Clearance and tissue distribution of staphylococcal enterotoxin A in the rat and potential use of adsorbents for removal from plasma

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Summary. Many of the profound effects of staphylococcal septicaemia are thought to be the result of entry of enterotoxins into the systemic circulation. The aim of this study was to investigate the disposition of staphylococcal enterotoxin A (SEA) in the rat and its possible removal from blood. SEA labelled with ^{125}I was administered intravenously ($250\text{ }\mu\text{g/kg}$) to rats. The blood clearance of SEA showed a biphasic pattern; an initial fast disappearance (half-life *c.* 3 min) was followed by a slower one (half-life *c.* 2 h). Thirty minutes after injection of ^{125}I -labelled SEA, most of the radioactivity was concentrated in the kidneys, indicating that renal excretion was the main route of elimination of SEA. The adsorption capacities of polymer-coated activated charcoal (DHP-1 and Adsorba 150C), uncharged resin (Amberlite XAD-7), anion exchange resin (Dowex-1) and polymyxin B matrix were assessed by measurement of the equilibrium adsorption isotherms for SEA. DHP-1 charcoal, Amberlite XAD-7 resin and Dowex-1 resin adsorbed similar amounts of SEA in human plasma. Plasma perfusion experiments were performed *in vitro* with small columns containing either charcoal or resin adsorbents. Over 4 h perfusion, DHP-1 charcoal removed 50% of the initial amount of ^{125}I -SEA, Adsorba 150C charcoal 8.1% of SEA and Amberlite XAD-7 resin 32.5% of SEA. These results suggest that it may be feasible to develop the adsorbent columns for direct removal of SEA from the plasma of patients with staphylococcal septicaemia.

Introduction

The exotoxins released by *Staphylococcus aureus* include enterotoxins (SEs) A, B, C1–3, D and E, toxic shock syndrome toxin 1 (TSST-1) and staphylococcal exfoliative toxin (ExFt). These toxins are structurally closely related^{1,2} and are known to be responsible for human diseases such as food poisoning,³ shock⁴ and scalded skin syndrome.⁵ In mice and monkeys, these toxins produce rapid weight loss and death.^{6,7} Staphylococcal sepsis in man could cause potent enterotoxins to circulate throughout the body, resulting in damaging effects. Studies have been performed in animals to characterise the toxemia produced by parenteral administration of staphylococcal enterotoxins. Intravenous administration of SEB produced fever and leucopenia followed by leucocytosis and shock.⁸ Shock appeared to develop through pathophysiological mechanisms similar to those which accompany bacterial endotoxaemia,⁹ which is mediated by cytokines released from macrophages or monocytes as a result of stimulation by lipopolysaccharide (LPS).^{10,11} The staphylococcal toxins also increase susceptibility to endotoxic shock,¹² which may be important clinically.

The specific mechanisms involved in the toxicity of staphylococcal enterotoxin A (SEA) have been elucidated only recently, in studies which have demonstrated that nearly all of the toxins bind to major histocompatibility complex (MHC) proteins.^{13,14} These complexes of toxin and MHC proteins stimulate T cells,^{15,16} causing the release of large amounts of cytokines, including tumour necrosis factor (TNF) and γ -interferon (IFN- γ).^{17,18} These responses are beneficial to the host in small quantities, but damaging in excess. Direct removal of these enterotoxins from the circulation may be of potential therapeutic value in preventing the consequences of staphylococcal septicaemia.

In the present study, the clearance and tissue distribution of SEA was investigated in rats and the capacities of various adsorbents to bind this toxin *in vitro* assessed with the aim of directly removing the toxin from the circulation.

Materials and methods

Purified staphylococcal enterotoxin A was provided by the Centre for Applied Microbiology and Research, PHLS, Porton Down, Wilts. Radioactive sodium (^{125}I)iodide (100 mCi/ml) was obtained from Amer-

sham International plc, Buckinghamshire. DHP-1 charcoal, coated with polyhema, was purchased from Kuraray Co. Ltd, Osaka, Japan; Adsorba 150C charcoal, coated with cellulose, from Gambro Ltd, Sidcup, Kent; Amberlite XAD-7 resin (425–850 μm fraction) from Rohm and Haas Ltd, Croydon, Surrey; Dowex-1 resin (50–100 μm fraction) from Sigma; and Affi-Prep Polymyxin Matrix from BioRad.

