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EVALUATION OF PLATELET-INHIBITING DRUGS IN MODELS OF ATHEROSCLEROSIS

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INTRODUCTION

Numerous methods have been described to measure platelet function or responses and alterations thereof by drugs. These techniques have contributed greatly to our understanding of platelet biochemistry and morphology. From these studies evolved procedures to measure the effect of platelet-inhibiting drugs in thrombotic states which have been extensively investigated. Several recent reviews (1, 2) have very adequately covered this aspect and, therefore, discussions of these test systems will be purposefully omitted from this review.

The role of platelets in atherosclerosis is somewhat more controversial than their participation in thrombosis. Nevertheless, over the past decade a significant body of evidence has accumulated suggesting that platelets assume a pivotal aspect of the early vessel wall lesion development. New techniques and approaches are being formulated in which established antiplatelet drugs may be inappropriate.

PLATELETS AND ATHEROGENESIS

Proliferation of intima smooth muscle cells is one aspect of early atherogenesis in the response to injury theory (3). Ross and co-workers (4) identified that a platelet-derived growth factor (PDGF) was required for optimal proliferation of primate arterial smooth muscle cells in culture. Subsequently PDGF was found to also stimulate the growth of skin fibroblasts (5), mouse 3T3 (6), and human glial cells (7), but not endothelial cells (5, 6). Thus PDGF may fit within the atherogenesis schema as a vessel wall mitogen. Endothelial injury may lead to desquamation, platelet adherence,

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release of PDGF, and intima smooth muscle cell proliferation (8). Re-endothelialization occurs over the lesion which then tends to regress. Repeated or chronic injury to the endothelium may lead to complicated lesions and the clinical sequelae associated with coronary heart disease (8).

The response to injury hypothesis and the proposed platelet role has been evaluated using numerous forms of endothelial injury, i.e.: homocysteine infusion (9); cholesterol feeding (3, 10–14); balloon catheter (15–18); air desiccation (19, 20); and an indwelling aortic catheter (21). A consistent finding in the above models is platelet adherence to the subendothelium and myointimal thickening. The myointimal thickening results from proliferation of medial smooth muscle cells rather than blood cell colonization (17). Major reduction of the platelet count will prevent the proliferative response (21).

Another model frequently cited to support platelet involvement in atherosclerosis is the swine homozygous for von Willebrand's disease. These pigs have prolonged bleeding times (22) and a deficiency of the von Willebrand factor necessary for normal platelet adherence to subendothelial tissue (23). These swine are less susceptible to aortic atherosclerosis induced by either atherogenic diet alone (24) or atherogenic diet and balloon endothelial injury (23).

Platelet involvement with human atherosclerosis has not been well documented. A suggestion of this association is presented within accelerated graft arteriosclerosis which is a consistent occurrence in human heart transplantation (25, 26). The ethics of treatment and the low number of recipients have not allowed controlled clinical drug trials with heart transplant patients. Retrospective investigations, however, have revealed that the incidence of graft arteriosclerosis was dramatically reduced by the addition of anticoagulant and antiplatelet therapy (25).

Sinzinger et al (27) hypothesized that prostacyclin (PGI_2), the most potent known platelet aggregation inhibitory agent, is an important factor within the response to injury hypothesis. These investigators suggest that atherosclerosis occurs at sites of reduced PGI_2 synthesis, perhaps as a result of vessel injury. Reduced PGI_2 synthesis would allow greater platelet interaction with the altered vessel surface. Szczeklik et al (28) have had limited but exciting clinical success at reversing ulcer lesions and pain associated with advanced arteriosclerosis of the lower extremities by the infusion of PGI_2. Reversal, however, of the atherosclerotic lesions has not been demonstrated, and enhanced local circulation is assumed to be the mechanism by which PGI_2 demonstrates improvement in these patients.

