ТЕОРЕТИЧНА І ЕКСПЕРИМЕНТАЛЬНА МЕДИЦИНА

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ASSESTMENT OF INFLUENCE OF SYNTHETIC MATRIX METALLOPROTEINASES INHIBITOR DOXYCYCLINE ON THE TOTAL PROTEOLYTIC ACTIVITY OF BLOOD IN EXPERIMENTAL THERMAL BURNS IN RATS

The effect of synthetic inhibitors of matrix metalloproteinases (MMP) doxycycline on the total proteolytic activity (TPA) in blood in an expiremental thermal ingury in rats has been studied. It is shown that thermal burn without treatment, in experimental rats, accompanied by increased levels of TPA throughout the study period. The use of reference drugs thio-triazoline and methyluracil leads to a reduction of these indicators by the end of the experiment (28-th day). Synthetic MMP inhibitor doxycycline (especially in a dose of 30 mg/kg) was superior to comparative preparations by the ability to normalize TPA level in blood of the thermal damage.

Keywords: *synthetic inhibitors of matrix metalloproteinases, doxycycline, general proteolytic activity.*

Introduction

Despite the all achievements of modern medicine, the treatment of thermal burns represents one of the most complicated specific problems and is relevant not only to the medical but also to the social and economic problem [1, 2]. This is due to the high incidence of burns among all age groups of the population, severe clinical picture with the development of the syndrome of a systemic inflammatory response, metabolic disorders and also the activation of pathological processes in cells [3, 4]. It is known that the burn wound is prone to chronic flow due to the long-term inflammation in the body [5, 6]. Active participants in the regeneration of tissues are MMPs, which play a central role in the metabolism of connective tissue proteins in healthy and damaged organs. It is believed that the main source of MMP in inflammation are neutrophils and macrophages [7], and the excessive activity of proteolytic enzymes in the inflammation zone leads to the prolongation of the pathological process, which prevents the successful healing of the wound, in particular, the burn injury [8]. It is also known that doxycycline is an inhibitor of MPP and is able to suppress excessive proteolytic activity in the treatment of rheumatoid arthritis [9]. We suggested a possible suppressive effect of doxycycline (as a synthetic inhibitor of MMP) on the healing processes of a burn wound, namely, the possible reduction of proteolysis during thermal damage. Therefore, the purpose of our study of the mechanisms of the doxycycline action was to study its effect on the level of GPA under experimental thermal burn.

Materials and methods

Studies were performed on 144 white mongrel mature nonlinear albino rats of both sexes weighing 200–250 g. Experiments were conducted in the laboratory of Department of Pharmacology and Prescription writing (Kharkiv National Medical University (KhNMU), Kharkiv, Ukraine).

On the shaved part of the back thigh under the thiopental anesthesia a thermal burn was caused (A.V. Krivoshapka, T.V. Zvyagintseva, 2010). All experiments were conducted according to the European convention for the protection of vertebrate animals used for experimental and other

scientific purposes (Strasbourg, 1986) and according to the guidelines of the State Expert Center Ministry of Health of Ukraine (Protocol № 9 meeting of the Commission on Ethics and Bioethics KhNMU, 03.12.2014) [10–14].

The animals were divided into 6 groups of 24 individuals in each group. The first group – intact animals, the second (control) – rats with thermal burn without treatment, rats of the third group were administered thiotriazoline at a dose of 30 mg/kg (reference drug), the fourth group – methyluracil at a dose of 0.126 mg/kg (reference drug), the fifth and the sixth group – the synthetic inhibitor of MMP – doxycycline at doses of 2.5 mg/kg and 30 mg/kg, respectively. Preparations were administered orally in starch suspension immediately after thermal exposure and daily during the entire experiment period (28 days). Observations of the healing processes of burn wounds were carried out on the 7th, 14th, 21st and 28th days (six rats in each series). Determination of total proteolytic activity in blood serum and skin homogenates was carried out by the method of K.N. Veremeenko and O.P. Golo-borodko by the amount of cleaved arginine [15]. Statistical processing of the obtained data was carried out by standard statistical methods [16].

Results and discussion

During studying of the TPA level in the blood serum of animals in the control group increasing during the entire experimental period in comparison with intact animals was found. The maximum values in the first 14th days of observation were recorded (table). In these terms, the TPA exceeded the level of intact animals in 2 times (7th day) and 1.8 times (14th day).

Under the influence of a synthetic inhibitor of MMP doxycycline at a dose of 2.5 mg/kg, a significant decrease of TPA in blood started from the 14th day after the burn (at this time, the TPA decreased by 19.7% compared to thiotriazoline), and continued to decrease progressively by 21 day (18.0% below the control). By the end of the experiment (on the 28th day), the TPA in the blood did not exceed the indices of intact animals.

