

LOCAL ANTIOXIDANT EFFECT OF ORIGINAL DERMAL FILM WITH MELATONIN IN THERMAL INJURY

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Oxidative stress (OS) escalation associated with thermal trauma (TT) and pleiotropic effects of melatonin (MT) suggest a study of protective properties of the latter when applied as part of a novel dermal film (DF) to skin burns. This work aimed to assess the content of OS markers in the skin subjected to experimental TT and treated with DF with MT. Third A degree TT (area of 3.5%) were modeled by immersing a patch of skin in boiling water. Twelve cm² of DF with 5 mg/g of MT were applied daily for 5 days. The parameters calculated were wound's area and epithelialization rate. The products monitored in the burn wound were lipid peroxidation (LPO) products in heptane and isopropanol phases of the lipid extract and protein oxidative modification (POM) products, the modification being spontaneous and metal-dependent. With TT in the wound, the content of secondary and end LPO products in heptane and isopropanol phases increased on the 5th and 10th days; the total content of POM products grew on the 5th day (primary products, neutral) and on the 10th day (primary and secondary products, neutral). Application of DF to a TT wound reduced the burn area, increased the epithelialization rate (by the 10th day, the median went from 1.90% to 6.57%; $p < 0.05$), reduced the content of secondary and end LPO products in isopropanol phase (by the 10th day, the median went from 0.007 to 0.004 u.o.i.; $p < 0.05$), reduced the total content of OMP products, namely that of primary neutral products — on the 5th day, of primary and secondary neutral products — on the 10th day. With TT present in the context of MT application, the burn area showed presence of secondary LPO products in heptane and isopropanol phases, LPO end products in isopropanol phase, POM products in the wound (basic and neutral primary/secondary POM products).

Keywords: thermal trauma, oxidative stress, melatonin, dermal film**Author contribution:** MV Osikov — study concept and design, integrated analysis of the data obtained, authoring, manuscript editing; EV Simonyan — experimental material collection, analysis of the data obtained; AA Ageeva — experimental material collection, statistical processing and analysis of the data obtained, authoring; Yul Ageev — experimental material collection, statistical processing and analysis of the data obtained; AA Fedosov — analysis of the results, manuscript editing; AI Sinitsky — experimental material collection, manuscript editing.**Compliance with ethical standards:** the study was approved by the Ethics Committee of the South Ural State Medical University, Chelyabinsk (Minutes #10 of November 15, 2019), carried out in standard vivarium conditions with strict adherence to the requirements for animal keeping and care, as well as withdrawal of animals from the experiment and subsequent disposal in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental or Other Scientific Purposes (ETS № 123 of March 18, 1986, Strasbourg), EC Recommendations 2007/52/EC of June 18, 2007 outlining procedures of keeping and care for animals used for experimental and other scientific purposes, as well as the European Parliament and EU Council Directive 2010/63/EU of September 22, 2010 on protection of animals used for scientific purposes as governed by the rules of humane treatment of animals, guidelines for their withdrawal from experiments and euthanasia.✉ **Correspondence should be addressed:** Anna A. Ageeva
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ЛОКАЛЬНЫЙ АНТИОКСИДАНТНЫЙ ЭФФЕКТ ОРИГИНАЛЬНОЙ ДЕРМАЛЬНОЙ ПЛЕНКИ С МЕЛАТОНИНОМ ПРИ ТЕРМИЧЕСКОЙ ТРАВМЕ

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Эскалация окислительного стресса (ОС) при термической травме (ТТ) и плейотропные эффекты мелатонина (МТ) являются предпосылкой для изучения его протекторных свойств в составе новой дермальной пленки (ДП) при ожогах кожи. Цель работы — оценить содержание маркеров ОС в коже при экспериментальной ТТ и применении ДП с МТ. ТТ IIIA степени площадью 3,5% моделировали погружением участка кожи в кипящую воду. ДП площадью 12 см² с МТ в концентрации 5 мг/г наносили ежедневно в течение 5 сут. Вычисляли площадь раны и скорость ее эпителизации. В ожоговой ране определяли продукты перексидного окисления липидов (ПОЛ) в гептановой и изопропанольной фазах липидного экстракта, продукты окислительной модификации белков (ОМБ) в спонтанном и металл-зависимом режимах. При ТТ в ране на 5-е и 10-е сут. увеличилось содержание вторичных и конечных продуктов ПОЛ в гептановой и изопропанольной фазах, суммарное содержание продуктов ОМБ за счет первичных продуктов нейтрального характера на 5-е сут., первичных и вторичных продуктов нейтрального характера на 10-е сут. Применение ДП при ТТ уменьшило площадь ожога, увеличило скорость эпителизации раны (на 10-е сут. по медиане с 1,90% до 6,57%; $p < 0,05$), снизило содержание в изопропанольной фазе вторичных и конечных (на 10-е сут. по медиане с 0,007 до 0,004 е.и.о.; $p < 0,05$) продуктов ПОЛ, снизило суммарное содержание продуктов ОМБ на 5-е сут. за счет первичных продуктов нейтрального характера, на 10-е сут. за счет первичных и вторичных продуктов нейтрального характера. При ТТ в условиях применения МТ площадь ожога ассоциирована с содержанием в ране вторичных продуктов ПОЛ в гептановой и изопропанольной фазах, конечных продуктов ПОЛ в изопропанольной фазе, суммарным содержанием продуктов ОМБ, содержанием первичных, вторичных, основного и нейтрального характера продуктов ОМБ.