Male Wistar rats (190–200 g) were housed in an air-conditioned room with a 12-h light-dark cycle and maintained on a commercial stock diet and tap water *ad libitum*. For experiments *in vitro*, SEA was added to citrated human plasma, obtained from four patients with haemochromatosis who were undergoing weekly venesection therapy and who all had normal serum tests of liver function.

Iodination of SEA

SEA was radiolabelled with carrier-free Na^{125}I by immobilised lactoperoxidase according to the method of David and Reisfeld.¹⁹ Briefly, 100 μg of SEA in 50 μl of pyrogen-free water was mixed with 50 μl of azide-free phosphate-buffered saline (PBS), pH 7.4, 10 μl of 0.1 mM NaI, 25 μl of immobilised lactoperoxidase suspension and 10 μl (1 mCi) of carrier-free Na^{125}I . The reaction was started by the addition of 10 μl of 1 mM H_2O_2 and incubation was for 30 min at room temperature. The reaction was terminated by diluting the mixture to 0.5 ml with PBS containing sodium azide 0.02%, and the lactoperoxidase was removed by centrifugation at 3500 *g* for 3 min. Radiolabelled SEA was separated from the NaI by fractionation on a Sephadex G-50 column and purified by extensive dialysis with PBS. The concentration of SEA was determined by an ELISA specific for SEA with a sensitivity limit of 7.8 ng/ml. This sandwich assay incorporated a guinea-pig polyclonal antibody to SEA on the plate and a rabbit polyclonal second antibody to SEA, both provided by the Centre for Applied Microbiology and Research; detection was with antibody to rabbit IgG conjugated with horseradish peroxidase (Dako). The specific activity of ^{125}I -SEA was 220 Ci/mmol.

^{125}I -SEA administration to rats

SEA (50 μg containing 2.25 μCi of ^{125}I -labelled SEA in 200 μl of PBS) was injected as a bolus into the femoral vein of the rats ($n = 9$) over a period of 1 min. Rats were kept anaesthetised throughout the experiment with sodium pentobarbitone. Clearance of SEA was determined from the radioactivity in 100- μl blood samples taken at various intervals, starting at 2 min after injection (three rats were sampled at each time point). Distribution of SEA in tissues was studied in rats killed 5, 30 or 120 min after injection. Whole organs were weighed, and blood, urine and tissue samples were placed into pre-weighed labelled vials, and sample weights were determined. Tissue, blood or

urine samples were distributed into plastic tubes and the radioactivity was measured in a γ scintillation counter (Canberra Packard). The total amount of SEA in $\mu\text{g/g}$ of tissue was calculated from ^{125}I cpm/g of tissue and the specific activity of the injected SEA. To determine free iodine in blood and urine samples, membrane ultrafiltration was performed in an Amicon Stirred Cell (Amicon Ltd, Upper Mill, Stonehouse) with a PM10 membrane (10000 da cut off) as the M_r of SEA protein is 27078. Free ^{125}I present passes through the membrane and is detected in the filtrate.

In vitro binding of SEA

The two polymer-coated activated charcoals (DHP-1, Adsorba 150C), uncharged resin (Amberlite XAD-7), anion exchange resin (Dowex-1) and polymyxin matrix (Affi-Prep) were tested for their capacities to bind ^{125}I -labelled SEA *in vitro*. To determine the effects of plasma protein binding of SEA on adsorption, two different media were used: PBS, pH 7.4, with bovine serum albumin (BSA) 1 mg/ml (to prevent adhesion to plastic) and human plasma; 1 g (wet weight) of the charcoals or resin, or 1 ml of polymyxin matrix (50% suspension) was added to 5 ml of the media containing ^{125}I -SEA at a concentration of 1, 5, 10, 50, 100, 500 or 1000 ng/ml, and mixed on a roller mixer for 48 h at 4°C. The supernate was aspirated and assayed for free SEA by measurement of γ radioactivity. A sample of the wet charcoal and resin used was dried at 60°C to obtain the dry weights of adsorbent used. The binding capacity of each adsorbent was determined from the amount of SEA bound/g of dry adsorbent at equilibrium.

Adsorption characteristics during column perfusion

Columns (7.2 cm length, 2.2 cm internal diameter) cut from polystyrene tubes (Sterilin, Teddington, Middlesex) were packed with DHP-1 charcoal, Adsorba 150C charcoal or Amberlite XAD-7 resin. Human plasma containing ^{125}I -labelled SEA (100 ng/ml, 11 Ci/mmol) was perfused through the columns. The perfusions were performed in a scaled-down recirculating perfusion circuit for 4 h at a flow rate of 30 ml/min. Plasma was simultaneously sampled from the column inlet and outlet at different time points. The serial concentrations of SEA were determined by measurement of the ^{125}I radioactivity in the plasma samples in a γ scintillation counter. At the end of perfusion, the columns were washed thoroughly with saline and samples of the adsorbents were counted to determine directly the amount of the substance bound to the adsorbent.

