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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

4 °C for 15 min at 2500 × *g*. The plasma was then stored at −20 °C until analysis.

Laboratory samples recorded belonged to routine daily clinical measurements in the ICU and consisted of white blood cell count, C-reactive protein, plasma creatinine, platelet count and plasma bilirubin.

Concentrations of doxycycline in plasma samples were measured after protein precipitation by use of an API 3000 liquid chromatography–triple quadrupole mass spectrometry system (Sciex Division of MDS, Toronto, Ontario, Canada), as previously described [32,33].

The limit of quantification was 0.005 mg/l and coefficients of variation were between 7% and 10% at concentrations ranging from 0.3 to 3 mg/l.

We determined MMP-8 levels in plasma by a time-resolved immunofluorometric assay (IFMA, Medix Biochemica, Kauniainen, Finland). Monoclonal MMP-8-specific antibodies 8708 and 8706 were used as a catching antibody and tracer antibody, respectively [34]. Tracer antibody was labelled using europium chelate (Eu) [35]. The assay buffer contained 20 mmol/l Tris–HCl, pH 7.5, 0.5 mol/l sodium chloride, 5 mmol/l $CaCl_2$, 50 μmol/l $ZnCl_2$, 0.5% bovine serum albumin, 0.05% sodium azide and 20 mg/l diethylenetriamine penta-acetic acid (DTPA). The assays were performed on microtitration plates in two steps. Twenty microliters of samples and 80 μl of assay buffer with 2 μl/ml normal mouse serum were pipetted into the wells and after incubation wells were washed and filled with 100 μl of assay buffer containing 8706-Eu-labelled antibody. After incubation and washing 100 μl of enhancement solution was added and after 5 min fluorescence was measured using 1234 Delfia Research Fluorometer (Wallac, Turku, Finland). The level of MMP-8 was expressed as ng/ml. Plasma concentrations of healthy controls and non-septic critically ill patients determined by this method have been reported [36].

We measured plasma concentrations of MMP-9 and TIMP-1 by enzyme-linked immunosorbent assay (ELISA) as previously described [37] using commercial kits and according to the instructions from the manufacturer (Biotrak ELISA System; Amersham Biosciences, Buckinghamshire, UK). According to the manufacturer's information this method measures active, pro-, complexed- and fragmented forms of MMPs and TIMP-1. The interassay variability coefficients for the Biotrak ELISA System kit are 2.5% and 4.1% for MMP-8 and 9.3% and 12.4% for TIMP-1, respectively. The activity of MMP-8 and MMP-9 was measured by two different methods. A commercially available fluorometric kit Sensolyte Plus 520 Assay Kit (Anaspec, Fremont, CA) utilising a FRET (fluorescence resonance energy transfer) peptide as a MMP substrate for cleavage was used for both MMP-8 and -9. The fluorescence of the 5-FAM created by cleavage is monitored at wavelengths of 490/520 nm. In this method presence of other MMPs in the sample may interfere with the results. The second method, QuickZyme Human MMP-8 and -9 activity assay (QuickZyme Biosciences, Leiden, NL) used, was a quantitative colorimetric direct measurement of active and total MMP-8 and -9 at 405 nm. Both methods were used as instructed by the manufacturer.

2.5. Statistics

Statistical analyses were performed using SPSS 19.0 (IBM, Chicago, IL). Comparisons of non-normally distributed variables between groups were performed using Mann–Whitney's test for two groups and Kruskal–Wallis's test for three or several groups. Correlations were tested by using Spearman's correlation, due to the non-normally distributed parameters. A *p*-value less than 0.05 was considered statistically significant.

