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MEDICINAL PLANTS

OPTIMIZATION OF THE CONDITIONS FOR ASSAY OF THE ANTI-COMPLEMENT ACTIVITY OF HUMAN IMMUNOGLOBULIN PREPARATIONS FOR INTRAVENOUS ADMINISTRATION

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Anti-complement activity is a measure of the specific safety of human immunoglobulins characterizing one of the unwanted effect - spontaneous activation of the complement system. In compliance with the specifications of the European Pharmacopeia and WHO recommendations, the anti-complement activity of human immunoglobulin preparations must not be greater than 50%, which corresponds to a maximum of 1 CH_{50}/mg of immunoglobulin. The measurement method was based on the ability of human immunoglobulins to produce "non-specific" (in the absence of an antigen-antibody complex) binding of complement, preventing lysis of sensitized sheep red blood cells. We present here the results of analysis of the critical parameters of the method for measuring anti-complement activity, along with assessment of existing methodological approaches to its optimization. We report experimental studies of the effects of components of the hemolytic system and complement in guinea pigs on the results of measurements of anti-complement activity. The optimum conditions for the method of measuring the anti-complement activity of human immunoglobulins are based in evidence and the anti-complement properties of human immunoglobulin preparations for intravenous use of Russian origin are evaluated.

Keywords: human immunoglobulin, anti-complement activity, specific safety, standard immunoglobulin.

Human immunoglobulin preparations for intravenous use (IGIV) have a stringent requirement for specific safety, one parameter of which is its anti-complement activity [1]. According to the specifications of the European Pharmacopeia (Eur. Ph.) and the recommendations of the World Health Organization (WHO), the anti-complement activity (ACA) of IGIV preparations must not be greater than 50%, which corresponds to a maximum of 1 CH_{50}/mg of IgG [2, 3]. This level of ACA prevents patients from developing potential adverse reactions associated with effects on the complement system and supports the therapeutic efficacy of IGIV preparations [4]. The need to obtain significant values of ACA for IGIV preparations, allowing clinical results to be predicted, makes the present study relevant.

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The aim of the present work was to optimize the conditions for measuring the anti-complement activity of human immunoglobulin preparations for intravenous use.

EXPERIMENTAL SECTION

Guinea pig hemolytic sera and complement were obtained from Siemens, Germany, ZAO ÉKOlab, and FGUP NPO Mikrogen, Russian Ministry of Health; pooled (in-house) guinea pig complement [3] was used; experiments used normal human immunoglobulin with protein contents of 50 mg/ml in therapeutic formulations: lyophilisate for preparation of solutions for intravenous administration; solution for intravenous administration; solution for infusions, and standard human immunoglobulin BRP (Biological Reference Preparation) batch 1 (Y0001504).

