Anti-Inflammatory Effects of Alkaline Phosphatase in Coronary Artery Bypass Surgery with Cardiopulmonary Bypass

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Abstract: Laboratory and clinical data have implicated endotoxin as an important factor in the inflammatory response to cardiopulmonary bypass. Alkaline phosphatase prevents endotoxin-induced systemic inflammation in animals and humans. We assessed the effects of the administration of bovine intestinal alkaline phosphatase on surgical complications in patients undergoing coronary artery bypass grafting. In a double blind, randomized, placebo-controlled study, a total of 63 patients undergoing coronary artery bypass grafting were enrolled. Bovine intestinal alkaline phosphatase or placebo was administered as an intravenous bolus followed by continuous infusion for 36 hours. The primary endpoint was reduction of post-surgical inflammation. No significant safety concerns were identified. The overall inflammatory response to coronary artery bypass grafting with cardiopulmonary bypass was lower in both placebo and bovine intestinal alkaline phosphatase patient group. Five patients in the placebo group displayed a significant TNFα response followed by an increase in plasma levels of IL-6 and IL-8. Such a TNFα response was not observed in the bovine intestinal alkaline phosphatase group, suggesting anti-inflammatory activity of bovine intestinal alkaline phosphatase. Other variables related to systemic inflammation showed no statistically significant differences. Bovine intestinal alkaline phosphatase can be administered safely in an attempt to reduce the inflammatory response in coronary artery bypass grafting patients with a low to intermediate EuroSCORE. The anti-inflammatory effects might be more pronounced in patients developing more fulminant postoperative inflammatory responses. This will be investigated in a further trial with inclusion of patients undergoing complicated cardiac surgery, demanding extended cardiopulmonary bypass and aortic cross clamp time. In this review article some recent patents related to the field are also discussed.

Keywords: Cardiopulmonary bypass, inflammatory response, endotoxin, alkaline phosphatase, coronary artery bypass grafting.

1. INTRODUCTION

A significant portion of morbidity and mortality observed following cardiac surgery is due to an inflammatory response [1]. This inflammatory response is initiated by contact of heparinized blood and endothelial surfaces. This leads to an acute body’s defense and thus activation of primary blood constituents like, complement, neutrophils, monocytes and platelets. Other mediators of the inflammatory response are anaphylatoxins, cytokines, reactive oxidants and endotoxins. Endotoxin is produced by intestinal flora and is normally confined to the lumen of the intestine by a barrier of endovascular cells. During cardiopulmonary bypass (CPB) mesenteric hypoperfusion occurs which results in a loss of barrier function and as a consequence bacterial endotoxins may enter the systemic circulation [2,3]. The amount of endotoxin in the systemic circulation appears to be related to CPB time and also to cross clamp time [4]. An endotoxin molecule consists of four different parts: a lipid A moiety, an inner core, an outer core and an O-antigen. The lipid A moiety of the endotoxin molecule is composed of two phosphorylated glucosamine saccharides. The two phosphate groups attached to the saccharides are essential for the toxic activity of lipid A [5]. Alkaline phosphatase is an endogenous ecto-enzyme, widely expressed in many organs that are exposed directly or indirectly to the external environment, like the gastrointestinal tract and the lungs. Exposure of cells to endotoxin results in upregulation of alkaline phosphatase, indicating that alkaline phosphatase serves a role in the natural defense system against an endotoxin insult [6]. The phosphorylated lipid-A moiety of endotoxin is a substrate for alkaline phosphatase, which enzymatically dephosphorylates the toxic lipid-A part into monophosphoryl lipid-A, a non-inflammatory metabolite, and inorganic phosphate [6,7]. In the intestine alkaline phosphatase detoxifies endotoxines and prevents inflammation in response to gut microbiota [8]. Bovine intestinal alkaline phosphatase (bIAP) has been used in an animal model in sepsis and inflammatory bowel disease [9]. In a clinical study in severe sepsis patients continuous infusion of bIAP significantly improved their renal function [10,11]. Another composition comprising of anti-TNF and anti-IL-6 antibodies and polyclonal antibodies is described by Kink for the treatment of sepsis [12].

