Volkovoy V. A., Sevrukov O. V., Bereznykova M. E., Reshetniyk N. V., Fomina G. P., Karabut L. V. Investigation antiradical and antioxidative action dezapur in experements in vitro. Journal of Education, Health and Sport. 2016;6(6):291-299. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.55501
http://ojs.ukw.edu.pl/index.php/johs/article/view/3579

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 755 (23.12.2015).
755 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 05.05.2016. Revised 25.05.2016. Accepted: 10.06.2016.

UDC 615.275.4:616-36-002:616.127

INVESTIGATION ANTIRADICAL AND ANTIOXIDATIVE ACTION DEZAPUR IN EXPEREMENTS IN VITRO

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Abstract

This article presents the results of a study of antiradical and antioxidative action a new chemical compound 1-fenetil-5,7-dihydro-1H-pirolo-[2,3-d]pyrimidine-2,4,6-trione (code name dezapur). Antiradical activity was investigated on model of interaction dezapur with a stable radical dyphenilpykrylgydrazyn (DPPH) by used method deoxyribose and conversion reaction of adrenalin in adrenochrom. The results showed that dezapur in 10^{-5} M concentration after 10 min reduces the concentration of DPPH on 25,7%, and after 120 minutes on 64,7%. The reference-compound mexidol within 12 hours did not reduce the concentration of DPPH.

Antioxidative activity of dezapur was determined in a model system for the egg yolk lipoprotein inhibition of accumulation of products which react with 2-thiobarbituric acid (TBA-active products) as well as indicators of Fe^{2+}-biochemiluminescence initiated. The results suggest that concentrations in the range dezapur 0,2-100,0 mkg/ml exhibits antioxidant properties inhibiting the formation of TBA-active products on 72,6-97,9%. The antioxidative activity determined by parameters of biochemiluminescence, dezapur exceed mexidol, reducing the area ratio of light sum reaction BCL in a wide range of concentrations.

Studies in vitro on several models showed that dezapur has a high antiradical and moderate antioxidative activity.

Key words: antiradical and antioxidative action, spectrophotometry, chemiluminescence (BCL), dezapur, mexidol.

ИССЛЕДОВАНИЕ АНТИРАДИКАЛЬНОГО И АНТИОКИСЛИТЕЛЬНОГО ДЕЙСТВИЯ ДЕЗАПУРА В ОПЫТАХ IN VITRO

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Реферат

В данной работе приведены результаты исследования антирадикального и антиокислительного действия нового химического соединения 1-фенетил-5,7-дигидро-1Н-пироло-[2,3-d]пиримидин-2,4,6-трион (условное название дезапур).

Антирадикальную активность исследовали на модели взаимодействия дезапура со стабильным радикалом дифенилпикрилгидразином (ДФПГ) используя дезоксирибозный метод и реакцию превращения адреналина в адренохром. Результаты исследований показали, что дезапур в концентрации 10^{-5} М уже через 10 мин снижает концентрацию ДФПГ на 25,7%, а через 120 мин на 64,7%. Препарат сравнения мексидол на протяжении 12 часов не снижал концентрацию ДФПГ.

Антиокислительную активность дезапура определяли в модельной системе липопротеинов яичного желтка за угнетением накопления продуктов, которые реагируют с 2-тиобарбитуровой кислотой (ТБК-активные продукты) а также за показателями Fe^{2+}-инициированной биохемилюминесценции. Полученные результаты свидетельствуют о том, что дезапур в диапазоне концентраций 0,2-100,0 мкг/мл проявляет антиокислительные свойства угнетая образование ТБК-активных продуктов на 72,6-97,9 %. За антиокислительной активностью, определенной по параметрам биохемилюминесценции, дезапур превышал мексидол, уменьшая показатель площади светосуммы реакции БХЛ в более широком диапазоне концентраций.

Проведенные исследования in vitro на нескольких моделях показали наличие у дезапура высокой антирадикальной и умеренной антиокислительной активности.

Ключевые слова: антирадикальное и антиокислительное действие, спектрофотометрия, биохемилюминисценция (БХЛ), дезапур, мексидол.

ДОСЛІДЖЕННЯ АНТИРАДИКАЛЬНОЇ І АНТИОКИСНЮВАЛЬНОЇ ДІЇ ДЕЗАПУРА В ДОСЛІДАХ IN VITRO

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Реферат В даній роботі приведені результати дослідження антирадикальної і антиокиснювальної дії нової хімічної сполуки 1-фенетил-5,7-дигідро-1н-піроло-[2,3-d]піримідин-2,4,6-трион (умовна назва дезапур).

Антирадикальну активність досліджували на моделі взаємодії дезапура зі стабільним радикалом дифенилпікрилгідразином (ДФПГ), використовуючи дезоксирибозний метод і реакцію перетворення адреналіну в адренохром. Результати досліджень показали, що дезапур в концентрації 10^{-5} М вже через 10 хв знижує концентрацію ДФПГ на 25,7%, а через 120 хв на 64,7%, препарат порівняння мексидол на протязі 12 годин не знижував концентрацію ДФПГ.

