INFLUENCE OF GAMMA IRRADIATION ON THE FATTY ACID COMPOSITION OF TOTAL LIPIDS RABBIT SKIN

It is found that gamma radiation leads to a significant decrease in the skin of rabbits content of these fatty acids, as meristin and palmitoleic close to the physiological norm.

Key words: gamma-radiation, rabbits, lipids acids, skin, piridoxyn.

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Introduction. Platelet activation and aggregation play a crucial role in the maintenance of normal hemostasis [1, 2], but malfunction of these processes can lead to a loss of hemostatic equilibrium within the blood vessel resulting in the formation of occlusive platelet-rich thrombosis, responsible for the manifestations of atherothrombotic disease [3-5]. Pharmacologic modification of platelet function reduces the risk for the development of thrombotic diseases and their complications [6, 7]. Ideally, a clinically useful, platelet-modifying drugs should be nontoxic, orally effective, has sustained action and good antithrombotic potency without excessive risk of abnormal bleeding. None of the clinically available agents satisfy sufficient all these requirements. Moreover, despite the proven benefits of current antiplatelet agents, morbidity and mortality rates for thrombosis disease are remaining at high level. Therefore, there is much room for further improvement of antiplatelet treatment and development of novel antiplatelet agents with increased efficacy and safety profile.

Several studies have suggested that some quinone derivatives can significantly modify platelet functions [8, 9]. On the other hand, it was found that sulfur-rich compounds that contain R-SO2-CH2-S-S-R fragment are also known as pharmacologic modifiers of platelet aggregation. Hence, the search for new antiplatelet compounds with increased efficacy and safety profile continues.

The aim of the present study was to obtain more information about the effects of S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl)4-aminobenzenesulfonothioate on platelet aggregation function and mechanism of its action.

Fig. 1. Chemical structure of S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl)4-aminobenzenesulfonothioate

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Materials and methods. Studied compound, S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl)4-aminobenzensulfonothioate, was synthesized at the Department of Technology of Biologically Active Substances, Pharmacy and Biotechnology, of Lviv Polytechnic National University. The compound was synthesized according the scheme depicted in Figure 2.

Fig 2. Synthesis of S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl)4-aminobenzensulfonothioate

Melting points were determined on a Büchi capillary melting point apparatus and are uncorrected. Element analyses were performed by the centre of Microanalyse of the Aix-Marseille University. Both 1H and 13C NMR spectra were determined on a Bruker AC 200 spectrometer. The 1H and 13C chemical shifts are reported from CDCl3: peaks: 1H (7.26 ppm) and 13C (77.16 ppm) and from DMSO: 1H (2.50 ppm) and 13C (39.52 ppm).

Silica gel 60 (Merck, particle size 0.063–0.200 mm, 70–230 mesh ASTM) was used for column chromatography. TLC was performed on 5 cm × 10 cm aluminum plates coated with silica gel 60 F254 (Merck) in an appropriate solvent.

Procedure for synthesis of S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl)4-aminobenzensulfonothioate: Into a two-necked flask equipped with a nitrogen inlet cap and equipped with a nitrogen inlet, 100 ml THF (10 ml) and dissolved in portion of THF (10 ml) sodium salt of 4-amino-benzethiosulfonic acid (0.45 g, 2.50 ppm) and 13C (39.52 ppm).

Yellow precipitate, Mp. 215-217

1H NMR (200 MHz, DMSO-d6) δ, ppm: 3.83 (s, 3H, OCH3), 3.95 (s, 3H, OCH3), 4.35 (s, 2H, CH2), 6.09 (s, 2H, methyl)4-amino-benzenesulfonothioate: dihydroanthracen-2-yl)methyl)4-aminobenzenesulfonothioate.

S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl)4-aminobenzensulfonothioate: Yellow precipitate, Mp. 215-217 °C, yield 30 %.