The above cited reports suggest that platelets are involved in the early stages of experimental arteriosclerosis and that removal of platelets or interruption of platelet adherence may reduce the severity of the progressive

disease. Prevention of human atherosclerotic disease in properly controlled clinical trials is, of course, the ultimate goal. Prior to introduction of such trials, clinical evaluation endpoints will need to be devised, new sites for intervention of platelet function should be designated, and new drugs with these activities identified. The following discussion will focus on several key areas for modification of platelet involvement by drugs.

EVALUATION TECHNIQUES

The response to injury hypothesis as outlined by Ross & Harker (8) assumes involvement of: desquamation of endothelial cells; adhesion of platelets; release of platelet constituents, some of which enter the artery wall; migration and proliferation of medial smooth muscle cells into the intima; formation of a connective tissue matrix; and intra- and extra-cellular lipid accumulation. Sites for intervention within this sequelae are numerous but for the purpose of this review will be limited to platelet adhesion, platelet α-granule release, smooth muscle cell proliferation, and lesion (plaque) formation.

Platelet Adhesion

Previous reviews have outlined the complex interactions of platelets with components of the vessel wall (29, 30). A popular clinical-model for the adhesiveness of platelets has been platelet counts before and after aspiration of whole blood through a column of glass beads (31, 32). Although this technique is extensively used, factors such as transit time, flow rate, column length, and platelet-platelet interaction have left the results of such assays questionable. Platelet aggregation within the column can be minimized by the addition of pyruvate kinase and phosphoenolpyruvate which converts the released ADP to ATP (33).

Platelets do not adhere to normal endothelial cells. This lack of adhesion is assumed to be related to structural components rather than to the production of PGI_2 by endothelial cells since blockade of the synthesis of this anti-aggregatory hormone does not increase the platelet-endothelium interaction (34). Platelets will, however, adhere to a matrix of microfilaments formed beneath the endothelial cells in culture which is subsequently exposed by cell injury (35, 36). In static systems, the aspirin-induced decrease in PGI_2 synthesis by cultured vascular cells (endothelial or smooth muscle) allows greater platelet adherence when thrombin is used to induce platelet-cell interaction (37).

The effect of antiplatelet agents on the adherence to the subendothelium has been the subject of conflicting reports. Aspirin treatment of de-endothelialized rabbit aortas everted on probes rotated in radiolabeled platelet

suspensions did not inhibit the adherence of platelets to the subendothelium (34, 38). In the same system, some inhibition of platelet adherence was noted with the additon of dipyridamole, prostaglandin E_1, methylprednisolone, penicillin G, or indomethacin (38).

Baumgartner and co-workers (39–42) demonstrated that blood flowing over an everted rabbit aorta preparation could mimic the in vivo blood cell-wall relationship. The blood flow rate critically affected the amount and the duration of attachment of platelets to aortic subendothelial tissue (43). Subendothelial adhesion was found to be impaired with platelets derived from patients with von Willebrand's disease (44–46), storage pool disease (46), and Bernard-Soulier Syndrome (46). Sulfinpyrazone and aspirin inhibit platelet-platelet adherence or thrombi formation but not monolayer adhesion to the subendothelium with this technique (41). A similar effect with aspirin was observed by Silver et al (47) in the rabbit injured middle ear artery in situ. Essien & Mustard (48), in contrast, found that sulfinpyrazone, but not aspirin, reduced platelet adhesion to a perfused de-endothelialized rabbit aorta. The use of an indirect measure of platelet adherence by Essien & Mustard, rather than the direct microscopic measurement as utilized by Baumgartner and co-workers, tends to support the latter's findings of an absence of effect by sulfinpyrazone on platelet adhesion. Concentrations of dipyridamole (1 mM) which are not likely to be achieved in patients did inhibit adhesion in the Baumgartner model (41).

