

LETTERS to the EDITOR

Does aprotinin affect blood loss in liver transplantation?

SIR,—The kallikrein inhibitor aprotinin reduces blood requirements in orthotopic liver transplantation (OLT), although comparisons were with historical controls.¹⁻³ Because the perioperative transfusion requirement in OLT is influenced by various factors, including the experience of the transplantation team, we have done a prospective double-blind, placebo-controlled study of aprotinin.

20 patients undergoing primary elective OLT between February and September, 1991, were randomly divided. The groups were similar for demographic and preoperative laboratory data. Group I received a loading dose of 2 million kallikrein inhibiting units (KIU) of aprotinin after induction of anaesthesia, followed by a continuous infusion of 5×10^5 KIU until the end of the procedure. Group II was given placebo. All transplants were done with a standard anaesthetic regimen and by the same surgical team, with venovenous bypass. Haematological and haemostatic indices were monitored in theatre with a mobile laboratory unit.⁴ Red blood cells, platelets, and fresh frozen plasma were transfused to maintain a packed cell volume of 30%, a platelet count of $30 \times 10^9/l$, and a prothrombin ratio below 1.33. Antithrombin III was given at plasma concentrations below 70%. 2 patients had to be excluded because of massive surgical bleeding and death during the study period.

EFFECTS OF APROTININ ON TRANSFUSION REQUIREMENTS, AND DURATION OF OPERATION		
	Aprotinin	Placebo
Red cells (units)		
Intra	18 (6-35)*	20 (6-32)
Post	3 (0-14)	7 (0-20)
Plasma (units)		
Intra	28 (8-58)	30 (12-55)
Post	8 (2-29)	13 (2-43)
Platelets (units)		
Intra	2.0 (0-5)	0.4 (0-2)
Post	0.4 (0-2)	2.2 (0-8)
Total time	424 (335-550)	429 (355-530)
Time rep-end	122 (90-157)	143 (100-170)

*Mean and range Rep-end = time from reperfusion until end of operation

No significant differences (U test) were observed between the groups in the perioperative requirement of red cells, platelets, and plasma, or in duration of the procedure (table). In agreement with our previous study,⁴ fibrinolysis during OLT was uncommon compared with the reported frequency.^{1,3,5} Fibrinolysis was observed by thromboelastography in only 2 out of 18 patients (11%), both of whom received placebo. The low frequency of fibrinolysis is most likely due to the immediate treatment of coagulation disorders made possible by immediate laboratory monitoring. Under these conditions aprotinin did not significantly reduce "oozing" during OLT.

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IgE-mediated anaphylactic reaction to aprotinin during anaesthesia

SIR,—Allergic and pseudoallergic reactions to anaesthetics, especially muscle relaxants, are well known, but in general anaesthesia other drugs may be involved in anaphylactic reaction.^{1,2} We have described a case of anaphylactic shock due to mannitol during anaesthesia.³ We now report severe anaphylaxis to aprotinin in a boy during general anaesthesia. Aprotinin is now used routinely during cardiac surgery to reduce operative blood loss.⁴

The 3-year-old boy required open-heart surgery because of a congenital heart defect and received high-dose intravenous aprotinin during the procedure. 2 months later, in August, 1991, pleural decortication was necessary because of bilateral chylothoraces, and we decided to give aprotinin again. Immediately after the dose, facial flushing, severe bronchospasm, and decrease in arterial pressure to 30 mm Hg occurred. The infusion was stopped and, after intravenous treatment with adrenaline, noradrenaline, and corticosteroids, and fluid administration, cardiovascular stability was restored, bronchospasm was resolved, and the operation was concluded successfully.

A serum sample taken 1 month after the first aprotinin infusion and 1 month before the reaction had been frozen. Anti-aprotinin IgE antibodies could be detected by radioallergosorbent test (RAST)⁵ in this sample and in samples taken 8 days and 3 months after the last surgical intervention. The earlier sample (July 25, 1991) was RAST class 2 positive (3.4 U/ml), indicating the presence of specific IgE antibodies against aprotinin. Thus, these antibodies were likely to be responsible for the hypersensitivity reaction immediately after receiving aprotinin again. We assume that sensitisation was induced by the first infusion in June, 1991. The serum samples taken after the anaphylactic shock (Aug 28 and Nov 21, 1991) were weakly positive as class 1 (0.6 and 0.4 U/ml, negative ≤ 0.35). This indicates depletion of circulating IgE antibodies and no new synthesis in the absence of antigenic stimulation as seen, for example, in immediate hypersensitivity to penicillin.⁶

The close relation between the intravenous administration of the drug and the typical anaphylactic symptoms shows that the reaction was probably IgE mediated. A non-allergic, anaphylactoid reaction due to histamine has been linked to the adverse reaction to aprotinin.^{7,8} IgE and IgG antibodies to aprotinin have been detected in a high proportion of sera from patients receiving aprotinin therapy and who had adverse reactions (10/18), and also in the absence of clinical symptoms (14/44).⁹ However, these immunological findings have not been confirmed. There has been one case of anaphylactic reaction to aprotinin, in which specific IgE serum antibodies could be detected with a strong urticarial reaction with diluted aprotinin in skin testing.¹⁰ Because the frequency of hypersensitivity reactions after repeated aprotinin administration is about 10%,¹⁰ anaesthetists and surgeons especially should be aware of this potential side-effect. We recommend RAST with aprotinin

before repeated administration, or at least serum sampling before operation, to enable serological examination later.

