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Effect of Aprotinin (Trasylol) on the Inflammatory and Thrombotic Complications of Conventional Cardiopulmonary Bypass Surgery

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ABSTRACT

Before the discovery of its hemostatic properties, aprotinin was thought of as a potential anti-inflammatory agent. Its clinical introduction in 1987 to prevent blood loss during cardiac surgery [Royston, 1987, van Oeveren 1987] led to its anti-inflammatory benefits being largely overlooked in favor of a vigorous debate centering on whether aprotinin may be pro-thrombotic when given to patients. In this article, we summarize evidence for the anti-inflammatory activity of aprotinin and discuss our recent contributions in this area. We also summarize the state of the thrombosis debate and discuss our recent evidence from purified platelets which shows that aprotinin is simultaneously hemostatic yet anti-thrombotic.

DISCUSSION

Inflammation in CPB

The systemic inflammatory response syndrome

Cardiopulmonary bypass (CPB) has been known since the early days of cardiac surgery to be associated with inflammatory complications, particularly with respect to the lung [Kolff 1958, Byrick 1978]. The factors causing this inflammatory response include surgical trauma, ischemia/reperfusion injury, contact activation of the circulating blood compartment and endotoxemia [Am. Coll. Chest Phys. 1992]. Exposure of leukocytes and platelets to the extracorporeal bypass circuit is thought to be a major contributing factor in CPB-related inflammation, resulting in elaboration of systemic humoral inflammatory mediators, including tumor necrosis factor α, Interleukin (IL)-1, IL-6 and IL-8 [Lahat 1992, Butler 1993a, Boyle 1997, Fujiwara 1997, Kotani 2000]. Elevated levels of pro-inflammatory cytokines cause systemic activation to vascular lining endothelial cells, resulting in enhanced expression of adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 [Albelda 1994, Asimakopoulos 1998]. Contact activation of both classical and alternative complement pathways leads to the production of the chemoattractant factors C3a and C5a, and the membrane attack complex C5b-C9, all of which exacerbate leukocyte activation and lead to vascular injury [Chenoweth 1981, Hansch 1987, Tennenberg 1990, Gillinov 1994b, Boyle 1997].

Trapping of large numbers of leukocytes within the tissues can give rise to the Systemic Inflammatory Response Syndrome (SIRS). The organs affected and the severity of this condition can differ widely between patients, but the ischemic lung appears to be particularly susceptible [reviewed in Butler 1993b, Taylor 1996]. In its extreme form SIRS can lead to multiple organ failure that often includes Adult Respiratory Distress Syndrome (ARDS), which is associated with massive leukocyte infiltration into the lungs resulting in high mortality [Bernard 1994, Connelly 1997]. The significance in acute lung injury of specific inflammatory mediators has been investigated in animal models of CPB-related injury and also in trials involving humans. Analysis of the pathophysiological mechanism involved in these studies suggests that activation of complement components and subsequent neutrophil and monocyte activation are followed by pulmonary endothelial injury, production of IL-8 and platelet activating factor (PAF), and migration of neutrophils and monocytes into the alveoli [Jorens 1992, Johnson 1994, Dreyer 1995, Warner 1996, Miotla 1998]. When neutrophils and monocytes have gained access to the alveoli, acute lung injury is mediated by the release of oxygen free radicals and histotoxic

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contents from neutrophils, such as elastase and myeloperoxidase (MPO) [Weiss 1989, Hashimoto 1992, Nagamine 1994, Eppinger 1997].

Leukocyte activation following CPB

Although serious clinical manifestations of SIRS are rare, systemic activation of leukocytes is readily detectable at 15-60 minutes following bypass [Gillinov 1993, Hill 1994]. This was observed measuring expression of the neutrophil activation marker Mac-1. Mac-1 is an adhesion molecule of the β2 integrin family, which appears early upon leukocyte activation and mediates binding to endothelial ICAM-1. Taking time-points out to six days beyond bypass, we found no evidence for expression of the very late antigen (VLA) family of integrins, which mediate binding to extracellular matrix proteins [Asimakopoulos 2000a]. An unremarked fact in the cardiothoracic literature is that systemic activation is not limited to neutrophils and monocytes but may include lymphocyte activation. Shedding of L-selectin is a hallmark of cell activation which, unlike Mac-1, is not restricted to the inflammatory leukocyte subpopulation [Kishimoto 1989, Asimakopoulos 2000a]. Using this pan-reactive marker, we detected evidence for lymphocyte activation in the first 15-60 minutes following the onset of bypass, which returned to baseline levels by 24 hours (R.C.L., G.A., M.P., D.O.H., K.M.T.—submitted). However, this initial activation event was not followed by the appearance of later markers of activation, such as CD69 and CD25, suggesting that CPB did not lead to a lymphoproliferative state.

