Dose-Rsponse Relationship for the Anti-inflammatory Activity of Doxycycline in Experimental Models of Chronic Inflammation

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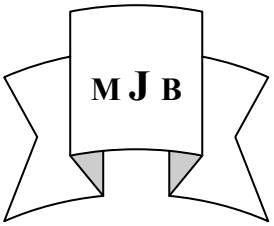

Abstract

Recently, progress has been made to utilize the matrix metalloproteinase inhibitory properties of some drugs for developing anti-inflammatory drugs that are efficacious and relatively free of side effects, and can be used effectively for a long time. The present study was designed to evaluate the dose-response relationship of the anti-inflammatory activity of doxycycline in experimental animal models of chronic inflammation and compared to that produced by the standard drugs dexamethazone and methotrexate. Seventy two rats were allocated into nine groups, each containing 8 rats, for the study of the anti-inflammatory activity of doxycycline in experimental animal model of formaldehyde-induced chronic inflammation; another 16 rats were used, and allocated into two groups, each containing 8 rats, for the study of the anti-inflammatory activity of (doxycycline 2.5 mg/kg) when used in combination with dexamethasone or methotrexate. The result of the present study reported that doxycycline in the dose range of (0.1, 0.2. 0.4, 0.6, 1.2, 2.5 mg/kg) significantly suppress inflammation in experimental animal model of formaldehyde induced chronic inflammation. Combination of doxycycline with dexamethasone (1 mg/kg body weight) significantly suppresses inflammation which was higher than all of the effects produced by other approaches of treatment. In conclusion, doxycycline, in a dose dependent pattern, was effective in attenuating formaldehyde-induced chronic inflammation in rats and has the ability to potentiate the anti-inflammatory activity of dexamethasone and methotrexate.

Runing title: *Anti-inflammatory activity of doxycycline*

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الخلاصة

لقد حصل تقدم هام لتطوير أدوية فعالة مضادة للالتهاب باستعمال الخصائص المثبطة للبروتيناز الفلزي للقالب البين خلوي لبعض الأدوية خالية من الآثار الجانبية نسبياً، يمكن استعمالها بصورة فعالة لفترة طويلة، صممت هذه الدراسة لتقييم العلاقة بين الجرعة و الاستجابة للفعالية المضادة للالتهاب لمادة الدوكسيسيكلين في النماذج الحيوانية التجريبية للالتهاب المزمن مع مقارنتها بالفعالية المضادة للالتهاب لمادتي الديكساميثازون والميثوتريكسيت و التي استخدمت كأدوية قياسية، وتقييم الفعالية المضادة للالتهاب للأدوية القياسية عند إعطائها مؤتلفة مع مادة الدوكسيسيكلين. تم استخدام 72 جرذ مختبري قسمت إلى 9 مجموعات ، كل مجموعة تحتوي على 8 جرذان لدراسة الفعالية المضادة للالتهاب لمادة الدوكسيسيكلين والمركبات القياسية في النموذج التجريبي للالتهاب المزمن المستحدث بمادة الفورمالديهايد. تم استخدام 16 جرذ مختبري قسمت إلى مجموعتين، كل مجموعة تحتوي على 8 جرذان لدراسة الفعالية المضادة للالتهاب لمادة الدوكسيسيكلين(2.5 ملغم/كغم)عند إعطائها مع مادتي الديكساميثازون والميثوتريكسيت. بينت نتائج هذه الدراسة ان الجرع المستخدمة لمادة الدوكسيسيكلين(0.1, 0.2, 0.4, 0.6, 1.2, 2.5 ملغم/كغم) قد أحدثت تأثيرا ذا فرق معنوي في كبت الالتهاب في النموذج الحيواني التجريبي للالتهاب المزمن المستحدث بمادة الفورمالديهايد، كما بينت الدراسة انه عند إعطاء مادة الدوكسيسيكلين(2.5ملغم/كغم) مؤتلفة مع مادة الديكساميثازون (1.0ملغم/كغم) تعطي فعالية ذات فرق معنوي في كبت الالتهاب في النموذج الحيواني التجريبي للالتهاب المزمن المستحدث بمادة الفورمالديهايد بصورة أعلى من التأثيرات المنتجة بواسطة الطرق الاخرى

للعلاج. ويمكن الاستنتاج من نتائج الدراسة بان مادة الدوكسيسيكلين تعمل وفق نموذج ارتباط الجرعة بالفعالية في تقليل لالتهاب المزمن المستحدث بمادة الفورمالديهايد في الجرذان، كما إن لها القابلية على تقوية الفعالية المضادة للالتهاب لمادتي الديكساميثازون والميثوتريكسيت.

