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Targeting matrix metalloproteinases with intravenous doxycycline in severe sepsis – A randomised placebo-controlled pilot trial

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ABSTRACT

An overwhelming inflammatory process is the hallmark of severe sepsis and septic shock. Matrix metalloproteinases (MMPs)-8 and -9 are released from neutrophils and activated in sepsis to participate in inflammation in several ways. High levels of MMP-8 may associate with increased ICU mortality. The activity of MMP-8 and -9 is regulated by a natural inhibitor, tissue inhibitor of metalloproteinases-1 (TIMP-1). Moreover, MMPs are chemically inhibited by tetracycline-group antibiotics, such as doxycycline. We therefore aimed to study plasma concentration and MMP inhibition after intravenous doxycycline in critically ill patients with severe sepsis and septic shock in a prospective, randomised, placebo-controlled double-blinded pilot trial. Twenty-four patients with severe sepsis or septic shock were randomised in 3 groups. Group 1 received 200, 100 and 100 mg, group 2 100, 50 and 50 mg of intravenous doxycycline and group 3 placebo on three consecutive days. We measured doxycycline concentrations from baseline up to day 5. MMPs and TIMP-1 concentrations were measured from baseline up to day 10 of study and we compared their changes over time from baseline to 72 h and from baseline to 120 h. Data from 23 patients were analysed. At 72 h all patients in group 1 showed doxycycline concentrations >1 mg/l, whereas none in group 2 did. No serious adverse effects of the drug were recorded. We observed no differences over time up to 72 or up to 120 h in the concentrations or activities of MMP-8, -9 or TIMP-1 in any of the groups. We found intravenous doxycycline 100, 50 and 50 mg to be adequate to achieve a sub-antimicrobial concentration in patients with severe sepsis or septic shock but having no impact on MMP-8, -9 or TIMP-1 concentrations or activities.

1. Introduction

The incidence of severe sepsis is increasing [1,2] and associated with a remarkable mortality of approximately 20–30% [3,4]. Large clinical trials with the attempt to pharmacologically modify the inflammatory response in severe sepsis have failed to demonstrate survival benefit [5–8]. In experimental rodent studies, inhibition of matrix metalloproteinases (MMPs) with specific chemically modified tetracyclines has resulted in improved survival and a blunted inflammatory response [9,10]. MMPs are Zn^{2+} dependent endopeptidases involved in extracellular matrix degradation and turnover. Cell proliferation, adhesion, angiogenesis, apoptosis, cell differentiation and inflammation are examples of biological processes facilitated by MMPs [11,12]. In inflammation, neutrophil granulocytes secrete MMP-8 and -9 [11]. In addition to degradation of matrix components, MMP-8 and -9 are capable of activating and inactivating chemoattractant chemokines and cytokines and are therefore important in the regulation of the inflammatory process and cell migration to the inflammatory site [11,13,14]. The activity

Abbreviations: MMP, matrix metalloproteinase; TIMP, tissue inhibitor of matrix metalloproteinase; SIRS, systemic inflammatory response syndrome; RRT, renal replacement treatment; APACHE II, Acute Physiology and Chronic Health Evaluation II; IL-1β, interleukin-1-beta; IL-6, interleukin 6; TNFα, tumour necrosis factor alpha; GCF, gingival crevicular fluid.

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of MMPs is strictly regulated by naturally occurring tissue inhibitors of MMPs (TIMPs), α_2-macroglobulin, α_1-antiprotease, tissue factor pathway inhibitor-2 and numerous other proteins [11,15,16].

Compared to healthy controls, elevated levels of MMP-8, -9 and TIMP-1 in urine, serum, skin blister- and peritoneal fluid have been found in critically ill patients [17–20] and higher concentrations of MMP-8 and TIMP-1 have been associated with increased ICU mortality in severely septic patients [18]. In addition to TIMP-1 and other endogenously produced molecules, tetracycline antibiotics act as MMP inhibitors. Doxycycline, a tetracycline antibiotic, downregulates the transcription of MMP-8 and -9 and inhibits the protease activity of the enzyme [21,22] by chelating the Zn^{2+} and Ca^{2+} ions needed for the enzymatic activity of MMPs [11,23]. This effect is independent of its antimicrobial effect, as seen in studies utilising chemically modified tetracyclines devoid of antimicrobial activity [21]. In clinical studies doxycycline has both been shown to reduce the concentrations and the activity of MMP-8 and -9 in chronic inflammatory states [24] as well as to relieve clinical symptoms. The inhibition of MMP-8 and -9 occurs at lower doses than needed for antimicrobial effect and has been clinically utilised in several disease states [25]. Data from preclinical studies on MMP inhibition in acute inflammatory states together with findings of elevated MMP-8-levels in septic patients has revealed the need for a clinical trial [10,26–28]. Intravenous doxycycline administration with the aim to influence the concentration and activity of MMP-8 and -9 in severely septic patients has not been studied and the appropriate dose is not known.