3. Results

3.1. Patients

We screened 62 patients for inclusion to the study of which 38 were excluded. Twenty-four patients or their next of kin consented to our study. The flow chart demonstrating the reasons for exclusion is illustrated in Fig. 1. There was one withdrawal from the study in group 2 after administration of the first dose of the study drug. Therefore, only 23 patients were included in the final analyses. Main characteristics of the patients in the three groups are presented in Table 1. In 2 patients in group 2 adverse effects were reported; diarrhoea and gout-like symptoms. One patient in group 1 inadvertently received three 200 mg doses of doxycycline. The use of ventilator-, hydrocortisone- and renal replacement treatment (RRT) together with ICU- and 28 days mortality are shown in Table 1. None of the patients were treated with tetracycline antibiotics for infection.

3.2. Doxycycline concentrations

Five minutes after the first dose of doxycycline the peak concentration measured was above 1 mg/l in all patients (*group 1; 1.99–8.28 mg/l, group 2*; *1.56–3.51 mg/l*). In group 1 all patients showed steady state plasma concentrations >1 mg/l at 72 h, whereas in group 2 all patients showed plasma concentrations <1 mg/l. 120 h after the initial dose (=72 h after the last dose) doxycycline was still measurable in all patients in group 1 and in 6 out of 7 patients in group 2. Median doxycycline concentrations are shown in Fig. 2. We did not observe any effect of RRT use on the plasma concentration of doxycycline (*data not shown*).

3.3. MMPs and TIMP-1

The median and interquartile ranges (IQR) for admission MMP-8, -9 and TIMP-1 are shown in Table 2. As baseline concentrations of MMP-8 and -9 differed significantly between the study groups, we calculated and compared the change over time (72 h – baseline and 120 h – baseline) in these concentrations ($\Delta MMP8_{72h}$, $\Delta MMP8_{120h}$, $\Delta MMP9_{72h}$ and $\Delta MMP9_{120h}$) and found no differences between the groups. $\Delta MMP8_{72h}$, $\Delta MMP9_{72h}$ are depicted in Fig. 3. Compared to placebo, the change in concentration over time was not influenced by doxycycline. In patients with baseline MMP-8 concentrations >100 ng/ml, the concentration in the treatment groups declined at 72 h (*electronic supplementary material*).

Baseline activities for MMP-8 and -9 did not differ between treatment groups. There was, however, a remarkable heterogeneity in the activity levels of the MMPs within and between treatment groups throughout the study period. For MMP-8 the relative fluorescence for 1 h ranged from 0 to 333,900 RFU/min (SensoLyte Plus 52) and when using the QuickZyme activity assay (absorbance at 2 h – baseline/120 min) values ranged from 0 to 0.0058 Δ Abs/min. The corresponding values for MMP-9 at baseline using SensoLyte Plus 520 were 0–40.7 and 0–0.00206 using QuickZyme activity assay. There was no correlation between the activity results of MMP-8 analysed by QuickZyme or Sensolyte Plus. At baseline MMP-8 and -9 activities showed no negative correlations with TIMP-1. We observed no statistically significant differences in activities of either enzyme at 72 h or 120 h after the first dose of doxycycline in any of the groups. Neither did any statistically significant change occur at 72 h or 120 h when using MMP-8/TIMP-1 and MMP-9/TIMP-1 molar ratios as surrogate markers for activity (*data not shown*).