Results

Intravenous bolus injection of 250 μg of SEA/kg body weight was associated with an immediate peak

Fig. 1. Concentration of ^{125}I -labelled SEA in blood of rats following i.v. bolus injection of SEA $250\text{ }\mu\text{g/kg}$ body weight. Each point represents the mean value and SEM for three to six rats.

and rapid decline in blood concentrations of SEA (fig. 1). The elimination consisted of two exponential components: an initial fast one with an average half-life of *c.* 3 min and a second slower one with a half-life *c.* 2 h. To determine whether the radioactivity detected in blood was associated with SEA, plasma samples obtained 30 min after injection of the dose were ultrafiltered; < 4% of the radioactivity was ultrafiltrable through a PM 10 membrane, indicating that most of the radioactivity in blood was still associated with SEA.

Measurement of the time course of the distribution of tissue radioactivity within different tissues following the i.v. injection of ^{125}I -labelled SEA showed that the

greatest concentration of radioactivity/g wet tissue weight was found in the kidney at all time points (table I). Comparison of the concentration of SEA in tissue (uncorrected for blood contamination) with the concentration of SEA in blood at 30 and 120 min showed that SEA was at significantly higher concentrations in the kidney and spleen than in blood. The concentrations in other tissues and organs (liver, lung and muscle) were equal to or less than that in blood. On a whole organ basis, at 5 min, the liver accounted for 16% of the injected dose of radioactivity, kidney 8% and spleen 1%, at 30 min liver 8%, kidney 36% and spleen 2%, and at 120 min liver 6%, kidney 20% and spleen 2%. A large amount of ^{125}I -labelled SEA was observed in urine at 30 and 120 min. Ultrafiltration with a PM 10 membrane showed that > 70% of radioactivity excreted in the urine appeared to be still associated with SEA. Significant amounts of ^{125}I -labelled SEA were also found in the duodenum at 2 h, which indicates the possibility that ^{125}I -SEA was excreted into bile.

Effects of adsorbents on removal of SEA

All adsorbents tested except for the polymyxin matrix adsorbed SEA. The polymyxin matrix, which has a specific affinity for LPS, did not bind any SEA from either PBS solution or human plasma. For the other adsorbents, a higher affinity for adsorption of SEA from PBS solution was found than from human plasma (figs. 2a and 2b). Comparing the binding abilities of the different adsorbents for SEA, the rank order of amount bound/g of dry adsorbent in PBS solution was: DHP-1 charcoal > Amberlite XAD-7 resin > Dowex-1 resin > Adsorba 150C charcoal. With plasma, DHP-1 charcoal, Amberlite XAD-7 resin and Dowex-1 resin adsorbed similar amounts of SEA. During 4 h in-vitro perfusion of human plasma through the adsorbent columns, DHP-1 charcoal removed 50% of the initial amount of SEA, Adsorba 150C charcoal removed 8.1% of SEA and Amberlite XAD-7 resin removed 32.5% of SEA (fig. 3). When the binding capacities were determined directly from the amount of radioactivity bound to the adsorbents and expressed as the amount/g dry weight (table II),

Table I. Time course of distribution of SEA following a single i.v. injection of ^{125}I -labelled SEA ($250\text{ }\mu\text{g/kg}$) in rats

Site	Mean (SD) SEA $\mu\text{g/g}$ wet weight at			Mean (SD) SEA $\mu\text{g/whole organ}$ at		
	5 min	30 min	120 min	5 min	30 min	120 min
Liver	0.71 (0.11)	0.43 (0.05)	0.34 (0.03)	8.07 (2.09)	4.05 (0.15)	2.90 (0.21)
Kidney	2.60 (0.34)	13.00 (1.5)	16.87 (1.06)	4.12 (0.48)	17.96 (0.46)	9.79 (1.25)
Spleen	0.49 (0.01)	1.48 (0.04)	1.23 (0.06)	0.32 (0.04)	0.96 (0.10)	0.78 (0.05)
Lung	0.94 (0.17)	0.56 (0.25)	0.24 (0.05)	...	...	...
Intestine	0.23 (0.04)	0.25 (0.02)	1.17 (0.26)	...	...	...
Muscle	0.07 (0.02)	0.10 (0.04)	0.08 (0.02)	...	...	...
Blood	1.86 (0.20)*	0.43 (0.01)*	0.25 (0.06)*	...	...	...
Urine	...	1.20 (0.05)*	2.02 (0.17)*	...	...	...

