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Blood Groups in Toxinology and Reactive Oxygen Species Generation

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Introduction

At present, there are 34 blood group systems recognized by the International Society for Blood Transfusion (ISBT) [6, 17]. Blood group antigens may reflect polymorphisms on red cell glycoproteins or are carbohydrate epitopes (ABO and Lewis) on glycoproteins and glycolipids [2, 17]. Many blood groups reside on proteins critical for red cell maturation and function. Several blood group proteins are clustered at the red cell band 3-ankyrin metabolon (Diego, MNSs, Duffy, Colton, and LW) and junctional complexes (Diego, Gerbich, and MNSs), which anchor the membrane to the underlying cytoskeleton [1]. Interestingly, many of these same proteins are receptors for intraerythrocytic pathogens (malaria, *Bartonella* species, and *Babesia* species), with a loss of red cell deformability upon infection [6]. Other blood groups are associated with membrane microdomains (for example, Pk, P, Cromer, GIL, Colton, and Raph) and play a role in endocytosis, cell signaling, and the immune response. Some systems, such as ABO, have multiple associations with infectious disease [6]. Likewise, many pathogens can utilize or interact with several different blood group antigens. This is particularly true of malaria, which has potential interactions with 8 to 9 different blood group systems [10].

The ABO histo-blood group consists of two antigens (A and B antigens) and four blood types (types A, B, AB, and O). The A and B antigens are the products of the ABO gene and are autosomal codominant. The group O phenotype is an autosomal-recessive phenotype due to the homozygous inheritance of two null ABO alleles. Group O individuals express the H antigen, the biosynthetic precursor to A and B antigens. ABO, therefore, is the blood type, whereas A, B, and H refer to the antigens. The relative distribution of ABO types can vary among different ethnic populations, although group O tends to be the most common [6, 16]. ABO is unique among all human blood

groups by the fact that it requires the typing of both red cells and plasma or serum [5]. In forward of red cell typing, red cells are typed for the expression of A and B antigens on red cells by hemagglutination using monoclonal or polyclonal antibodies. In general, the individual should possess antibodies against any missing A/B antigens. For example, a group O person possesses both anti-A and anti-B, whereas group AB, which expresses both antigens, is negative for ABO antibodies. Clinically, the forward and reverse grouping results must match for a blood type to be determined. There are several known variant ABO alleles that are associated with weak A/B antigen expression, accompanied by elevated H antigen expression. For example, 20% of group A individuals belong to the A2 subgroup, which has only 25% of normal A expression on red cells and virtually no A antigen in platelets, the endothelium, and secretions [7]. ABO is also an oncofetal antigen with altered expression in certain populations. For example, ABO is markedly depressed on newborn red cells due to developmental delays in I blood group gene expression, which is responsible for branching and multivalent ABO expression [4]. In addition, newborns lack ABO antibodies for the first 4 to 6 months of life and achieve adult titers only at 5 to 10 years of age [6, 7]. ABO grouping problems can also occur in patients with cancer, congenital or acquired immunodeficiencies, protein-losing enteropathies, recent transfusion, and other conditions.

ABO blood type influences numerous aspects of biology and medicine, such as susceptibility to infection by various pathogens and potential for complications due to blood transfusions. Infections can stimulate naturally occurring antibodies, sometimes leading to hemolysis and blood grouping problems. Agglutinins, which tend to react to A/B precursors (anti-i, anti-I, anti-H, and anti-HI), are not uncommon following infection with *Mycoplasma*, mononucleosis, and other viral illnesses [5]. In recent studies, it was reported that ABO blood type correlates with survival on prostate cancer vaccine therapy [14]. Particularly, pre-vaccination IgM antibody levels to blood group A trisaccharide significantly correlate with survival of patients treated with PROSTVAC-VF [3]. The importance of immune recognition of an ABO antigen raised the possibility that a patient's blood type may influence their response to the vaccine.

Lastly, blood group antigens can be displayed on many different vaccines. For example, whole cell vaccines derived from human tissue can display blood group antigens depending on the blood type of the original donor. Since immune recognition of these blood group antigens would depend on the blood type of the individual receiving the vaccine, blood type may potentially influence responses and efficacy for other vaccines [21].

Materials and Methods

Materials Packed red blood cells of four ABO groups (RBCs) were obtained from Haematology Center after Prof. R. Yeolyan (Ministry of Health, Republic of Armenia). Snake venoms *Naja kaoutia (NK), Macrovipera lebetina obtusa* (MLO) and *Montivipera raddei* (MR) were purchased from Latoxan (France), and all other chemicals are from Sigma and Roth.