We hypothesized that modulating the host response to endotoxin by intravenous administration of alkaline phosphatase may prove to be an effective way of reducing the adverse post-operative inflammatory effects of cardiopul-
monary bypass surgery. Furthermore, we hypothesized that a limited ability to neutralize endotoxins, measured by the amount of circulating anti-endotoxin antibodies (IgM EndoCAb) [13], may play a role in a poor outcome after cardiac surgery. Primary endpoint of this study was reduction of the post-surgical inflammatory reaction. Next to that we studied the effect of bIAP on post-surgical complications.

2. MATERIALS AND METHODS

In this double blind, placebo-controlled study, patients undergoing coronary artery bypass grafting (CABG) were randomized to receive either bovine intestinal alkaline phosphatase (bIAP) or matching placebo. The study was approved by the Institutional Review Board on the 16th of March 2006. The study drug bIAP was manufactured by Biozyme ltd (Bleanavon, Wales, UK) and Alloksys Life Sciences B.V. (Bunnik, The Netherlands).

2.1. Patient Selection

After written informed consent was obtained, male or non-pregnant female patients aged ≥18 and with a EuroSCORE ≥ 2 and ≤ 6, scheduled to undergo non-emergent coronary artery bypass grafting with the use of cardio-pulmonary bypass, were enrolled. Exclusion criteria were: redo or emergency operations, baseline alkaline phos- phatase levels > 100 IU/L, evidence of significant hepatic disease or levels of total bilirubine > 34 μmol/L, ALT > 120 U/L or AST > 135 U/L, history or signs of pre-operative infections, immunomodulating medication (i.e. steroids) or patients who were scheduled to receive ‘stress doses’ of glucocorticoids, renal failure, creatinin > 177 μmol/L or patients with chronic renal insufficiency requiring dialysis, planned use of leucoyte depletion filtration, preoperative ventilatory support, Body Mass Index > 30, history of idiopathic thrombocyto- penia and vegetarians, possibly intolerant of bovine proteins.

2.2. Schedule of Assessments

Relevant medical history, concomitant medication and physical examination were obtained at baseline and throughout the study until postoperative day 30. Blood samples (haematological parameters, clinical chemistry, cytokines e.g. IL-6, IL-8 and TNFα, anti-endotoxin anti-body) were collected at several time points before, during and after treatment to evaluate the primary efficacy endpoints. Clinical parameters like length of ICU stay, duration of ventilation and length of hospital stay were recorded. Adverse events were documented. All clinical laboratory measurements were performed in our hospital. Cytokine measurements based on the Luminex method [14] were performed at the National Institute of Health reference laboratory University Medical Centre, Wilhelmina Children’s Hospital, Utrecht, the Netherlands.

2.3. Study Drug Administration, Rationale for Safety and Randomization

The study drug bovine Intestinal Alkaline Phosphatase (bIAP) or matching placebo, a sterile solution for infusion containing no bIAP (content 1 ml) in a 2 ml vial in an aqueous buffer containing 20 mM Tris-HCl, 5 mM Magnesium Chloride, 0.1 mM Zinc Chloride, pH 7.3, with 25 % glycerol and human serum albumin as stabilizer, was administered as an intravenous bolus of 1000 International Units (IU), just prior to induction of anaesthesia, directly followed by intravenous continuous infusion of 5.6 units per kilogram bodyweight per hour at a flow rate of 4 ml per hour for 36 hours in order to maintain supranormal levels of alkaline phosphatase in blood. A phase I bIAP study demonstrated that 72-hour continuous infusions of up to a total of 16,000–48,000 IU (at 80 kg bodyweight) of bIAP was safe and well tolerated. No immune incompatibility was found as evidenced by lack of induction of specific antibodies to bIAP over a period of 90 days after administration. No drug-related adverse events were reported [15]. The responsible trial pharmacist at the pharmacy department performed randomized of the study drugs. In the post-operative period routinely assayed alkaline phosphatase results were blinded by the clinical laboratory.