Ключевые слова: термическая травма, окислительный стресс, мелатонин, дермальная пленка**Вклад авторов:** М. В. Осиков — концепция и дизайн исследования, интегральный анализ полученных данных, написание текста, редактирование рукописи; Е. В. Симонян — набор экспериментального материала, анализ полученных данных; А. А. Агеева — набор экспериментального материала, статистическая обработка и анализ полученных данных, написание текста; Ю. И. Агеев — набор экспериментального материала, статистическая обработка и анализ полученных данных; А. А. Федосов — анализ результатов, редактирование рукописи; А. И. Синицкий — набор экспериментального материала, редактирование рукописи.**Соблюдение этических стандартов:** исследование одобрено этическим комитетом Южно-Уральского государственного медицинского университета г. Челябинск (протокол № 10 от 15 ноября 2019 г.), выполнено в стандартных условиях вивария при строгом соблюдении требований по уходу и содержанию животных, а также выводу их из эксперимента с последующей утилизацией в соответствии с Европейской конвенцией о защите позвоночных животных, используемых для экспериментов или в иных научных целях (ETS № 123 от 18 марта 1986 г., Страсбург), Рекомендациями Европейской комиссии 2007/526/ЕС от 18 июня 2007 г. по содержанию и уходу за животными, используемыми в экспериментальных и других научных целях, а также Директивой 2010/63/EU Европейского парламента и совета Европейского союза от 22 сентября 2010 г. по охране животных, используемых в научных целях в соответствии с правилами гуманного отношения к животным, методическими рекомендациями по их выведению из опыта и эвтаназии.✉ **Для корреспонденции:** Анна Алексеевна Агеева
ул. Воровского, д. 64, г. Челябинск, 454092; anne.ageeva.r@yandex.ru**Статья получена:** 03.10.2020 **Статья принята к печати:** 23.10.2020 **Опубликована онлайн:** 10.11.2020**DOI:** 10.24075/vrgmu.2020.070

There are about 250,000 burn cases registered in the Russian Federation annually. About 80% of them are associated with thermal trauma (TT) [1]. The most common causes of TT are hot liquids and flame; two-thirds of patients have the burn area smaller than 10% of the body surface [2]. Studying pathophysiology of burn wounds allows finding ways to halt their progression and develop new, pathogenetically valid methods of safe necrectomy and wound closure, modern substitutes. Burn wound healing rate, adverse outcome and development of TT-related complications depend not only on the injury's area and depth, severity of inflammation, acute phase response and immune reactions, but also on the local redox status [3]. For free radicals, the key targets in the skin are lipids and proteins. The "attacks" produce metabolites of lipid peroxidation (LPO) and protein oxidative modification (POM), respectively. As reported earlier, LPO products contribute to DNA damage, mutagenic and carcinogenic effects, modification of membrane proteins, enzymes, signaling molecules [4]. In the TT pathogenesis, the relationship between inflammation and redox status under the OxInflammation concept describes the prooxidative potential of the lesion with positive feedback from inflammatory process events, cross-interactions of inflammatory mediators and redox status with possible progressions into SIRS [5]. Among the biomarkers of OxInflammation are lipid hydroperoxides 4-hydroxy-2-nonenal and malonic dialdehyde, carbonyl derivatives of 3-nitrotyrosine proteins, hydroxyguanosine etc. In pathological situations, identification of aldehyde- or ketone-containing carbonyl derivatives of proteins (POM products) and LPO products (as markers of oxidative stress and performance of antioxidants) is of great interest [6].

Currently, there is a number of types of wound dressings applied as treatment of localized thermal skin injuries. Such dressings ease pain during bandaging, make the wound moist and thus promote cell differentiation and effective intercellular interaction, and shorten treatment time. There are absorbent dressings, atraumatic (usually tulle) dressings of natural fibers, powder (xerogel) dressings, spongy dressings made of porous material, hydrogel dressings made of insoluble polymers, hydrocolloidal dressings made of gel-forming substances, film dressings made of semipermeable polymer materials, etc [7]. No dressing is universal; its choice depends on the wound's stage, the degree of exudation and complications, if any. About 80% of wound dressings are made by foreign manufacturers, which makes research and development of an original dermal film (DF) relevant and promising. The films may incorporate various pharmacologically active substances (antimicrobial, antiseptic, analgesic, etc.); endogenous regulators of homeostasis with pleiotropic properties are particularly interesting [8–11]. In addition to its capacity to regulate rhythms of sleep and wakefulness, melatonin (MT) can produce multitropic effects and possesses antioxidant, pro-

and anti-inflammatory, immunomodulatory, antiapoptogenic, cell proliferation and differentiation regulating, anti-aging and other properties, which make it attractive as a therapeutic agent [12]. There are no papers published and available that mention local use of MT as a part of DF applied to TT.

This study aimed to assess the content of oxidative stress markers in the skin subjected to experimental TT and treated with original DF with MT.

