International Journal of Advanced Biotechnology and Research (IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-7, Issue-3, 2016, pp1111-1114
http://www.bipublication.com

Research Article

Study the effect of compound of phenolic nature on the formation of blood clots using intravital microscopy and fluorescent labels

MV Pokrovsky, MV Korokin, KV Kudryavtsev, TG Pokrovskaya, LV Korokina, YA Hoschenko, GA Batishcheva, TA Denisyuk and AA Shaposhnikov

Belgorod State University,
Medical School. Supervisor MV Pokrovsky

[Received-05/09/2016, Accepted-12/09/2016, Published-27/09/2016]

ABSTRACT

This article presents the results of research of influence of phenolic compounds on the formation of a blood clot by using intravital microscopy and fluorescent labels. Established in varying degrees a marked positive effect on the formation of a blood clot in the test compounds under laboratory code KUD259, KUD970, KUD971, KUD972, KUD973, KUD974, KUD975 and KUD976. Statistically significant positive impact on the process of thrombus formation in the simulation FeCl3 induced microvascular thrombosis observed when using compounds KUD259, KUD974 and KUD975 at a dose of 3 mg / kg, which was reflected in the reduction of the peak size of a blood clot, increasing the time to reach the peak size of the clot. reducing time and steady state thrombus occlusion percent reduction as compared to the animals in which simulated thrombosis without correcting the test compounds.

Keywords: compound of phenolic nature, thrombosis, of FeCl3, vital microscopy, fluorescent labels.

INTRODUCTION

Interconnection endothelial dysfunction and oxidative stress demonstrated in many experimental studies in vitro and in vivo, including clinical studies on humans [2, 3, 4, 7]. Consequently, a rational approach to the development of new drugs with endotelioprotektive action may be the use of organic compounds having antioxidant properties. In addition, the compounds developed should have acceptable physical and chemical properties which are characteristic for the drug compounds - so-called ADMET-profile. Active ingredients with unacceptable ADMET-properties will not be able to muster the necessary physiological effects in vivo. In the present study developed a new class of compounds, some representatives of which had previously been demonstrated antioxidant activity for a model of lipid peroxidation (LPO), and that contain privileged substructure - structural fragment heterocyclic thioacetamide [1, 6, 8].

Search for virtual targets were several available for noncommercial online services. The primary assessment used predictor of activity from the company Molinspiration [9]. Classifier was used, based on these classes of possible active as activity against GPCR-receptors, ion channel modulation, inhibiting kinase activity for nuclear receptors, protease activity and the total enzyme activity. The results of the search target virtual possible to identify phenolic compounds that selectively inhibit arginase II and thrombin. Next was an extensive 100K ckrining to find compounds that are both inhibitors of arginase II and thrombin. Found a number of small molecules with pIC50> 5 [6].

The purpose of this study was to investigate the effect of the synthesized compounds of phenolic nature, containing heteroatomic directly related and heterocyclic structural moieties under laboratory code KUD259, KUD970, KUD971, KUD972, KUD973, KUD974, KUD975 and KUD976 to processes of thrombosis with the use of complex vital microscopy and fluorescent labels.

The study was supported by the Ministry of Education and Science of the Russian Federation (agreement №14.578.21.0012, a unique identifier of the agreement RFMEFI57814X0012).

The study was supported by grant of the President of the Russian Federation № MD-4711.2015.7.

Research Methodology

The experiment was to evaluate the effect of phenolic compounds on the processes of thrombosis with the use of fluorescent labels was conducted on the model of thrombosis branch microvascular m.cremaster male C57BL thrombosis model induced applique solution FeCl3, to conduct the analysis of clinical and functional characteristics of vascular occlusion and study the mechanism of intravital thrombus formation.

To simulate thrombosis branch microvascular m.cremaster animals were anesthetized (chloral hydrate 300 mg / kg), were transferred to controlled breathing, by intubation and connection to the ventilator, jugular vein was cannulated for administration of fluorescent labels, performed surgical release of the fragment microvascular m.cremaster.

Surgery was performed with the use of dissection stereomicroscope Leica M60. 10% FeCl3 solution was applied onto filter paper at a concentration of 0.3-0.8 M and carrying out the application on the prepared portion of the vessel. The duration of application is 5 minutes. [5]

Immediately after completion of the application in the jugular vein were fluorescent markers for marking platelet Rhodamine 6G in a dose of 3 mg per mouse weighing 20-22 grams, DiOC6 (dihexaoxacarbocyanine iodide) at a dose of 3 mg per mouse weighing 20-22 grams.