1H NMR (200 MHz, DMSO-d6) δ, ppm: 3.83 (s, 3H, OCH3), 3.95 (s, 3H, OCH3), 4.35 (s, 2H, CH2), 6.09 (s, 2H, NH2), 6.69 (d, J = 8.6 Hz, 2H, Ar-H), 7.28 (s, 1H, Ar-H), 7.67 (d, 2H, J = 8.6 Hz, Ar-H), 7.71-7.74 (m, 2H, Ar-H), 8.12-8.17 (m, 2H, Ar-H). 13C-NMR (50 MHz, DMSO-d6) δ, ppm: 34.5 (CH2), 56.8 (OCH3), 62.7 (114.0) (2CH), 120.4 (C), 125.1 (C), 126.4 (CH), 126.6 (CH), 127.1 (C), 129.6 (CH), 133.0 (C), 133.4 (CH), 133.6 (C), 133.8 (C), 133.8 (CH), 134.2 (C), 138.3 (C), 151.1 (C), 152.3 (C), 156.2 (C), 182.7 (CO), 183.0 (CO).

Calculated for (C23H19NO6S2), %: C 58.84; H 4.08; N 2.76; S 12.85.

Found, %: C 58.02; H 4.41; N 2.76; S 12.85.

RESULTS AND DISCUSSION. Platelet function can be regulated by various agonists. A major signaling molecule causing platelet aggregation is adenosine-5'-diphosphate (ADP) which activates platelets and is known to play an important role in hemostasis and thrombosis. Moreover, ADP receptor antagonists are in wide clinical use [12, 13]. Therefore, we investigated the effect of the test quinoid thiosulfonate derivative on platelet disaggregation, samples of PRP were stimulated with ADP (5 μM) at 37°C with continuous stirring for the formation of aggregates in PRP. Studied compound (50, 100 μM) or 1% DMSO alone were added 90 seconds after the addition of inducer and changes in light transmission were recorded by aggregometer. Results were expressed as means±SEM. The difference between groups was analyzed by standard Student's t-test. P values less than 0.05 were considered statistically significant.

Assay of antiplatelet activity:
Preparation of platelet rich plasma (PRP) was done according to the method described previously [11]. All procedures were conducted at room temperature. Blood was collected from the auricular artery of healthy rabbit into 3.8 % citrate in a ratio of 9:1 and than centrifuged at 150 g for 15 min in order to obtain PRP. The PRP was carefully removed and placed into a plastic tube. Platelet-poor plasma (PPP) was prepared by further centrifugation of the remaining plasma at 1500 g for 40 minutes. Throughout all experiments, the platelet number was adjusted to 250x10^9/L by diluting PRP with PPP. Platelet aggregation in PRP was recorded under constant stirring conditions (500 rpm) at 37°C for 10 min by aggregometer (AT-02, Belarus). The baseline value was set using PRP while PPP served as full transmission control. PRP suspension was incubated with the studied compounds (final concentration: 5, 10, 25, 50 and 100 μM) or with dimethylsulphoxide (DMSO) alone for 2 minutes. To minimize the effect of DMSO, the solvent, on aggregation, the final concentration of DMSO was fixed at 1% (v/v). The aggregation was induced by adding ADP (final concentration: 5 μM) and monitored for the maximal changes in light transmission for 8 minutes, measuring the maximal increase after the addition of the inducer. The concentration at which the test compound showed 50% inhibition was taken as the IC50. To study the time-dependent inhibitory effect of the test quinoid thiosulfonate derivative on ADP-induced platelet aggregation, samples of PRP were preincubated with 50 μM DMSO and monitored for the maximal changes in light transmission. To investigate the effect of the test quinoid thiosulfonate derivative on platelet disaggregation, samples of PRP were stimulated with ADP (5 μM) at 37°C with continuous stirring for the formation of aggregates in PRP. Studied compound (50, 100 μM) or 1% DMSO alone were added 90 seconds after the addition of inducer and changes in light transmission were recorded by aggregometer. Results were expressed as means±SEM. The difference between groups was analyzed by standard Student's t-test. P values less than 0.05 were considered statistically significant.

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ADP to induce aggregation. Obtained results suggested that 1% DMSO had no effect on platelet aggregation induced by ADP. Test agent inhibited ADP-induced aggregation and the degree of inhibition was proportional to its concentration (Figure 3). As shown in Figure 3 the inhibition increased linearly from 5 to 100 μM with the half maximal inhibitory concentration (IC50) – 50 μM.