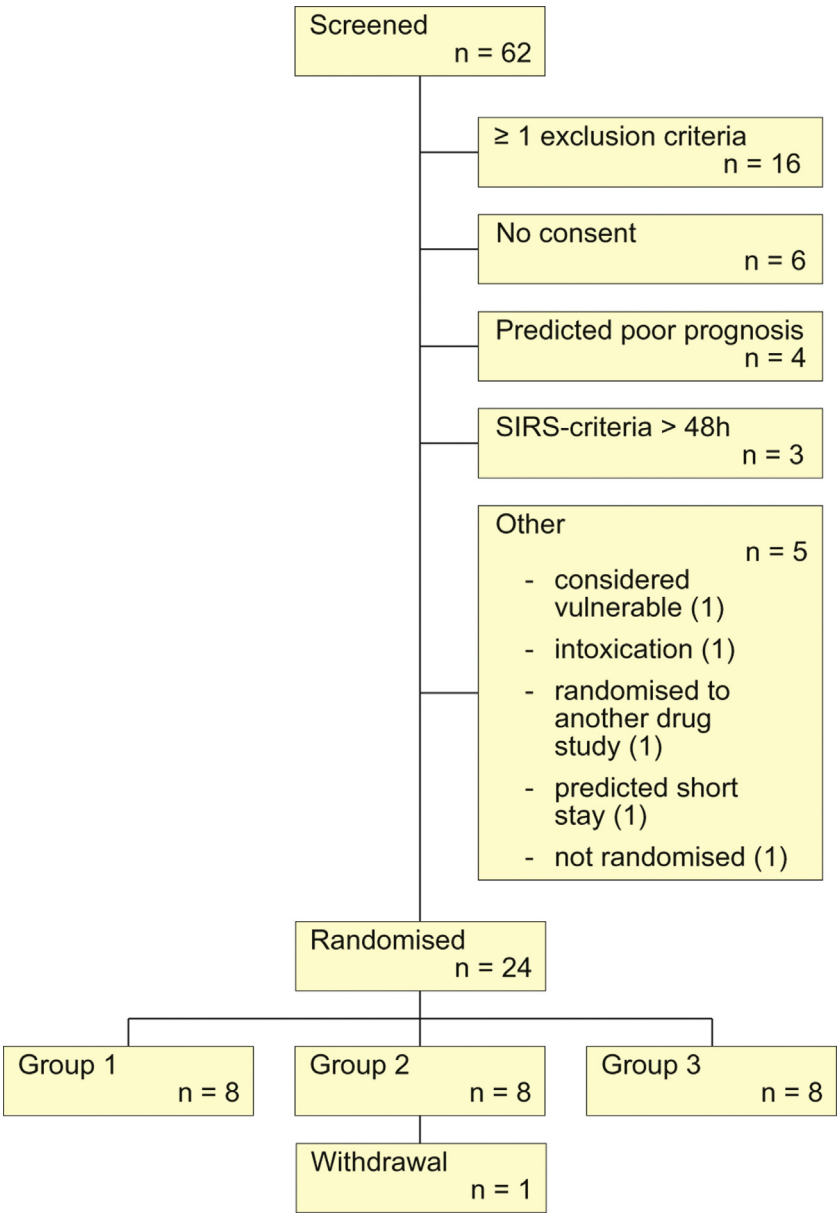

Fig. 1. Flowchart of the screening process.

4. Discussion

In this study we assessed the plasma concentrations of i.v. doxycycline in patients with severe sepsis and septic shock after two dose regimens. We found no impact of doxycycline on the concentrations or activity of MMP-8 or -9. Doxycycline administration in our study was feasible and safe.

Doxycycline is an old drug with known adverse effects and it has been successfully used orally as an antimicrobial drug in clinical practice and as a modifier of the inflammatory process in several studies [22,25,38]. Inhibition of MMP release and activity occur independently from the antimicrobial dose of doxycycline [39]. Low dose doxycycline is approved by the US FDA as per oral adjunctive treatment for periodontitis [22] and is now being widely utilised for this purpose [40]. In addition to inhibiting MMPs, tetracyclines suppress the release of cytokines such as interleukin-6 (IL-6) and IL-1β and tumour necrosis factor alpha (TNFα) in inflammation [41]. A previous study in periodontitis patients utilising per oral doxycycline considered steady-state serum levels <1 mg/l to be sub-antimicrobial [40]. In critically ill patients per oral dosing of medication is not always feasible and intravenous administration is more commonly used. Septic patients receive broad-spectrum i.v. antibiotics as treatment for the causative microbe(s) and with sub-antimicrobial concentration targeting we wanted to avoid interference with this treatment. No previous studies assessing the feasibility and safety of intravenous doxycycline administration with the aim of achieving sub-antimicrobial concentrations to septic patients exist. In order to assess possible side effects more specifically a larger study population would be needed. However, the risk of adverse effects is probably smaller when targeting lower plasma concentrations, as we did. The two adverse effects reported in our study were mild, suggesting that doxycycline can be considered relatively safe in this group of patients.

