**ESTIMATION OF THE LIPID PEROXIDE OXIDATION PROCESSES AND ANTIOXIDANT PROTECTION IN THE TREATMENT OF RATS TERMAL BURNING BY DOXICYCLINE AS A SYNTHETIC INHIBITOR OF MATRIX METALLOPROTEINASES IN EXPERIMENT**

**Iermolenko T.I., Aleksandrova A.V.**

*Kharkiv national medical university, Ukraine*

Free radical oxidation under physiological conditions refers to normal metabolic processes, reactive oxygen species (ROS) in low concentrations have a various physiological effect [1-3]. Oxygen radicals contribute to the induction of lipid peroxidation (LPO), the function of which is to update cell membrane phospholipids as well as regulate their permeability [4-6]. One of the primary physiological functions of ROS is the activation of a number of membrane proteins, immunoglobulins, as well as enzymes involved in the metabolism and synthesis of macroergic compounds in the cell [7,8]. Products of free radical reactions and LPO are involved in the biosynthesis of prostaglandins, hormones, leukotrienes, thromboxane A2. However excessive generation of reactive oxygen species with depletion of antioxidant reserves resulting in lipid peroxidation have been implicated in programmed cell death pathways and development of various pathological processes [9-11].

Thermal injury is a severe form of pathology and is manifested not only by the development of local vascular tissue changes, but also by the formation of a systemic inflammatory response syndrome associated with increased formation of reactive oxygen species [12-14]. As a result of excessive activity of free radical processes and the lack of adequate growth of antioxidant protection, toxic decomposition products accumulate, which leads to serious metabolic disorders, the formation of oxidative stress, and can also be a factor that inhibits the activity of proteinase inhibitors [15-17]. Therefore, the problem of studying the mechanisms of correction of free radical oxidation processes and the activity of antioxidant system (AOS) enzymes in a burn wound is relevant [18-21].

Doxycycline as an synthetic inhibitor of matrix metalloproteinases (IMMP) is known to inhibit protein breakdown. Excessive proteolysis leads to disruption of wound healing processes, including burns [22,23]. However, the state of the prooxidant system and the activity of AOS enzymes in the treatment of thermal burns with doxycycline as an IMMP have not been studied in modern literature. In this regard, the purpose of our study was to study the effect of the investigational drug on LPO and changes in the activity of the AOS.

**Materials and methods:** Studies were performed on 144 rats of the WAG population weighing 200-250 g. Еxperiments were conducted in the laboratory of Department of Pharmacology and Prescription writing (Kharkiv National Medical University, Ukraine).

On the shaved part of the back thigh under the thiopental anesthesia a thermal burn was caused. 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).

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 IMMP - 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.

The state of LPO was assessed by the level of diene conjugates (DC) according to the method of. Stalnoy I.D in modification Skornyakova V.I. et al. and the level of TBA - active products (TBA-AP) according to the method of Uchiyma M., Michara M. modified by Volchegorsky I.A. et al. on the test with TBA. Catalase activity was determined by the method based on the ability of hydrogen peroxide (H2O2) to form a stable colored complex with molybdenum salts, the activity of ceruloplasmin (CP) in serum was determined by Houchin O. in a modification of Mzhelskaya T.I. et al. Statistical processing of the data was performed by standard statistical methods. Research results were processed by variation biomedical statistics methods using t-Student criteria by computer program "Statistica 6,0".

Doses of referent drugs were determined according to the instructions for medical use of the considering a specific sensitivity factor according to Yu. R. Rybolovlev's method. The introduction of doxycycline was carried out according to the recommended earlier studies of Brown D.L. (2004) at a dose of 2.5 mg / kg and Michelle P.B. (2002) at a dose 30 mg/kg .

**Results and their discussion.**

According to the obtained experimental data, presented in Table 1, the level of DC in the serum of intact animals was 19.31 ± 1.02 mmol / l.

The study of the level of DC in the serum of animals with thermal burn without treatment, showed its increase during the whole time of the study compared with intact animals. So, on the 7th day the level of DC increased by 69.0%, on the 14th - 28th day on average by 36.0%.

Doxycycline in both doses unidirectionally influenced the level of DC in the blood: it did not significantly change it in the first three weeks of observation compared to the control, but by the 28th day there was a sharp decrease in the primary LPO products. Only under the influence of doxycycline on the 28th day after the thermal damage, the level of DC in the blood was significantly lower than in the group without treatment (by 25.0%), which may indicate a positive effect of the studied drug on excessive activity of LPO.