METHODS

The experiment involved 88 male Wistar rats weighing 200–240 g. The animals were randomly divided into three groups: group 1 ($n = 20$) — intact control; group 2 ($n = 36$) — animals with TT; group 3 ($n = 32$) — animals with TT and DF with MT applied to the wound. Third degree A TT burns with a relative area of 3.5% were imposed in the interscapular area by immersion in purified water at 98–99 °C for 12 s. Depth of the burn was verified by morphological methods. Experimental model relying on hot water is most often considered by researchers as the TT standard. Zoletil 100 (tiletamine, zolazepam) (Virbac Sante Animale; France), dose of 20 mg/kg, was the anesthetic used in the experiment. Subjects of the 3rd group received a 12 cm² patch of the film with MT immediately after TT was inflicted. The film was fixed with an aseptic bandage, changed daily for 5 days. Preliminary studies yielded DF composition based on sodium carboxymethylcellulose (poly-1,4-β-O-carboxymethyl-D-pyranosyl-D-sodium glycopyranose). The film was enriched with MT (5 mg/g), after which its pharmacological and technological parameters were evaluated, namely: organoleptic indicators (type, color, transparency, elasticity, presence of impurities and microcracks), adhesiveness, mechanical tensile strength, thickness (patent application #2020118766). A Nikon Coolpix S2800 camera (Nikon; China) and Microsoft Office Visio software package (Microsoft; USA) enabled digital planimetry that allowed establishing the wound area 24 hours later, on the 5th and 10th days after TT infliction. The epithelialization rate (VS) was calculated using the following formula: $VS = S - S_n / t$, where S is the initial wound area before treatment (hereinafter, area at previous measurement); S_n is the area at subsequent measurement; t is the number of days between measurements. The area of the wound at subsequent measurements was determined in %, with 100% being the area before treatment. The results of the measurement were expressed in %/day.

To prepare 10% skin homogenate, the burn wound was excised, immersed in a cooled 0.1 M phosphate buffer solution (pH 7.4), after that about 40 mg of tissue was homogenized in a glass mechanical homogenizer (1 : 10 ratio) for 3 min at 4 °C, which finally yielded 1 ml of the homogenate. SF-56 spectrophotometer (LOMO-Spectr; St. Petersburg) was used to determine the content of LPO products in the homogenate;

Table 1. Influence of MT as part of DF on the parameters of healing of the wound, with experimental TT (Me (Q₂₅; Q₇₅))

Indicator	Group 2 TT, 5 th day ($n = 16$)	Group 2 TT, 10 th day ($n = 20$)	Group 3 TT + MT, 5 th day ($n = 16$)	Group 3 TT + MT, 10 th day ($n = 16$)
Burn area, cm ²	11.66 (11.50; 11.94)	9.48 (9.28; 9.93)*	10.33 (10.17; 10.56)#	8.34 (8.19; 8.51)#
Relative area, %	3.34 (3.25; 3.39)	3.17 (3.10; 3.29)*	3.36 (3.23; 3.42)	3.02 (2.91; 3.13)#
Epithelialization rate, %/day	0.89 (0.86; 0.89)	1.90 (1.88; 1.95)*	1.33 (1.29; 1.35)#	6.57 (5.92; 6.93)#
Wound area shrinkage, %	2.61 (2.59; 2.64)	3.68 (3.53; 4.23)*	9.80 (9.64; 10.08)#	16.10 (14.62; 17.73)#

Note: * — statistically significant ($p < 0.05$) differences with group 2 on the 5th day; # — with group 2 on the corresponding day.

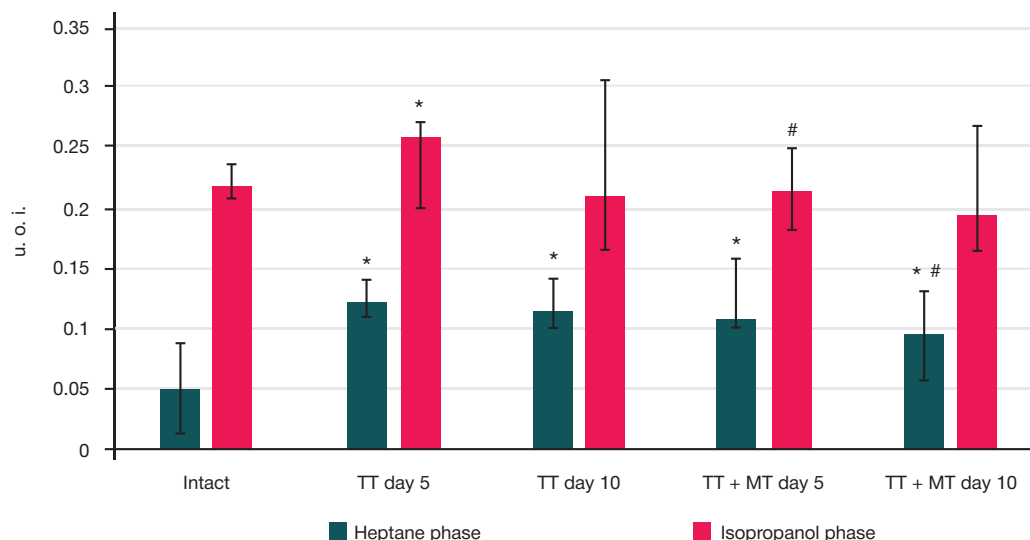


Fig. 1. Effect of MT as part of DF on the content of ketodienes and conjugated trienes in the heptane and isopropanol phases of skin homogenate, experimental TT (Me (Q_{25} ; Q_{75})). * — statistically significant ($p < 0.05$) differences with group 1; # — with group 2