Hemoglobin-free erythrocyte ghosts

Erythrocyte membranes were obtained by the method of Dodge, Mitchell & Hanahan [8, 9]. Protein was measured by the method of Lowry as described (Lowry et al., 1951) using bovine serum albumin as a standard. To obtain erythrocyte ghosts, after the last wash the RBC pellet was mixed with nine volumes of ice-cold lysis buffer (5 mM sodium phosphate) and stirred for 15 min at 0°C. Subsequently, the unsealed erythrocyte ghosts were pelleted by centrifugation at 37 000 × *g* for 10 min at 0°C. After the centrifugation the ghosts were washed with ice-cold lysis buffer until residual hemoglobin was not visible. The RBC ghosts were suspended in about 0.5 volume of PBS and were kept frozen at −30°C until use.

Chemiluminescence analysis and lipid peroxidation

ROS' levels have been measured by chemiluminescence (ChL) analysing system Junior LB 9509 portable tube luminometer (BERTHOLD TECHNOLOGIES, Germany). Lipid peroxides are unstable and decomposed to a complex series of compounds. The most abundant compound is malonic dialdehyde (MDA). MDA level of tissues has been determined by spectrophotometric measurement [20], using the TBA-test, based on the reaction of a chromogenic reagent, thio-barbituric acid (TBA) with MDA at 100°*C* and two molecules of MDA, reacting with one molecule of TBA to yield a stable threemethin complex dye. MDA concentration has been measured at 532 *nm*, using the B01-CT-8 spectrophotometer ("E-ChromTech", Taiwan).

Superoxide dismutase activity

Determination of superoxide dismutase (SOD) activity has been done using method of the adrenaline autoxidation reaction in pH 10.2 [13]. The method is based on the inhibition of adrenochrome formation in epinephrine autoxidation in aqueous alkaline solution (pH>8.5) to yield a chromophore with a maximum absorbance at 480 *nm*, using the B01-CT-8 spectrophotometer. Kinetic measurement of the 480 *nm* absorbance change (adrenokhrom concentration) has been preformed after the addition of adrenalin. SOD activity has

been determined from ratio of the autoxidation rates at the presence and absence of SOD.

Statistical analysis

For quantitative analysis results are reported as means ± SEM. The significance of differences between the means was assessed by ANOVA followed by Bonferroni's test when various experimental groups were compared with the control group. A value of $P < 0.05$ indicated significance.

Results and Discussion

On the first stage of investigation we have studied influence of three different venoms on the lipid peroxidation of the erythrocyte ghosts obtained from the four different Rhesus positive blood groups (Fig. 1). The most interesting phenomenon is that the levels of MDA formation was also noticeably various in control groups. The lipid peroxidation normal level in the blood group O(I) is significantly lower than in all others. The pre-incubation of the EGs with the venom of cobra, the venom of which is not hemolytic does not demonstrate any significant differences depending on the blood groups, while the picture is dramatically different in case of the vipers' venom. The intriguing fact is that for blood group B(III) both viper venoms demonstrated antioxidant properties, while for three others the pre-incubation with MLO and MR venoms increased the levels of lipid peroxidation.

To check whether the overall ROS generation following the same regularities as the lipid peroxidation the ChL analysis has been done with further enhancing by Luminol and hydrogen peroxide (Fig 2). Again we see the big differences of the dynamic levels of ROS generation in the EGs depending of the blood groups: the lowest light generation is in the O group, but the differences of this group from the A(II) and B is not significant, while the spontaneous chemiluminescence in group AB(IV) ghosts is noticeably higher.

The spontaneous chemiluminescence levels are different also in case of different venoms, but all of them work as prooxidants. The pattern of the chemiluminescence in a real-time mode in course of the subsequent additions of the Luminol and then the H_2O_2 is also similar for different groups of blood: fast peak, then some fall of the ROS production during next 10 min, then the higher peak after the H_2O_2 addition. It is interesting to witness that for the overall ROS generation abilities the venoms demonstrate the similar tendencies both in case of the neurotoxic venom of cobra and hemolytic viper venoms. However, for all venoms the observation showed, that in EGs of group B and AB ROS the generation is significantly more intense.

