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Molecular structure alteration of IgG increased anticomplementary activity of intravenous immunoglobulin

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AIM: To study the cause of anticomplementary activities (ACA) of intravenous immunoglobulins (IVIG). **METHODS**: ACA were determined by two assays (limit test and $100CH_{50}$ test), distributions of IgG molecular size (polymer, dimer, monomer and fragment) by HPLC, IgG subclasses and IgA contents by radial immunodiffusion, prekallikrein activator (PKA) and kallikrein (KK) activities by chromogenic assay, potency of antibody against hepatitis B surface antigen (Anti-HBs) by enzyme-linked immunosorbent assay (ELISA). **RESULTS**: The two ACA assays showed good correlation. However, ACA levels were not associated with distributions of IgG molecular size, IgG subclasses and IgA contents, PKA and KK activities. After heating incubation, ACA levels increased markedly and Anti-HBs decreased notably, distributions of IgG molecular size remained relatively constant. **CONCLUSION**: Molecular structure alteration of IgG increased spontaneous complement activation of IVIG.

KEY WORDS intravenous immunoglobulins; complement activation; IgG; prekallikrein; kallikrein; polymer

In the past decade an increasing number of human intravenous immunoglobulins (IVIG) have been developed[1]. The availability of sufficient quantities and safe IVIG have established their importance and superiority over intramuscular immunoglobulins for antibody replacement in the immunodeficiency states for the prevention and treatment of diseases[1-3]. The immunomodulatory effects of IVIG on complex immunological network are currently receiving a great attention[4-6]. However, IVIG sometimes produced side effects (dyspnea, fever, flushing, hypotension, and bronchospasm), especially in hypogammaglobulinemic patients. It is proposed that complement activation by IgG aggregates in IVIG, so called anticomplementary activity (ACA), be responsible for these adverse reactions during iv injection[7,8]. But this proposal was controversial[9]. In this paper, the cause of ACA of IVIG was studied.

MATERIALS AND METHODS

IVIG Four different IVIG preparations, Venoglobulin-S (Lot GL2901A, Alpha Therapeutic Co, USA), Sandoglobulin (Lot 291S0089, Sandoz Pharmaceuticals Co, Switzerland), Gamimune-N (Lot 40X118, Cutter Biological Inc, USA), and Sclavo (Lot 140, Sclavo Co, Italy) were within their period of validity for clinical use. All IgG concentrations were adjusted to 50 g L^{-1} with the diluent recommended by the manufacturers.

ACA assays Two ACA assays[8,10]: 1) $100CH_{50}$ test consisted of one volume of $100CH_{50}$ of guinea pig complement, one volume of test material containing IgG 50 g L^{-1}, and three volumes of buffer solution, allowed to react at 37 C for 1 h. The amount of complement was determined. Result of ACA value

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was expressed in units of complement inactivated by per mL of product, CH_{50}/mL. 2) limit test used one volume of two CH_{50} units of guinea pig complement and one volume of sequential dilutions of test material, allowed to react at 37 ℃ for 2 h. After incubation, sensitized sheep red blood cells were added and inhibition of hemolysis was determined. Result of ACA value was expressed in mg of product needed to inactivate per unit of complement, mg/CH_{50}.

HPLC Analytical exclusion chromatography was carried out with a Hewlett Packard HPLC equipped with a variable wavelength spectrophotometer and an automated integrator. A TSK-Gel GSWP Precolumn (7.5 mm × 75 mm) and a TSK-Gel G3000SW column (7.5 mm × 600 mm) were connected in series. The column buffer (NaAc 50 mmol L^{-1} + NaCl 67 mmol L^{-1} + 0.02 % NaN_3, pH 6.6) was pumped at a flow rate of 0.8 mL min^{-1}, and the wavelength was set at 280 nm. For chromatography, IVIG solutions were injected via a 20 μL Loop. All experiments were performed at 20—22 C.

Antibodies determination Anti-HBs was determined by enzyme-linked immunosorbent assay (ELISA) (Ausab Test kit, Abbott, USA) and compared with the WHO standard (1st reference preparation). IgA and IgG subclasses were determined by radial immunodiffusion (Accra Assay Test kit, ICN, USA).

Prekallikrein activator (PKA) and kallikrein (KK) activities determination PKA and KK activities were determined[11] with S-2302 as a synthetic chromogenic substrate (Chromogenix AB, Sweden) and compared the activity with WHO PKA standard (1st international standard).