the extraction-spectrophotometric method applied was described in earlier works [13]. Optical density was the parameter measured in the heptane and isopropanol phases of the lipid extract. The measurement wavelengths were 220 nm (content of isolated double bonds), 232 nm (content of diene conjugates, DC), 278 nm (content of ketodienes and conjugated trienes, KT and CT), 400 nm (Schiff bases, SB). The relative content of LPO products was expressed in units of oxidation indices (u.o.i.): E232/E220 (DC), E278/E220 (KD and CT), and E400/E220 (SB). The POM products in the homogenate were determined by the reaction between protein carbonyl derivatives and 2,4-dinitrophenylhydrazine in spontaneous and metal-dependent modes, according to the Fenton reaction, with the following step being registration of aldehyde dinitrophenylhydrazones (ADNPH) and ketondinitrophenylhydrazones (KDNPH) in UV and visible parts of the spectrum [14]. The results were expressed in optical density units per 1 mg of protein (c.u./mg) or in relative values (%). The reserve adaptive potential (RAP, %) was calculated as the ratio of difference between the total content of POM products in the induced and spontaneous modes to the content of POM products in the metal-dependent mode. IBM SPSS Statistics 19 software (SPSS: An IBM Company; USA)

was used for statistical processing of the data. The indicators were presented as median (Me) and quartiles (Q_1 ; Q_3). Kruskal–Wallis, Mann–Whitney, Wald–Wolfowitz tests enabled assessment of significance of differences between the groups. Spearman's correlation coefficient (R) allowed identification of relationship between the studied parameters. The differences were considered significant at $p < 0.05$.

RESULTS

Assessment of the burn wound healing parameters revealed that its absolute and relative areas decreased, with the result of this decrease greater on the 10th day compared to the 5th day. This process boosts the monitored indicators, i.e. wound epithelialization rate and proportion of decrease of its area (Table 1). On the 5th and 10th days, the content of LPO products registered in the wound skin homogenate changed (Fig. 1, 2). Thus, on the 5th day the content of ketodienes, conjugated trienes and Schiff bases increased significantly in the heptane and isopropanol phases. It is predominantly reserve lipids (triacylglycerides) that accumulate in the heptane phase, while those accumulating in the isopropanol phase are membrane phospholipids. Assessment of the LPO product

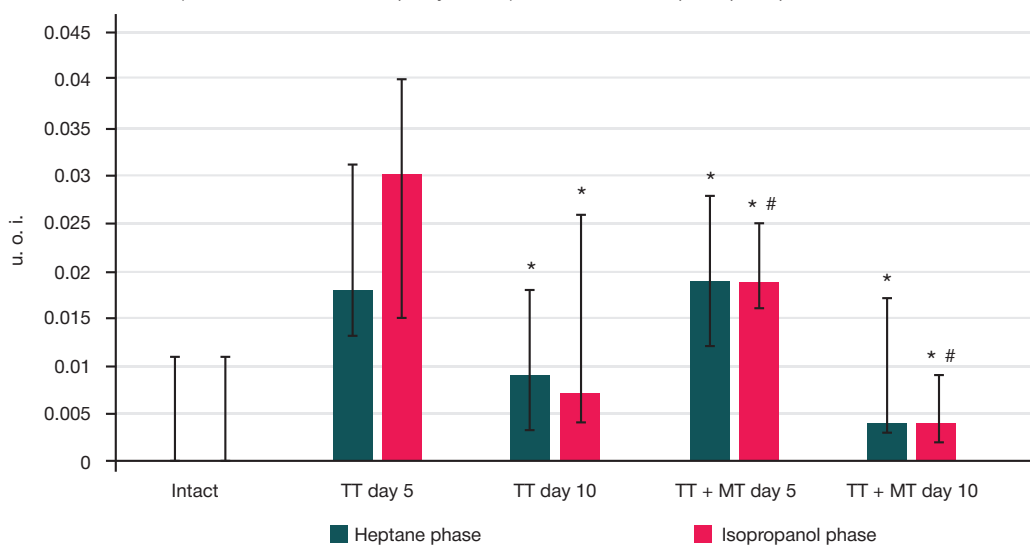


Fig. 2. Effect of MT as part of DF on the content of Schiff bases in the heptane and isopropanol phases of skin homogenate, experimental TT (Me (Q_{25} ; Q_{75})). * — statistically significant ($p < 0.05$) differences with group 1; # — with group 2