Fig. 3. Maximal ratio of ADP-induced platelet aggregation after 2 min incubation of PRP with different concentration of studied quinoid thiosulfonate derivative (QTD) or with 1% DMSO (M±m, n=6)

* – P<0.05 vs 1% DMSO

The inhibitory effect of studied compound was inversely associated with preincubation time (Figure 4). For this experiment, samples of PRP were preincubated with 50 μM of test quinoid thiosulfonate derivative for 0, 2, 3, 5, 20, 40, 60 min and then stimulated with ADP (5 μM) to induce aggregation. As shown in Figure 4 the inhibition levels of ADP-induced aggregation observed after preincubation of PRP with 50 μM of compound for 0, 2, 3 or 5 minutes did not differ from each other. On the other hand, the inhibitory effect of S-(1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl 4-aminobenzenesulfonothioate was significantly reduced after more than twenty minutes of incubation. Moreover, after preincubation of PRP with 50 μM of the derivative for 60 minutes, the level of aggregation was identical to that in untreated PRP (Figure 4).

Fig. 4. Maximal ratio of ADP-induced platelet aggregation after preincubation of PRP with 50 μM of studied quinoid thiosulfonate derivative for 0, 2, 3, 5, 20, 40, 60 min (M±m, n=5)

* – P<0.05 vs untreated PRP

Our results suggest that test compound at the concentration 100 μM could also effectively disaggregate the preformed platelet aggregate caused by ADP as the inducer. As shown in Figure 5 where PRP was incubated with ADP for 90 seconds, as soon as studied compound was added into the mixture, disaggregation occurred rapidly and profoundly. In contrast, the addition of lower concentration of tested derivative of thiosulfonate (50 μM) as well as vehicle 1% DMSO did not affect the aggregate (Figure 5).
Platelet aggregation is a result of complex signal transduction cascade reactions brought about by stimulants. One of the components in the cascade is ADP that is an important mediator of platelet aggregation and activation. ADP elicits its effects on the platelet through the membrane bound P2Y1 and P2Y12 receptors [14]. The P2Y1 receptor is coupled to Gq which regulates phospholipase C and intracellular Ca2+ mobilization and leads to aggregation and shape change. P2Y12 is coupled to Gi and leads to subsequent inhibition of adenylate cyclase and regulation of phosphoinositide 3-kinase. The importance of ADP in the process of thrombus formation has been demonstrated both by antiplatelet drugs that target the ADP receptors [14] and by patients with dysfunctional P2Y1/P2Y12 receptors [15]. ADP receptors may be important therapeutic targets. Moreover ADP receptor antagonists are in wide clinical use [11-13, 16-18]. However, several limitations of known synthetic ADP receptors antagonist have recently been discussed including inter-patient variability in antiplatelet effects and a relatively slow onset of action [19-21]. So, additional studies are needed and new selective platelet inhibitors with increased anti-thrombotic efficiency and safety profile must be developed. The results reported here indicate that S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl) 4-aminobenzensulfonothioate can inhibit ADP-induced effects on platelet aggregation. But detailed studies are needed and new selective platelet inhibitors including inter-patient variability in antiplatelet effects and a relatively slow onset of action [19-21].

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References


ВЛИЯНИЕ НОВОГО ТИОСУЛЬФАНОВОГО ПРОИЗВОДНОГО ХИНОНА НА АДФ-ИНДУЦИРОВАННУЮ АГРЕГАЦИЮ ТРОМБОЦИТОВ

Авторами обнаружено, что S(1,4-диметилокс-9,10-диоксо-9,10-дихиноантрацен-2-илметили) 4-аминоцензисульфонат (дофамин) способствует влиянию антитромбоцитарную активность. Для выяснения механизма его антагонического действия, проведена серия дополнительных экспериментов. Установлено, что соединение индуцирует АДФ-индуктуемую агрегацию тромбоцитов. Индуцирующий эффект зависит от концентрации препарата и времени преконхуления. Производное также оказывает дисагрегационное действие на агрегацию тромбоцитов предшественником формированной в результате АДФ-стимуляции.

