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Esmolol: immunomodulator in pyelonephritis by *Pseudomonas aeruginosa*

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ABSTRACT

Background: Based on previous animal studies showing promising immunomodulatory efficacy esmolol, a selective β1-blocker, it was assumed that administration of esmolol in experimental pyelonephritis by multidrug-resistant *Pseudomonas aeruginosa* would prolong survival and modulate immune response.

Methods: Acute pyelonephritis was induced in 80 rabbits and assigned to eight groups receiving normal saline (controls), esmolol, amikacin, or both agents as pretreatment and as treatment. Blood was sampled for measurement of malondialdehyde and tumor necrosis factor alpha. Animals were followed up for survival, and after death quantitative tissue cultures were performed. The *in vitro* effect of esmolol on bacterial growth and on the oxidative burst of neutrophils of healthy controls and of sepsis patients was studied.

Results: Survival of pretreatment groups administered single esmolol or esmolol and amikacin was prolonged compared with that of controls ($P = 0.018$ and $P = 0.014$, respectively); likewise, survival of treatment groups administered single esmolol or both agents was prolonged compared with that of controls ($P = 0.007$ and $P = 0.014$, respectively). Circulating malondialdehyde was significantly lower in pretreated animals administered esmolol or esmolol and amikacin compared with that in controls and in treated animals administered both agents compared with in controls ($P = 0.020$). In these groups, the bacterial load of the lung was significantly lower compared with controls. Serum tumor necrosis factor alpha did not change. Amikacin was increased in serum of esmolol-treated animals at levels which inhibited the *in vitro* growth of the studied isolate. Esmolol did not modify the *in vitro* growth of *P aeruginosa* and the oxidative burst of neutrophils.

Conclusions: It is concluded that esmolol prolonged survival after experimental infection by multidrug-resistant *P aeruginosa*. Survival benefit may be related with pleiotropic actions connected with modulation of pharmacokinetics and attenuation of inflammation.

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1. Introduction

Sepsis is an acute inflammatory response characterized by a complex cascade of cellular and chemical interactions and leading to increased mortality in the intensive care units around the world [1,2]. The problem is further aggravated by the increasing emergence of multidrug-resistant (MDR) bacteria, which generates the question on the appropriateness of the available antimicrobials to control sepsis episodes in the hospital setting [3]. Despite existing management guidelines [4], the economic burden of direct hospitalization costs and indirect morbidity-associated costs warrants the need for further research in the management of sepsis, leading to the investigation of promising novel therapies, such as immunomodulatory drugs [5].

Beta-adrenergic blockers might have a significant role in immunomodulation because they have long been considered beneficial to patients with endotoxin shock. In the pioneer experimental endotoxin shock animal model assessing the impact of nonselective beta-blockade in sepsis [6], propranolol infusion resulted in four times higher survival rate compared with the control group. This was achieved through hemodynamic stabilization eliminating the late phase of hypotension of septic shock; maintenance of arterial oxygenation; prevention of the congestive tissue disorders and subsequent bleeding complications; and reduction of the amount of fluids needed for the resuscitation of the animals. In a contemporary approach of the endotoxin shock model, esmolol, a selective β_1-blocker, prevented the hemodynamic collapse occurring in sepsis by limiting tachycardia and preserving stroke index [7]. Using one cecal ligation and puncture rodent model, esmolol infusion efficiently preserved cardiac function in peritonitis-induced septic rats and its effects were mediated through improved myocardial oxygen consumption as a result of blockade of beta-adrenergic stimulation; administration was accompanied by significant decrease of circulating tumor necrosis factor alpha (TNFα) [8]. In the most recent study by the same group, esmolol showed similar immunomodulatory properties; administration prolonged survival without hemodynamic deterioration and minimized structural and functional changes of the intestinal mucosa thus inhibiting bacterial translocation to the mesenterial lymph nodes [9].

The present experimental study was designed to assess the immunomodulatory role of esmolol in a setting resembling everyday practice where resistant pathogens emerge. It was hypothesized that administration of esmolol in a rabbit model of pyelonephritis and sepsis by a multidrug-resistant isolate would result in prolonged survival and modulate immune response. Infection was induced by one MDR isolate of *Pseudomonas aeruginosa*; esmolol was infused in parallel with bacterial challenge to assess its effect on a culminating infection, while it was also infused in some animals after the development of signs of sepsis.

2. Materials and methods

2.1. Bacterial isolate

One blood isolate of *P aeruginosa* derived from a female patient with acute pyelonephritis and severe sepsis was studied. Minimal inhibitory concentrations of ticarcillin and/or clavulanate, piperacillin, ceftazidime, imipenem, meropenem, ciprofloxacin, clarithromycin, and amikacin were >256/2, >512, >512, >256, >256, >512, >512, and 256 mg/L, respectively.

The isolate was stored as multiple aliquots in skimmed milk (Oxoid Ltd, London, United Kingdom) at −70°C. Before each experiment, one aliquot was thawed and cultured onto MacConkey agar plates (Becton–Dickinson, Cockeysville, MD). Single colonies were suspended in Mueller–Hinton broth (Oxoid) and incubated for 12 h at 37°C in a shaking water bath. The resulting inoculum was washed three times with 0.9% NaCl to remove any free endotoxins.

2.2. Animals

A total of 80 white New Zealand male rabbits, mean (±standard deviation) weight 2.88 ± 0.25 kg, were studied. The study received a permit (which inherently includes an ethics approval) from the Veterinary Directorate of the Prefecture of Athens according to the Greek legislation in conformity to the 160/91 Council Directive of the EU (license number K/8952). All aspects of the protocol were approved by the Directorate. Male mice were chosen (over females) to avoid confounding effects posed by varied expression of biomarkers and circulating cells associated with different phases of the estrous cycle. Animals were housed in single metal cages and had access to tap water and standard balanced rabbit chow *ad libitum*. Room temperature ranged between 18 and 22°C, relative humidity between 55 and 65%, and the light–dark cycle was 6 AM–6 PM.

2.3. Study design

Acute pyelonephritis was induced according to a protocol previously used by our group [10]. Animals were initially sedated by the intramuscular injection of 25 mg/kg ketamine and 5 mg/kg xylazine. Anesthesia was maintained by the intramuscular administration of 15-mg/kg xylazine at 30-min time intervals. The peritoneal cavity was entered through an upper midline abdominal incision, and the intestines were displaced to the left. The right ureter was recognized and ligated with a 3.0 suture just below the pelvis. A total of 1×10^7 cfu of the pathogen, in a volume of 0.1 mL, was injected by a 26-gauge needle into the renal pelvis, proximal to the suture. The peritoneal cavity and the abdominal wall were closed in layers. A catheter was inserted under aseptic conditions into the right ear vein of all animals for drug administration. Paracetamol suppositories (125 mg each) were administered to animals to minimize pain and suffering due to laparotomy.