Use of Aprotinin in Patients Undergoing Deep Hypothermic Circulatory Arrest: A Review

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Abstract: Hemostatic derangements continue to be a major clinical challenge during thoracic aortic surgery using deep hypothermic circulatory arrest despite advances in surgical and pharmacologic therapy. Aprotinin, a broad-based, nonspecific serine protease inhibitor has been advocated for prophylactic use in cardiac surgery to decrease perioperative blood loss and blood transfusions. Its efficacy has been documented in several studies throughout the United States and Europe. Currently, aprotinin is advocated for use in patients undergoing cardiopulmonary bypass in the course of coronary artery bypass graft surgery. A review of current studies is provided that examines aprotinin usage under deep hypothermic circulatory arrest. Keywords: aprotinin, cardiopulmonary bypass, circulatory arrest, renal insufficiency, thrombosis. JECT. 2003;35:339–345

Although remarkable advances have been made in the surgical treatment of thoracic aortic surgery, coagulation disturbances are a common source of morbidity and mortality (1). Deep hypothermic circulatory arrest (DHCA) in concert with cardiopulmonary bypass (CPB) is routinely utilized in these major aortic procedures that lie in the complex and high-risk end of the spectrum of cardiac procedures. The problem of altered hemostasis remains a major challenge during thoracic aortic surgery. These hemostatic derangements are a result of multiple factors: disruption of vascular integrity and extensive surgical dissection, temporary need for complete inhibition of the coagulation process, large fluid and blood product usage, hemodilution, hypothermia, ischemia and reperfusion, extracorporeal circulation systems, and the resulting activation of systemic inflammatory response and inhibition of fibrinolysis (2,3). Often, bleeding can be attributed to the degree of aortic dissection as well as the friable attributes of pathological aortic tissue in these cases. The use of impervious vascular grafts (4) impregnated with collagen, gelatin, or albumin, as well as an array of biological glues have contributed greatly to a decrease in bleeding and the often vicious cycle that follows: transfusion of bank blood and an abnormal coagulation profile (4–6).

Aprotinin (Trasylol) is a broad-based, nonspecific serine protease inhibitor isolated from bovine lung. It was first used in cardiac surgery to inhibit plasmin-induced complement activation during CPB. Serendipitously, significant reductions in blood loss and blood transfusion requirements were noted in treated patients, and it is now widely advocated for prophylactic use in cardiac surgery to reduce perioperative blood loss and subsequent blood transfusions (7–11). Subsequent investigation showed that aprotinin preserves hemostatic function for CPB (12), especially in high-risk cardiac procedures where such coagulopathies as platelet dysfunction may be expected. The mechanism by which aprotinin achieves its hemostatic effect is still unknown. Several mechanisms seem to contribute to the hemostatic effect, including a decrease in fibrinolysis (13), inhibition of neutrophil activation (14–15), and preservation of platelet membranes via a reduction in glycoprotein loss (Gplb/Gpplb/Gplla) (15,16). Aprotinin, through its inhibition of kallekrien, attenuates the effects of contact activation that occurs when blood meets the nonendothelized CPB circuit. The dose for inhibition of different enzymes varies. For example, about 50 KIU per mL is required to inhibit plasmin and about 200 KIU per mL is necessary to inhibit kallikrein. Aprotinin's main pharmacokinetic actions are based on its ability to inhibit proteases such as trypsin, plasmin, and tissue kallikrein (17). Furthermore, its molecular weight is low (6500 Daltons), and it is rapidly eliminated ($1/2 = 2.5$ h) from the
circulation by glomerular filtration with temporary partial storage of the administered dose (89–90%) by the proximal tubular cells (18).

The efficacy of aprotinin in reducing bleeding and the need for homologous blood transfusions in cardiac surgery with the use of CPB is documented in a large number of randomized, double-blind, placebo controlled studies performed throughout the United States and Europe (19–21). These results have inevitably meant that the use of aprotinin would be extended to any situation in cardiac surgery where bleeding may be a problem. Thus, patients undergoing aortic repair with DHCA with its increased risk of coagulopathy would be expected to benefit from aprotinin. It is logical to suggest that aprotinin would offer similar benefits in hypothermic circulatory arrest cases, as has been documented in patients undergoing CPB in the course of coronary artery bypass graft (CABG) surgery.

