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The effects of adrenomedullin in traumatic brain injury

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ABSTRACT

Traumatic brain injury (TBI) is a common cause of death and disability throughout the world. A multifunctional peptide adrenomedullin (AM) has protective effects in the central nervous system. We evaluated AM in an animal model as a therapeutic agent that reduces brain damage after traumatic brain injury. A total of 36 rats was divided into 3 groups as sham, head trauma plus intraperitoneal (ip) saline, and head trauma plus adrenomedullin ip. The diffuse brain injury model of Marmarou et al. was used. Blood samples were taken from all groups at the 1st, 6th and 24th hours for analysis of TNF-α (tumor necrosis factor-α), IL-1β (interleukin-1β) and IL-6 (interleukin-6) levels. At the end of the study (at the 24th hour) a neurological examination was performed and half of the rats were decapitated to obtain blood and tissue samples, the other half were perfused transcardiacally for studying the histopathology of the brain tissue. There were no statistically significant changes in plasma levels of IL-1β, IL-6 and TNF-α relative to the sham group. Also, changes in tissue levels of malonedialdehyde, myeloperoxidase and glutathione were not statistically significant. However, neurological scores and histopathological examinations revealed healing. AM individually exerts neuroprotective effects in animal models of acute brain injury. But the mechanisms of action remain to be assessed.

1. Introduction

Traumatic brain injury (TBI) is a common cause of death and disability throughout the world. An immune response to brain injury gives a timeline to the pathological cascades that have multiple cellular, metabolic and immune pathways and are activated from the moment of injury [4]. TBI includes primary and secondary injuries. Primary injuries result directly from the traumatic event. This is unavoidable brain damage. Secondary injuries result from delayed processes associated with the trauma, such as oxidative stress, neuroinflammatory responses due to both CNS (central nervous system) and systemic immunoactivation [18]. It is thought that avoiding these secondary injuries may reduce the damage to the brain and improve the prognosis.

Adrenomedullin (AM) is a multifunctional peptide that was discovered during high throughout screening of pheochromocytoma extracts for novel biologically active peptides. Today, it is well established that AM functions as a circulating hormone and local paracrine mediator with multiple biological activities. The AM gene is expressed in peripheral blood monocytes and is rapidly up-regulated during their transformation to macrophages. AM has been demonstrated to inhibit proinflammatory cytokine secretion in several studies. Apart from acting on inflammatory cells, AM reduces endothelial permeability that has been increased by reactive oxygen species, endotoxins or cytokines and thus may limit the formation of inflammatory exudates [16]. Also it has been shown that AM prevents neuronal damage due to brain ischemia [21]. These findings have strong implication for investigating AM as therapeutic agent to reduce brain damage in TBI.

There have been limited experimental studies of the therapeutic effects of AM in acute TBI. Thus, the aim of this study was to evaluate the effects of AM in an animal model of TBI.

2. Material and methods

2.1. Animals

Sprague–Dawley rats of both sexes (250–300 g; $n = 36$) were fasted for 12 h, but allowed free access to water before the experimental protocol. Animals were kept in a controlled environment. This study was approved by Animal Use and Care Committee, School of Medicine, Marmara University. Animal care was in compliance with the current regulations of the European Union (O.J.

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of E.C.L 358/1, 12/18/1986) on the protection of animals used for experimental and other scientific purposes.

2.2. Experimental protocol

2.2.1. Experiment groups

A total of 36 rats were divided into 3 groups first (n = 12/group). Group 1, named as Sham Operated-Isotonic NaCl(SF) Group. Scalp dissection was done without head trauma (HT) and isotonic NaCl(SF) 0.12 ml/100 g intraperitoneally (ip) was given. Group 2, named as Head Trauma (HT)-SF Group. Scalp dissection was done with head trauma (HT) and isotonic NaCl(SF) 0.12 ml/100 g ip was given. Group 3, named as Head Trauma-Adrenomedullin (AM) Group. Scalp dissection was done with head trauma (HT) and adrenomedullin, 12 μg/100 g (0.12 ml/100 g) ip was given. Then each group was divided into 2 subgroups as A and B (n = 6/each group). After decapitation at 24 h, from the brain parenchyme MPO (myeloperoxidase), GSH (glutathione), MDA (malonedialdehyde) levels were studied from Group A; histopathological analysis was done from Group B. Rats that had a fracture or died were excluded from the study.

2.2.2. Adrenomedullin treatment

Adrenomedullin was obtained from Peptanova, Germany. Cerulein was obtained from Sigma Chemical Co., St. Louis, MO, USA. 12 μg/100 g adrenomedullin was given to the HT-AM (Head Trauma – Adrenomedullin) group intraperitoneally after the head trauma described below.

