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Extracorporeal circulation causes release of neutrophil gelatinase-associated lipocalin (NGAL)

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Extracorporeal circulation (ECC) used during cardiac surgery causes activation of several inflammatory systems. These events are not fully understood but are responsible for complications during the immediate postoperative period. Neutrophil gelatinase-associated lipocalin (NGAL), a member of the expanding lipocalin family, has recently been described as an inflammatory protein. In this study, the release of NGAL into the circulation in 41 patients undergoing heart surgery with ECC was evaluated. A 4- to 5-fold elevation of the concentration of NGAL in plasma was observed during the immediate postoperative course with a rapid elimination during the first postoperative day. Four patients undergoing lung surgery (without ECC) were also studied. The plasma concentration of NGAL only increased with a factor of 1.1–2.2 over the operation. We conclude that NGAL is released into the circulation during heart surgery, probably as a result of the inflammatory activation of leukocytes initiated by the extracorporeal circulation.

Key words: Cardiac surgery, Extracorporeal circulation, Leukocyte activation, Neutrophil gelatinase-associated lipocalin

Introduction

Extracorporeal circulation (ECC) is fundamental for cardiac surgery but it also induces a systemic inflammatory response which may cause morbidity and mortality.[1] Various inflammatory cascade systems are activated and effects are seen on coagulation and fibrinolysis. Activation of neutrophil leukocytes occurs and proteolytic and lysosomal enzymes are released, contributing to the increased vascular permeability.[2]

Neutrophil gelatinase-associated lipocalin (NGAL) is a member of the recently described lipocalin family and was named after co-purification with leukocyte gelatinase from secretory granules in human polymorphonuclear leukocytes.[3–4] It has later also been given the name human neutrophil lipocalin, HNL.[5] NGAL is a 24-kDa protein and exists in two main forms, as a monomer and as a dimer.[4–5] The function of NGAL has yet to be clarified. Production of NGAL has been demonstrated in *in vitro* inflammatory models. Our purpose was to elucidate whether NGAL is released during extracorporeal circulation.

Patients

Forty-one elective patients undergoing elective heart surgery with extra-corporeal circulation were studied regarding possible release of NGAL. The male/female ratio was 31/10 and the median age was 62 years (range, 36–79). The surgical procedures included coronary bypass (32 patients), valve replacement (seven patients) and coronary bypass+valve replacement (two patients). The median time of extracorporeal circulation was 83 min (range, 31–220) and the median aortic cross-clamp time was 50 min (range, 14–118). The temperature during extracorporeal circulation was 28°C.

Four patients undergoing major thoracic surgery without the use of ECC were also studied. These patients had lung resections via thoracotomy.

Methods

ECC patients

Blood samples were drawn off into EDTA-containing tubes preoperatively, 3 h after removal of the aortic clamp and on the first and second postoperative days. After centrifugation, plasma was separated and stored at –30°C until analysed. Three samples were missing from the second postoperative day.

Non-ECC patients

The corresponding samples were drawn but the second sample was collected 3 h after closure of the skin.

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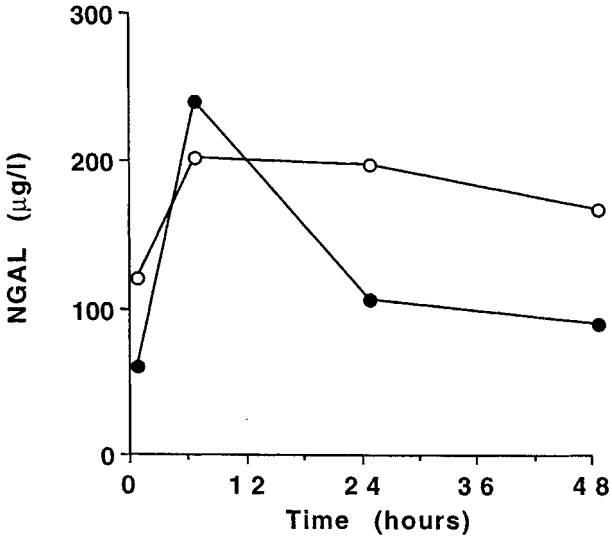

FIG. 1. Median concentration of plasma-NGAL in samples from patients operated with ECC (●) and without ECC (○).

Laboratory analysis

The concentration of NGAL was determined by an enzyme-linked immunoassay.[4]

For the phagocytosis experiment 2 ml human serum and 4×10^9 yeast particles were incubated for 15 min at 37°C. The mixture was washed in 0.15 M NaCl, centrifuged and resuspended to a volume of 1 ml. The yeast was then incubated with 6.5 ml human venous blood in constant shaking for 30 min at 37°C followed by centrifugation at 300 × *g* for 5 min. A total of 200 µl of the supernatant was separated on Superose 12 using an HPLC system (LKB). Two patient samples, with high concentration of NGAL, were also selected for separation. The column was run at a flow rate of 0.8 ml/min and elution volume 0.5 ml. The fractions were tested with ELISA for NGAL content and measured in a spectrophotometer at 405 nm. The identification of the monomer and dimer was made using Western blot as described previously.[4]

Statistics

Median values were calculated. Correlation between time on cardiopulmonary bypass and levels of NGAL was tested using the Spearman rank correlation test. Differences in immediate NGAL release between ECC and non-ECC patients was calculated using the Mann–Whitney *U*-test.