However, the safety of aprotinin when used with DHCA is still a matter of intense debate, despite its presumed salutary effects on blood loss. The questions to be addressed when deciding whether or not to use aprotinin must be based on the perceived as well as the documented benefit and then any safety issues concerning its use. A review of current studies is provided that examines aprotinin utilization in hypothermic circulatory arrest, beginning with a review of hypothermic effects on blood coagulation.

Hypothermia and Effect on Blood

Hypothermia itself exerts several anticoagulant effects, the most basic of which is kinetic: decreased temperatures slow the enzyme reactions of the coagulation cascade resulting in prolonged clotting times, regardless of clotting factor levels. Prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombin time (TT) display a negative exponential correlation with temperature (22,23). These kinetic effects are presumably the major factors responsible for the prolonged ACT seen in hypothermic blood independent of heparin concentration. The kinetic effects on blood temperature are reversible with warming of the blood and do seem to be affected by aprotinin. Hypothermia also activates kallikrein, causing an increase in the conversion of kininogen to kinins. Kinins are vasoactive peptides that induce vasodilation and increased vascular permeability. Kinins are normally degraded in a single pass through the lungs by a pulmonary-converting enzyme on the luminal surface of the vascular endothelial cells. This is obviously greatly decreased on CPB, with blood flow to the lungs diverted through the extracorporeal circuit. By inhibiting kallikrein, aprotinin may block the conversion of high molecular weight kininogen to the inflammatory mediator bradykinin, as well as inhibit the activation of C1 of the complement system (24). Thus, the kallikrein-activating effects of CPB and hypothermia and loss of the normal degradation pathways make the kallikrein-inhibiting effects of aprotinin attractive when CPB is employed (25).

Similarly, hypothermia and CPB are associated with many well-described platelet defects (26–29). Hypothermia induces temperature-dependent morphologic alterations that result in changes in platelet membrane and function (30), including increased adhesiveness, inhibition of ADP-induced aggregation, and decreased synthesis of both thromboxane and prostacyclin (31). Platelet dysfunction, both qualitative and quantitative, are the most predictable and consistent disturbances in hemostasis and the most common cause of intraoperative and postoperative bleeding (16). Platelets are integral for adequate hemostasis, because they are the first blood elements to arrive at a vascular injury site, which then enables the hemostatic system to recognize the injury.

Once on bypass, the contact of the platelets with the foreign surfaces of the extracorporeal circuit results in expression and subsequent loss of platelet surface glycoproteins. The surface glycoprotein GpIb is always exposed on the platelet surface and takes part in adhesion (16,32). Thrombocytopenia occurs as a result of hemodilution, hypothermic-induced splancnic sequestration of platelets, and platelet destruction form blood/gas and blood/tissue interfaces created by cardiotomy suction and filters.

The use of intraoperative aprotinin under conditions of moderate hypothermia significantly reduces the effects of CPB on platelet aggregation and function (16). Recovery of platelet function is as rapid as in normothermic patients (33).

Numerous alterations to the fibrinolytic system on CPB have also been documented. These include elevated fibrinogen degradation products (34) decreased plasminogen (35), and increased fibrinolytic activity (36). Hypothermia alone also alters fibrinolytic activity by reducing the enzymatic rate of conversion of fibrinogen to fibrin. The antifibrinolytic action of aprotinin should be effective against the cumulative antifibrinolytic effects of CPB and hypothermia.