Introduction

Tetracyclines are indicated for the use as adjunctive therapy in infections with severe inflammatory manifestations like acne; doxycycline and minocycline are the most commonly prescribed[1]. Doxycycline treatment at doses of 100-200 mg/kg has been shown to reduce the number and severity of inflammatory lesions[2]. However, these anti-microbial doses are often associated with the emergence of resistant bacteria[3], and adverse effects such as Gram-negative folliculitis, vaginal candidiasis, gastrointestinal upsets and dose-related phototoxic effects[4], sequalae that adversely influence the use of these doses in treatment of such type of infections. With emerging evidence suggesting that tetracycline family members can function as matrix metalloproteinase (MMP) inhibitors, there is a renewed interest in studying tetracycline analogues, such as doxycycline, minocycline and especially non-antimicrobial tetracycline analogues[5]. Application of the anti-MMP properties of tetracycline family members has extended into multiple medical fields, such as neurological diseases, in inflammatory diseases and tumor metastases[6]. Doxycycline has known as being a safe drug, and it is the only FDA approved MMP inhibitor for the treatment of periodentitis[7]. Moreover, it has been reported that doxycycline exerts an anti-angiogenesis effect independent of being an MMP inhibitor[8]. The present study was designed to evaluate the dose-response relationship of the anti-inflammatory activity of doxycycline in animal models of chronic inflammation.

Materials and Methods

Animals' selection

Eighty eight Sprague-Dawley rats weighing 180-240 gm of both sexes were brought from the animal house of the College of Pharmacy/University of Baghdad. The animals were maintained there on normal conditions of temperature, humidity and light/dark cycle. They were fed standard rodent pellet diet and have free access to water except when starvation needed during the investigations. The research protocol was approved by the Ethic Committee for Animal Care and Experiments at the College of Pharmacy, University of Baghdad.

Preparation of Doxycycline solution

Doxycycline hydrochloride powder (SDI, Iraq) was dissolved in distilled water to produce solution with concentration of 0.5mg/ml, which is utilized as a standard solution for the preparation of the doses used in the study.

Study design

The study protocol were divided into 2 stages: First stage, in which 72 rats were used, and allocated into nine groups, each containing 8 rats, for the study of the anti-inflammatory activity of doxycycline in experimental animal model of formaldehyde-induced chronic inflammation. These groups represent control, standard drugs and test drug groups. Second stage**,** includes 16 rats allocated into two groups for the study of the anti-inflammatory activity of the maximum effective dose of doxycycline when used in combination with the standard anti-inflammatory agents, dexamethasone (American regent Inc. USA) and methotrexate (Ebewe Pharma, KG, Austria) in experimental

animal model of formaldehyde-induced chronic inflammation.

Formaldehyde-induced chronic inflammation

The effect of doxycycline in chronic inflammation was evaluated by formaldehyde-induced paw edema[9]. In this model, chronic inflammation was induced by injecting 0.1ml of 2% formaldehyde into the sub planter area of the right hind paw of ether anaesthetized rat. All drugs including doxycycline (0.1, 0.2, 0.4, 0.6, 1.2, and 2.5 mg/kg), dexamethasone 1 mg/kg and methotrexate 0.075 mg/kg, in addition to the vehicle normal saline (0.1 ml/100 gm) which is given to the control group, were given 30 minutes prior to formaldehyde injection and continued for seven consecutive days. All drugs and the vehicle were given as once daily intraperitoneal doses.

In this model, the increase in paw edema was measured by the vernier caliper method. The paw thickness was measured before and 6 days after induction of inflammation, and presented as mean increase in paw thickness (mm)[10].

The ability of the anti-inflammatory drug to suppress paw inflammation was expressed as a percentage of inhibition of paw edema and calculated according to the following equation[11]:

Percentage of inhibition (%) = (1 - X / Y) × 100

Where X= mean increase in paw thickness of treated group of rats and Y= mean increase in paw thickness of control group of rats.