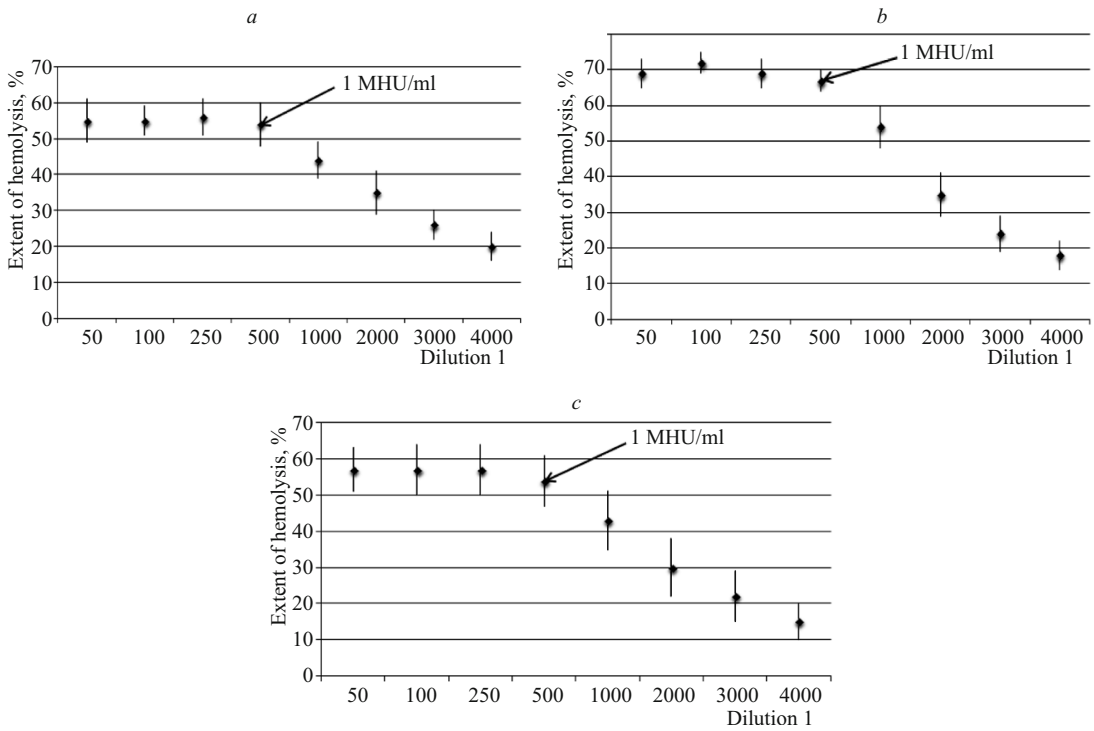

Fig. 1. Results of assays of 1 MHU/ml hemolytic serum from: *a*) Siemens, Germany; *b*) ZAO ÉKOlab, Russia; *c*) FGUP NPO Mikrogen, Russian Ministry of Health.

ACA was measured as described in the Eur. Ph. (monograph 01/2010:20617) [3] and Methodological Instructions MUK 3.3.2.1063 – 01 [5]. Experimental data were processed using statistical methods in Microsoft Office Excel. Results are presented as mean and standard deviation ($\bar{x} \pm s_x$).

Studies used a PD-303S digital spectrophotometer (Apel, Japan), a model 5810R multifunction centrifuge (Eppendorf AG, Germany), an Adventurer AR-5120 precision balance (Ohaus, USA); a BD 115 incubator (Binder, Germany), and a model S220 SevenCompact pH meter (Mettler Toledo AG, China).

RESULTS AND DISCUSSION

The method for measuring ACA was based on the ability of human immunoglobulins to bind complement, preventing lysis of sensitized sheep red blood cells. Complement activity was expressed in hemolytic units (CH_{50}). A value of 1 CH_{50} was the quantity of complement which in defined experimental conditions induces the hemolysis of 50% of 5×10^8 optimally sensitized red blood cells. Binding of complement requires the presence of the required concentrations of Ca^{2+} and Mg^{2+} ions in buffer solutions. In addition, CH_{50} values depend on pH and the ionic strength of the system, the red blood cell concentration, and the quantity of antibodies used for sensitization.

An information-analytical search showed that the critical parameters influencing the final results of measurements of the ACA of IGIV preparations include the activity of the hemolytic serum used, the sheep red blood cells, guinea pig complement, the composition of the buffer solution, and its temperature and pH [6, 7]. Variability in these parameters generates the need to use a standard sample, positive and negative controls for which confirm the significance of the results obtained at different ranges of ACA values. Current world practice uses the BRP human immunoglobulin standard, ratified by the European Directorate for the Quality of Medicines, though WHO experts recommend using national standard samples [3].

Assays of ACA in IGIV preparations in Russia currently employ a method [5, 8] not involving use of a standard. The method described in [8], introduced in 1988, does not evaluate whether immunoglobulins meet current international specifications, i.e., interpret ACA as “retention of the activity of 2 hemolytic units in the presence of 10 mg of immunoglobulin protein.” The method described in [5], in contrast to non-Russian pharmacopoeia methods [3, 9], uses barbital in the buffer solution, dried (commercial) complement (Technical conditions TU 9398-016-14237183-2007), and a large reaction volume, and does not calculate ACA as a percentage.