The study of the formation of a blood clot produced by a complex vital microscopy based microscope Carl Zeiss Examiner Z1 c unit Vivo DSC, Hamamatsu C9300 camera and control units manufactured by 3i, the Netherlands.

The evaluation parameters of clot formation and their changes under the influence of the compounds produced by a computer program SlideBook 5,0.

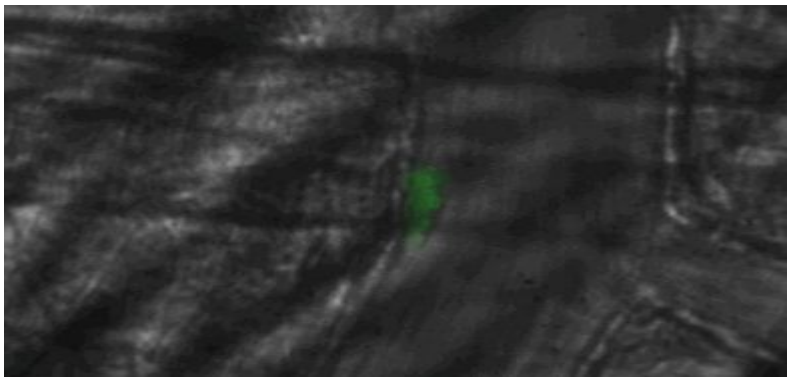

Figure 1. The accumulation of the fluorescent dye at the site of thrombus formation after exposure to a solution of solution of FeCl3. Place the accumulation of the dye highlighted in green, X80

To assess the effect of test compounds on thrombus formation was conducted research peak thrombus size, time to maximum size thrombus formation and clot time stable state before the process of regression and recanalization vessel occlusion percentage within 10 minutes after the start of the study.

Significant changes in absolute parameters determined by a difference method of variational statistics with finding the average values of the shifts (M), an error arithmetic mean (± m) and the likelihood of possible errors (p) tables Student. Differences were evaluated as valid when $p < 0,05$. Statistical calculations were

performed using the program Microsoft Excel 7.0.

STUDY RESULTS

The results of studies of the effect of test compounds on thrombus formation was conducted research peak thrombus size, time to maximum size thrombus formation and clot time stable state before the process of regression and recanalization vessel occlusion percentage within 10 minutes after the start of the study are presented in Table 1 and Figure 2.

Table 1: Changes in the peak size of the thrombus, the time before the onset of the peak size of the thrombus and thrombus vrmeni stable state in the modeling of thrombosis induced by FeCl3 solution applique on the background of administration of the compounds KUD259, KUD970, KUD971, KUD972, KUD973, KUD974, KUD975 and KUD976 (M ± m)

Group of animals	Peak size of the thrombus, cond.	The time to peak size of thrombus, seconds.	Time steady state blood clot, seconds
Intact	0	0	0
Model of thrombosis	6855,1±965,2	133,3±6,0	278,0±16,4
KUD-259 (3 mg/kg)	2661,1±596,2*	191,8±9,1*	69,5±6,*8
KUD-970 (3 mg/kg)	6862,1±827,8	136,8±5,8	119,7±11,0*
KUD-971 (3 mg/kg)	7113,5±652,6	126,2±6,0	125,7±5,5*
KUD-972 (3 mg/kg)	7500,4±485,5	128,5±4,4	144,3±13,0*
KUD-973 (3 mg/kg)	6391,9±834,7	157,0±5,2*	90,8±6,8*
KUD-974 (3 mg/kg)	3596,5±668,2*	183,0±10,3*	90,4±9,2*
KUD-975 (3 mg/kg)	2356,2±259,2*	172,9±9,1*	83,1±11,7*
KUD-976 (3 mg/kg)	8951,2±672,7	134,5±5,6	86,4±8,0*

Note: * - p <0,05 - in comparison with a group of animals, "Model of Thrombosis"