2.4. Anti-Endotoxin Levels

Based on the correlation between anti-endotoxin titers (EndoCAb titers) and post surgery inflammation we evaluated the pre-surgical EndoCAb titers at the end of the inclusion period. The patients were grouped on the basis of low (< 70 mIU/mL), normal (70 -150 mIU/mL) or high (>150 mIU/mL) anti-endotoxin titers. Cytokine levels, clinical parameters and outcome were compared among the different groups.

2.5. CPB Technique

After median sternotomy and preparation of the internal mammary artery, all patients received 3 mg/kg heparin (Leo Pharma, the Netherlands) intravenously. Because we used low dose (200 ml, 10000 KIU/mL) aprotinin (Bayer Health Care Pharmaceuticals) added to the prime in all patients we repeated heparin administration 1 mg/kg every hour during CPB, regardless of the ACT. The CPB circuit consisted of a Biomedicus BP80 centrifugal pump (Medtronic, Minneapolis, MN, USA), a membrane oxygenator (Sorin Srl. Avant, Mirandola, Italy or Medtronic Affinity, Minneapolis, MN, USA, or Gish Biomedical, Rancho Santa Margarita, California, USA), a custom made collapsible venous reservoir (Sorin Biomedica, Mirandola, Italy) and a D980 Avant dual chambered hard-shell venous cardiomyocardium reservoir (Sorin Srl., Mirandola, Italy). Priming fluid consisted of 800 ml NaCl 0,9%, 500 ml Voluven® (Fresenius Kabi, the Netherlands), 200 ml Mannitol 20% (Baxter Health Care, the Netherlands), 200 ml Aprotinin 10000 KIU/mL, 25 ml NaHCO3 8,4% and Heparin 7500 IU. Normothermic cardio-pulmonary bypass was applied in all patients. For myocardial protection, depending on the surgeon’s preference, either warm blood cardioplegia or st. Thomas cold crystalloid cardioplegia was used. At the end of cardiopulmonary bypass heparin was neutralized with protamine chloride (Valeant Pharmaceuticals, the Netherlands).

2.6. Statistical Analysis

Evaluation was performed with help of the SAS® System (Software Release 9.13). Data were checked for completeness and a second plausibility check was performed. The
Wilcoxon signed rank test was used to compare continuous variables of two groups, the Pearson’s chi-square test was used to investigate the frequency (percentage) to parameters, and a probability of $p<0.05$ was considered to be statistically significant. However, apart from the primary endpoint (frequency of major pro-inflammatory reaction) all $p$-values given are descriptive only.

3. RESULTS

3.1. Patients and Procedures

A total of 63 patients was enrolled in this study. No significant safety concerns were identified. The patients’ clinical data are listed in Table 1. In the bIAP group BMI was significantly higher than in the placebo group. No statistical significant differences in other demographic data were observed. Mean EuroSCORE was 3.6 and 3.7 for bIAP and placebo treated group respectively. Analysis of the subpopulations of patients per EuroSCORE showed a notable difference in their pro-inflammatory peak values between EuroSCORE 2 and EuroSCORE 4. Peak IL-6 values for bIAP were 40% less compared to placebo peak IL-6 levels. The two groups were similar with regard to the number of grafts, CPB- and cross clamp time and type of cardioplegia used. In both groups, one patient underwent concomitant pulmonary vein isolation. There were no new stroke.

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statistical significant differences in postoperative length of stay and postoperative complications.