*Table 1. Influence of doxycycline on the DC level in blood serum (mmol /h × l) of rats with burn wound (n = 6)*

|  |  |
| --- | --- |
| **Group** | **Time of observation (days)** |
| **7-th** | **14-th** | **21-st** | **28-th** |
| Intact | 19,31±1,02 |
| Control | 32,61±1,27 а | 27,73±2,18 а | 25,92±3,17 а | 24,86±2,59 а |
| thiotriazoline 30 mg/kg | 27,43±1,20 а, b | 26,46±0,71а | 25,57±0,74 а | 20,64±0,73 |
| methyluracil,0,126 mg/kg | 29,06±2,19 а | 26,30±1,56 а | 23,85±1,05а | 20,14±0,64 |
| doxycycline,2,5 mg/kg | 27,76±3,66 а | 24,85±1,81а | 22,79±1,39а | 18,70±1,41b |
| doxycycline,30 mg/kg | 28,42±3,55 а | 26,56±0,57 а | 23,07±0,91 а | 18,57±1,03b |

Notes:

1. а - the reliability of differences in comparison with intact rats, р<0,05;

2. b- the reliability ofdifferences in comparison with control, р<0,05;

3.c- the reliability of differences in comparison with thiotriazoline, р<0,05;

4.d- the reliability of differences in comparison with methyluracil, р<0,05.

The use of thiotriazoline resulted in a decrease in the level of DC in the first week compared with the control (by 16.0%). On the 14th - 21st day, the concentration of primary LPO products was at the control level, on the 28th day it was not significantly different from that of intact animals.

Under the influence of methyluracil, there was no significant decrease in the concentration of DC in the blood compared with the group without treatment. Only on the 28th day, the level of DC decreased, reaching the level of intact rats.

The level of TBA-AP in the serum of animals not treated was increased during the whole experiment period (7th day - by 63.0%, 14th day - by 42.0%, 21 day - by 20.0%, day 28 - by 12.0%).

Doxycycline at a dose of 2.5 mg / kg contributed to a significant decrease in the level of TBA-AP by day 28 (by 19.0%), that is, it showed an effect at the level of methyluracil.

The level of TBA-AP was most actively reduced under the influence of doxycycline at a dose of 30 mg / kg. This was confirmed by the fact that starting from the 2nd week and until the end of the experiment, it did not differ from that of intact animals, while remaining below the level of TBA-AP in the control group throughout the entire observation period, which may indicate a positive effect of the studied drug on excessive activity of the LPO (Table 2).

*Table 2. Influence of doxycycline on the TBA-AP level in blood serum (mmol /h × l) of rats with burn wound (n = 6)*

|  |  |
| --- | --- |
| **Group** | **Time of observation (days)** |
| 7-th | 14-th | 21-st | 28-th |
| Intact | 3,50±0,29 |
| Control  | 5,70±0,09а | 4,97±0,35а | 4,20±0,19 а | 3,92±0,18 а |
| thiotriazoline 30 mg/kg | 5,15±0,36а | 4,50±0,28а | 3,88±0,30 | 3,59±0,20 |
| methyluracil,0,126 mg/kg | 5,18±0,29 а | 4,51±0,44 а | 4.36±0,61 а | 3.31±0,10 b |
| doxycycline,2,5 mg/kg | 5,19±0,42 а | 4,60±0,27 а | 4,47±0,24 а | 3,18±0,13 b |
| doxycycline,30 mg/kg | 4,97±0,28 а, b | 3,75±0,40 b | 3,32±0,16 b | 3,11±0,33b |

Notes:

1. а - the reliability of differences in comparison with intact rats, р<0,05;

2. b- the reliability of differences in comparison with control, р<0,05;

3.c- the reliability of differences in comparison with thiotriazoline, р<0,05;

4.d- the reliability of differences in comparison with methyluracil, р<0,05.

Under the influence of thiotriazoline, the level of TBP-AP decreased from the 21st day. During this period, as well as on the 28th day, the concentration of TBA-AP did not differ from the values of intact rats.

Methyluracil resulted in the normalization of the secondary products of LPO only by the 28th day, while their level was significantly lower than the control group by 1.2 times.

Thus, based on the above, we can assume a positive therapeutic effect of doxycycline in correcting the excessive activity of free radical oxidation during a thermal burn by the most active decrease in the level of DC (regardless of dose) and TBA-AP of peripheral blood (at a dose of 30 mg / kg) by the end of the experiment.

In the study of the activity of the antioxidant system enzyme - catalase in the blood of animals in the control group, a decrease in the activity of this enzyme was detected during the first three weeks of the experiment in comparison with intact values. The lowest indicator was registered on the 7th day and amounted to 2.12 ± 0.16 USD, which is 1.9 times lower than the similar indicator of intact rats (Table 3).

Under the influence of a synthetic IMMP at a dose of 2.5 mg / kg, the recovery of the activity of the enzyme under study was more intense and earlier than in groups using reference drugs. This was confirmed by a significant increase in catalase activity already on the 14th day in comparison with the control group (by 17.0%). Subsequently, catalase continued to increase its activity and reached the level of intact rats.