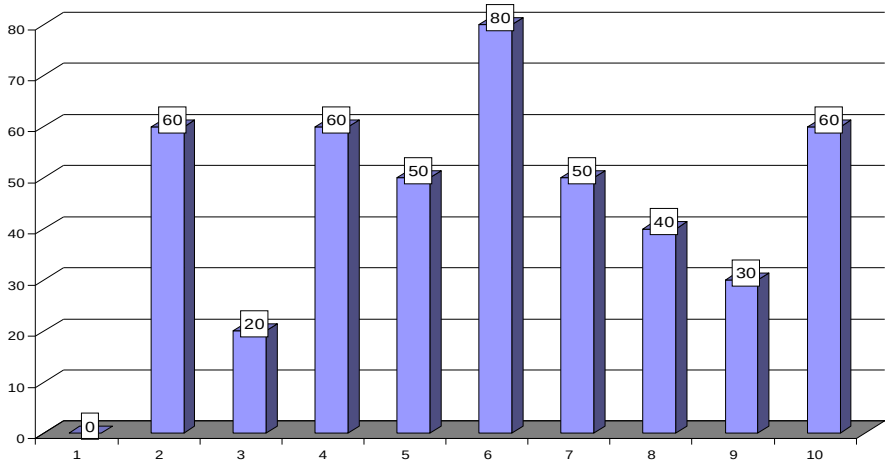

Figure 2. Percentage of vascular occlusion of the microvasculature in the modeling of thrombosis induced by FeCl3 solution applique on the background of administration of the compounds of of phenolic nature (s)

Note: 1- Intact; 2 - model of thrombosis; 3 - KUD259; 4 -KUD970; 5 - KUD971; 6 - KUD972; 7 - KUD973; 8 - KUD974; 9 - KUD975; 10 - KUD976; * - P <0,05 - in comparison with a group of animals "model of thrombosis" The results of previous in vitro studies we confirmed that the obtained compound have nanomolar inhibitory activity against thrombin and arginase II. Thus, the present study shows that the resulting computer

modeling and high skrinininga library phenolic active compounds hits have a pronounced effect on the formation of blood clots in an experiment in vivo. The study of influence of phenolic compounds on the formation of a blood clot on the chosen model of pathology using intravital microscopy and fluorescent labels have established in various degrees of positive impact on the formation of a blood clot in the test compounds under laboratory code KUD259, KUD970, KUD971, KUD972, KUD973, KUD974, KUD975 and KUD976. A statistically significant positive impact on the process of thrombus formation in the simulation FeCl3 induced microvascular thrombosis found for the compounds KUD259, KUD974 and KUD975 at a dose of 3 mg / kg, which was reflected in the reduction of the peak size of the clot, increasing the time to reach the peak size of the clot. reducing time and steady state thrombus occlusion of the vessel percent decrease compared to the animals in which simulated thrombosis without correcting the test compounds. The most active compound in this study should recognize the connection to the laboratory code KUD259

LITERATURE

1. Pat. RU2473540 Russian Federation, IPC C07S 323/18; A61K 31/095; A61P 39/06. Amides of 2- (2-hydroxyphenylthio) acetic acid derivatives having antioxidant activity, and their method of preparation. Published on 01/27/2013. Bull. No. 3.
2. Endothelio- and cardioprotective effects of vitamin B6 and folic acid in modelling methionine-induced hyperhomocysteinemia / Provotorov V.Y., Korokin M.V., Pokrovskii M.V. et all // Research result: pharmacology and clinical pharmacology. – 2016, - V.2, - № 1(2), - P. 16-20
3. Endothelium and cardioprotective effects of HMG-Co-A-reductase in combination with L-arginine in endothelial dysfunction modeling / Denisyuk T.A., Lazareva G.A., Provotorov V.Y., Shaposhnikov A.A // Research result: pharmacology and clinical pharmacology. – 2016, - V.2, - № 1(2), - P. 4-9
4. Higashi Y., Maruhashi T., Noma K., Kihara Y. Oxidative stress and endothelial dysfunction: Clinical evidence and therapeutic implications // *Trends Cardiovasc. Med.* – 2014. – V. 24. – P. 165–169.
5. Critical Review of Mouse Models of Venous Thrombosis/ Jose Antionio Diaz et al. Arterioscler Thromb Vasc Biol. 2012 Mar; 32(3): 556–562. doi: 10.1161/ATVBAHA.111.244608
6. Synthesis of novel bridged dinitrogen heterocycles and their evaluation as potential fragments for the design of biologically active compounds / Kudryavtsev Konstantin V. et all. Tetrahedron, Pergamon Press Ltd. – 2014, - V. 70, - № 43, DOI 7854-7864.
7. Arginase inhibitor in the pharmacological correction of endothelial dysfunction/ Pokrovskiy M.V., Korokin M.V., Pokrovskaya T.G. et al., International journal of hypertension, DOI 515047 (2011).
8. Song Y., Zhan P., Liu X. Heterocycle-thioacetic acid motif: a privileged molecular scaffold with potent, broad-ranging pharmacological activities // *Curr. Pharm. Des.* – 2013. – V. 19. – P. 7141–7154.
9. www.molinspiration.com