Expression of heat shock protein 70 mRNA in polymorphonuclear cells responding to surgical stress

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Abstract
Purpose. This study was performed to investigate the expression of heat shock protein (HSP) 70 mRNA in polymorphonuclear neutrophils (PMN) as a possible new biomarker for surgical stress.

Methods. The HSP70 mRNA in PMN of 10 patients who underwent lobectomy was evaluated by Northern blot analysis. Their leukocyte counts, including white blood cells (WBC) and PMN, plasma cortisol levels, and plasma interleukin-6 (IL-6) levels, were obtained by cell counting, radioimmunoassay, and enzyme-linked immunosorbent assay, respectively.

Results. The level of HSP70 mRNA in PMN slightly increased at the end of surgery and showed a significant increase 6h after surgery. It promptly decreased at 24h postoperatively and returned to the basal preanesthetic level 48h after surgery. On the other hand, WBC/PMN counts, plasma cortisol, and IL-6 significantly increased at the end of surgery. WBC/PMN counts remained at increased levels until 48h postoperatively. Cortisol peaked at 6h postoperatively and gradually decreased. IL-6 reached a maximum at 1h postoperatively, then tapered down to its basal level at 48h postoperatively.

Conclusion. Expression of HSP70 mRNA in PMN that is induced after thoracic surgery appears to be a promising candidate as a marker for evaluating surgical stress.

Key words: Heat shock protein, Surgical stress, Interleukin-6, Cortisol

Introduction
Surgical stress induces various physiopathological responses in patients after surgery. The most common response to surgical stress is an increased count and activation of polymorphonuclear neutrophils (PMN) [1]. To evaluate surgical stress and prognosis of the patient, PMN functions have been widely investigated. It has been found that excessive activation of PMN causes deleterious complications such as adult respiratory distress syndrome (ARDS) and multiple organ failure (MOF) [2,3], whereas insufficient function of PMN results in serious bacterial infection with sepsis [4].

Heat shock protein (HSP) 70 is one of the family of HSP that has an important function in protecting hosts from various biohazardous effects [5]. HSP70 is induced in human host cells by thermal stress, hypoxia, ischemia, oxidative injury, heavy metal intoxication, and infections [6,7]. Recently, it was shown that HSP70 was induced in PMN in patients with sepsis or ARDS [8,9]. The induction of HSP70 has also been reported in the adrenal cortex and the greater vessels in rats in response to surgical stress. However, the expression of HSP70 in human PMN after surgery has not been studied [10].

In the present study, we investigated the significance of HSP70 as a new biomarker for surgical stress by analyzing the expression of HSP70 mRNA in PMN and comparing it with some widely accepted biomarkers of surgical stress: cortisol and interleukin-6 (IL-6) plasma levels and cell counts of PMN in the circulation.

Materials and methods
Patients
Ten patients with lung cancer aged 62–77 years, ASA I-II, who were scheduled for lobectomy were enrolled in the study. This study was approved by the Institutional Ethical Committee, and informed patient consent was obtained. Patients with metabolic or hormonal diseases were excluded. No patients were taking corticosteroids. All patients received oral premedication with 5mg of
diazepam 1 h before arrival in the operating room. After arrival in the operating room, an intravenous cannula was inserted and an arterial cannula was placed into the radial artery for continuous measurement of arterial pressure and blood sampling. Oxygen saturation, end-tidal CO$_2$, and inspired and end-tidal concentrations of anesthetics were monitored by a respiratory gas monitor (Ohmeda RGM 5250, Ohmeda, Louisville, CO, USA). After placement of a thoracic epidural catheter into the epidural space at the T4-7 intervertebral level, anesthesia was induced with 5 mg kg$^{-1}$ thiamylal sodium, 0.1 mg fentanyl, and 0.1 mg kg$^{-1}$ vecuronium. The trachea was intubated with a double-lumen endobronchial tube (Broncho-Cath, Mallinckrodt, St. Louis, MO, USA) under fiberoptic control. Anesthesia was maintained by inhaled isoflurane with either a mixture of O$_2$ and air or O$_2$ and N$_2$O, and epidural was maintained by inhaled isoflurane with either a 

**Blood Samples**

Five-milliliter samples of arterial blood were collected for WBC, PMN, cortisol, and IL-6 analysis at the following perioperative times: before induction of anesthesia (preanesthesia), immediately after skin incision (operation), at the end of the operation, and 1, 2, 4, 6, 24, and 48 h after the end of the operation. Twenty milliliters of arterial blood was necessary for HSP70 mRNA analysis, and blood samples were collected from the patients at five intervals: preanesthetic, end of operation, and 6, 24, and 48 h after the operation.