Anti-inflammatory mechanisms of aprotinin

Randomized trials have previously demonstrated that aprotinin can significantly reduce neutrophil activation at 15-60 minutes post-bypass, as assessed by diminished expression of the activation marker Mac-1 [Hill 1995, Alonso 2000, Asimakopolous 2000]. But does diminished leukocyte activation translate into diminished leukocyte sequestration into tissues? This supposition has been partially validated using broad spectrum inhibitors of the β2 integrin adhesion molecules, which demonstrated improved pulmonary function in animal models of CPB due to diminished leukocyte-endothelial cell interaction [Gillinov 1994a, Dreyer 1995]. However, the anti-inflammatory and cytoprotective mechanism of protease inhibitors remains unresolved: Is it due to diminished leukocyte adhesion to the vessel wall, diminished endothelial cell activation, or diminished cytotoxicity of sequestered neutrophils within organs, or all of the above? A role for neutrophil elastase has been identified in mucociliary dysfunction, but whether this is due to effects on leukocyte sequestration or neutralization of neutrophil elastase within the airways is not known [O'Riordan 1997]. We therefore carried out intravital microscopy experiments in rats to measure the effect of aprotinin on the three main stages of the leukocyte-endothelial cell adhesion cascade [Asimakopoulos 2000b]. Intravital microscopy permits visualization of leukocyte trafficking through the transparent mesenteric tissue and has the power to discern specific effects at each of the three steps in the adhesion cascade, which are: (1) initial rolling of leukocytes on endothelium, (2) firm adhesion, and (3) extravasation [Ley 1996].

The representative movie presented (◎) illustrates the effect of aprotinin on leukocyte responses elicited by the topical application of the chemoattractant N-Formyl L-Methyl L-Leucyl L-Phenylalanine (fMLP). The leukocyte rolling step can be seen in the first movie clip (◎) (prior to application of fMLP), which reveals no detectable difference in rolling between control and aprotinin treatment groups. The second movie clip (◎) shows firm adhesion of leukocytes to the vessel wall at 20 minutes following fMLP addition, with again no difference in the number of leukocytes firmly adhered to the vessel wall between the control and aprotinin treatment groups, but already a discernible drop in the number of extravasated leukocytes in the aprotinin treatment group. By 40 minutes a large number of leukocytes have transmigrated into the tissues in the control group but few if any have in the aprotinin group. The total dose of aprotinin infused into rats during this procedure, which lasted approximately 75 minutes from the point of incision to exteriorize the mesentery to the final 40-minute time-point on the video, was approximately 60,000 KIU/kg, compared to the total dose given to a patient throughout a CPB operation of between 60,000-120,000 KIU/kg.

The above intravital microscopy study demonstrates that aprotinin, infused at concentrations relevant to cardiac surgery, exerts no effect on either rolling or adhesion of leukocytes, but significantly inhibits the passage of leukocytes through the endothelial barrier. This observation was further supported by in vitro transmigration studies with human neutrophils and passage through cultured endothelial cells. Transendothelial migration was dose-dependently inhibited by aprotinin in response to several chemoattractant stimuli, including IL-8 and PAF, that are considered important in acute lung injury. By pretreating the neutrophil or endothelial cell populations separately, aprotinin was found to target each cell type, with maximum effects being achieved when both were targeted [Asimakopoulos 2000b]. Our demonstration that aprotinin can target the extravasation step may explain the previous observation that aprotinin can prevent neutrophil accumulation in the bronchial alveolar fluid of CPB patients [Hill 1996].

One final level of anti-inflammatory action has been discovered at the level of neutrophil degranulation. Thus we and others have shown that neutrophil degranulation is significantly inhibited by aprotinin in vitro, resulting in diminished secretion of potentially cytotoxic azurophilic granule constituents, such as IL-8, MPO and elastase [Wachtfogel 1993, Hill 1996, Asimakopoulos 2000b]. Overall, these findings suggest that not only does aprotinin act to prevent neutrophils from being sequestered into tissues, but any cells that have gained access to tissues are less capable of causing tissue damage.

In summary, aprotinin has been shown to exhibit an anti-inflammatory effect by suppressing leukocyte activation and leukocyte extravasation both in vitro and in vivo.

Aprotinin would therefore be expected to provide a significant clinical benefit in the prevention of SIRS following conventional bypass surgery. This expectation has been borne out in clinical practice, particularly in the case of high-risk patients, in which the length of hospital stay following CPB surgery was significantly reduced by aprotinin compared to other anti-inflammatory strategies, thereby more than offsetting the initial cost of the drug during surgery [Gott 1998].

Inflammation in OPCAB surgery

Initial trials that measured circulating inflammatory markers have suggested that off-pump surgery elicits an attenuated inflammatory response compared to conventional bypass surgery [Brasil 1998, Gu 1998, Struber 1999, Wan 1999, Matata 2000]. Yet despite the elimination of the artificial bypass circuit, a certain level of inflammation is still evident due to the stress response to surgery [Kilger 1998, De Paulis 1999, Gu 1999]. The early trials did not directly assess levels of leukocyte or platelet activation and should therefore still be considered as preliminary. Yet even at this stage, it seems safe to assume that any residual inflammatory response elicited by OPCAB surgery will be suppressed by the anti-inflammatory activities of aprotinin detailed above. When the anti-inflammatory properties of aprotinin are considered in conjunction with its established hemostatic properties, a strong case can be made that aprotinin will be beneficial in the management of the inflammatory response to OPCAB, as it has been to conventional bypass surgery.