3.2. Cytokine Levels

In the placebo group 5 patients displayed a significant TNFα response. The mean peak level of TNFα in these 5 patients was 108.1 ± 205.9 pg/ml and was observed at 4 hours post induction of surgery. This TNFα response was followed by an increase in plasma levels of IL-6 (682.6 ± 965.0 pg/ml) and IL-8 (641.9 ± 836.7 pg/ml). In contrast, in these 5 patients the anti-inflammatory cytokines IL-10 (56.58 ± 44.03), IL-2 (0.15 ± 0.12 pg/ml) and IL-4 (0.12 ± 0.05 pg/ml) did not show any response. Such a post-surgical TNFα response was not observed in the bIAP group (p<0.02). Figure 1 shows the plasma levels of IL-6, IL-8 and TNFα of all patients in the bIAP and placebo group. Figure 2 shows the anti-inflammatory cytokines IL-2 and IL-10 of all patients. Interestingly, the overall inflammatory response was low both in the bIAP and the placebo group. However, the peak level for cytokine IL-6 was observed at 4 hours post induction of surgery and was 64.4 ± 133.7 pg/ml in the bIAP group and 140.3 ± 428.2 pg/ml in the placebo group (p = ns). At 4 hours post induction of surgery also the peak level for cytokine IL-8 was observed and was 27.2 ± 35.8 pg/ml in the bIAP group and 117.3 ± 385.1 pg/ml in the placebo group (p = ns). In the total study population, we did not observe statistically significant differences in haematological data outcome, especially inflammatory parameters like white blood cell count and CRP levels, between the bIAP and the placebo group.
Fig. (1). Plasma levels of TNFα, IL-6 and IL-8 in all patients.

Fig. (2). Plasma levels of anti-inflammatory cytokines IL-2 and IL-10 in all patients.
3.3. Anti-Endotoxin Levels

There were no statistically significant differences in distribution of bIAP and placebo between the low endocab level (< 70 mU/ml) group and intermediate endocab level (between 70 and 150 mU/ml) group. In the high endocab level (> 150 mU/ml) group one patient was treated with placebo. This patient hardly showed any reaction of IL-6 and IL-8, 9.1 and 4.6 pg/ml respectively Table 2. Although there is an obvious trend that low endocab levels are followed by a higher cytokine level, this can only be explained by the 5 patients in the placebo group that showed a significant TNFα response.

4. DISCUSSION

A significant portion of morbidity and mortality observed following cardiac surgery has been proposed to be due to an inflammatory response induced by endotoxin [2-4]. Endotoxin is produced by intestinal flora and is normally confined to the lumen of the intestine. CABG is associated with increased LPS translocation from the intestine [16], and is recognized to be a major stimulus for the development of the systemic inflammatory response syndrome. Hence, Brands et al. describe the use of alkaline phosphatase for the prophylaxis or treatment of LPS mediated diseases [17]. However, the reported circulating endotoxin concentrations and the duration of exposure to endotoxin and subsequent pro-inflammatory response vary among different articles and may depend on methodology used at the clinical site [3,18]. Once in the circulation, excess of endotoxin levels induces inflammatory cytokines provoking an inflammatory response and ultimately activation of e.g. neutrophils. Neutrophil activation is associated with organ hypoperfusion resulting in multiorgan dysfunction including cardiovascular dysfunction, kidney dysfunction and acute respiratory distress syndrome [1,19]. Reduction of endotoxin levels may result in an attenuation of the postoperative inflammatory response.

Alkaline phosphatase has been shown to be able to detoxify endotoxin [6-8]. In recent animal studies promising therapeutic effects of alkaline phosphatase were described [9,20]. In these preclinical studies it was demonstrated that during an inflammatory insult endogenous alkaline phos-
Nevertheless the administration of aprotinin was primary focused on the reduction of postoperative blood loss. But with regard to the use of aprotinin in our patients it is also important to point out that aprotinin might not only play a role in the reduction of blood loss but also in the reduction of the inflammatory response [27]. This, however, was not confirmed in recent studies, albeit carried out under different conditions, which may underlie the impact of the methodology applied [28,29]. Another example is described by Ladner et al. They provide methods for reducing blood loss and systemic inflammatory response after cardiopulmonary bypass [30].

5. CURRENT & FUTURE DEVELOPMENTS

In this phase II study we show that bIAP can be administered safely in CABG patients with a low to intermediate EuroSCORE. bIAP might be effective in reducing overall post surgical inflammation and ischemic reperfusion damage. The anti-inflammatory effect of bIAP might be more pronounced in patients developing more fulminant postoperative inflammatory responses. This will be investigated in a further trial with inclusion of patients undergoing complicated cardiac surgery, demanding extended CPB and aortic cross clamp time.

7. CONFLICT OF INTEREST

There is no conflict of interest in the current study.

6. ACKNOWLEDGMENTS

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REFERENCES