*Table 3. Influence of doxycycline on the catalase level in blood serum (mmol /h × l) of rats with burn wound (n = 6)*

|  |  |
| --- | --- |
| **Group** | **Time of observation (days)** |
| **7-th** | **14-th** | **21-st** | **28-th** |
| Intact | 3,97±0,18 |
| Control | 2,12±0,16 а | 2,82±0,09 а | 3,18±0,31а | 3,60±0,44 |
| thiotriazoline 30 mg/kg | 2,36±0,13 а | 2,78±0,56 а | 3,53±0,23 | 3,95±0,16 |
| methyluracil,0,126 mg/kg | 2,21±0,35 а | 3,07±0,12 а | 3,68±0,21 | 3,92±0,17 |
| doxycycline,2,5 mg/kg | 2,41±0,33 а | 3,30±0,23 а,b | 3,54±0,18 | 4,14±0,11 |
| doxycycline,30 mg/kg | 2,37±0,17 а | 3,11±0,28а | 3,44±0,46 | 4,16±0,15 |

Notes:

1. а - the reliability of differences in comparison with intact rats, р<0,05;

2. b- the reliability of differences in comparison with control, р<0,05;

3.c- the reliability of differences in comparison with thiotriazoline, р<0,05;

4.d- the reliability of differences in comparison with methyluracil, р<0,05.

The effect of doxycycline at a dose of 30 mg / kg resulted in an identical effect with the group receiving doxycycline at a lower dosage. This was manifested by an increase in catalase activity to the norm from the 21st day of observations.

Under the influence of thiotriazolin, enzyme activity was restored, starting from the 21st day of observation. During this period and until the end of the experiment (28th day), the activity of the enzyme was not significantly different from intact animals.

The use of methyluracil also restored catalase in the blood serum on the 21st and 28th day.

Thus, the highest levels of catalase activity in the serum of rats were registered in the group using doxycycline, regardless of the dose, which obviously can help reduce the healing time of thermal damage.

The study of the activity of CP in the serum of animals with thermal burns that did not receive treatment revealed a decrease in its activity throughout the entire period of the experiment in comparison with intact rats. The minimum value was recorded on the 14th day and was 81.96 ± 6.64 y. e., which was 28.0% lower than that of intact animals (Table 4).

The most informative indicators were changes in the activity of CP in the serum in the group of animals using doxycycline at a dose of 30 mg / kg. This was confirmed by the restoration of its activity to physiological values already from the 14th day of the experiment. At the same time, on the 21st day, the activity of the CP is higher than that of the control group by 37.0%.

*Table 4 Influence of doxycycline on the catalase level in blood serum (mmol /h × l) of rats with burn wound (n = 6)*

|  |  |
| --- | --- |
| **Group** | **Time of observation (days)** |
| **7-th** | **14-th** | **21-st** | **28-th** |
| Intact | 114,40±2,80 |
| Control | 86,77±4,38 а | 81,96±6,64а | 84,88±12,14а | 100,52±4,53а |
| thiotriazoline 30 mg/kg | 97,42±15,62а | 90,27±19,44а | 102,32±6,58 | 120,46±18,74 |
| methyluracil,0,126 mg/kg | 85,70±7,36 а | 99,55±5,43 а, b | 110,44±10,66 | 114,26±10,42 |
| doxycycline,2,5 mg/kg | 93,02±15,97а | 104,01±12,29 | 111,61±11,74 | 121,73±8,28 b |
| doxycycline,30 mg/kg | 98,07±9,28а | 110,40±21,28 | 116,15±8,62b | 123,92±13,41 |

Notes:

1. а - the reliability of differences in comparison with intact rats, р<0,05;

2. b- the reliability of differences in comparison with control, р<0,05;

3.c- the reliability of differences in comparison with thiotriazoline, р<0,05;

4.d- the reliability of differences in comparison with methyluracil, р<0,05.

In the treatment with doxycycline at a dose of 2.5 mg / kg, the positive dynamics was more active than in the groups using comparative drugs. So, already from the second week of the experiment, the activity of CP in this group did not differ from that of intact rats. On the 28th day, the activity of CP significantly increased in comparison with the control group by 23.0%.

The study of the studied enzyme in the group of rats with the use of thiotriazolin showed that the activity of the CP was restored gradually, starting from the 21st day, reaching physiological values only on the 28th day.

Under the influence of methyluracil, the activity of CP increased more intensively than in the previous group, as evidenced by the lack of significant differences in comparison with intact animals from the 21st day. At the same time, already on the 14th day, an increase in the activity of the CP was recorded in comparison with the control by 1.3 times.