In terms of volume of distribution (Vd) and clearance (Cl), remarkable alterations in the pharmacokinetics of antimicrobial agents occur in septic conditions [42]. The capillary leakage and concomitant fluid resuscitation in sepsis lead to expansion of extracellular fluid space, consequently increasing the Vd of hydrophilic

Table 1
Demographic characteristics.

	Group 1 ($n=8$)	Group 2 ($n=7$)	Group 3 ($n=8$)
Age median [IQR]	57 [53–65]	58 [52–72]	58 [49–70]
Gender male *n* (%)	5 (62.5)	6 (85.7)	1 (12.5)
Co-morbidity *n* (%)			
Cardiovascular	3 (37.5)	5 (71.4)	4 (50)
Diabetes	1 (12.5)	4 (57.1)	3 (37.5)
COPD	0 (0)	1 (14.3)	1 (12.5)
Cancer	1 (12.5)	0 (0)	2 (25)
Other	5 (62.5)	2 (28.6)	3 (27.5)
APACHE II max	23.5 [19.5–27.0]	18 [12–30]	21 [17.3–27]
Site of infection *n* (%)			
Respiratory	5 (62.5)	3 (42.8)	3 (37.5)
Urinary	2 (25.0)	0 (0)	1 (12.5)
Gastrointestinal	1 (12.5)	1 (14.2)	3 (37.5)
Soft tissue	0 (0)	1 (14.2)	1 (12.5)
Other	0 (0)	1 (14.2)	0 (0)
Leucocyte count (10^6/l)	9.4 (3.9–19.9)	12.2 (1.8–26.1)	12.2 (5.8–18.3)
CRP (mg/l)	266 (170–366)	189 (89–314)	328 (224–482)
Noradrenaline (μg/kg/min)	0.22 (0.11–0.55)	0.08 (0.03–0.39)	0.23 (0.10–0.32)
Mechanical ventilation *n* (%)	7 (87.5)	5 (71.4)	7 (87.5)
RRT *n* (%)	5 (62.5)	1 (14.3)	1 (12.5)
Hydrocortisone *n* (%)	4 (50)	2 (28.6)	2 (25)
ICU survival *n* (%)	7 (87.5)	6 (85.7)	6 (75)
28 days survival *n* (%)	7 (87.5)	6 (85.7)	6 (75)

Group 1: Doxycycline 200-, 100- and 100 mg. Group 2: Doxycycline 100-, 50- and 50 mg. Group 3: Placebo. Categorical variables are expressed as numbers and percentages. Continuous variables are expressed as median and interquartile range [IQR]. APACHE: Acute Physiology and Chronic Health Evaluation Score. Site of infection and co-morbidity are expressed as number and percentage. Leucocyte count at admission. CRP: C- reactive protein at admission. Noradrenaline dose at admission in μg/kg/min. Use of ventilator, renal replacement therapy (RRT) and hydrocortisone during study expressed as number and percentage. Survival at the end of ICU treatment and at 28 days from admission expressed as number and percentage.