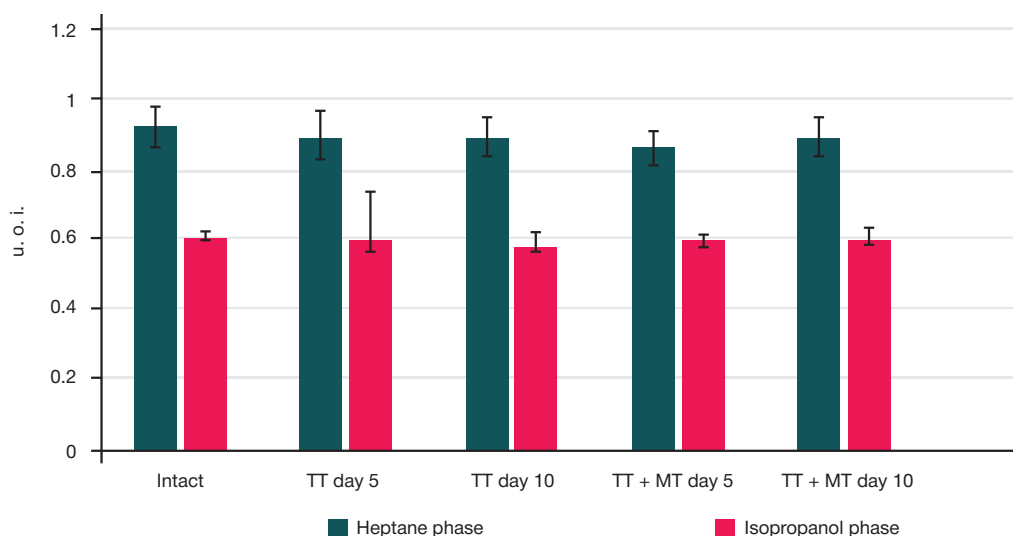


Fig. 3. Effect of MT as part of DF on the content of diene conjugates in the heptane and isopropanol phases of skin homogenate, experimental TT (Me (Q_{25} ; Q_{75}))

content on the 10th day of TT revealed a significant increase in ketodienes and conjugated trienes and Schiff bases in the lipid extract's heptane phase, while in the isopropanol phase only the Schiff bases have grown. As for the primary LPO products (diene conjugates), their content in the heptane and isopropanol phases of the burn wound skin homogenate's lipid did not change significantly by the 5th and 10th days (Fig. 2). With TT changing over time, the content of LPO products in the burn wound decreases: on the 10th day, as compared with the 5th day, the share of Schiff bases was smaller in the lipid extract's heptane phase ($p < 0.01$), and in the isopropanol phase ($p < 0.01$) the content of ketodienes, conjugated trienes and Schiff bases went down.

Table 2 shows the results of examination of POM products in the burn wound skin homogenate. The total amount of POM products increased on the 5th and 10th days of the experiment. Considered in the context of TT dynamics, the total content of POM products increased on the 10th day in comparison with that registered on the 5th day ($p < 0.01$). Analysis of POM components in the spontaneous mode burn wound skin homogenate revealed that on the 5th day the share of ADNPH increased significantly in the UV part of the spectrum, which is also true for the total ADNPH content and the overall amount of POM products. As for KDNPH, their content did not change significantly in the burn skin wound homogenate on the 5th day (as verified in both UV and visible part of the spectrum). On the 10th day of TT existence, there were registered ambiguous changes in the shares of various OMP products: the amount of ADNPH and KDNPH as seen in the UV part of the spectrum have grown, while that in the visible part of the spectrum has decreased significantly. Accordingly, on the 10th day the total content of ADNPH and KDNPH has increased, as did the total content of POM products in the UV part of the spectrum, and the total content of POM products in the visible part of the spectrum has gone down. It should also be noted that the total content of POM products in the visible part of the spectrum on the 10th day is significantly ($p < 0.01$) higher than that on the 5th day.

RAP of the burn wound skin homogenate was assessed through calculation of the difference between the content of POM products in the metal-induced and spontaneous modes and its share in the content of POM products in the metal-induced mode (see Table 2). This stage required studying the content of POM products in the burn wound skin homogenate after induction of protein oxidation by the components of

reaction mixture containing Fe^{2+} and H_2O_2 , with the reaction also yielding formation of a highly reactive radical OH — in the Fenton reaction. The approach allows estimation of uplift in carbonyl derivatives *in vitro*, as influenced by the Fenton's reagent, and calculation of the ratio of results of measurement of spontaneous oxidation and induced oxidation. It was found that on the 5th and 10th days, POM indicators in the burn wound skin homogenate (metal-induced mode) have grown in relation to the total content of POM products, content of ADNPH and KDNPH, with the assessment carried out for both UV and visible parts of the spectrum. In addition, the total content of ADNPH and KDNPH in the UV and visible spectra, as well as the total content of POM products therein, have increased. The changes in POM indicators peculiar to the metal-induced mode largely align with the POM indicator changes registered for the spontaneous mode on the 5th day. With the exception of POM products in the visible part of the spectrum, same is true for the 10th day of TT existence. The total RAP, assessed by the total content of POM products, increased significantly on the 5th day and did not change on the 10th day. The change of the 5th day results from the RAP's growth in relation to ADNPH and KDNPH in the UV and visible spectra, with ADNPH growing predominantly in the visible part of the spectrum, and KDNPH in both UV and visible spectra. On the 10th day, the overall RAP was significantly ($p < 0.01$) lower than on the 5th day of the experiment.