Protein C is one of the most important natural anticoagulant mechanisms that might be exerting a protective effect in the setting of stasis during DHCA. Protein C is activated by the endothelial factor thrombomodulin through a calcium-dependent interaction. Thrombomodulin binds to thrombin, which reverses the procoagulant effect of thrombin. This thrombomodulin/thrombin complex activates the protein C system that accelerates its anticoagulant activity (37) through destruction of factors Va and VIIIa, which prevents the formation of Factor Xa and thrombin. Activated protein C induces fibrinolysis by releasing tissue plasminogen activator (t-PA) from the en-
dothelial cellsurface. Smith and Spanier surmise that since activated protein C is a serine protease, its anticoagulant activity might be attenuated by aprotinin. However, this action of aprotinin is, in all likelihood, taking place in non-DHCA procedures without obvious sequelae, and as of yet, there is no evidence to support that this net effect is altered at lower temperatures (25). Furthermore, aprotinin has not been shown to decrease protein C activity in patients undergoing CPB (38), but this has been demonstrated in vitro (39).

The mechanism by which aprotinin achieves its hemo-
static effect remains to be fully elucidated. It may be caused by interference with contact activation of the intrinsic coagulation pathway that occurs with exposure of blood to the bypass circuit as well as preservation of plate-
let function, inhibition of fibrinolysis and the kinin-
kallikrein pathway. Aprotinin’s main pharmacokinetic ac-
tions are based on its ability to inhibit proteases such as trypsin, plasmin, and tissue kallikrein. Aprotinin also ap-
ppears to exhibit both coagulant and anticoagulant prop-
erties.

Aprotinin and Deep Hypothermic Circulatory Arrest: A Decade in Review

In 1993, in a retrospective study investigating renal dys-
fuction with high-dose aprotinin use, Sundt and col-
leagues (40) identified 20 patients undergoing thoracic aorta operations using profound hypothermic circulatory arrest and compared the results to 20 age-matched control patients undergoing similar operations without aprotinin dosing. They found that the use of aprotinin in elderly patients undergoing such complex procedures was associ-
ated with an increased risk of renal dysfunction (defined as serum creatinine levels in the first postoperative week to a level 1.5 times the preoperative value or higher) as com-
pared to the age-matched control group. Renal failure occurred in 65% of the aprotinin-treated patients, all of whom were 65 years of age or older. Widespread intra-
vascular coagulation was also noted in the kidneys, heart, lungs, and brain on autopsy. The study further failed to find a reduction in blood loss when comparing the two groups. In the discussion section of this study, the authors mention that additional antifibrinolytic therapy with the use of alpha-aminocaproic acid (Amicar) was used in six patients, three of whom developed renal failure and three of whom died.

Westaby (41) and colleagues in 1994 reported results in a series of 80 consecutive patients operated on between 1987 and 1992 for Type A dissection, before and during the aprotinin era. Twenty-seven patients were operated on without aprotinin, and 53 patients received high-dose aprotinin. This group reported a “significant” increase in blood loss for patients who had undergone DHCA with aprotinin (control: 837 mL/24 h ± 90; aprotinin: 1929 mL/24 h ± 90) as well as a greater incidence of thrombosis-related deaths. They also admitted to adjusting the hepa-
rinization protocol after about 2 years to account for aprotinin’s effect on ACT, but still did not provide any data relating results to the time period or new heparin protocol. Furthermore, this study was not without design errors: patients were not matched, and almost half of the patients were studied retrospectively. Standard errors were calculated, but statistical significance tests were not performed. Westaby concluded that aprotinin association with DHCA may promote a coagulopathic state and actually increase bleeding.

Although these observations were real in the Sundt and Westaby investigations, an alternative explanation for the coagulopathic state was most likely attributable to inade-
quate heparinization during the surgery (42). Clarifica-
tions for these phenomena are as follows. The first reason was that during this time period, the recommended ACT with aprotinin use was greater than 700 seconds, but this was later shown to be too low a value during hypothermia. The second reason was that these studies were carried out before the distorting effects of aprotinin on the celite ACT
was appreciated. It was not until 1992 that the first reports of better monitoring of anticoagulation levels with use of kaolin ACTs were published (43,44). When celite is the activating agent in the presence of aprotinin, the ACT is artificially elevated varying from 29–71% in several reports (43,45). In addition, hypothermia alone tends to increase the ACT artificially (43,46) which can result in aprotinin-treated patients receiving less heparin than patients who do not receive aprotinin. As a result, Sundt’s initial target ACT of 480 seconds combined with the temperature effect further reduces the possibility of reaching an ACT low enough to trigger administration of additional heparin, resulting in inadequate heparinization of these patients. Westaby reported an increase in morbidity and mortality between the aprotinin-and nonaprotinin-treated patients, but no statistical significance is given. In Westaby’s study, the ACT was maintained at greater than 700 seconds for aprotinin treated patients, but the activator used for measuring ACT, celite or kaolin, is not mentioned.