Антиокиснювальну активність дезапуру досліджували у модельній системі ліпопротеїнів яєчного жовтка за пригніченням накопичення продуктів, які реагують з 2-тіобарбітуровою кислотою (ТБК-активні продукти), а також за показниками Fe^{2+}-ініційованої біохемілюмінісценції. Одержані результати свідчать про те, що дезапур в діапазоні концентрацій 0,2-100,0 мкг/мл проявляє антиокиснювальні властивості пригнічуючи утворення ТБК-активних продуктів на 72,6-97,9 %. За антиокиснювальною активністю, визначеною за параметрами біохемілюмінесценції, дезапур перевершував мексидол, зменшуючи показник площі світлосуми реакції БХЛ, в більш широкому діапазоні концентрацій.

Проведені дослідження in vitro на декількох моделях показали наявність у дезапура високої антирадикальної і помірної антиокиснювальної активності.

Ключові слова: анти радикальна та антиокиснювальна дія, спектрофотометрія, біохемілюмінісценція (БХЛ), дезапур, мексидол.

Introduction. One of the major causes of development human's disease is inflammation. For the treatment of inflammatory process using anti-inflammatory drugs that are with antiexudative activity should exercise antioxidative effect [1, 2]. To evaluate the antiradical and antioxidant action dezapur (code name for a new chemical compound 1-fenetil-5,7-dihydro-1H-pirolo-[2,3-d]pyrimidine-2,4,6-trione) in vitro using classic methods

are spectrophotometry and biochemiluminescence [3].

The aim of our work – the investigate of antioxidative and antiradical activity of the new compounds 1-fenetil-5,7-dihydro-1H-pirolo-[2,3-d]pyrimidine-2,4,6-trione.

Material and methods. Antiradical activity was investigated on model of interaction dezapur with a stable radical dyphenilpykrylgydrazyn (DPPH). The ability to «capture» the hydroxyl and superoxide radicals dezoxyribose is determined by the reaction and the conversion of adrenalin on adrenochrom [3, 4].

Antioxidative activity of dezapur was determined in a model system for the egg yolk lipoprotein inhibition of accumulation of products which react with 2-thiobarbituric acid (TBA-active products), as well as indicators of Fe^{2+}-biochemiluminescence initiated [5, 6].

Also, besides the effect of the compound was determined by the rate of accumulation of TBA-active products in isolation membranes of rat liver microsomal fraction in a spontaneous and induced lipid peroxidation (LPO) [7, 8].

Experiments conducted on white non-linear rats mass 170,0 – 200,0 g. All animals were divided into 3 groups (6 animals in every group): 1 group – intact animals which were prepared distillate water in volume 1 ml; 2 group – animals which administrated the investigative compound – dezapur in effective dose 10 mg/kg; 3 group - animals which administrated the reference-compound – mexidol in effective dose 100 mg/kg [9, 10].

Results and discussion. The obtained results of interaction the radical forms of DPPH with dezapur and mexidol given in table 1. These dates showed that dezapur interaction with DPPH reaction takes place very soon.

Table 1

Indicators absorbance alcohol solutions stable radical DPPH (D^0_{520}), DPPH + dezapur (D^t_{520}) and DPPH + mexidol (D^t_{520}), t = 25^0 (M ± m)

Alcohol solution	Concentration, M	Amount of measurement, n	The optical density for the period of time from the start of reaction (D^0_{520}).				
			1 min	10 min	60 min	120 min	180 min
DPPH	10^{-5}	12	1,006 ±0,003	0,976 ±0,004	0,972 ±0,004	0,970 ±0,003	0,971 ±0,003
DPPH + mexidol	10^{-5}	6	1,005 ±0,003	0,977 ±0,003	0,980 ±0,002	0,982 ±0,003	0,979 ±0,002
DPPH + dezapur	10^{-5}	6	0,864 ±0,003*	0,726 ±0,003*	0,524 ±0,003*	0,342 ±0,003*	0,214 ±0,003*

Note. * - p <0.05 relative to the alcohol DPPH solution without the addition of a physiologically active systems (D^0_{520}).

Thus, dezapur in 10^{-5} M concentration after 10 minutes, the initial concentration of DPPH reduced by nearly 25,7% after 60 min - 46%, after 120 minutes – 64,7%. At the same time, the addition to the alcohol solution of DPPH mexidol equal in concentration over 24 hours of observation is no reduction in the concentration of the radical forms of DPPH.

In this model, antiradical activity dezapur significantly exceeds mexidol activity equal concentrations. The presence of high dezapur antiradical activity, namely the ability to «intercept» superoxide and hydroxyl radicals has been confirmed in other models in vitro.

The effect of dezapur to «catching» of hydroxyl radicals determined by dezoxyribose method. Analysis of the results (table 2) showed that antiradical action of dezapur suppression to accumulation of TBA-active products by 50%, according to a concentration of 0,3 mkg/ml.