RESULTS

ACA assays For the limit test, the ACA levels were from 0.81 mg/CH_{50} to 3.25 mg/CH_{50}, the higher the values were, the higher the qualities of products were. The order of quality of ACA appeared to be Sandoglobulin > Sclavo > Gamimune-N > Venoglobulins. For the 100CH_{50} test, the ACA levels were from 8.55 CH_{50}/mL to 43.20 CH_{50}/mL, the higher the values, the lower the qualities. The order of quality of ACA was the same as that of the limit test. A good correlation was found between the two ACA assays ($r = -0.9216$) (Tab 1).

Effect of IgG aggregates on ACA level The IVIG preparations contained 0.07 %— 3.02 % IgG polymer, 0 %— 16.92 % IgG dimer, 82.47 %— 99.54 % IgG monomer, and 0.09 %— 0.68 % IgG split product. There was no relationship between IgG aggregates concentration and ACA level (Tab 1). Moreover, ACA showed no relation with IgG subclasses and IgA contents, PKA and KK activities in IVIG preparations (Tab 2).

Effect of heating on ACA level Venoglobulin-S, Gamimune-N, and Sclavo IVIG samples were heated at 60 ℃ in Capped polypropylene tubes (17 mm × 100 mm). The distributions of IgG polymer, dimer,

Tab 1. Anticomplementary activity (ACA) level and distribution of IgG molecular size in four intravenous immunoglobulin (IVIG) preparations.

IVIG preparation	ACA		IgG			
	Limit test (mg/CH_{50})	100CH_{50} test (CH_{50}/mL)	Polymer (%)	Dimer (%)	Monomer (%)	Fragment (%)
Venoglobulin-S	0.81	43.20	0.22	7.25	92.35	0.18
Gamimune-N	2.20	9.36	0.07	0.00	99.54	0.39
Sclavo	2.98	8.74	3.02	1.88	94.42	0.68
Sandoglobulin	3.25	8.55	0.52	16.92	82.47	0.09

Tab 2. IgG subclasses and IgA contents, prekallikrein activator (PKA) and kallikrein (KK) activities in four intravenous immunoglobulin (IVIG) preparations.

IVIG preparation	IgG_1 (%)	IgG_2 (%)	IgG_3 (%)	IgG_4 (%)	IgA (mg L^{-1})	PKA (IU L^{-1})	KK (IU L^{-1})
Venoglobulin-S	75.8	18.5	4.8	0.9	34	370	1.55
Gamimune-N	63.6	29.2	5.0	2.2	936	260	1.03
Sclavo	57.9	35.7	4.2	2.2	214	1000	1.79
Sandoglobulin	61.9	29.8	5.6	2.8	607	150	1.72

monomer, and fragment remained relatively constant. The ACA levels increased markedly and Anti-HBs concentrations decreased notably (Tab 3).

DISCUSSION

Barandun and his co-workers reported[7] that adverse side reactions caused by iv injection of conventional intramuscular immune globulin were due to immunoglobulin aggregate which had an intrinsic ability to activate complement system by a similar mechanism to antigen-antibody complexes, and that the safety of immune globulins for iv injection could be predicated by *in vitro* measurement of ACA. Up to now, two ACA assays have been taken for IVIG, and little informations are available on comparison of these two assays. In this paper, a good correlation was found between these two ACA assays, contrary to result of Rousell research[8].

The proposal established by Barandun, which has a profound effect on the development of IVIG[8,9], has been controversial in recent years[9]. We analyzed the IgG molecular size distributions of four different IVIG preparations by HPLC. The data showed that all preparations except Sandoglobulin contained relatively high levels of monomeric IgG (>92 %), relatively low amounts (0.09 % — 0.68 %) of IgG split product, and variable quantities of IgG polymer and dimer. Gamimune-N contained 99.54 % IgG monomer levels, because it was formulated at low pH, but its ACA quality was not the best. In contrast, although Sandoglobulin contained the highest IgG aggregates, the units of complement inactivated by this product was the lowest. Compared the IgG distributions with their ACA levels, there was no relationship between these two respects, suggesting that the main cause of ACA might not be the IgG

Tab 3. Effect of heating on intravenous immunoglobulin (IVIG) preparations.