It was established that, in absolute figures, application of DF with MT to a TT wound decreases the burn wound significantly on the 5th and 10th days, and in relative values — on the 10th day (see Table 1). The wound epithelialization rate and the relative wound area shrinking process accelerated on the 5th and 10th days of observation. On the 5th day of TT existence, the absolute area of the burn surface decreased by 11% (median). The maximum change was recorded on the 10th day, when the absolute area of the wound decreased by 12% and the epithelialization rate have grown by 246% (median) relative to the group that had no DF with MT applied after TT infliction. Dynamics of experimental TT have shown that on the 10th day, as compared to the 5th day, the burn area has decreased significantly ($p < 0.01$), and the rate of wound epithelialization and relative wound area shrinkage have increased. As shown in Table 2, we have registered changes in LPO products content in the burn wound skin homogenate's lipid extract in the group that had DF with MT applied to the TT (see Table 2). On the 5th day of observation, the content of ketodienes and

Table 2. Effect of MT as part of DF on the content of POM products in skin homogenate, with experimental TT (Me (Q₂₅; Q₇₅))

Indicators	Group 1 Intact (n = 20)	Group 2 TT, 5 th day (n = 16)	Group 2 TT, 10 th day (n = 20)	Group 3 TT + MT 5 th day (n = 16)	Group 3 TT + MT 10 th day (n = 16)
S _{ADNPH} uv (sp), c.u./mg	29.85 (24.69; 32.84)	51.49 (48.03; 55.81)*	60.50 (52.95; 93.13)*	32.87 (31.04; 48.35) [#]	53.71 (45.11; 59.16) [#]
S _{ADNPH} vs (sp), c.u./mg	6.93 (5.32; 8.71)	6.91 (5.72; 9.75)	3.53 (2.09; 5.07)*	7.09 (5.17; 12.27)	5.24 (3.10; 7.17) [#]
S _{KDNPH} uv (sp), c.u./mg	8.19 (7.37; 10.59)	7.79 (7.34; 9.43)	15.19 (9.05; 25.63)*	9.29 (7.56; 16.39)	8.22 (6.48; 12.61) [#]
S _{KDNPH} vs (sp), c.u./mg	0.89 (0.69; 1.14)	0.91 (0.69; 1.41)	0.50 (0.35; 0.66)*	0.79 (0.66; 1.42)	0.45 (0.27; 0.77)
S POM (sp), c.u./mg	47.83 (41.94; 55.40)	66.87 (60.56; 76.11)*	79.30 (62.59; 122.34)*	49.97 (41.94; 79.07) [#]	64.65 (56.91; 76.64) [#]
S _{ADNPH} (sp), c.u./mg	38.54 (30.64; 41.39)	59.19 (52.29; 62.31)*	65.04 (54.51; 96.45)*	40.77 (35.11; 64.89) [#]	57.90 (48.93; 64.39) [#]
S _{KDNPH} (sp), c.u./mg	10.12 (8.23; 11.31)	8.81 (8.09; 10.67)	15.49 (9.56; 26.11)*	10.28 (8.23; 18.01)	8.97 (6.76; 13.15) [#]
S uv (sp), c.u./mg	38.47 (34.05; 45.31)	59.10 (53.57; 68.66)*	74.97 (60.81; 118.45)*	40.94 (36.99; 64.74) [#]	61.28 (52.69; 70.97) [#]
S vs (sp), c.u./mg	7.87 (6.02; 9.73)	7.81 (6.41; 11.16)	4.05 (2.35; 5.85)*	7.88 (5.84; 13.69)	5.67 (3.37; 7.89) [#]
S _{ADNPH} uv (ind), c.u./mg	90.68 (70.81; 94.67)	386.82 (279.79; 542.03)*	178.71 (128.45; 239.17)*	260.81 (94.29; 50.90) [#]	211.60 (140.76; 235.78)*
S _{ADNPH} vs (ind), c.u./mg	18.81 (16.01; 21.09)	156.21 (90.34; 244.15)*	37.79 (29.25; 60.75)*	91.49 (19.62; 264.02) [#]	68.35 (45.15; 100.18) [#]
S _{KDNPH} uv (ind), c.u./mg	28.73 (24.72; 37.55)	208.12 (128.85; 320.23)*	76.51 (45.82; 94.77)*	125.89 (29.35; 333.99) [#]	93.56 (60.57; 127.56) [#]
S _{KDNPH} vs (ind), c.u./mg	2.07 (1.62; 2.32)	14.53 (8.45; 22.58)*	3.84 (2.93; 6.02)*	8.84 (2.32; 26.09) [#]	6.61 (4.68; 9.76) [#]
S POM (ind), c.u./mg	140.38 (113.93; 155.46)	771.61 (507.38; 1128.99)*	312.84 (207.79; 393.90)*	487.04 (145.58; 1175.01) [#]	378.89 (262.70; 476.87) [#]
S _{ADNPH} (ind), c.u./mg	109.49 (87.20; 115.77)	548.02 (370.12; 786.18)*	230.51 (157.87; 301.83)*	352.30 (113.90; 814.93) [#]	277.53 (197.90; 338.84)*
S _{KDNPH} (ind), c.u./mg	30.82 (26.73; 39.69)	223.59 (137.26; 342.81)*	80.12 (48.56; 101.26)*	134.74 (31.67; 360.08)*	99.88 (64.40; 138.03) [#]
S uv (ind), c.u./mg	119.47 (95.53; 132.23)	594.94 (408.64; 862.26)*	252.79 (175.39; 344.84)*	386.71 (123.64; 884.89) [#]	304.79 (218.89; 363.53) [#]
S vs (ind), c.u./mg	20.91 (17.63; 23.23)	170.73 (98.74; 266.73)*	41.63 (32.13; 66.99)*	100.33 (21.94; 290.12) [#]	74.96 (49.83; 109.94) [#]
RAP, %	61.81 (53.98; 72.55)	91.66 (86.44; 94.78)*	69.22 (62.76; 74.76)	83.77 (71.19; 96.33)*	81.15 (74.35; 86.09) [#]