Accordingly, the aim of our study was to (1) investigate the feasibility and safety of intravenous administration of doxycycline in severe sepsis and septic shock patients, (2) determine the appropriate i.v. dose to achieve sub-antimicrobial plasma levels in order to avoid interference with antimicrobial treatment of sepsis and (3) to determine the effects of the tested dose regimens of doxycycline on the plasma concentration and activity of MMP-8 and -9.

2. Patients and methods

2.1. Study design

This pilot study was a single centre randomised, double-blind, placebo-controlled clinical trial. The ethical principles of the study were based on the World Medical Association's Declaration of Helsinki [29]. The study was approved by the Ethical Committee of the Department of Surgery at Helsinki University Central Hospital. An amendment for recruitment of patients from another unit in the same hospital was approved by the same Ethical Committee during the study. Our study was registered at EU Clinical Trials Register (EUDRA CT2012-000748-8). Before entering the study we obtained written informed consent from all patients or their next of kin.

2.2. Study participants

Our study was conducted at the medical-surgical intensive care unit at Helsinki University Central Hospital between September 2012 and August 2013 and at the medical high dependency unit of the same hospital (March–August 2013). All patients with severe sepsis or septic shock were screened for eligibility by an intensivist.

The inclusion criteria were: age 18 years or older and severe sepsis or septic shock with systemic inflammatory response syndrome (SIRS) criteria fulfilled within 48 h. The SIRS-criteria, severe sepsis and septic shock were defined according to the American College of Chest Physicians/Society of Critical Care Medicine criteria [30].

Exclusion criteria were as follows: age <18 years, pregnancy, concomitant malignancy, active liver disease, porphyria, known immunological deficiency, human immunodeficiency virus infection, hepatitis B or C infection, chronic corticosteroid- or immunosuppressive therapy at the time of inclusion, use of tetracycline antibiotics or bisphosphonates within 3 months prior to inclusion, known allergy towards tetracycline antibiotics or severe allergic reaction to any drug.

The sample size was calculated based on a previous study on admission MMP-8 -levels in ICU-treated peritonitis patients [20]. Our study was designed to have an 80% power to detect a 50% change in the plasma levels of MMP-8 of doxycycline-treated patients, with a p-value of <0.05 to designate statistical significance. Hence, a minimum of 24 patients with 8 patients per each of the three groups had to be enrolled in the study.

2.3. Randomisation and interventions

After informed consent we randomised 24 patients to receive either: (1) Doxycycline hyclate 200 mg i.v. on day 1 and 100 mg i.v. on days 2 and 3 once daily (OD), (2) Doxycycline hyclate 100 mg i.v. on day 1 and 50 mg i.v. on days 2 and 3 OD or (3) Placebo consisting of 100 ml 0.9% sodium chloride on days 1, 2 and 3 OD. Randomisation was performed in advance with SPSS 20.0 (IBM, Chicago, IL) by an independent person and the group allocation sheets were stored in sealed envelopes numbered 1–24. Preparation of the study drug was done by a pharmacist or a nurse from another ICU not otherwise involved in the study. The study drug, doxycycline hyclate (Doximycin 20 mg/ml, Orion Pharma Ltd, Espoo, Finland), was diluted in 0.9% sodium chloride solution (Natriumklorid Baxter Viaflo 9 mg/ml) to a total volume of 50 ml in an opaque, black syringe and infusion line labelled only with patient study code. A 15 min infusion of the study drug was administered either through a peripheral intravenous route or an opaque tape-covered central intravenous route. The nurse caring for the patient administered the study drug at the same time of the day on three consecutive days (days 0, 1, 2). Any adverse effect attributed to the use of doxycycline was recorded.

As corticosteroids may inhibit the synthesis of MMPs [11] we recorded use of corticosteroids during the study. Use of antimicrobial therapy was recorded in order to detect possible use of tetracyclines. The Acute Physiology and Chronic Health Evaluation II (APACHE II) [31] was derived from the routine intensive care data set (Finnish Quality Consortium, Tieto Oy, Helsinki, Finland) to assess severity of disease.

2.4. Blood sampling and laboratory investigations

We collected blood samples for plasma doxycycline concentration measurements as follows: before administration of the study drug (baseline), 5 min, 6 h, 24 h, 72 h and 120 h after administration of the first dose of the study drug. The sample at 24 h was drawn before administration of the second dose of the study drug. Samples for analyses of MMP-8, -9 and TIMP-1 were collected similarly (baseline, 5 min, 6 h, 24 h, 72 h and 120 h) and further on day 7 and day 10 of the study. All blood samples during ICU stay were drawn from an arterial line by an intensive care nurse. If the study patient was transferred to a regular ward before the end of the study period blood sampling was performed by the laboratory personnel in conjunction with routine morning rounds on the ward. At each time point 10 ml of blood was sampled in heparinised plasma tubes for MMP-8, -9, and TIMP-1 analyses. For doxycycline concentration measurement 5 ml of blood was sampled in EDTA (ethylenediamine tetraacetic acid) plasma tubes. The blood samples were transported on ice to the laboratory and centrifuged at