Endothelial cells produce the factor VIII-associated von Willebrand factor required for platelet adhesion at sites of vascular injury (49). Fuster and co-workers (50) demonstrated a reduced platelet-arterial wall interaction in pigs with homozygous von Willebrand's disease (vWD). Radioactivity associated with the carotid artery after air desiccation was reduced in von Willebrandt pigs 24 hours after infusion of ^{111}Indium-labeled platelets when compared to similarly treated normal pigs. No difference in platelet adherence to denuded coronary artery segments from pigs with vWD compared to normal pigs was observed by Reddick et al (51). Interestingly, this noted difference between these two research centers in observed platelet adherence in vWD pig vessels is related to a similar difference in observed susceptibility to atherosclerosis within these same vessels as described within the Lesion Development section of this review.

Prostacyclin has recently been shown to inhibit the ristocetin-induced interaction of platelets with human or bovine von Willebrand factor (52, 53), which would suggest that prostacyclin modulates platelet adhesion. The physiological role of prostacyclin as an inhibitor of platelet adhesion has been questioned by an investigation utilizing rabbits with balloon catheter-damaged aortas (34). Aspirin administered to the rabbits in doses (100 mg/kg) sufficient to inhibit prostacyclin production did not alter the

amount of platelet accumulation on the injury site. The converse has been observed (54) with the same model when the carotid artery is the site of injury, suggesting that results may vary with the arterial segment chosen for investigation.

Hypercholesterolemia induced by diet in cynomolgus monkeys can be used to influence the quantity of vessel wall-platelet adherence (55). After 100 days of diet, increased platelet adherence was observed throughout the aorta and branch arteries. In vivo images (scintiphotos) with a gamma camera have been utilized to quantitate labeled platelet adhesion to saphenous vein bypass grafts in dogs (56). Platelet-graft adhesion was significantly reduced at 32 hours after surgery by administration of dipyridamole for 2 days pre-operative and dipyridamole plus aspirin at 1 and 24 hours post-operative.

An ex vivo evaluation of antiplatelet drug effect on platelet adherence to vascular prosthesis was conducted by McCollum and co-workers (57). Human volunteers took aspirin, dipyridamole, sulfinpyrazone or aspirin plus dipyridamole for 1 week. Blood from the volunteers was perfused through a vascular prosthesis of preclotted, woven Dacron. The number of adhering platelets was counted from scanning electron micrographs of the luminal surface of the prosthesis. Aspirin and dipyridamole reduced platelet adhesion to the prosthesis, whereas sulfinpyrazone did not. The combination of aspirin and dipyridamole was not better than either medication alone. The effect of aspirin or dipyridamole ingestion by normal volunteers on the subsequent adherence of their platelets from native blood circulated over everted segments of de–endothelialized rabbit aortas was measured by Weiss et al (58). Aspirin decreased platelet build-up, but not adhesion, whereas dipyridamole did not alter either thrombi formation or platelet adhesion to the superperfused segments.

Variable drug effects on platelet adhesion are suggested by the above reports. Adhesion is influenced by the type of material or artery utilized, the blood flow rate, stasis vs movement, and in vivo vs in vitro experiments. A summation of the above reports indicates that antiplatelet drugs do not alter in vivo adhesion to subendothelium at doses achievable in the clinic. A bleeding diathesis, such as severe cases of von Willebrand's disease, might be expected if platelet adhesion to subendothelium was effectively inhibited.

α-Granule Release

The α-granules of the platelet contain fibrinogen, platelet derived growth factor (PDGF), platelet factor-4 (PF_4), β-thromboglobulin (βTG), low affinity PF_4 (LA-PF_4), and platelet basic protein (PBP) (59, 60). The importance of PF_4 is uncertain at present, although it is known to have heparin-

neutralizing activity. PBP is suggested to be a mitogenic peptide (61) which may be the precursor to LA-PF_4. LA-PF_4 may in turn be converted to βTG by the deletion of four amino acids (62). These three peptides are immunologically identical (62); therefore, Niewiarowski has recommended the use of the term βTG/LA-PF_4/PBP antigen (63) rather than just βTG, as currently is practiced. The relationship, if any, of the mitogen PBP to PDGF remains to be established. At present most investigators have focused on PDGF as the predominant platelet mitogen.