Doxycycline (in a dose of 30 mg/kg) showed more pronounced efficacy, which was confirmed by a decreasing of the TPA level in the serum on the 7th day (by 9.0% compared to the control). On the 14th day, the decline of this indicator continued (by 21.0% in comparison with the control and by 21.3% in comparison with thiotriazoline). It should be noted that only in this group of animals, by the 21st day, the parameters of the TPA were reduced to the level of physiological fluctuations, i. e. authentically not differing from similar indices of intact rats. The tendency to decrease was also maintained at the 28th day.

Conclusions

1. A thermal burn in rats in an experiment that occurs without treatment is accompanied by an excessive increase of the total proteolytic activity level in the blood serum.

2. The use of thiotriazoline and methyluracil leads to normalization of the total proteolytic activity parameters by the end of the experiment (on 28th day).

3. The synthetic matrix metalloproteinases inhibitor doxycycline (especially at a dose of 30 mg/kg) is superior to the action of the reference drugs thiotriazoline and methyluracil by the ability to normalize of the total proteolytic activity level in the serum of the thermal damage.

Influence of doxycycline on the TPA level in blood serum (mmol / h·l) of rats with burn wound (n=6)

Group	Time of observation (days)			
	7th	14th	21th	28th
Intact	2,09±0,07			
Burn without treatment, (control)	4,25±0,10 [a]	3,85±0,07 [a]	3,18±0,11 [a]	2,55±0,20 [a]
Thiotriazoline 30 mg/kg	4,04±0,09 [a]	3,70±0,09 [a]	2,84±0,11 [a,b]	2,14±0,04 [b]
Methyluracil, 0,126 mg/kg	3,98±0,08 [a,b]	3,08±0,08 [a,b]	2,70±0,10 [a,b]	2,12±0,09 [b]
Doxycycline, 2,5 mg/kg	4,01±0,09 [a]	3,09±0,17 [a,b,c]	2,61±0,08 [a,b]	2,04±0,14 [b]
Doxycycline, 30 mg/kg	3,87±0,08 [a,b]	3,05±0,13 [a,b,c]	2,50±0,21 [b]	2,08±0,13 [b]

Notes. p<0,05: [a] the reliability of differences in comparison with intact rats; [b] the reliability of differences in comparison with control; [c] the reliability of differences in comparison with thiotriazoline.

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ОЦІНКА ВПЛИВУ СИНТЕТИЧНОГО ІНГІБІТОРА МАТРИЧНИХ МЕТАЛОПРОТЕЇНАЗ ДОКСИЦИКЛІНУ НА ЗАГАЛЬНУ ПРОТЕОЛІТИЧНУ АКТИВНІСТЬ КРОВІ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ ТЕРМІЧНОМУ ОПІКУ ЩУРІВ

Вивчено вплив синтетичного інгібітора матричних металопротеїназ доксицикліну на загальну протеолітичну активність в крові при експериментальному термічному опіку у щурів в експерименті. Показано, що у експериментальних щурів термічний опік без лікування супроводжується підвищенням рівня загальної протеолітичної активності протягом усього періоду досліджень. Застосування препаратів порівняння тіотриазоліну та метилурацилу призводить до зниження активності цього показника до кінця експерименту (на 28-му добу). Інгібітор матричних металопротеїназ доксициклін проявляє високий антипротеазний ефект, особливо в дозі 30 мг/кг, що перевищує референтні препарати.

Ключові слова: *синтетичний інгібітор матричних металопротеїназ, доксициклін, загальна протеолітична активність.*

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ОЦЕНКА ВЛИЯНИЯ СИНТЕТИЧЕСКОГО ИНГИБИТОРА МАТРИЧНЫХ МЕТАЛЛОПРОТЕИНАЗ ДОКСИЦИКЛИНА НА ОБЩУЮ ПРОТЕОЛИТИЧЕСКУЮ АКТИВНОСТЬ КРОВИ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ТЕРМИЧЕСКОМ ОЖОГЕ У КРЫС

Изучено влияние синтетического ингибитора матричных металлопротеиназ доксициклина на общую протеолитическую активность в крови при экспериментальном термическом ожоге у крыс в эксперименте. Показано, что термический ожог без лечения у экспериментальных крыс сопровождается повышением уровня общей протеолитической активности на протяжении всего периода исследований. Применение препаратов сравнения тиотриазолина и метилурацила приводит к снижению активности этого показателя к концу эксперимента (на 28-е сутки). Ингибитор матричных металлопротеиназ доксициклин проявляет высокий антипротеазный эффект, особенно в дозе 30 мг/кг, превышающий референтные препараты.

Ключевые слова: *синтетический ингибитор матричных металлопротеиназ, доксициклин, общая протеолитическая активность.*

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