IVIG preparation	Heating (h)	ACA $100CH_{50}$ test (CH_{50}/mL)	Anti-HBs (IU L^{-1})	IgG Polymer (%)	IgG Dimer (%)	IgG Monomer (%)	IgG Fragment (%)
Venoglobulin-S	0	43.2	570	0.22	7.25	92.35	0.18
	6	61.7	390	0.22	8.82	90.82	0.14
Gamimune-N	0	9.3	390	0.07	0.00	99.54	0.39
	6	42.9	130	0.13	0.00	99.57	0.30
Sclavo	0	8.7	500	3.02	1.88	94.42	0.68
	6	40.9	290	3.22	1.66	94.68	0.44

aggregates. Moreover, ACA levels had no relationship with IgG subclasses, PKA and KK. This conclusion was similar to previous reports[12,13]. Although clear correlation between IgA concentrations of IVIG preparations and anaphylactic reactions in patients with antibodies to IgA has been demonstrated, no antibody to IgA presented in our *in vitro* ACA test system, so complement system could not be activated by antibody-antigen complex and ACA levels also showed no association with IgA concentrations.

The concentration of Anti-HBs of IVIG decreased after the heating incubation, suggesting that part of antibody activities disappeared and structure of antibody molecule might have been altered. ACA levels increased markedly after incubation, meanwhile the distribution of IgG polymer, dimer, mononer and fragment remained relatively constant, suggesting that structure alteration of IgG molecule, not IgG aggregates, might play a key role in spontaneous complement activation of IVIG preparations.

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415-41

IgG 分子结构变化增强静注免疫球蛋白抗补体活性

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目的：研究静注免疫球蛋白(IVIG)产生抗补体活性(ACA)的原因. **方法**：用二种方法测定ACA活性，高效液相色谱测定IgG多聚体、双聚体、单体及碎片组成，环状免疫扩散法测定IgG亚类和IgA含量，显色反应测定前激肽释放酶激活因子(PKA)和激肽释放酶(KK)活性. 酶联免疫吸附试验测定针对乙肝表面抗原的特异抗体(Anti-HBs). **结果**：二种ACA方法有相关性，但ACA活性与各种IgG分子组成、IgG亚类和IgA含量、PKA和KK无关. IVIG热培养后ACA活性显著升高，Anti-HBs活性则明显下降，而各种IgG分子组成则基本未变. **结论**：IgG分子结构变化增强IVIG自发抗补体活性.

关键词 静脉注射免疫球蛋白；补体激活；免疫球蛋白G；前激肽释放酶；激肽释放酶；多聚体

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Effect of ginsenosides against anoxic damage of hippocampal neurons in culture[1]

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AIM: To study the protective effect of ginsenosides (Gin) against anoxic injury in brain cells. **METHODS**: On d 12 after plating of the hippocampal cells from newborn rat, the cultures were exposed to anoxic atmosphere (95 % N_2+5 % CO_2) for 4—24 h. The cellular morphology, survival rate, and effluxes of lactate dehydrogenase (LDH) and K^+ from cells were observed. **RESULTS**: After 24-h anoxia, the survival rate of cells was decreased from 92 %±4 % before anoxia to 1.0 %±2.0 %; the effluxes of LDH and K^+ were increased from 2.3±0.6 U L^{-1} and 5.56 ±0.16 mmol L^{-1} before anoxia to 36±5 U L^{-1} and 8.5±0.7 mmol L^{-1}, respectively. In the Gin group exposed anoxia for 24 h, the survival rate of cells was 4 %±4 %; the effluxes of LDH and K^+ were 30±3 U L^{-1} and 7.9±0.8 mmol L^{-1}, respectively. All these changes were lower in Gin group than those of control. **CONCLUSION**: Under anoxia the cultured hippocampal neurons were seriously damaged, which may be protected by Gin.

KEY WORDS ginseng; saponins; anoxia; hippocampus; cultured cells

Ginsenosides (Gin), the main active component of ginseng, potentiated the nerve growth facter-mediated nerve fiber production in organ cultures of chicken embryonic dorsal root ganglia and sympathetic ganglia[1]. The crude saponin fraction of ginseng root had a proliferative effect on neurite extension of primary cultured neurons of the rat cerebral cortex[2]. The Gin has the protective effect against acute cerebral ischemia/reperfusion injury in rats[3]. Our previous data showed that the Gin delayed the onset of irreversible

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