In contrast to Sundt and Westaby’s study, Goldstein (47), in a 1995 study, claimed benefit with high-dose aprotinin use in DHCA. This study compared 23 DHCA patients who received aprotinin with 24 matched historical controls, and found a significant (p < .01) reduction in requirements for postoperative homologous blood transfusions, but no difference was seen in intraoperative blood requirements. The aprotinin-treated DHCA patients showed a significant difference in the number of patients who did not receive any transfusions (16.7 vs. 4.1%) as well as the number of patients who required less than 10 total units of homologous blood products (45.8 vs. 12.5%). No difference was noted between the two groups in total chest tube drainage (488 vs. 495 mL). Furthermore, there were no differences in the incidence of neurological dysfunction or postoperative myocardial infarctions and a trend toward reduced mortality in the aprotinin-treated patients. Although the aprotinin-treated patients had renal dysfunction 3.5 times more frequently than the control group, as defined by Sundt et al., this did not reach statistical significance. Furthermore, none of the surviving patients in either group required permanent hemodialysis. The authors conclude that aprotinin is safe in DHCA when an ACT of greater than 1000 seconds is used.

As with previous studies, there are methodological weaknesses with the Goldstein study, most notably the lack of randomization and the potential for bias. The authors state in the discussion that a randomized study design was not acceptable to the surgeons, which could be interpreted as bias in favor of aprotinin. In addition, because of its retrospective nature, the control group overlapped with the aprotinin-treated patients in time. Could this mean that certain populations of patients (renal dysfunction or those patients at high risk for thromboembolism) were not given aprotinin? The trigger for homologous blood transfusions was also not stated, and this could lead one to different conclusions based on this information.

A prospective study by Regragui (48), published in November 1995, prospectively investigated a consecutive series of 19 patients undergoing surgery of the ascending aorta and/or the aortic arch with DHCA in conjunction with high dose aprotinin from January 1993 to October 1994. Activated clotting times were kept greater than 750 seconds utilizing a celite activator. No clinically evident neurologic or cardiac events were detected. Mean total chest tube drainage was 878 ± 548 mL (range 300–2000 mL) with a mean usage of homologous blood products of 2328 ± 1600 mL. There was one patient death, but no intravascular thrombosis was found. However, significant early postoperative renal dysfunction was seen in more than 30% of patients, but no dialysis or other renal support was necessary. In patients discharged from the hospital with an elevated serum creatine (15%), this had fallen to normal limits within 6 weeks of the surgery. Overall, this study reports excellent early survival in a consecutive series of patients undergoing aortic surgery with DHCA and aprotinin use with no comparison made to a control group.

Okita and colleagues (49) reported a study published in 1996 that examined 112 consecutive patients who underwent aortic surgery. Sixty of these patients underwent DHCA, and 39 of these 60 patients received aprotinin, at a dose of 2 × 10^6 U in the pump prime only. The study started out as a prospective, randomized study, but a bias toward the use of aprotinin began at some unspecified point, because the authors felt “...aprotinin was effective for preventing ... bleeding after lung manipulation.” Blood loss after CPB (p = .047), chest tube drainage (p = .039), and homologous blood transfusions in the intensive care unit (p = .009) were significantly less in the aprotinin treated patients.