All the results were expressed as mean±SD. The data were analyzed by using computerized SPSS program. The significance of difference among the studied groups was determined using one-way analysis of variance (ANOVA). *P* values less than 0.05 are considered significant.

Results

Using vernier caliper method, the suppressive effects of different doses of doxycycline, when used alone, and in combination with standard anti-inflammatory agents (dexamethasone and methotrexate) on formaldehyde-induced chronic inflammation were shown in table 1 and figure 1.

All doxycycline doses (0.1, 0.2, 0.4, 0.6, 1.2, and 2.5 mg/kg body weight) significantly reduced the increase in paw thickness (in a dose-dependent pattern) compared to control group, with maximum effect produced by 1.2 mg/kg (31%) and 2.5 mg/kg (32.8%) respectively, where both are not significantly different when compared with each other. Meanwhile, both 1 mg/kg dexamethasone and 0.075 mg/kg methotrexate significantly inhibited the increase in paw thickness compared to controls, with maximum effect produced by dexamethasone (36.4%). Doxycycline (2.5 mg/kg body weight) in combination with dexamethasone (1 mg/kg body weight) resulted in 39.0% inhibition in paw thickness, which was significantly higher than all of the effects produced by others; while doxycycline (2.5 mg/kg body weight) in combination with methotrexate (0.075 mg/kg body weight) resulted in 35.5% inhibition in paw thickness, an effect comparable to that produced by dexamethasone alone and significantly higher than the effects produced by other approaches of treatment. In figure 2, the dose-response relationship for the anti-inflammatory activity of doxycycline was found to be relatively linear within the dose ranges utilized in the study, with best linearity between 0.1–1.2 mg/kg.

Discussion

Pioneering work done by Gloub *et al*, (1983) with minocycline[12] and other chemically modified

tetracyclines[13] led to the discovery that these compounds could inhibit matrix metalloproteinases (MMPs) and down regulate connective tissue destruction in inflammatory diseases independent of their anti-microbial activity(14). In the present study, the dose-dependent anti-inflammatory activity of doxycycline in chronic model of inflammation is quite evident, and found to be comparable with those reported previously, where different doses of the drug produced different levels of interference with the inflammatory cascades[15,16]; this anti-inflammatory activity may be due to inhibition of IL-6, IL-1β and TNF-α, inflammatory cytokines up-regulated in response to inflammatory challenge; in turn, this inhibits the inflammatory pathway that leads to further recruitment of cellular components, such as neutrophils and their proteolytic enzymes like collagenase and matrix metalloproteinase-8[17,18]. Doxycycline doses used in the present study are much more less than those required for antibacterial activity, because the MMP-inhibition, supposed to be involved in the anti-inflammatory activity of doxycycline, can occur within concentration ranges below that required therapeutically for the treatment of bacterial infections[19], which makes it a very attractive agent for further examinations as a potential adjuvant treatment for chronic inflammations. Our observation of doxycycline's dose-dependent effect in chronic model of inflammation is considered as a first evidence for the potential role of this antibacterial agent in the treatment of many inflammatory disorders, suggesting that it may be a promising anti-inflammatory agent in many chronic inflammatory diseases like rheumatoid arthritis. Doxycycline is considered as a non-specific MMP inhibitor, although this dose-response relationship was not correlated with its effect on MMPs (especially on MMP-9), it has been reported that doxycycline inhibits TGF-β1-induced MMP-9 expression via smad and MAPK pathways in human corneal epithelial cells[8]. Based on the currently used model, although there was no significant difference among some groups, utilization of a more sensitive biochemical or enzymological techniques (including the MMP activity and the serum level of the inflammatory mediators like TNF-α, IL-1β, and IL-6) may give more evidence in this respect. With emerging evidence suggesting that tetracycline family members can function as MMP inhibitors, there is a renewed interest in studying tetracycline analogues, such as doxycycline, minocycline, and especially non-antimicrobial tetracycline analogs. The anti-MMP properties of tetracycline family members has extended into multiple medical fields, such as neurological diseases, inflammatory diseases and tumor metastases[6]. Moreover, these results supported our proposal in using a combination of doxycycline with corticosteroids or NSAIDs as adjuvant therapy for resistant cases of chronic inflammatory disorders like RA, or for reducing the dose of corticosteroids or NSAIDs to decrease the chance of side effect production when used in combination with doxycycline. In conclusion, doxycycline in a dose-dependent pattern produced anti-inflammatory activity in experimental model of chronic inflammation in rats.