With the aim of optimizing the conditions for determining ACA, the effects of the hemolytic system (the hemolytic serum and the sheep red blood cell suspension) were studied. The method uses a dilution of hemolytic serum containing two minimum hemolytic units per ml (MHU/ml). A value of 1 MHU/ml corresponds to the lowest dilution at which fur-

ther increases in the quantity of hemolytic serum do not produce any significant increase in the extent of hemolysis. Assessment of three batches of hemolytic serum from different manufacturers did not identify any differences in their activity, i.e., 2 MHU/ml was present in a dilution of 1:250 (Fig. 1).

The sheep red blood cell suspension could be prepared from sheep blood defibrinated with sodium citrate. Because of the instability of defibrinated sheep blood, it could be used for only 14 days, after which spontaneous hemolysis started, so there was a preference to use sheep blood defibrinated with sodium citrate.

Studies of the effects of guinea pig complement from different manufacturers, (two commercial and pooled (in-house) complement) showed that the ACA of the BRP human immunoglobulin standard corresponded to the verified values only when pooled (in-house) complement was used (Table 1).

Assessment of the ACA of human immunoglobulin preparations using the method described in [5], as regulated by the normative documentation, provided evidence that of all the samples studied, the greatest ACA was obtained using pooled (in-house) complement (Table 2).

Use of dried guinea pig complement as specified in MUK 3.3.2.1063-01 did not detect significant levels of ACA and cast doubt on the significance of the results obtained for predicting the safety of using preparations. The results of these studies confirm the need to use pooled (in-house) complement in the ACA measurement method.

The use of sodium barbital in the buffer solution is currently restricted by the need for the relevant licensing. Methodological recommendations [8] previously required the use of a gelatin-salt solution. Evidence was obtained for the suitability of using this in the method harmonized in the EU by finding ACA values for the BRP human immunoglobulin standard which corresponded to the verified values (Fig. 2).

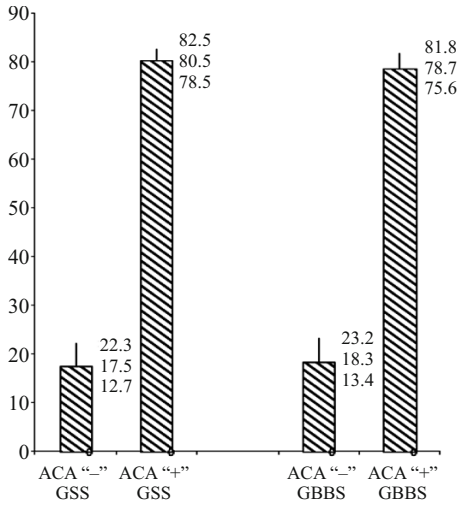

Fig. 2. Anti-complement activity of positive (ACA "+") and negative (ACA "–") controls for BRP human immunoglobulin standard using the gelatin-salt solution (GSS) and gelatin-barbital buffer solution (GBBS).

The studies reported here provided evidence for the optimum conditions for ACA determinations and for developing a general pharmacopeia monograph "Determination of the anti-complement activity of medicinal formulations of human immunoglobulins for intravenous administration" [10]. Studies of the anti-complement properties of IGIV preparations from Russian manufacturers using the optimized

TABLE 1. ACA of BRP Standard Human Immunoglobulin Using Guinea Pig Complement from Different Manufacturers