Ключевые слова: общехимическая агрегация плазмы, тиосульфоновы производные хиноны, АДФ-индуктуемая агрегация тромбоцитов, антитромбоцитарные средства.

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EFFECTS OF NOVEL QUINOID THIOSULFONATE DERIVATIVE ON ADP-INDUCED PLATELET AGGREGATION

Recently, in a large scale screening test, we have found that S(1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methylb4-aminothiophenolsulfonate, a chemically synthesized thiosulfonate derivative of quinone, possessed an antiplatelet activity. To elucidate the mechanism of its antiplatelet action, a series of experiments were performed. The compound was found to inhibit the ADP-induced platelet aggregation. The inhibitory effect was dose-dependent on concentration and preincubation time. The derivative also disaggregated the preformed platelet aggregates induced by ADP.

Key words: platelet rich plasma, quinoid thiosulfonate derivative, ADP-induced platelet aggregation, antiplatelet agents.

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ВПЛИВ МЕЛАТОНІНУ НА ГІПОТАЛАМО-АДРЕНАЛОВУ СИСТЕМУ ПТАХІВ: ЕФЕКТ БЛОКАДИ ДОФАМІНОВИХ D1-РЕЦЕПТОРІВ

Показано, що ввечері та вдень (але не вночі та вранці) дофамін через D1-рецептори може опосередковувати вплив мелатоніну на синергію надгіпофізо-адренодрінінового комплексу птахів. Також дофамін через D1-рецептори може бути залучений до раптового здиктуння втрачення функції гіпофіз-адреналової системи птахів у відповідь на стрес. Було вивчено 16 експериментальних груп по птахів у кожної групи. Ім'я у відповідний час доби одночасно добували: 1.) фізіологічний розчин відомих (7:00) (контрольна група); 2.) мелатонін в дозі (7:00) в дозі 10 мкг (тут і далі всі дози вказані з розрахунку на 100 г маси тіла); 3.) R(+)SCH 23390 гідроклорид (блокатор дофамінових D1-рецепторів) в дозі (6:00) в дозі 8 мкг; 4.) R(+)SCH 23390 гідроклорид в дозі 6 мкг в дозі 8 мкг, а через годину (7:00) в дозі 10 мкг; 5.) фізіологічний розчин відомих (7:00) (контрольна група); 6.) мелатонін в дозі 10 мкг (7:00) (контрольна група); 7.) R(+)SCH 23390 гідроклорид в дозі 12 мкг (7:00) в дозі 12 мкг; 8.) R(+)SCH 23390 гідроклорид в дозі 12 мкг (в дозі 8 мкг, а через годину (13:00) мелатонін в дозі 10 мкг; 9.) фізіологічний розчин відомих (19:00) (контрольна група); 10.) мелатонін в дозі 10 мкг (19:00) в дозі 10 мкг; 11.) R(+)SCH 23390 гідроклорид в дозі 18 мкг (19:00) в дозі 18 мкг; 12.) R(+)SCH 23390 гідроклорид в дозі 18 мкг (19:00) в дозі 18 мкг; 13.) фізіологічний розчин відомих (1:00) (контрольна група); 14.) мелатонін в дозі 10 мкг; 15.) R(+)SCH 23390 гідроклорид в дозі 18 мкг (в дозі 8 мкг, а через годину (0:00) мелатонін у дозі 10 мкг; 16.) R(+)SCH 23390 гідроклорид в дозі 18 мкг (в дозі 8 мкг, а через годину (1:00) мелатонін у дозі 10 мкг. Фізіологічний розчин вводили перорально в кількості 0,2 мл на птаха. R(+)SCH 23390 гідроклорид розчиняли у фізіологічному розчині і вводили за допомогою стеретаксичного приладу в порожнину третього шлуночка мозку. Мелатонін замішували у борошняному комбікормового заводу, вода – ad libitum, температура – +22-23°С. Світло-тінь: 14 годин – світло (з 7 до 21 години), 10 годин – темрява (з 21 до 7 години).

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