Note: * — statistically significant ($p < 0.05$) differences with group 1; [#] — with group 2.

conjugated trienes, as well as Schiff bases, in the isopropanol phase of the lipid extract has decreased significantly. As for the content of diene conjugates, ketodienes and conjugated trienes, as well as Schiff bases in the heptane phase, and Schiff bases in the isopropanol phase of the lipid extract, these did not differ significantly from the same indicators registered in the group of animals that had TT inflicted but no DF with MT applied. On the 10th day of the experiment, we have identified a significant decrease in ketodienes and conjugated trienes in the heptane phase, Schiff bases in the isopropanol phase of the burn wound skin homogenate's lipid extract. The data obtained indicate that the effect MT produces on TT as part of DF is maximal in the isopropanol phase of the burn wound skin homogenate's lipid extract. As for the content of secondary and end LPO products in the heptane and isopropanol phases, it was lower on the 10th day ($p < 0.01$) than on the 5th day of TT evolution, which is same as registered in the group that did not receive DF with MT.

The use of MT in the composition of DF applied to TT leads to a change in the content of POM products in the burn

wound (see Table 2). On the 5th day of the experiment, in the spontaneous mode, the content of ADNPH in UV spectrum, the total content of ADNPH, the total content of POM products in UV spectrum have decreased; consequently, the total content of POM products has grown down. It should be noted that the values of these indicators do not differ from the values captured in the group of intact animals, which allows stating their complete recovery. On the 10th day, in the spontaneous mode, the total content of POM products seen in the TT groups has decreased and did match the values peculiar to the group of intact animals, i.e. the recovery was only partial. Against this background, the content of ADNPH and KDNPH in the UV region, the total content of ADNPH and KDNPH, POM products in the UV region have decreased and completely recovered in relation to KDNPH in the UV region, and POM products in the UV region. When assessing the RAP, its decrease on the 5th day was found to depend on the approximately equivalent contribution of the studied primary and secondary POM products in the visible and UV light range. On the 10th day, RAP

Table 3. Correlation between burn area (cm²) and FRO indicators in the skin homogenate, experimental TT, application of DF with MT

Indicators	5 th day	10 th day
DC (heptane phase), u.o.i	R = 0.21	R = 0.18
KD and CT (heptane phase), u.o.i.	R = 0.34	R = 0.52
SB (heptane phase), u.o.i	R = 0.17	R = 0.27
DC (isopropanol phase), u.o.i.	R = 0.17	R = 0.15
KD and CT (isopropanol phase), u.o.i.	R = 0.68	R = 0.21
SB (heptane phase), u.o.i	R = 0.53	R = 0.51
S POM (spont. mode), c.u./mg	R = 0.74	R = 0.67
S POM (ind. mode), c.u./mg	R = 0.53	R = -0.25
S ADNPH (spont. mode), c.u./mg protein	R = 0.51	R = 0.39
S KDNFH (spont. mode), c.u./mg protein	R = 0.07	R = 0.47
S uv (spont. mode), c.u./mg protein	R = 0.57	R = 0.37
S vs (spont. mode), c.u./mg protein	R = 0.18	R = 0.51

Note: significant ($p < 0.05$) connections are highlighted in bold.

has increased in the DF with MT group, mainly driven by the RAP growing in relation to ADNPH and KDNPH in the visible spectrum, KDNPH in the UV spectrum.

DISCUSSION

We found that in the locus of thermal damage to the skin, the amount of secondary and end LPO products increases in the heptane and isopropanol phases of lipid extract. With TT, the total amount of carbonyl derivatives of proteins goes up, and they are irreversible products of oxidative stress formed due to the oxidation of several amino acid residues, as well as interactions with LPO products and reducing sugars. Since neutral carbonyl derivatives accumulate in the UV spectrum, and basic carbonyl derivatives accumulate in the visible part of the range, a relative analysis of the total content of carbonyls in the UV and visible spectra allows assessment of the nature of the products formed against the background of TT dynamics. On the 5th and 10th days of TT existence, the POM products accumulating predominantly are neutral, as evidenced by an increase in the total amount of products in the UV region of the spectrum. In the TT groups, the total ADNPH content in the skin increases on the 5th and 10th days, that of KDNPH — on the 10th day. This fact allows registering primary POM products accumulation on the 5th and 10th days, these products being the early markers of oxidative protein destruction, with another phenomenon registered being predominant aggregation of proteins under the influence of OH. On the 10th day, it is the secondary POM products that accumulate, which are late markers of oxidative protein destruction, and the second phenomenon registered is predominant fragmentation of proteins under the influence of the combined action of OH[•] and O₂⁻ radicals. Protein fragments are highly resistant to proteolysis, have toxic properties and can initiate apoptosis or cell necrosis, expanding the zone of secondary alteration. Skin is the largest organ with intensive LPO and POM processes, and the LPO and POM products formed therein can have a local and distant cytotoxic effect [15]. Oxidative stress in burns is registered not only in the locus, but also in the heart, lungs, kidneys and other organs, where protein, lipid and DNA damage products are found [16]. The main inducers of oxidative stress after TT are activated neutrophils, monocytes/macrophages, endotheliocytes with known systems for generating reactive oxygen species (ROS): NADPH oxidase and MPO, xanthine oxidase, NO synthase [17]. Increased production of endogenous glucocorticoids plays an important part in escalation of oxidative stress in the presence