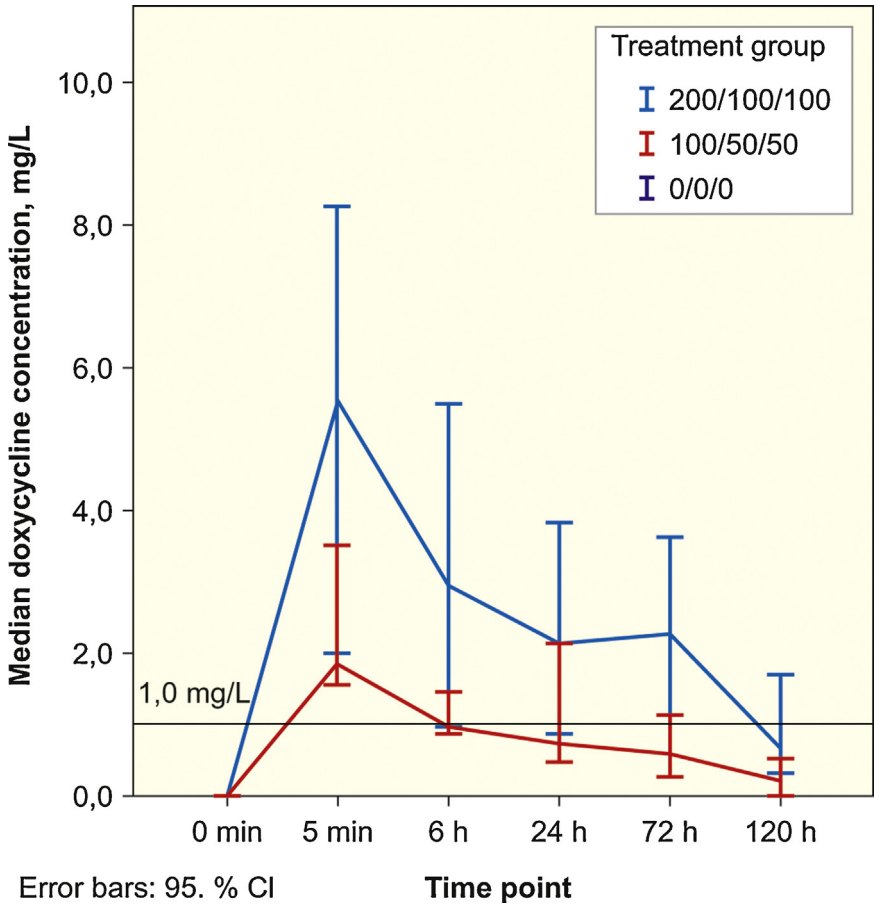

Fig. 2. Median doxycycline concentration of all patients in group 1 (*blue line*) and group 2 (*red line*). Error bars: 95% CI. *Y-axis*: Median doxycycline concentration in mg/l. *X-axis*: Time points for plasma samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Median MMP-8, -9 and TIMP-1 – concentrations at admission.

	Group 1	Group 2	Group 3
MMP-8 (ng/l)	90.0 [28.0–178.8]	75.6 [39.4–377.4]	307.8 [157.7–511.7]
MMP-9 (ng/l)	43.5 [10.7–59.6]	27.3 [0.0–109.1]	34.0 [19.2–108.3]
TIMP-1 (ng/l)	1787.1 [988.1–3226.7]	1508.7 [578.0–2076.1]	1661.0 [788.7–108.3]

Group 1: doxycycline 200, 100 and 100 mg; Group 2: doxycycline 100, 50 and 50 mg; Group 3: placebo; MMP-8, matrix metalloproteinase-8; MMP-9, matrix metalloproteinase-9; TIMP-1, tissue inhibitor of matrix metalloproteinase-1. Concentration is expressed as nanograms per litre (ng/l). Values are expressed as median and interquartile ranges [IQR].

antimicrobials [43]. Doxycycline is a highly lipophilic tetracycline antibiotic drug which implies a large Vd for the drug [42,44]. In severe sepsis the expansion of Vd might be of less importance for lipophilic antimicrobials due to rapid redistribution of the drugs from intracellular compartments to the interstitium [43]. We did not observe any effect of RRT on the plasma concentration of doxycycline, which is in accordance to the large Vd of the drug. By administering 100, 50 and 50 mg of doxycycline (group 2) intravenously on three consecutive days sub-antimicrobial concentrations were achieved at steady state 72 h after the first dose. This suggests the lower dosing, as in group 2, to be more appropriate for the purpose of inhibition of MMPs. We did not, however, find any constant overall decline of MMP-8 concentration in either of the treatment groups at 72 or 120 h.