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Table 1 Effect of different doses of doxycycline and its combination with dexamethasone or methotrexate on paw thickness and inhibition of paw edema (%) in formaldehyde-induced chronic inflammation in rats.

Treatment Groups	Mean paw thickness (mm) at zero time	Mean paw thickness (mm) after 7 days	Mean increase in paw thickness (mm) after 7 days	Inhibition of paw edema (%)
Normal saline (0.1 ml/100gm)	4.01± 0.06	7.42 ± 0.05	3.41 ± 0.04	—
Dox (0.1 mg/kg)	4.03 ± 0.08	7.14 ± 0.06	3.11 ± 0.07^{*a}	8.80
Dox (0.2 mg/kg)	3.93 ± 0.09	6.88 ± 0.08	2.95± 0.08^{*b}	13.49
Dox (0.4 mg/kg)	3.91± 0.08	6.44 ± 0.09	2.53 ± 0.09^{*c}	25.81
Dox (0.6 mg/kg)	4.09 ± 0.07	6.56 ± 0.08	2.47 ± 0.08^{*c}	27.56
Dox (1.2 mg/kg)	4.11± 0.07	6.46 ± 0.05	2.35 ± 0.06^{*d}	31.09
Dox (2.5 mg/kg)	4.05 ± 0.08	6.34 ± 0.09	2.29 ± 0.08^{*d}	32.84
Dox (2.5 mg/kg) + Dex (1mg/kg)	4.08 ± 0.07	6.16 ± 0.08	2.08 ± 0.07^{*f}	39.00
Dox (2.5 mg/kg) + MTX (0.075mg/kg)	3.94 ± 0.07	6.14 ± 0.09	2.20 ± 0.09^{*e}	35.48
Dex (1mg/kg)	4.02 ± 0.08	6.19 ± 0.09	2.17 ± 0.07^{*e}	36.36
MTX(0.075 mg/kg)	4.09 ± 0.07	6.41± 0.08	2.32 ± 0.08^{*d}	31.96

Data were expressed as mean ± SD; number of animals = 8 in each group; *P<0.05 with respect to control group; values with non-identical superscripts (a, b, c, d, e, and f) are considered significantly different (P<0.05).

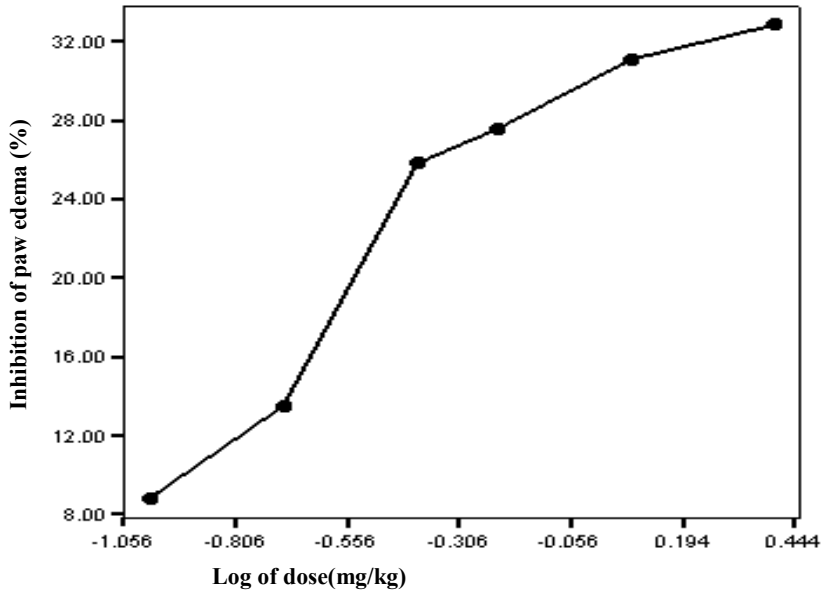

Figure 1. Dose-response effect of different doses of doxycycline on inhibition of paw edema (%) in formaldehyde-induced chronic inflammation in rats.