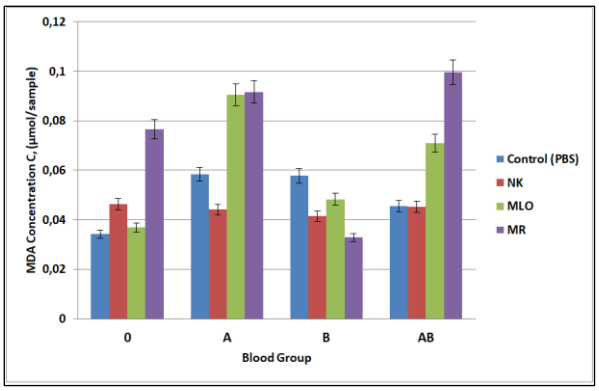

Fig.1. Changes in the concentration of malonic dialdehide of erythrocyte ghosts in the course of *Naja kaoutia*, *Macrovipera lebetina obtusa* and *Montivipera raddei* venom *in vitro* processing.

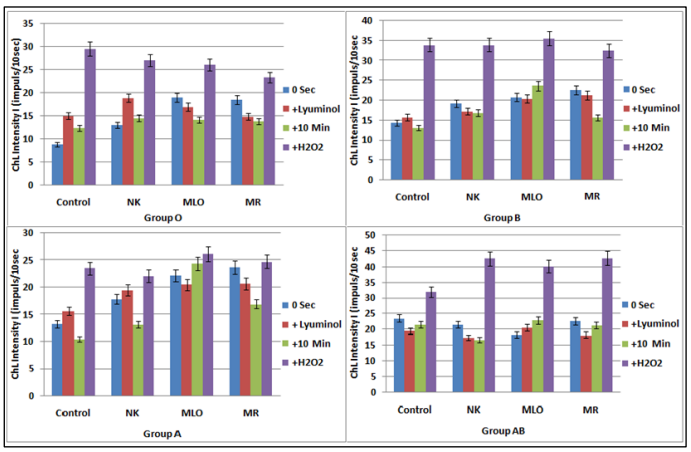

Fig.2. Changes in the spontaneous and enhanced chemiluminescence of erythrocyte ghosts in the course of *Naja kaoutia*, *Macrovipera lebetina obtusa* and *Montivipera raddei* venom *in vitro* processing.

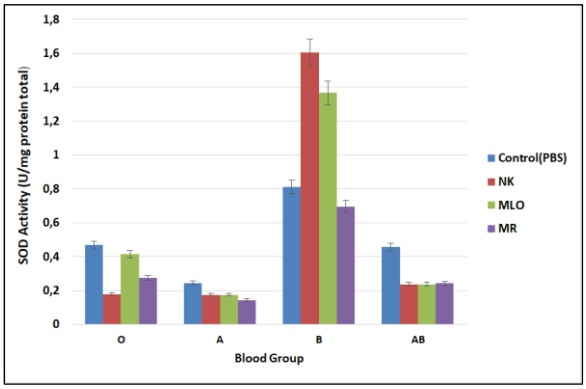

Fig.3. Changes of the superoxide dismutase activities of erythrocyte ghosts in the course of *Naja kaoutia*, *Macrovipera lebetina obtusa* and *Montivipera raddei* venom *in vitro* processing.

During the other series of experiments we also have investigated how NK, MLO and MR venoms changed the activities of the superoxide dismutase, which is one of the main enzymes of the antioxidative defense system of the organism. The addition of venom is remarkably decreasing the activities of the ferment, except of the blood group B case, the reaction of which was unique, but quite significant: for the venom of NK and MLO the intensification of the SOD activities was absolutely drastic, while the MR venom slightly decreased it. Again these differences are also concerning the control assays which means that the levels of SOD activities following the intensities of the ROS generation and lipid peroxidation processes within the erythrocyte membranes are quite different in accordance of the presence or absence of the A or B agglutination factors (antigens) in it.

Viperidae snakebites with their heavy intoxication by the hemolytic venom produce notable morbidity and mortality and have a significant impact on health care. In spite of some new research trends for snakebites envenoming neutralization, antibody technologies still remain the best treatment applied in the field of toxinology.

