SHORT COMMUNICATION

Sepsis induces DNA fragmentation in rat skeletal muscle

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ABSTRACT. Recent studies have demonstrated the activation of skeletal muscle DNA fragmentation in some catabolic conditions [1]. In an attempt to elucidate if sepsis (a catabolic state) was also associated with muscle apoptosis, sepsis was induced by cecal ligation and puncture, and the results clearly show an induction of DNA fragmentation in gastrocnemius muscle following the induction of the septic state. Administration of rolipram (an inhibitor of tumour necrosis factor-α (TNF-α) synthesis) to septic rats clearly prevented the increased DNA fragmentation, suggesting that TNF-α is involved in the activation of the apoptotic events in septic rat skeletal muscle.

Keywords: sepsis; cecal ligation; skeletal muscle; DNA fragmentation; TNF-α; apoptosis

INTRODUCTION

Metabolic alterations associated with sepsis include increased liver gluconeogenesis [2-4], increased adipose tissue fat mobilization [5], skeletal muscle proteolysis [6], and increased nitrogen loss [7]. These metabolic alterations are associated with profound changes in whole-body amino acid metabolism. Endotoxins or lipopolysaccharides (LPS) from Gram-negative bacteria are often the molecules that trigger the septic state. Other compounds can also be mediators of sepsis, such as enterotoxin, toxic shock syndrome toxin-1, Gram-positive peptidoglycans and viral or fungal antigens. Once in the circulation, LPS binds to and can be incorporated into phagocytic cells, endothelial cells and platelets. The interaction results in the release of several types of mediators of septic shock (such as cytokines), the blood clothing cascade, the complement system and the kallikrein-kinin system [8].

The loss of body weight and development of cachexia are common signs associated with several diseases. Muscle wasting associated with infection, trauma or tumour growth results in large part from accelerated protein breakdown [9], this process leading to weight loss [10, 11]. Tumour necrosis factor-α (TNF-α) and interleukin-1 (IL-1) are cytokines synthesized and released by blood monocytes and tissue macrophages in response to invasive stimuli, and exert diverse metabolic effects [12]. Although a large body of evidence suggests that cytokines participate in the protein wasting and loss of nitrogen associated with cachectic situations [13], the mechanisms underlying such actions still remain obscure.

Apoptosis, a kind of programmed cell death, is an important physiological process in the development and homeostasis of multicellular organisms. Apoptotic cell death is characterized by a common pattern of morphological alterations such as chromatin condensation, membrane blebbing, DNA fragmentation and cell shrinkage [14]. In cardiac muscle, apoptosis has been recognized as a component of many common pathologies, including chronic heart failure, cardiac sudden death, viral myocarditis and ischemia [15-17]. Moreover, during chronic heart failure, rat skeletal muscle atrophy has been related to apoptosis [18]. Indeed, apoptosis has already been described associated with skeletal muscle atrophy [19-21].

During cachexia, the activation of the ubiquitin-dependent proteolytic pathway seems to be responsible for the muscle protein mobilization [22]. Recently, a link between the apoptosome and the proteasome pathway has been described [23]. In addition, we have demonstrated that during experimental cancer cachexia, DNA fragmentation is increased in skeletal muscle [1]. Therefore, it was the aim of the present investigation to examine if, during the septic state, skeletal muscle DNA fragmentation was activated. We have also attempted to examine the involvement of TNF-α in the process.

EXPERIMENTAL STUDIES

Animals

All animals (male Wistar rats weighing 150-200 g) were fed on a diet consisting (by weight) of 54% carbohydrate, 17% protein and 5% fat (the residue was non-digestible

material), with free access to drinking water. They were housed in individual polypropylene cages, maintained at 22-23 °C with a 12 h-light/12 h-dark cycle. All animal manipulations were made in accordance with the European Community guidelines for the use of laboratory animals.

Chemicals

They were all reagent grade, and obtained either from Roche (Barcelona, Spain) or from Sigma Chemical Co. (St. Louis, MO, USA). Rolipram [4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone] was a kind gift from Dr. Wachtel, Schering AG, Berling (Germany).

Induction of sepsis

Sepsis was induced by cecal puncture and ligation. The animals were anesthetized using ketamine/xylacine. When unconscious, an incision was made into the mesocaecum, the caecum being ligated using a silk ligature. After this, the antimesenteric surface of the caecum was punctured ten times with a 0.5 × 16 mm needle. The bowel was then returned to the abdominal cavity, and its wall sewn using silk ligature. After the operation, the animals had no access to food although they were given drinking water *ad libitum*. The animals were used 16 hours after the operation, and at this time they displayed piloerection, a lack of active movement and often diarrhoea. A group of sham-operated animals were also used for comparison purposes. Rolipram (15 mg/kg bw) was administered immediately after the surgical intervention.

DNA fragmentation assay

Gastrocnemius muscles were homogenized, and DNA was extracted with phenol/chloroform. After ethanol precipitation, the pellets were resuspended and the DNA integrity was checked in a 2% agarose gel electrophoresis and ethidium bromide staining. The percentage of DNA fragmentation was quantified by scanning densitometry. Liver from 8-hour-anti-Fas antibody-treated mice [24] was used as a positive control of DNA fragmentation.

Statistical analysis

Statistical analysis of the data was performed by means of Student's *t* test (two-tailed, unpaired).