Efficiency dezapur to «catching» of superoxide radicals evaluated by their ability to inhibit the conversion reaction of adrenalin to adrenochrom that occurs as a result of the participation of superoxide anions at pH 10,2 [4].

Table 2

Dezapur ability to «capture» the hydroxyl radicals dezoxyribose methods in vitro experiments (M ± m), n=6

Concentration of substances, мkg/ml	Level of inhibition, %		
	Ionol	Manit	Dezapur
0,10	43,6±0,248	39,3±0,445	41,4±0,298*
0,15	54,4±0,294	44,6±0,495	43,6±0,313*
0,20	56,2±0,319	47,9±0,524	46,3±0,358*
0,30	71,6±0,567	53,4±0,556	51,6±0,387*
0,40	79,3±0,628	58,7±0,596	56,8±0,432*
0,60	86,4±0,659	61,3±0,607	59,3±0,458*
0,80	89,1±0,682	67,8±0,629	64,8±0,509*

Note: * - $p < 0.05$ relative to ionol.

The dates of table 3 showed that dezapur in concentration of 5 mkg/ml of the reaction medium inhibits the conversion of adrenalin to adrenochrom on 57,6% and not inferior to reference-compound, which the inhibitory effect is 54,3%.

The dates can be concluded that dezapur can «catching» of superoxide radicals at the level of reference-compound.

Table 3

The ability of dezapur to «cathing» of superoxide radicals in vitro (M±m), n = 6

Concentration of substances, мkg/ml	Level of inhibition, %	
	Reduced glutathione (GSH)	Dezapur
3	39,1±1,4	34,0±1,1
5	51,6±1,1	56,4±1,2
6	69,9±1,2	64,8±1,3
12	86,7±1,4	81,5±1,2
25	89,8±1,5	83,9±1,3
50	92,9±1,6	87,0±1,4
100	98,9±1,8	92,1±1,6

However, in the study of antioxidative activity, which determined by the parameters of Fe^{2+}-initiation biochemiluminescence in the model system of egg yolk lipoprotein. Dezapur and mexidol in the range of concentrations of 10^{-6}-10^{-4}M did not cause to rapid changes of possible light intensity but dezapur exceed mexidol in influence to the rate of the reaction area light sum BCL (tab. 4), and the data obtained in this series of experiments make it possible to conclude that dezapur as mexidol can interact ferrous ions. This in turn leads to a reduction in radical formation in the studied model system. For the antioxidative effect that determined by parameters biochemiluminescence, dezapur exceed mexidol reducing the area ratio light sum reaction BCL in a wide range of concentrations.

Antioxidative activity of dezapur compared to mexidol and ionol also studied on the model of spontaneous and induced lipid peroxidation in isolated rat liver microsomal membranes. In these experiments evaluated the effect dezapur and reference drugs in the concentration range of 10^{-6}-10^{-3} M on the rate of accumulation of TBA-active products in terms uninitiated lipid peroxidation (POL spontaneous) and induction of Fe^{2+} ascorbate (ascorbate lipid peroxidation POL,) and Fe^{2+}-NADFN (NADPH-dependent lipid peroxidation, PHN).

Table 4

Influence dezapur on the level of TBA-active products of Fe^{2+} induced lipid peroxidation in suspension yolk lipoprotein in vitro (M ± m), n=6

Concentration of substances, мkg/ml	Level of inhibition, %	
	Ionol	Dezapur
0,2	74,8±0,229	72,6±0,291
0,4	96,1±0,250	84,2±0,307*
0,8	97,2±0,254	91,4±0,364*
1,0	98,4±0,286	96,8±0,389*
50,0	99,2±0,417	97,9±0,418
100,0	98,3±0,284	97,4±0,407

Note. * - P <0,05 with respect to the ionol.

The rate of accumulation of TBA-active products was calculated in nmol/mg protein•min and expressed as a percentage of the control. The mexidol and dezapur have not effect on the rate of accumulation of TBA-active products and unlike ionol not show antioxidant properties.

The presence of high dezapur antiradical activity can play a positive role in terms of excessive activation of free radical oxidation in vivo, which resulted in the need for such research. The experiments in vitro showed that dezapur has a high antiradical activity and moderate antioxidative effect. He concedes to the antioxidant ionol, but exceeds reference compound – mexidol.

Conclusions:

1. Dezapur showed expression antiradical activity according to the DPPH test and exceeds the antiradical properties of mexidol.

2. Dezapur for data suppression peroxide oxidation of lipoproteins egg yolk that was monitored by the method of Fe^{2+}-induced biochemiluminescence, shows more antioxidant activity than mexidol.

3. The mexidol and dezapur have not effect on the rate of accumulation of TBA-active

products and unlike ionol not show antioxidant properties

4. The experiments in vitro showed that dezapur has a high antiradical activity and moderate antioxidative effect.

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