Release of platelet α-granules is related to surface contact, platelet spreading, and calcium ions (41). The hypothesis that α-granule contents released from platelets in contact can permeate the vessel wall has been strengthened by the observations of Goldberg et al (64). These investigators found PF_4 localization in balloon-catheter injured vessel walls within 30 min after injury using a specific immunofluorescent stain for PF_4.

The potential for independent secretion mechanisms of the α-and dense (δ) granules is currently a point of debate. The majority of investigators (65, 66) have concluded that the α-and δ-granules are released by similar stimuli at only slightly different time intervals. A few investigators have observed differences between α-and δ-granule release such as: sensitivity of gel filtered human platelets to collagen (67) or thrombin concentration (68); with baboons or humans undergoing cardiopulmonary bypass surgery (69); platelets subjected to shear stress injury (70); and stirred human platelet-rich-plasma in the presence of δ-granule release inhibitors (71). A specific inhibitor of α-granule release has not been identified but would be desirable to control release of platelet mitogens without altering platelet adhesion or aggregation.

Elevation of plasma levels of platelet α-granule contents (PF_4 or βTG) has been observed associated with clinical states of peripheral vascular disease (72, 73), diabetes mellitus (72–74), hyperlipidemia (73), coronary artery disease (72, 74, 75), and patients with heart valve prosthesis (76). The effect of antiplatelet drugs on plasma PF_4 or βTG levels in the above mentioned clinical conditions has received only limited evaluation. Dipyridamole was observed to lower plasma βTG levels in ten juvenile-onset, insulin-dependent diabetics with proliferative retinopathy (72, 77). Plasma PF_4 levels were determined in patients with and without aspirin usage who had recent myocardial infarction, coronary artery disease, or diabetes mellitus (74). Separation of the data from these patients by aspirin medication indicates that this cyclooxygenase inhibitor does not alter plasma PF_4 levels. Aspirin ingestion by normal volunteers had no effect on plasma βTG levels (78).

Caution in the use of these peptides as markers for clinical disease has been stated (59). PF_4 has been shown to bind to endothelial cells (79, 80)

and to be released from this association by heparin (80, 81). The clearance of plasma PF_4 is extremely rapid and plasma levels may reflect secondary release from binding sites as well as primary release from platelets. A recent report of PF_4 location within tissue mast cells will add to the potential artifacts of the observed plasma level (82). Renal dysfunction has been shown to reduce βTG clearance and thus result in elevated βTG plasma levels (83, 66). Kaplan & Owen (84) have concluded that measuring both PF_4 and βTG will circumvent most difficulties in the interpretation of in vitro vs in vivo release. Increased βTG with normal PF_4 suggests in vivo platelet activation. An increase in both βTG and PF_4 in the absence of heparin therapy suggests in vitro release.

Cellular Proliferation

The effect of potential drugs on smooth muscle cell proliferation is an integral aspect of the anti-atherogenic evaluation process. Numerous arterial smooth muscle cell mitogens have been identified (4, 85–89). Most investigators have focused on PDGF located in the α-granule and apparently synthesized prior to platelet release in the megakaryocyte (90, 91). PDGF can be conveniently assayed using a fibroblast cell line (Balb/c-3T3). PDGF added to cultured human skin fibroblasts induces an increase in cholesterol ester formation (92) and transient exposute to PDGF induces quiescent 3T3 cells to become competent to replicate their DNA (93). PDGF alone is not sufficient to induce propagation and plasma is required for the cells to enter the S phase (DNA synthesis) of proliferation (93). A serum or plasma-free medium which will induce propagation was recently described (89).

Inhibitors of smooth muscle cell proliferation induced by platelet mitogens would be useful to validate the response to injury hypothesis and to prevent lesion formation if the latter is correct. Limited testing has been conducted using quiescent 3T3 cell cultures with ^{3}H-thymidine incorporation into DNA after mitogen addition as an indicator of proliferation. In this system, dipyridamole, aspirin, prostacyclin, papaverine, isoproterenol, nifedipine, and nitroglycerine had no effect on PDGF-induced ^{3}H-thymidine incorporation (94).