2.2.3. Brain injury

The widely used diffuse brain injury model of Marmarou et al. [12] was used. Briefly, a device which works by dropping a constant weight from a specific height was used. Rats were placed on a 5 cm foam rubber platform and a 300 g weight was dropped from a 1 m height which induces a mild trauma, as shown by Ucar et al. [20]. For the induction of trauma the scalp of each of the anaesthetized (100 mg/kg ketamine + 2 mg/kg chlorpromazin ip) rat was shaved, a midline incision was made and the periosteum was retracted. A 2 mm thick steel disc was fixed to the central portion of skull by using bonewax. The animals were placed in a prone position on a foam bed. The lower end of a steel tube was then positioned directly above the steel disc. The injury was produced by dropping the freely falling steel 300 g weight from a 1 m height down the tube. An inflexible rope was tied to the weight to prevent repeated impacts. After the head trauma, the rats were observed for their pattern of respiration. Stabilized rats were taken to the cages and fed by chow with free access to water.

Blood samples were taken from all groups at the 1st, 6th and 24th hours for measurement of TNF-α, IL1-β and IL-6 levels. At the end of the study (at the 24th hour) a neurological examination was performed and half of the rats were decapitated to obtain blood and tissue samples from right parietal lobe, the other half were perfused transcardiacally for studies of the histopathology of the brain tissue.

2.2.4. Neurological examination

The neurological examinations were scored according to Bederson's modified neurological examination test [2,19]. We used a 20

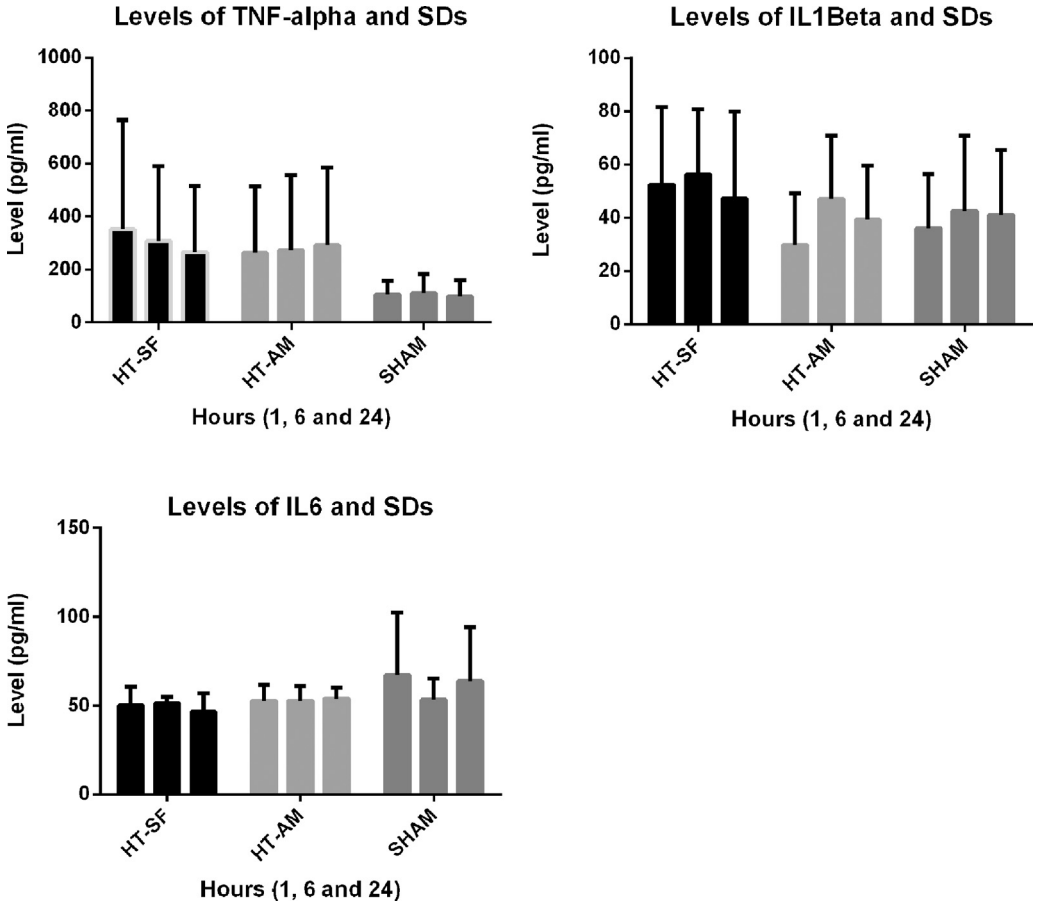

Fig. 1. Levels of TNF-α, IL-1β, IL-6 and standard deviations (pg/ml). TNF-α: tumor necrosis factor-α, IL-1β: interleukin-1β, IL-6: interleukin-6. HT-SF: Head Trauma-Isotonic NaCl Group, HT-AM: Head Trauma-Adrenomedullin Group, Sham: Sham Operated-Isotonic NaCl(SF) Group.