A prospective study by Godet and associates (50) from Paris, France, on 475 consecutive patients over a 12-year period ending in 1996, looked at risk factors for acute renal failure in thoracic or thoracoabdominal aortic surgery. In 255 patients operated on from January 1991, the procedure was carried out with aprotinin administration in a modified protocol (2 million KIU at the start of CPB followed by 500,000 KIU/h). The authors were surprised to find that multivariate analysis showed that the use of CPB, DHCA, and the administration of aprotinin were not found to influence the onset of acute renal failure. Such perioperative variables as age, preoperative renal dysfunction, and homologous blood transfusions were significantly associated with the onset of acute renal failure.

Parolari and colleagues (51) reviewed charts of 39 consecutive patients who underwent surgery for thoracic aor-
tic procedures requiring DHCA. Eighteen (46.2%) of these DHCA patients received high-dose aprotinin between 1990 and 1994; whereas, 21 (53.8%) patients operated on from 1987 to 1989 did not receive aprotinin. Activated clotting times utilizing the celite tubes were kept at greater than 800 seconds for the aprotinin treated patients and greater than 400 seconds in patients who did not get aprotinin. Blood loss and transfusion parameters were not significantly different between the two groups. They also claimed a slightly higher, but not statistically significant, incidence of neurological injury in aprotinin-treated patients. The authors were surprised to find an increased rate of permanent neurological deficits in the aprotinin-treated patients, because 11 of the 18 patients in the aprotinin group had been protected with the use of either antegrade or retrograde cerebral perfusion technique during DHCA. Furthermore, as pointed out by Smith (25) and the authors of this study, some caution may be appropriate before accepting conclusions from this particular study on the use of aprotinin in DHCA as this averages out to an experience of 3.6 procedures per year (Type 2 statistical error).

Okita and colleagues (52) retrospectively measured coagulation and fibrinolysis in 94 patients undergoing aortic surgery with DHCA and full dose aprotinin. Patients were divided into two groups: 45 patients were given heparin to keep the kaolin ACT greater than 500 seconds, and 49 patients received a continuous heparin drip of 1 mg/kg/h regardless of the ACT. No differences in morbidity and mortality were measured between the two groups, and no differences in the amount of bleeding in the first 24 hours postoperatively were noted. Blood coagulation tests demonstrated similar prothrombin and activated partial thromboplastin times as similar. The fibrinogen titer was higher in the aprotinin group after bypass, and the anti-thrombin III was identical in both groups. Fibrin degenerative products showed no difference but the d-dimer titer was lower in the aprotinin treated patients. In regard to fibrinolytic activity, plasminogen, alpha 2-plasmin inhibitor, and plasmin-alpha 2-plasmin complex, there was no significant difference between the two groups. Platelet count after the surgery was higher in the aprotinin-treated group with lower levels of platelet factor-4 and beta-thromboglobulin as compared to control. This study also found a higher incidence of transient cerebral dysfunction and delirium in the control group. They concluded that a policy of repeated heparin administration is necessary, irrespective of ACT measurements.

Ehrlich et al. (53) finally performed a prospective, double blind randomized study in 1997. This study evaluated the efficacy and safety of low-dose aprotinin on renal function in 50 patients undergoing operations on the thoracic aorta under conditions of DHCA compared to a placebo-controlled group. Heparin dosing for bypass was calculated at 3 mg/kg and measured with kaolin activated clotting times (ACT). Renal dysfunction was defined as an elevation of the serum creatinine level in the first postoperative week to a level 1.5 times the preoperative value or higher. This study found that in the first 48 hours postoperatively, no increase in renal dysfunction was found. The investigators measured the specific markers beta-N-acetyl-D-glucosaminidase (beta-NAG) and beta-2-microglobulin that are reflective of damage to the proximal tubule cells of the kidney, and found no significant difference between the study and control groups. In addition, no differences in blood urea nitrogen, creatinine, creatinine clearance, and excretion fractions of potassium and sodium were found. This study found no deleterious effects of low-dose aprotinin on renal function, or any statistically significant difference in the incidence of transient or permanent neurologic deficits or myocardial infarction. The low-dose aprotinin regime still afforded a decrease in blood loss ($p = .04$) and red blood cell transfusion requirements ($p = .0001$).