Trapidil (Rocornal®), developed as a coronary artery vasodilator and observed to be a weak inhibitor of collagen-induced aggregation [(94); D. S. Cohen, unpublished information] when added to the Balb/c-3T3 cell proliferation assay, prevented propagation induced by PDGF but not that induced by fibroblast growth factor (FGF) or calcium phosphate (94). This observation was not reproduced in Ross's laboratory using a Swiss 3T3 cell proliferation assay (P. Dicorleto, unpublished information).

Heparin added to the 3T3 cell proliferation assay inhibited the ^{3}H thymi-

dine incorporation induced by platelet basic protein (PBP) but not the incorporation induced by epidermal growth factor (EGF) (61). The effect of heparin on PDGF-induced proliferation has not been reported.

Several in vivo models involving proliferation of medial smooth muscle cells have evolved. Spaet et al (95) adapted a technique described by Baumgartner (15) for extensive endothelial cell removal from the aorta of rabbits using a balloon catheter. The denuded surface is rapidly covered by platelets which spread, discharge a variable amount of their granular contents, and detach within 3 hours (96). Within 1 week after injury, the de-endothelialized area was covered by modified smooth muscle cells which had migrated through the internal elastic lamina (95). The administration of sulfinpyrazone at a relative high dose (67 mg/kg) significantly reduced the neointima of the rabbit iliac artery after balloon catheterization (97). Dipyridamole at a high dose (50 mg/kg twice daily) and aspirin (30 mg/kg) did not alter the intimal lesions in this model.

A similar sequence of fibromusculoelastic intimal thickening was observed in rats following balloon-catheter aorta endothelial cell removal (98). Hypophysectomy 2 weeks prior to the balloon-catheter injury completely inhibited the smooth muscle proliferative response (98). This observation suggested that a pituitary factor was required for the smooth muscle cell proliferative response. A clever procedure was used to define the important missing proliferative agents in hypophysectomized plasma (89). The propagation of density-arrested Balb/c-3T3 cells briefly exposed to PDGF and then transferred to medium containing plasma from hypophysectomized rats supplemented with pituitary or pituitary synthesis-controlled factors was measured. The addition of somatomedin C to the hypophysectomized rat plasma restored the cellular progression towards proliferation post PDGF-induced competence. Somatomedin C plasma levels are regulated by growth hormone released from the pituitary (89).

A model of endothelial injury resulting from air desiccation of the common carotid artery in rats was described by Fishman et al (19). Intimal thickening in an asymmetrical distribution with endothelial regeneration at 14 days post injury was observed with this model. No effect on the morphology of the lesion development by the administration of aspirin, flurbiprofen, or reserpine to the rats prior to injury was observed by Clowes & Karnovsky in this model (99). Karnovsky (100) observed that heparin infusion into the rat reduced the amount of proliferation from carotid artery injury by desiccation. The potential exists for the development of heparin-like molecules which retained the antiproliferative activity but not the anticoagulant properties (100). It is interesting to note that heparin surprisingly induced a dose-dependent relaxation of coronary artery strips bathed in vitro (101).

Preliminary studies by Tiell (M. Tiell, unpublished observations) indicate that trapidil will greatly reduce the proliferative response in the aorta of the rat to balloon catheter injury. Trapidil within the same experiments did not influence the rate or extent of re-endothelialization as measured by the Evan's blue technique.

Hauss (102) describes an interesting technique for an induction and measurement of arterial smooth muscle cell proliferative response utilizing rats or minipigs. A risk factor for atherosclerosis, such as hypertension, diabetes, or atherogenic diet, is applied to the whole animal. After 10 days an explant of the aorta from the animal is cultured twice. A portion of the smooth muscle cells within the second subculture has been observed to proliferate at a faster rate when the whole animal was subjected to a risk factor. The addition of aspirin to the second subculture of the aortic explant reduced the rate of growth of the smooth muscle cells (102). The effect of antiplatelet drug administration to the whole animal during application of the atherogenic risk factor on the rate of subculture smooth muscle cell growth would be interesting.

