but also on an increased understanding of the mechanisms of thrombogenesis in general and of those active in the individual patient in particular.