*Blood and urine values are $\mu\text{g/ml}$.

Fig. 2. Equilibrium adsorption isotherm of SEA to DHP-1 charcoal (●), Adsorba 150C charcoal (○), Amberlite XAD-7 resin (■) and Dowex-1 resin (▲) versus concentration of SEA remaining in solution at equilibrium; a, PBS supplemented with BSA 0.1%; b, human plasma.

Amberlite XAD-7 resin was better than DHP-1 charcoal. This reflected the lower dry weight of resin than charcoal in the column.

Discussion

This study has demonstrated that after i.v. injection of SEA the clearance from the vascular compartment is characterised by two markedly different phases. This

biphasic clearance is characteristic of the pharmacokinetic behaviour of many proteins,^{20, 21} reflecting first the rapid tissue uptake of SEA followed by the catabolism and excretion of SEA. Initially, ¹²⁵I-labelled SEA disappeared very rapidly from blood, having an average half-life of 3 min, which is in agreement with reports that radio-iodinated SEB exhibited a half disappearance time of 2–4 min in rats²² and 5–9 min in monkeys.²³ Similar results were obtained also by Melish *et al.*,²⁴ who measured the concentrations of TSST-1 in plasma of rabbits fol-

Fig. 3. Removal of SEA (initial concentration 100 ng/ml) from 250 ml of human plasma by columns containing DHP-1 charcoal (●), Adsorba 150C charcoal (○) or Amberlite XAD-7 resin (■) at a flow rate of 30 ml/min. Percentages of SEA remaining unadsorbed in the inlet lines are shown. Each point represents the mean of two experiments.

Table II. Amount of SEA bound to charcoal and resin adsorbents during 4 h plasma perfusion

Adsorbent	Dry weight (g)	SEA bound, ng/g of dry adsorbent*
DHP-1 charcoal	14.2	696.5
Adsorba 150C charcoal	12.7	165.7
Amberlite resin	6.4	940.2

*Results are the mean of two experiments.

lowing i.v. bolus injection of 100 mg of TSST-1. Examination of tissues containing ^{125}I -SEA revealed that the kidney was the most important site of accumulation of SEA and that SEA was excreted mainly into urine, with some excretion into bile.

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Normann *et al.*²² showed that SEB was filtered easily by the glomerulus and was then rapidly and completely re-absorbed from the tubular urine by the cells of the proximal renal tubule. The importance of the kidney in removing these toxins from the blood has been demonstrated further by the finding that SEB disappeared from the blood at a slower rate when studies were conducted in nephrectomised monkeys or rats.²⁵ The kidney has been shown previously to be an important site of accumulation and catabolism of other proteins including TNF- α ,²¹ IFN- α ,²⁶ IFN- γ ,²⁷ and insulin.²⁸ On the other hand, it has been reported that LPS (endotoxin) derived from gram-negative bacteria is taken up by the liver immediately after i.v. injection and is concentrated primarily in the Kupffer cells.²⁹ However, this is probably because the molecular size of LPS (*c.* 10^6 da) is too great for renal elimination.

Protection against the effects of staphylococcal enterotoxins *in vivo* can be provided by induction of an antibody response or by passive transfer of antibodies, although antibody protection could be overwhelmed by massive release of enterotoxins.²³ As an alternative approach to this problem, direct removal of the toxins from the circulation may be of potential therapeutic value. Activated charcoal and Amberlite resin have been shown to have a large capacity for removing endogenous and exogenous toxins and have been used previously for clinical treatment of fulminant hepatic failure without serious adverse effects.³⁰ The differences between binding of the various adsorbents in PBS solution and plasma indicate that SEA interacts with components of plasma. The in-vitro plasma perfusion experiments with small columns containing either charcoal or resin demonstrated that polyhema-coated activated charcoal and uncharged resin could remove a significant amount of SEA from human plasma. Use of these non-specific adsorbents also has the advantage that they remove a range of cytokines such as TNF- α , IFN- γ , interleukin (IL)-1 and IL-6 at the same time,³¹ some of which can be induced by staphylococcal enterotoxins. If the columns were scaled up for clinical use, it would thus be possible to remove both SEA and the inflammatory mediators released in response to SEA at the same time.

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