**RNA extraction and Northern blot analysis of HSP70 mRNA expression**

PMN for RNA extraction was obtained from 20 ml of heparinized arterial blood using the single-step centrifugal technique with Polymorphprep (NYCOMED, Oslo, Norway) [11]. Arterial blood sedimentation in Polymorphprep was performed at room temperature at 450 g for 30 min. After centrifugation, the PMN were harvested and diluted with 0.45% NaCl solution to restore normal osmolarity. The PMN were resuspended in phosphate-buffered saline (PBS) and counted. The purity and viability of PMN were >95% and >98%, respectively, as determined by trypan blue staining.

Total RNA of PMN was extracted by the acid guanidium thiocyanate-phenol-chloroform method using ISOGEN (Nippon Gene, Toyama, Japan). RNA was quantitated by absorbance at $\lambda = 260\, \text{nm}$. Northern blot analysis was performed according to standard procedures [12]. In brief, 15 $\mu$g of total RNA was analyzed by electrophoresis through 1% agarose/formaldehyde gels and transferred to a nylon membrane (Hybond-N$^+$; Amersham, Oakville, ON, Canada) overnight. The membranes were prehybridized with buffer (5 $\times$ Denhardt’s reagent, 1% sodium dodecyl sulfate [SDS], 100 $\mu$l ml$^{-1}$ salmon sperm DNA) for 5 h at 48°C. Hybridization was run overnight at 48°C with the same buffer, including the specific $^{32}$P-labeled 741-bp probe (StressGen Biotechnologies, Sidney, BC, Canada), encoding the HSP70 cDNA [13]. After hybridization, the membrane was washed with 2 $\times$ SSPE (2 $\times$ saline-sodium phosphate-EDTA) for 15 min at room temperature and then with high stringency buffer (2 $\times$ SSPE, 0.1% SDS) for 15 min at 65°C, followed by 0.5 $\times$ SSPE for 15 min at 65°C. The membranes were autoradiographed with Amersham Hyperfilm-MP for 48 h at -80°C in an x-ray film cassette and quantitated by computerized planimetry (NIH Image 1.6, public domain software). Expression of $\beta$-actin mRNA in PMN before and after surgery was used as an internal control for HSP70 gene expression. All samples exhibited a constant relative density as compared with the $\beta$-actin expressed at preanesthesia.

**Cell counting and assays for plasma cortisol and IL-6**

Two milliliters of arterial blood was used for counting the number of WBC and PMN. Three milliliters of arterial blood was centrifuged to extract plasma samples for cortisol and IL-6 analysis. Plasma samples were stocked at $-80^\circ\text{C}$ until use. Cortisol concentration was measured in duplicate using the Amerlex Cortisol Kit (Kodak Japan Diagnostics, Tokyo, Japan), also known as the $^{125}$I-direct radioimmunoassay (RIA) kit. This assay kit uses a cortisol antiserum that has negligible cross-reactivity with other endogenous corticosteroids and cortisol metabolites. The intra- and interassay coefficients of variation were 5.7% at 1.98 $\mu$g dl$^{-1}$ and 8.9% at 2.18 $\mu$g dl$^{-1}$, respectively.

The plasma concentration of IL-6 was measured in duplicate by an in vitro enzyme-linked immunosorbent assay (Endogen Interleukin-6 ELISA, Endogen, Cambridge, MA, USA). The lower sensitivity of the assay was 1 pg ml$^{-1}$.

**Data analysis**

All data were analyzed using two-way analysis of variance (ANOVA) with Dunnett’s test for comparison versus preanesthesia. The difference was considered significant if the $P$ value was less than 0.05. Values are expressed as means ± SEM.