**Use of Aprotinin After Circulatory Arrest Period**

Some groups have shown benefit with aprotinin during the rewarming phase of CPB (54). Rooney and Bonser (55), using a modified aprotinin protocol since 1993, do not give aprotinin until CPB has been re-instituted after DHCA. Use of this protocol is intended to avoid the initiation of intravascular coagulation during the period of circulatory arrest and gain the hemostatic benefit of aprotinin in conjunction with platelets, fresh frozen plasma, and cryoprecipitate (10 units of each) that are given after protamine administration. Although this study reports acceptable results in terms of postoperative blood loss and transfusion requirements, renal dysfunction, myocardial dysfunction, stroke, and mortality, they cannot claim superiority of this modified protocol over either not using aprotinin or the standard aprotinin regimens.

**CONCLUSION**

The use of aprotinin in DHCA continues to be a controversial topic. Early reports of aprotinin use in DHCA were associated with significant adverse events and no reduction in transfusion requirements (40,41). This has since been attributed to the use of celite ACTs and subsequent under heparinization prior to our understanding of the impact of aprotinin on celite ACT. More recent reports, including a prospective randomized study suggest that aprotinin in combination with DHCA is not associated with significant adverse events provided that patients are adequately heparinized, and may have some benefit in reducing blood loss and homologous blood transfusions (47–48,52–53,55).

Inevitably, the role for aprotinin utilization in DHCA.
will manifest as the spectrum of aprotinin’s mechanism of actions on the hemostatic, coagulation and inflammatory systems are more clearly delineated. The role of stasis, a major prothrombotic stimulus associated with DHCA, is not very well defined clinically or experimentally, and no evidence supports the belief that aprotinin becomes prothrombotic in DHCA.

Because approximately 90% of an administered aprotinin dose is known to collect in the brush border of the convoluted tubules and remains there for 12 to 14 hours, concern has been raised that high-dose aprotinin may adversely affect the kidney (57). However, the risk of renal failure following surgery for acute aortic dissection is reported to be 10–23% in contemporary series, irrespective of any antifibrinolytic therapy. The increase in serum creatinine in aprotinin-treated patients seems transient for the most part, returning to baseline anywhere from a few days to 6 weeks postoperatively.

The studies to date on aprotinin and DHCA have several limitations. The major obstacle in determining the efficacy of aprotinin in DHCA is there is only one formal randomized placebo-controlled study (n = 50) Most studies to date on aprotinin use in DHCA have been retrospective or historical controlled data. Only one trial in adults has been prospective and randomized (53). Another trial began with a randomized design but was later aborted, because the authors felt that the benefits of using aprotinin outweighed finishing the study to completion (49).

Almost certainly, the alarming incidence of complications in the first reported studies of aprotinin with DHCA resulted from inadequate heparinization. With the realization of aprotinin’s effect on celite and subsequent recommendation that kaolin ACTs be used instead, further studies did not demonstrate such complications. From a safety issue, the data thus far support use of aprotinin in DHCA, provided that adequate heparin concentrations are maintained.

The efficacy of aprotinin in DHCA is still questionable. This is mostly attributable to the lack of randomization studies, but can also be attributed to the nonhomogeneous methods of reporting blood loss and transfusion requirements. As a result, the evidence for reducing blood loss and transfusion requirements with the use of aprotinin in DHCA is not staggering.

To date, aprotinin, manufactured under the trade name Trasylol®, is only indicated for prophylactic use to reduce perioperative blood loss and the need for blood transfusion for patients undergoing CPB in the course of CABG surgery. Thus, it is an off label use in DHCA with aprotinin.

There is no reason to believe that aprotinin use in DHCA is associated with increased risk of morbidity and mortality. Prospective, randomized studies are still needed to determine the efficacy of aprotinin use in DHCA. Blood loss and transfusion requirements have not been uniformly demonstrated for a variety of reasons, and may depend on clinical factors not easily elucidated or isolated. Use of aprotinin in DHCA needs to be based on the same considerations applied in other cardiothoracic procedures.

REFERENCES