Reagent/manufacturer	Reagent batch (number of experiments)	ACA of BRP Human immunoglobulin standard, %, $\bar{x} \pm S_x$		Consistency with verified anti-complement activity of BRP human immunoglobulin standard	
		Negative control	Positive control	Negative control (10 – 40%)	Positive control (60 – 100%)
Dry complement/FGUP NPO Mikrogen, Russian Ministry of Health	785 (n = 3)	6.7 ± 1.3	13.2 ± 2.4	no	no
	806 (n = 3)	13.1 ± 1.7	Not determined	yes	no
	811 (n = 3)	10.9 ± 2.1	36.9 ± 7.2	no	no
	814 (n = 3)	11.3 ± 2.9	30.3 ± 6.4	no	no
COMPLEMENT / Siemens	42178 (n = 3)	14.1 ± 3.5	35.2 ± 8.1	yes	no
	42794 (n = 3)	12.9 ± 2.8	41.1 ± 7.6	yes	no
Pooled (in-house) complement	10.04.13 (n = 4)	21.5 ± 5.9	84.7 ± 2.3	yes	yes
	17.04.13 (n = 3)	16.2 ± 4.2	79.5 ± 2.1	yes	yes
	20.08.13 (n = 6)	17.5 ± 4.8	80.5 ± 2.0	yes	yes
	24.10.13 (n = 5)	18.1 ± 5.7	83.5 ± 1.8	yes	yes

Note. Experiments were run using the Eur. Ph. Method.

TABLE 2. ACA of Human Immunoglobulin Preparations for Intravenous Administration

Reagent/manufacturer	Solution for i.v. use		Lyophilisate for preparation of solution for intravenous administration		Solution for infusion	
	CH_{50}/mg protein, $\bar{x} \pm S_x$	%, $\bar{x} \pm S_x$	CH_{50}/mg protein, $\bar{x} \pm S_x$	%, $\bar{x} \pm S_x$	CH_{50}/mg protein, $\bar{x} \pm S_x$	%, $\bar{x} \pm S_x$
Dry complement/FGUP NPO Mikrogen, Russian Ministry of Health	0.06 ± 0.05 (n = 33)	3.3 ± 2.1 (n = 33)	0.1 ± 0.06 (n = 12)	5.1 ± 3.2 (n = 12)	0.44 ± 0.04 (n = 3)	21.9 ± 2.1 (n = 3)
"COMPLEMENT"/Siemens	0.18 ± 0.07 (n = 9)	8.9 ± 3.5 (n = 9)	0.23 ± 0.06 (n = 16)	11.6 ± 3.2 (n = 16)	0.42 ± 0.12 (n = 10)	20.8 ± 5.9 (n = 10)
Complement/Siemens	0.36 ± 0.09 (n = 57)	17.9 ± 4.4 (n = 57)	0.42 ± 0.13 (n = 74)	20.8 ± 6.4 (n = 74)	0.61 ± 0.17 (n = 26)	30.4 ± 8.6 (n = 6)

TABLE 3. Anti-Complement Activity of Human Immunoglobulin Preparations for Intravenous Administration Using the Optimized Method

Human immunoglobulin in medicinal form	Anti-complement activity		Normative specification
	CH_{50}/mg protein, $\bar{x} \pm S_x$	%, $\bar{x} \pm S_x$	
Lyophilisate for preparation of solution for intravenous administration	0.52 ± 0.09 (n = 9)	25.6 ± 4.4 (n = 9)	No more than 1 CH_{50}/mg protein (no greater than 50%)
Solution for intravenous administration	0.37 ± 0.06 (n = 17)	18.4 ± 3.1 (n = 17)	
Solution for infusion	0.57 ± 0.14 (n = 13)	28.6 ± 6.9 (n = 13)	

Notes. N is the number of batches of preparation; ACA of the BRP human immunoglobulin standard ($\bar{x} \pm s_{\bar{x}}$), n = 5: negative control (18.1 ± 5.7)%; positive control (83.5 ± 1.8)%.

method confirmed their compliance with international safety and efficacy requirements (Table 3).

Thus, the experiments reported here provide evidential grounds for the optimum conditions for determining ACA in IGIV preparations and developing the general pharmacopeia monograph "Determination of the anti-complement activity of medicinal formulations of human immunoglobulins for intravenous administration" which uses sheep red blood cells defibrinated with sodium citrate, hemolytic serum at the appropriate condition, pooled (in-house) guinea pig complement, and gelatin-salt solution as an alternative to gelatin-barbital buffer solution. Russian-manufactured IGIV preparations were shown to comply with international specification in terms of the ACA level when the optimized method was used.

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