RESULTS AND DISCUSSION

Cecal puncture and ligation results in septicemia (fever, shock, hypotension, among others), due to the invasion of intestinal microorganisms that release several types of endotoxins. There is increasing evidence that these changes are not direct effects of endotoxins, but are mediated by cytokines (mainly TNF-α and IL-1) released by macrophages and other cells, in response to stimulation by bacterial endotoxin. The strongest argument supporting a role for cytokines in sepsis is derived from the fact that circulating TNF-α and IL-1 levels markedly increase after LPS injection, and that anti-TNF-α antibody prevents the systemic effects and death caused by a lethal dose of LPS [25]. On the other hand, septicemia is characterized by severe muscle wasting resulting in an important release of muscle amino acids (mainly alanine and glutamine), which have an important role in sustaining liver gluconeogenesis and acute-phase protein synthesis. Indeed, increased muscle catabolism is an important component of the metabolic response to acute and chronic inflammatory processes accompanying infections [26], trauma [27], tissue injury [28], and cancer cachexia [29].

DNA fragmentation is a common feature of the apoptotic cell death and we have previously suggested that the muscle wasting that accompanies cancer cachexia could be linked to an apoptotic phenomenon by which muscle cells lose not only protein, but also DNA [1]. Apoptosis has already been described in human [18, 19] and rat [17] atrophic muscle and in insect muscle [30].

The results presented in Figure 1 clearly show that the induction of sepsis by cecal ligation and puncture caused a significant increase in DNA fragmentation as early as 5 hours following surgery. The magnitude of DNA fragmentation increased with the duration of sepsis. Sixteen hours after induction there was a 67% increase in DNA laddering. Since we have evidence that TNF-α could participate in the induction of the apoptotic events in skeletal muscle [31], we decided to inhibit TNF-α synthesis by using rolipram. Indeed, rolipram, a cAMP-specific phosphodiesterase type IV inhibitor [32], reduces serum levels of TNF-α in animal models [33]. Compared with pentoxifylline (another phosphodiesterase inhibitor), rolipram is 500-fold more potent at suppressing TNF-α synthesis [34], and has been suggested as a better alternative to pentoxifylline. The results obtained (Figure 1) clearly show that the inhibition of TNF-α synthesis resulted in an almost complete abolition of the increase in DNA fragmentation associated with sepsis.

In conclusion, septic rats, in addition to their increased protein catabolism in muscle [26], have increased DNA fragmentation, which seems to be activated by TNF-α release, either directly or indirectly, through the induction of other cytokines.
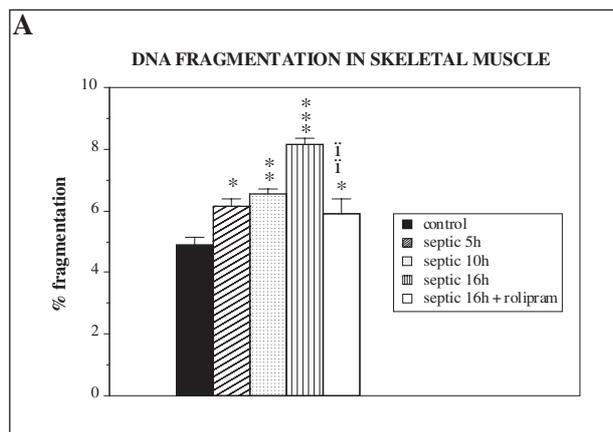
Figure 1

A) DNA fragmentation in skeletal muscle from septic rats. Time-course of DNA fragmentation in gastrocnemius muscle following induction of sepsis. For more details, see the Experimental section. The results are mean ± SEM for an average of five animals per group. Statistical significance of the results (Students's t test): *p < 0.05, **p < 0.01, ***p < 0.001 (vs. control); ••p < 0.01 (vs. septic). **B) DNA laddering of skeletal muscle in septic rats.** DNA fragmentation was assessed in gastrocnemius muscle 16 hours after cecal ligation. For more details, see the Experimental section. Lane 1: DNA molecular weight markers; lanes 2, 3 and 4: 40 μg of skeletal muscle DNA from control, septic and septic treated with rolipram, respectively; lane 5: 40 μg of liver DNA from anti-fas-treated mice (positive control).
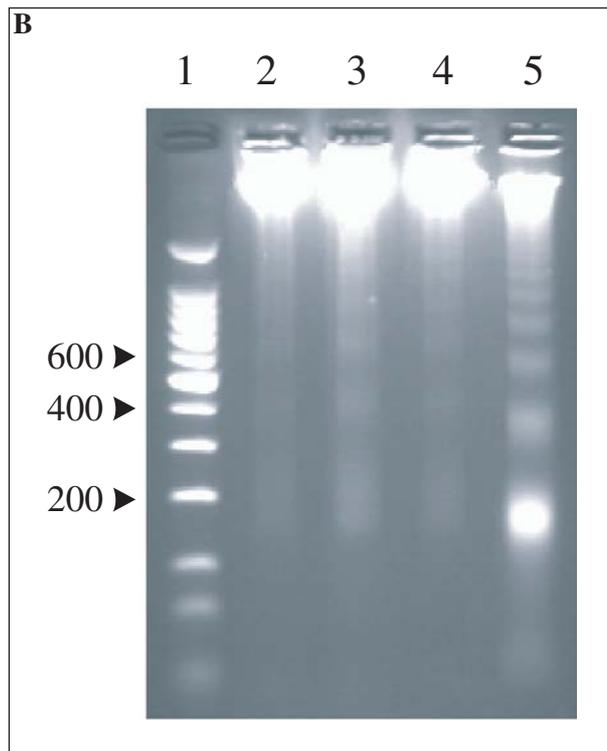
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