Is aprotinin pro-thrombotic? The evidence in platelets suggests not

The clinical use of aprotinin has been undoubtedly constrained due to the widely held concern that a strongly hemostatic agent such as aprotinin might also be pro-thrombotic when given to patients during their bypass surgery [Cosgrove 1992, Alderman 1998, Westaby 1998]. The weight of clinical evidence does not support a detrimental association between aprotinin use and loss of graft patency [Royston 1998]. The reason why aprotinin should be considered as potentially pro-thrombotic in the first place remains unclear. In fact, the cloning of the classical thrombin receptor on platelets in 1991 revealed a receptor that, on first principles, aprotinin would be predicted to antagonize, not potentiate [Vu 1991a].

PAR1, the major thrombin receptor on platelets

The classical thrombin receptor is a member of the seven transmembrane superfamily of receptors, but exhibits a unique feature that sets it apart from other receptors: It requires proteolytic cleavage to generate an intraplatelet activating-signal. This led to the receptor being termed the "protease-activated receptor," now referred to as PAR1, the prototypical member of a subfamily of four PAR receptors (PARs1-4). Receptor cleavage is by the serine protease activity of thrombin or, to a lesser affinity, by other serine proteases such as trypsin [Vu 1991a]. Signal transduction through PAR1 depends on the initial binding of thrombin to a hirudin-like domain (amino acids 53-64) on the receptor, followed by proteolytic cleavage between arginine 41 and serine 42 [Vu 1991b]. Proteolytic cleavage unmasks a so-called "tethered ligand" in the ectodomain of the receptor that interacts with distal sequences located in the transmembrane domain, causing G-protein translocation, Ca2+ fluxing and downstream platelet activation events, such as platelet aggregation [reviewed in Coughlin 1999].

Aprotinin inhibits PAR1 activation by thrombin

Since the serine protease thrombin is known to be competitively inhibited by aprotinin [Pintigny 1992], the prediction would be that aprotinin, far from potentiating the actions of thrombin, should antagonize thrombin-induced platelet activation. We tested this hypothesis using purified platelets in vitro and found that platelet aggregation was dose dependently and significantly inhibited by aprotinin at 50-160 KIU/ml [Poullis 2000], concentrations that correspond to the low dose and levels below the high dose of aprotinin employed in CPB surgery. Inhibition of platelet aggregation was 42.6 ± 21.6% at 50 KIU/ml aprotinin ($p = 0.0047$), 61.0 ± 25.2% at 100 KIU/ml ($p = 0.0001$), and 86.6 ± 8.9% at 160 KIU/ml ($p < 0.0001$).

Aprotinin is simultaneously hemostatic but anti-thrombotic

Interestingly, blockade of thrombin-induced platelet aggregation did not prevent reaggregation in response to alternative non-proteolytic agonists, such as collagen, ADP or epinephrine [Poullis 2000]. These observations suggest that aprotinin may simultaneously prevent the participation of platelets in the coagulation cascade and therefore have an anti-thrombotic function, but, on the other hand, maintain the hemostatic capacity of platelets in surgical wounds where collagen and ADP are likely to be generated.

"Platelet preservation" explained

The anti-thrombotic properties of aprotinin described above may also explain its "platelet preservation" properties seen clinically [Wildevuur 1989, Primack 1996, Shigeta 1997]. Platelet dysfunction is a well-recognized problem of CPB surgery that is caused by platelet exhaustion secondary to the generation of thrombin during surgery. Aprotinin may preserve platelet function through its ability to prevent thrombin activation during surgery, therefore allowing more functional platelets to participate in hemostasis.

CONCLUSION

Although our in vitro observations cannot be translated directly into a predicted clinical outcome, they strongly challenge the underlying assumption that a potent hemostatic agent per se should be pro-thrombotic. There have been numerous clinical trials to examine the relationship between aprotinin and graft patency in CPB surgery, the balance of which do not favor a detrimental association between aprotinin use and loss of graft patency [Royston

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1998]. We believe that one reason this assumption has been allowed to persist is that there has been a lack of mechanistic understanding to explain the diverse actions of aprotinin. Whereas much has been written about the effect of aprotinin on the coagulation cascade and fibrinolysis, there has been no basic science research to underpin arguments for or against a thrombotic action on platelets. By demonstrating that aprotinin can be simultaneously hemostatic but anti-thrombotic, we hope to have eased concerns over the safety of aprotinin and encouraged the clinical use of a drug that does so much to maintain the balance of the coagulation and inflammatory systems that are thrown into disequilibrium by cardiac surgery.

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