Lesion Development

Kincaid-Smith (103) demonstrated reductions in progressive arterial disease in human renal allografts by the additive therapy of anticoagulants and dipyridamole. This therapy prevented the progressive intimal thickening which is characteristic of rejecting allografts. This report prompted the cardiovascular surgery group at Stanford University to utilize warfarin and dipyridamole in cardiac transplant patients to reduce the accelerated atherosclerosis of the graft coronary arteries (104). A marked reduction of graft arteriosclerosis resulted but the lack of proper controls and multiple treatment changes between the two groups allow only speculation that the antiplatelet therapy was a contributor to this success (26, 104). To duplicate their clinical findings, the Stanford group developed an animal model using strongly mismatched rat cardiac allografts in which all rats develop graft artery disease within 20 days (105). Dipyridamole, in combination with the immunosuppressant cyclosporin A (Sandimmun®), completely prevented graft coronary vessel disease. In a similar experiment using the immunosuppressant azathioprine (Imuran®) and very high doses of sulfinpyrazone (160 mg/kg), a marked prolongation of graft survival time occurred (106). Sulfinpyrazone alone had a weak but significant effect on cardiac allograft survival time.

Moore et al (107) describe a rabbit model which develops aortic lesions from fatty streaks to lipid-rich, raised thrombo-atherosclerotic plaques from the placement of an indwelling catheter. Thrombocytopenia induced in this model by platelet antiserum significantly reduced the raised lesions.

An indwelling catheter to induce arterial injury and thrombosis concomitant with cholesterol feeding to rabbits produced greater intimal area lesion involvement than cholesterol feeding alone (108).

Several anti-inflammatory drugs demonstrated significant reductions in the extent of thoracic aorta atherosclerotic plaque formation in rabbits fed atherogenic diets (109). No apparent predictive index between thromboxane synthesis inhibition and anti-atherogenicity was observed in vivo. Phenylbutazone, flufenamic acid, and oxyphenylbutazone reduced plaque formation but aspirin and aminopyrine were ineffective (109). Sulfinpyrazone has also been reported (110) to reduce the arterial lesion formation in rabbits fed an atherogenic diet.

Sulfinpyrazone administered to minipigs with balloon-injured abdominal aortas did not alter lesion development nor collagen, elastin, or cholesterol content of the aorta (111). Clopath et al (111) suggest that metabolic transformation differences between the miniature swine and the rabbit account for the differences in effect on lesion formation between these species by sulfinpyrazone.

Fuster et al (24) used a 2% cholesterol diet to induce fibrous atherosclerotic plaques in the aortas of pigs. The response of dipyridamole and aspirin combination treatment was compared to untreated pigs and homozygous von Willebrand pigs on the diet. The platelet-inhibitor combination treatment and the von Willebrand pigs had significantly less aortic surface plaques than the normal untreated pigs (24). It is interesting and pertinent to note that similarities in the development of aortic lesion in vWD pigs have been found between the Mayo investigators (24) and the Chapel Hill investigators (23), but differences in coronary artery lesion formation were observed. Correlated with this difference is the relationship of platelet adherence to the denuded coronary artery surface. The Chapel Hill scientists (48) found platelet adhesion and subsequent lesion development, whereas the Mayo scientists (24) observed reduced platelet adhesion and no lesion development.

The infusion of homocystine into baboons for 5 days produced localized (1–2%) desquamation of vascular endothelium (9). The co-administration of dipyridamole with the homocystine prevented the shortened platelet survival rate but evaluations of fibromuscular plaque changes after drug treatment were not conducted.