Earlier experimental studies on specific MMP inhibitors in sepsis showed reduced activity of MMP-9 [9] and MMP-8 in plasma

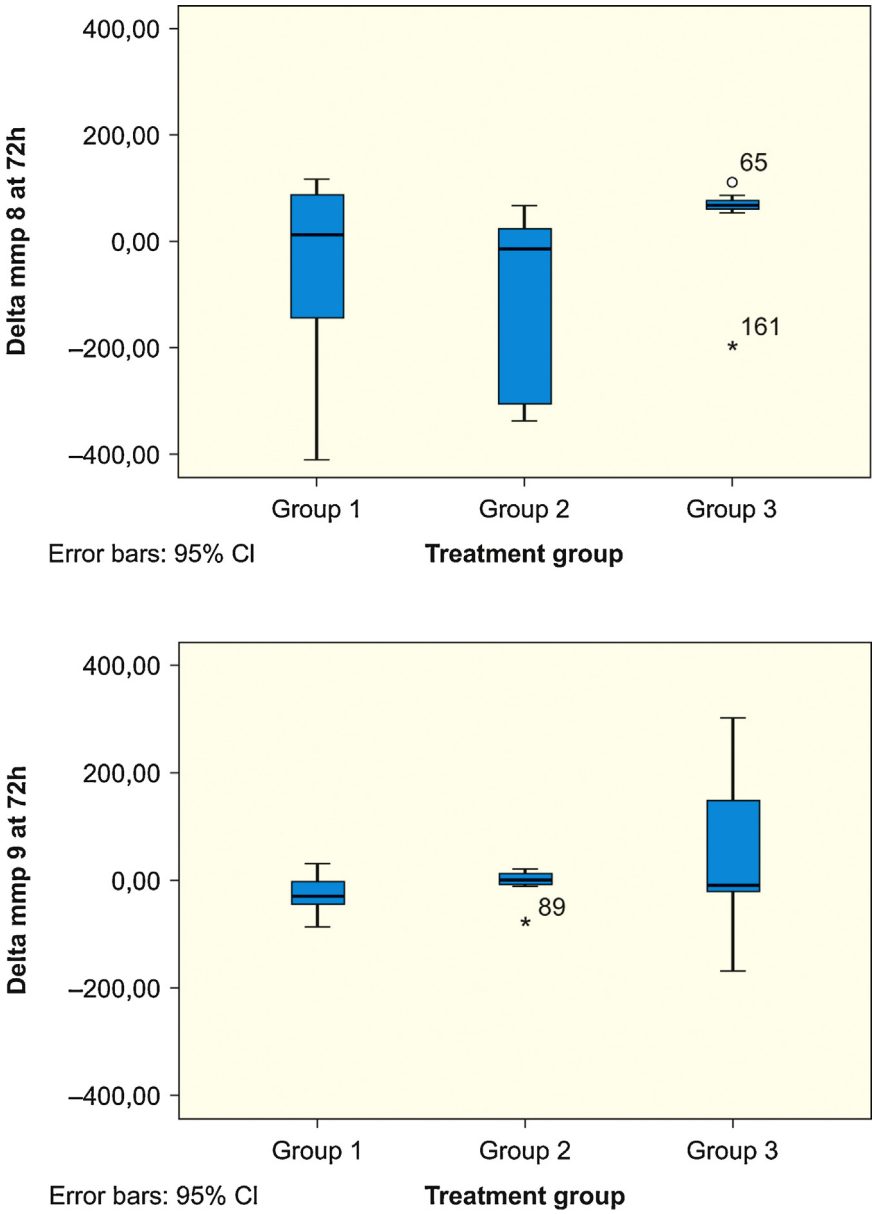

Fig. 3. (a) Change in MMP-8 concentration from baseline up to 72 h (ΔMMP-8) in group 1 (*left*), group 2 (*middle*) and group 3 (*right*). (b) Change in MMP-9 concentration from baseline up to 72 h (ΔMMP-9) in group 1 (*right*), group 2 (*middle*) and group 3 (*right*).