The study was approved by the local human investigations committee at Lund University and informed consent was obtained.

Results

A 4- to 5-fold elevation of the plasma concentration of NGAL was demonstrated after the use of cardiopulmonary bypass (Fig. 1). The preoperative median concentration was 55 µg/l (range, 23–191). The maximum concentration of NGAL, 234 µg/l (range, 82–574), was observed 3 h after removal of the aortic clamp. The level of NGAL at this point correlated to the time on cardiopulmonary bypass (r=0.37; P=0.02). The median concentration of NGAL on postoperative day 1 was 101 µg/l (range, 36–637) and 84 µg/l (range, 39–1011) on postoperative day 2. All patients but one had lower levels of NGAL on postoperative days 1 and 2 compared to the levels 3 h after removal of the

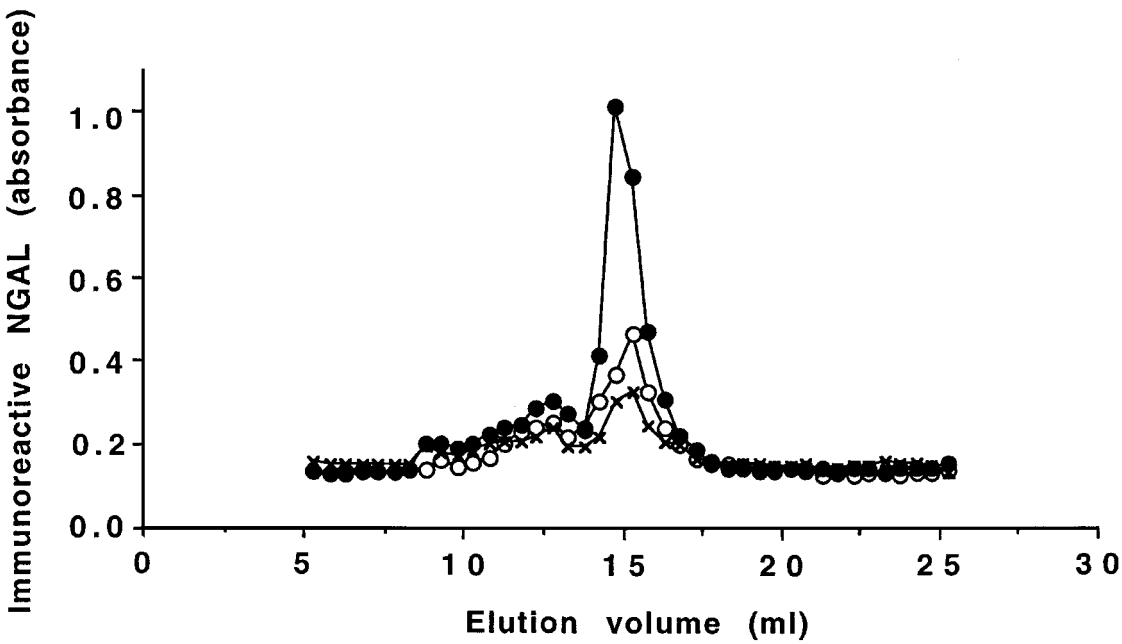

FIG. 2. Immunoreactive NGAL (measured as absorbance at 405 nm) after separation on HPLC Superose 12. Patient sample after 3 h (●) and postoperative day 1 (×) and in vitro activation (○).

aortic cross-clamp. The only exception was a patient with postoperative intermittent bleeding requiring a re-operation a few days later due to massive haemothorax. The preoperative concentration of NGAL in plasma in this patient was 169 μg/l and the postoperative values were 574, 637 and 1011 μg/l, respectively.

Patients undergoing thoracotomy and lung surgery had less pronounced increases in plasma NGAL concentration during the immediate postoperative course compared to patients operated with ECC ($P<0.01$). The 3-h levels were increased, compared to the preoperative levels, by a factor of 1.1, 1.3, 1.4 and 2.2, respectively.

After chromatography, immunoreactive NGAL eluted in two peaks. The first and smaller peak correlates to the monomer,[4] while the majority of immunoreactive NGAL was found as a dimer (Fig. 2).

Discussion

A few reports have been presented demonstrating the release of NGAL in inflammatory diseases. Levels of plasma-NGAL was elevated about 10 times in patients with peritonitis. Very high levels, 37 mg/l, were found in peritoneal exudates.[4] Local liberation of NGAL has been demonstrated in the gingiva during peridontitis[6] and in colonic epithelium during diverticulitis and appendicitis.[7]

Increased plasma levels of NGAL have also been found in patients with ischemic cerebrovascular diseases.[8] Ischemic cardiac disease does not seem to cause release of NGAL according to the normal preoperative values in our series where the majority of the patients included needed coronary re-vascularisation. However, heart surgery induces liberation of NGAL into the circulation. This is probably due to the use of extracorporeal circulation, causing an inflammatory response including the activation of leucocytes since the patients undergoing major thoracic surgery without ECC have a more limited initial release of NGAL.

NGAL was circulating mainly in its dimer form.[4] The significance of this finding has to be further evaluated.

The turnover of intravenously injected human NGAL in rats is fast.[4] In the present series, the peak plasma levels of NGAL, 3 h after removal of the aortic clamp, is more than halved on the first postoperative day. Thus, NGAL could be a useful indicator of the current degree of neutrophil activation.

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