Two groups of investigators have determined the effect of antiplatelet therapy on the progression of coronary atherosclerosis in cynomolgus monkeys consuming a diet containing 2% cholesterol and 10% butter. Pick and co-workers (13), in their primary study, observed a significant reduction with low dose aspirin treatment in the involvement of coronary vessels with atherosclerotic disease. Aspirin did not affect plasma cholesterol levels or

aortic atherosclerosis. However, from a second study with the same model, Pick (112) reports that aspirin or aspirin and dipyridamole in combination did not result in significantly less atherosclerosis involvement of the coronary arteries. Hollander et al (14), using a similar atherogenic diet, did not observe an effect of aspirin and dipyridamole co-administration on atherosclerosis involvement of the coronary, aorta, or other peripheral arteries. The antiplatelet therapy was ineffective during the atherosclerotic lesion development phase and during the regression phase.

CONCLUSIONS

The role of platelets in atherogenesis is as yet a hypothesis to be proven. The response to injury model of atherogenesis assumes cellular proliferation resulting from a blood-borne mitogen. Inhibiton of this induced proliferation and the subsequent reduction in atherosclerotic lesions would strengthen the response to injury hypothesis. Of course inhibition of the release or activity of the platelet mitogen(s) is but one aspect of this hypothesis evaluation since other mitogen sources have been identified. Current antiplatelet agents have been defined by their effects against platelet function in an antithrombotic sense and therefore may be inappropriate as anti-atherosclerotic agents. As potential inhibitors of atherogenesis, one would expect an affect upon platelet adhesion, α-granule release, or smooth muscle cellular proliferation leading to an ultimate reduction in vessel lesion formation.

The correlation between platelet adhesion and subsequent lesion formation seems well established, especially with the vWD pig investigations. Variable results depending upon the experimental conditions have been obtained with aspirin, dipyridamole, and sulfinpyrazone on platelet adhesion. Most investigators would assume that aspirin and sulfinpyrazone do not decrease platelet adhesion within in vivo conditions. Dipyridamole, although active in animal models as an inhibitor of platelet adhesion, may not be effective in humans because of the lower dose normally tolerated. Prevention of platelet adhesion may be the least acceptable site of intervention because of bleeding enhancement which would be expected as a side effect.

Selective inhibition of release of α-granule contents, or, more specifically, smooth muscle cell mitogens, has not had demonstrated success to date. Further advancement in the understanding of granule release mechanisms may be required. Extensive screening of potential inhibitors through existing models (69–71) may enhance our understanding of the possibility for selective α-granule release inhibition. Without consideration for the technical difficulties, this approach seems to pose the greatest potential for a long

term therapeutic approach if only platelet mitogens are considered within the content of atherogenesis.

Inhibition of cellular proliferation is the most direct and, perhaps, will be the most extensively investigated in the near future. Of the described approaches, the progressive evaluation from in vitro fibroblast cell culture effects to in vivo arterial injury models allows rapid screening and initial follow-up of potential agents. The traditional antiplatelet drugs do not seem to have a consistent effect in the in vitro or in vivo proliferative systems. The observations with heparin are interesting, especially since the activity seems to reside in the mucopolysaccharide structure and the potential exists for molecules of this type which lack the anticoagulant effects of heparin. The quantity required and route of administration may be limiting with heparin-like therapy. The observations with trapidil, although requiring extensive follow-up investigations, provide encouragement.

Dipyridamole in combination with cyclosporin A or aspirin has demonstrated some success in atherosclerotic lesion prevention. An equal number of experiments have shown ineffective results with dipyridamole, suggesting that the dose and lesion site may be important for anti-atherosclerotic effectiveness. Prevention of lesion formation in vivo is an extremely important indicator of an agent's therapeutic potential; however, the comparison of our atherosclerotic models with atherogenesis in man has been questionable at best. It may seem more appropriate at present to expect an agent to effectively reduce lesion formation in a model based on the injury-proliferation response in animals and then evaluate the effect on atherogenesis in man. One could assume on this basis that from the reported information, the current traditional antiplatelet agents do not deserve a clinical trial and that newer agents with more specific antiproliferative effects will need to be developed.

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