of TT [18]. The depletion of the antioxidant defense system contributes to the pathogenesis of oxidative stress with TT — the content of reduced glutathione, activity of SOD, catalase, and glutathione peroxidase (GPO) decrease [19]. We demonstrated a decrease in the body levels of zinc and copper, which are part of SOD, due to their loss with urine and burn wound exudate, as well as a deficiency of selenium, HPO component, due to a decrease in the intake through the gastrointestinal tract associated with burns [20]. We also demonstrated the role of iron in the activation of oxidative metabolism in the presence of TT. ROS in a burn wound are associated not only with tissue destruction and negative consequences: they also participate in activation of the pro-matrix metalloproteinase and skin repair after TT [21]. ROS play an important role in the modification of components of the extracellular matrix of connective tissue — glycosaminoglycans, collagens, and non-collagen glycoproteins. Also, they contribute to wound healing through activation of signaling pathways in stem cells [22]. ROS generated during inflammation associated with TT induce local synthesis of pro- and anti-inflammatory cytokines.

According to the results of the study, the use of MT in the composition of the DF applied to a TT injury accelerates healing of the burn wound, reduces its area and reduces the content of oxidative stress metabolites — LPO and POM products. On the 5th day of TT existence, the content of secondary and end LPO products in the lipid fraction of phospholipids decreases; on the 10th day, the content of secondary LPO products in the lipid fraction of triglycerides and end LPO products in the phospholipid fraction decrease. MT limits POM: on the 5th, this process relies on the neutral amino acid residues of the primary POM products and on the protein fragmentation decrease, on the 10th day it relies on the neutral amino acid residues of primary and secondary POM products and a decrease in protein fragmentation and aggregation. We believe there is a number of mechanisms that drive the decrease of content of LPO and POM products in a burn wound with experimental TT after application of DF with MT. MT can be rapidly distribute inside the cell and in the intercellular fluid through passive diffusion, as well as with the help of glucose transporters (GLUT1) and oligopeptides (PEPT1/2) [23, 24]. MT1 receptor is found in keratinocytes and fibroblasts of the skin, hair follicle cells; MT2 receptor is found mainly in the eccrine glands and blood vessels of the skin, melanocytes; ROR α nuclear receptor for MT was identified in keratinocytes, fibroblasts, melanocytes [25–27]. In the skin, MT can absorb ROS (OH, H₂O₂) directly, and one MT molecule is capable of

binding up to four or more ROS. MT's antioxidant effect is more pronounced than that of an equivalent dose of vitamins C and E [28]. There is also indirect antioxidant effect, which is the result of an increase in glutathione synthesis, activation of glutathione peroxidase, glutathione reductase, glutathione-S-transferase, SOD, catalase, hemoxidase-1, and a decrease in the activity of quinone reductase-2, NOS-1 [29]. Moreover, MT realizes its antioxidant effect by maintaining the mitochondrial membrane potential and increasing oxidative phosphorylation, production of ATP and not ROS [30]. Apparently, the antioxidant effect of MT in the TT locus, lighter damage to proteins and lipids that make up cytoplasmic membranes and cell organelles limit secondary alteration, reduce the term of vascular and exudative reactions, help activate reparative reactions earlier and reduce healing time of a burn wound. Correlation analysis of the burn surface area and content of LPO and POM products in the TT locus with DF with MT applied (Table 3) has shown that on the 5th day of TT existence there is direct average strength connection between the absolute area of the burn and content of secondary and end LPO products in lipid extract's isopropanol phase in the burn wound, as well as weak connection with the content of secondary LPO products in the heptane phase, direct average strength connection with the total content of POM products in spontaneous and induced modes, and the total content of primary POM products and the total content of neutral POM products. On the 10th day of TT existence, there was established a direct average strength connection between the content of secondary LPO products in the heptane phase and end POM products in the isopropanol

phase of the lipid extract, total content of basic POM products, content of POM products in spontaneous mode, a weak direct connection with total content of primary and secondary POM products, neutral POM products in spontaneous mode.

CONCLUSION

The study allowed achieving the goal set and establishing that with the evolution of TT, a burn wound sees growth of content of secondary and end LPO products in heptane and isopropanol phases of the lipid extract, same as the content of predominantly neutral primary and secondary POM products. It was demonstrated that application of DF with MT (original composition) to TT reduces absolute and relative areas of the burn, boosts wound epithelialization, and reduces the content of LPO and OMP products in the burn wound. When TT has DF with MT applied to it, the area of the burn is associated with the content of LPO products and OMP products in the burn wound. The results obtained expand the existing understanding of the role of redox status changes in TT pathogenesis, serve as a prerequisite for further studies of FRO in the skin of admitted patients with burns (clinical studies) in order to designate LPO and OMP products as diagnostic markers and predictors of complications, as well as indicators the effectiveness of therapy. The antioxidant and reparation stimulating effect of MT as part of DF, which we have demonstrated at the preclinical stage, is a prerequisite for further study of the action mechanism and the effectiveness of MT application in clinical settings.

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