**Conclusion.** Based on the results of a study of the effect of doxycycline as a synthetic inhibitor of matrix metalloproteinases on the activity of the LPO and AOS processes during thermal burn of rats, we can conclude that:

1. Doxycycline (especially at a dose of 30 mg / kg) exceeded the comparison drugs thiotriazolin and methyluracil in the ability to normalize the processes of lipid peroxidation in the blood of thermal damage, which was confirmed by the normalization of the level of DC by day 28 and TBA-AP as early as on the 14th day of the serum experiment animals.

2. All the drugs used in the study of the treatment of thermal burn restored antioxidant potential. According to the degree of increase in the activity of enzymes of antioxidant protection, the drugs were distributed as follows: thiotriazolin <methyluracil <doxycycline.

It can be assumed that the use of the study drug will help reduce the time of healing of thermal burns, as well as prevent the negative effects of excessive activity of the LPO by activating the antioxidant system.

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**РЕЗЮМЕ**

**ОЦЕНКА СОСТОЯНИЯ ПРОЦЕССОВ ПЕРЕКИСНОГО ОКИСЛЕНИЯ ЛИПИДОВ И АНТИОКСИДАНТНОЙ ЗАЩИТЫ ПРИ ЛЕЧЕНИИ ТЕРМИЧЕСКОГО ОЖОГА КРЫС ДОКСИЦИКЛИНОМ В КАЧЕСТВЕ СИНТЕТИЧЕСКОГО ИНГИБИТОРА МАТРИЧНЫХ МЕТАЛЛОПРОТЕИНАЗ В ЭКСПЕРИМЕНТЕ**

**Ермоленко Т.И., Александрова А.В.**

Харьковский национальный медицинский университет, Украина

Цель – оценить влияние доксициклина на состояние процессов перекисного окисления липидов и активности антиоксидантной системы.

Исследование было выполнено на 144 крысах популяции WAG массой 200-250 г. (6 крыс в каждой группе). Животным с термическим ожогом вводили исследуемый препарат доксициклин, а также референтные препараты – тиотриазолин и метилурацил внутрижелудочно в крахмальной взвеси с первого дня ожога и в течение 28-ми суток, выводили из эксперимента в соответствии с правилами биоэтики на 7, 14, 21 и 28-е сутки.

 В результате проведенных исследований было выявлено наиболее интенсивное влияние доксициклина на процессы ПОЛ в сыворотке крови крыс – снижение активности ДК к концу эксперимента (независимо от дозы) и ТБК-АП начиная с 14-х суток наблюдения достигая значения интактных крыс (в дозе 30 мг/кг). Вместе с тем было отмечено повышение активности ферментов антиоксидантной системы – уровня каталазы с 14-х суток (достоверно выше контрольной группы независимо от дозы) и уровня церулоплазмина (со второй недели наблюдений показатель достигал интактных значений в дозе 30 мг/кг). Референтные препараты уступали ему по своей эффективности.

 Полученные данные могут свидетельствовать о возможности сокращения сроков заживления термического ожога за счет подавления чрезмерной активности свободно-радикального окисления и активации антиоксидантной защиты.

**Ключевые слова:** синтетический ингибитор матричных металлопротеиназ, термический ожог, доксициклин.

**SUMMARY**

**ESTIMATION OF THE LIPID PEROXIDE OXIDATION PROCESSES AND ANTIOXIDANT SYSTEM IN THE TREATMENT OF RATS TERMAL BURNING BY DOXICYCLIN AS A SYNTHETIC INHIBITOR OF MATRIX METALLOPROTEINASES IN EXPERIMENT**

**Iermolenko T.I., Aleksandrova A.V.**

*Kharkiv national medical university, Ukraine*

Aim – to assess the effects of doxycycline on the state of lipid peroxidation processes and the activity of the antioxidant system.

The study was performed on 144 rats of the WAG population weighing 200-250 g (6 rats in each group). Animals with thermal burns were injected with the test drug doxycycline, as well as reference drugs — thiotriazolin and methyluracil orally in starch suspension after thermal exposure and daily during the entire experiment period (28 days). Animals were removed from the experiment in accordance with the rules of bioethics on the 7th, 14th, 21st and 28th day.

As a result of the research, the most intense influence of doxycycline on the processes of lipid peroxidation in the blood serum of rats was found - a decrease in DC activity by the end of the experiment (regardless of dose) and TBA-AP starting from the 14th day of observation reaching the value of intact rats (at a dose of 30 mg / kg). At the same time, an increase in the activity of enzymes of the antioxidant system was noted - the level of catalase from the 14th day (significantly higher than the control group regardless of dose) and the level of ceruloplasmin (from the second week of observation the indicator reached intact values ​​at a dose of 30 mg / kg). Reference drugs were inferior to doxycycline in their effectiveness.

The data obtained may indicate the possibility of reducing the healing time of a thermal burn due to the suppression of excessive free radical oxidation activity and activation of antioxidant protection.

**Key words:** synthetic inhibitor of nmatrix metalloproteinase, thermal burn, doxycycline.