[10]. There are clinical studies investigating the use of doxycycline for modulating inflammation in diseases of chronic nature, such as rheumatoid arthritis [38], periodontitis [22,39], rosacea as well as in different forms of cardiovascular disease and the rare fatal lung disease lymphangioleiomyomatosis [25]. The arthritis- and dental studies showed a decrease in MMP-8-levels in saliva and gingival crevicular fluid (GCF). However, in a recent randomised, placebo-controlled study of 66 patients measuring MMP-8 in GCF in doxycycline treated patients vs. placebo-treated no difference in MMP-8 levels was detected [45]. This is in accordance with our results with the exception that we did not measure MMP-8 from the effect site but from plasma. The variation over time in the MMP-levels in acute severe inflammation is presumably higher than in a relatively stable chronic disease and it is therefore difficult to do a direct comparison between these disease states. In acute inflammation, activation of neutrophil granulocytes lead to an early and rapid release of MMP-8 and -9 from their intracellular granules [11]. Concerning the degranulation process, doxycycline administration in our study might have occurred too late in some patients to have an effect. The variation in activities at baseline of both MMP-8 and -9 and the variation of measured values even within individual patients is perhaps reflective of the complex nature of the septic process and the timing of inclusion to our study patients. There was a notable heterogeneity concerning severity of disease in our study patients which might have influenced the measured MMP levels and activities. Studies in other patient groups have supported the use of a sub-antimicrobial dose of doxycycline for the inhibition of MMP-8 and -9 [25]. In periodontitis studies no changes in microbial flora or antibiotic resistance have been recorded [46]. Even with a doxycycline dosage equalling recommended doses for antimicrobial treatment, as in group 1, we found no effect on MMP concentration or activity. Use of a higher dose regimen may increase the risk of adverse drug effects and antimicrobial resistance. The IFMA method for measuring MMP-8 concentration in serum, although not being a commercially available product, has been successfully used in dental- and sepsis studies [17,20,22]. In a recent study conducted on burn patients using IFMA the healthy controls showed a median plasma MMP-8 concentration of 22.73 (1.66–59.02) ng/ml, whereas in the critically ill patients median MMP-8 was 92.49 (59.62–159.0) [36].

4.1. Strengths and limitations of study

Our study has some strengths. First, to the best of our knowledge this study is the first randomised, double-blinded, placebo-controlled clinical study to assess the use of intravenous doxycycline in order to influence the inflammatory process. Second, based on our results, we are the first to report a dosage of doxycycline for reaching sub-antimicrobial steady state plasma concentrations in patients with severe sepsis or septic shock. These data can be used for designing a larger clinical trial to investigate the effect of doxycycline on outcome in patients with severe sepsis. Third, unlike most clinical studies, we also measured the concentrations of MMPs as targets of inhibition depicting the biological response to the study drug.

There are acknowledged limitations in our pilot study. First, the studied population was small which precludes statistical processing of clinical outcome data. Second, the studied population was heterogeneous regarding severity of illness as well as the presumed start of the septic process, which may have contributed to the detected variation in baseline concentrations and activities of MMP-8, -9 and TIMP-1. The measured MMP activity levels between the two methods differed and did not inter-correlate and therefore the results considering MMP activity should be interpreted with caution.

5. Conclusion

We conclude that sub-antimicrobial concentrations at steady state (72 h) were achieved by a loading dose of 100 mg i.v. followed by 50 mg OD twice. However, we found no differences between the study groups in the overall change over time in concentrations of MMP-8, -9 or TIMP-1 or in the activity of MMP-8 and -9. Nevertheless, both dose regimens seemed to be feasible and safe.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phrs.2015.05.005

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