In Armenia, the majority of snake bites are due to *Macrovipera lebetina obtusa* (MLO) which is a subtype of viper family with venom containing the proteins belonging to few main families: Zn^{2+}- metalloproteinases (PIII and PI), phospholipase A2, serine proteinases, L-amino acid oxidase, disintegrins (short and dimeric), cysteine-rich secretory proteins, Bradykinin-potentiating Peptides and C-type Natriuretic Peptides [15, 18, 19]. According to the data provided by the Ministry of Health of Armenia (MOHA), during the timeframe of 2015-2019, there were recorded 89-146 cases per year of snake bites with a number of mortal cases. Despite the magnitude of this problem, there is no specific antivenom for Armenia. Unfortunately, the epidemiological situation with snakebites in our country is very patchy and scarce, but there is no doubt, that, although the incidence is not so high comparing to the tropical and sub-tropical world, severe envenomations often require antivenom, because of the strong inflammatory and necrotizing properties of this venom and its complicate impact on the blood system and cells of the organism. On the other hand, existing protocols for antivenom treatment of snake envenomations are generally not well optimized due to inadequate knowledge of the toxicokinetics of venoms in patients with different blood types. Our results suggest that both the severity of envenomation and the antivenom efficacy for humans could be defined also by the blood group of the patient.

Previously, we've investigated the membranotropic properties of the endemic viper's venom on the erythrocyte ghosts and also the comparative efficacy of the antivenom products of Uzbekistan and Orbeli Institute of Physiology [11, 12]. Current study describes in detail the specificity of *Macrovipera lebetina obtusa* venom on the human red blood cells with respect to the ABO groups and compared to the venom of other endemic viper

Montivipera raddei and Monocled cobra, the venom of which is neurotoxic and definitely should have less impact on the blood cells.

In addition to the fundamental importance of the present, our generated data may have biomedical applications in the healing of the damage of red blood cells in course of intoxication that lead to various pathologies and even to death. Developments of the necessary theories, in particular, a theory of membrane binding and ROS generation in accordance with the ABO system differences in course of intoxication, represent the perspective of this work and could be developed further for other pathological conditions and/or vaccine treatments.

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Роль группы крови в токсинологии и генерации реактивных форм кислорода

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Антигены групп крови, являясь полиморфными признаками, передающимися по наследству в популяциях, – частые цели в эпидемиологических исследованиях, в силу своей генетической детерминированности и известной полиморфной экспрессии у индивидуумов и популяций. Группы крови часто являются рецепторами для токсинов, паразитов и бактерий, способствуя колонизации и внедрению либо блокируя механизмы очистки организма. Антитела АВО можно рассматривать как часть врожденной иммунной системы против части бактериальных патогенов и вирусов, несущих АВО-активные антигены. Существующие протоколы лечения противоядиями и вакцинами недостаточно оптимизированы из-за плохого знания токсикокинетики ядов у пациентов с разными группами крови. Наши результаты предполагают, что тяжесть отравления, как и эффективность противоядия также могут определяться группой крови пациента. Настоящий проект посвящен влиянию ядов различных змей на эритроциты человека в зависимости от группы крови.

Արյան խմբերի դերը թունաբանության և թթվածնի ռեակտիվ տեսակների գեներացիայի ժամանակ

Գ.Վ. Ղուկասյան

Արյան խմբերի հակածինները պոլիմորֆ հատկանիշներ են, որոնք ժառանգում են անհատները կամ պոպուլյացիաները: Արյան խմբերը հաճախակի թիրախ են համաճարակաբանական հետազոտություններում, քանի որ գենետիկորեն որոշված հատկանիշներ են՝

հայտնի պոլիմորֆ էքսպրեսիայով: Դրանք տոքսինների, մակաբույծների և մանրէների ընկալիչներ են և կարող են նպաստել գաղութացմանը կամ ներխուժմանը, նաև խոչընդոտել օրգանիզմի ինքնամաքրումը: ABO-հակամարմինները կարելի է համարել բնածին իմունային համակարգի մաս որոշ բակտերիալ պաթոգենների, տոքսինների և ABO-հակածիններ կրող վիրուսների դեմ պայքարում:

Հակաթույներով և պատվաստանյութերով բուժման առկա մեթոդական ցուցումները բավարար օպտիմալացված չեն՝ մասամբ արյան տարբեր խմբեր ունեցող հիվանդների՝ տոքսիկոկինետիկայի թերի իմացության պատճառով: Մեր արդյունքները վկայում են, որ թե՛ թունավորման ծանրությունը, թե՛ հակաթույնի արդյունավետությունը կարող են պայմանավորված լինել նաև հիվանդի արյան խմբով:

Հետազոտության նպատակն է ուսումնասիրել տարատեսակ օձերի թույնի ազդեցությունը ABO-խմբերի էրիթրոցիտների վրա:

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