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Life-threatening reaction after viper bite: detection of venom-specific IgE by dot assay

SIR,—The only potentially dangerous snake in Western Europe is the viper, *Vipera aspis*.¹ Allergic and anaphylactic reactions have been reported.²⁻⁵ We report a near fatal reaction, which was at least partly IgE mediated, to a viper bite.

A 41-year-old man with no history of atopy had been bitten by a viper 2 years previously without systemic reaction. While hiking in the Alps, he was bitten a second time and lost consciousness for 10 min. On admission 2 h later he had abdominal pain, dyspnoea, cyanosis, urticaria, swelling of the face, and laryngeal oedema. Systolic blood pressure was 90 mm Hg. Despite rapid treatment with plasma expanders, intravenous adrenaline, and steroids he had to be intubated. Shock followed with haematemesis, rectal bleeding, haematuria, diffuse intravascular coagulation, and non-cardiac pulmonary oedema that required mechanical ventilation for 9 days. His bleeding was only controlled after 3 days of heparin and 75 ml specific anti-viper horse serum (Bern, Switzerland).

To determine whether the reaction was toxic or allergic, we developed a dot assay for anti-venom IgE. Serum was obtained at the start of the horse serum therapy (day 1), on day 7, and 9 months later (day 259). Serum from a female patient who had been bitten several times without reaction was used as a negative control. Crude venom from live *V aspis* and *V ammodytes* was obtained and kept at -80°C. 1 µl undiluted venom and 1/10, 1/30, and 1/60 dilutions in "tris"-buffered solution (TBS) were dotted on a nitrocellulose (NC) membrane (Millipore, 0.45 µm). The membrane was air-dried for 1 h at room temperature and blocked with 5% milk in TBS. NC strips were incubated overnight at room temperature with the test serum diluted 1/10 in TBS. The strips were washed five times with buffer and ¹²⁵I anti-IgE (Pharmacia) was added for 2 h at 125 000 counts per minute. The strips were washed five times, air dried, and autoradiographed for 10 days.

To detect horse IgG in the patient's serum an enzymeimmunosorbent assay was used. The first step was the same as for the radioimmunosorbent assay (with venom diluted 1/1, 1/10, 1/100, 1/1000). After overnight incubation, the NC strips were incubated for 2 h at room temperature with anti-horse rabbit IgG (Dako Z 309) diluted 1/1000 in 5% milk. Biotinylated anti-human IgE (Milan Analytica) at 1/500 in 5% milk was used to confirm the radioimmunosorbent assay. The strips were washed, incubated for 1 h with avidine alkaline phosphatase (1/1000 in NaHCO₃), washed

again with buffer, and incubated for 30 min in the dark in nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. The reaction was stopped with "tris"-edetic acid.

Specific IgE against *V aspis* but not against *V ammodytes* venom was detected in our patient's serum on day 1, 7, and 259 by the radioimmunosorbent assay. No IgE against the venoms of both snakes could be detected in control serum. With the enzymeimmunosorbent assay specific IgE was weakly detected on days 1 and 7 whereas on day 259 the reaction was strong. Detection on day 1 was probably inhibited by horse IgG in the patient's serum; horse anti-venom IgG was detected on days 1 and 7 but not on day 259.

Our patient probably had an anaphylactic reaction after a second bite by *V aspis*. Specific IgE against this venom did not crossreact with that of *V ammodytes*, a related species. Our assay had good specificity (other controls included serum from cord blood and from subjects not exposed to *V aspis*). The later clinical manifestations of the patient cannot all be attributed to an IgE-mediated reaction and were probably toxic, although coagulation defects have been described in anaphylaxis.⁶

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PSA-con-A binding ratio in benign prostate hyperplasia and prostate cancer

SIR,—Dr Marrink and colleagues (March 7, p 619) report prostate-specific antigen (PSA) binding ratios to concanavalin A (con A) sepharose in benign prostate hyperplasia and prostate cancer. Serum samples were incubated with con-A sepharose (Pharmacia), and the concentration of non-binding PSA was measured in the supernatant after centrifugation, as others have done.^{1,2} Marrink et al find no striking difference in glycosylation pattern of PSA in both conditions and conclude that the PSA-con-A binding ratio cannot be used to distinguish between these prostate lesions. However, the con-A sepharose was dried in air and subsequently reswollen, which is contrary to manufacturer's recommendations for both optimum sepharose swelling capacity and lectin-binding performance. Moreover, part of their argument is based on the results of varying the con-A concentration (ie amount of con A) during incubation with the serum samples. In the experiments described, fixed serum volumes (500 µl) seem to be incubated with swollen con-A sepharose gel, corresponding to 25, 75, 150, and 400 mg dry material, thus giving 5, 15, 30, and 80% weight/volume, respectively. However, a meaningless weight to volume unit is used, being the amount of dry con A divided by the serum volume, which is much higher than the true con-A concentration in the experiments. A striking correlation between the amount of con A present during incubation and the con-A binding ratio—defined as PSA after incubation divided by PSA in serum, × 100%—is found. In our view, this correlation is largely attributable to the dilution of PSA-containing serum with buffer bound in the con-A sepharose gel. Incubation with swollen 400 mg dry con-A sepharose would be much more dilute than an incubation with swollen 25 mg dry material, which is not corrected for.

To demonstrate the dilution effect we incubated 0.5 ml serum samples with varying amounts (0.15, 0.3, 0.6, 1.2, 2.4 ml) of