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PROPHYLACTIC AND THERAPEUTIC ANTIVIRAL EFFECT OF HUMAN GAMMA GLOBULIN

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Abstract—The antiviral effects of human gamma globulin, from pools of plasma obtained from 5000–10,000 donors, have been investigated in test systems using mice and tissue culture. In addition to excellent antiviral properties against adeno 3, herpes simplex, rhino and several influenza A2 virus strains a remarkable prophylactic and therapeutic effect was demonstrated against several viruses. For instance, a single intranasal application of gamma globulin to mice was prophylactically effective for about 72 hr against influenza A2 virus. A therapeutic effect could be demonstrated against intracerebral herpes simplex virus infection in these animals 48 hr post infection.

In mice, the route of administration of gamma globulin is of extreme importance for obtaining maximal protective effect.

The use of human gamma globulin as an internal, nontoxic reference antiviral substance in laboratory test systems is suggested.

ALTHOUGH there are numerous reports on the clinical effects of human gamma globulin (γG) on a large number of diseases, there are few published investigations on the effect of this serum protein fraction in animal test systems used in antiviral studies.[1] In our work with antiviral compounds a need was felt for a standard antiviral substance with a reasonably wide spectrum. Since human γG is commercially available and contains antibodies against a large variety of viruses[1–7] this substance seemed a possible candidate. During our subsequent experimental work with γG in mice and tissue cultures it was found that this substance is indeed an acceptable internal reference standard for a variety of viruses, such as influenza, herpes simplex, adeno 3 and rhino viruses.

During these experiments we also found that human γG has a pronounced therapeutic as well as prophylactic effect in mice against both influenza and herpes simplex virus.

MATERIAL AND METHODS

Gamma globulin. Samples from batches of γG, prepared from outdated blood, representing 5000–10,000 blood donors were obtained in a freeze dried state from AB Kabi's (Stockholm) blood fractionation plant. The samples were not quite representative of the composition of commercial γG, which is always a mixture of batches obtained from different sources. Each batch was prepared according to a modified Deutsch procedure[8] of Cohn method VI with additional batch chromatography according to Björling *et al.* (to be published). All solutions of γG used were freshly made in PBS or tissue culture medium (quantitation by weighing). According to immunoelectrophoresis the γG used contained about 80 per cent nonaggregated IgG.

The strains of viruses used were obtained from the National Bacteriological Laboratory, Stockholm, Sweden. Human amnion AV3 cells, were obtained from Flow Laboratories, Irwine, Scotland. Cells were grown in tissue culture glass tubes (12 × 100 mm), in Eagle's salt solution and 5% of calf serum (totally 1·5 ml). Tubes with a homogenous monolayer of cells were infected with virus suspension (0·15 ml) and the virus was allowed to absorb to cells for 1 hr at 33° for adenovirus 3 and rhinovirus type 17 (strain 33342) but 37° for herpes simplex.[9] The tubes were treated with the substance to be tested, dissolved in maintenance medium 24 and 0 hr before infection. The medium containing substance was then changed every second day throughout the experiment. The viral infection dose was chosen so that approximately 100 per cent cytopathogenic effect was obtained in the infected control tubes 5–6 days after infection. Usually four tubes were used for each concentration of substance to be tested. Uninfected and infected controls were also included in each experiment. Amount of viral infection was determined by daily estimation of the percentage of cells showing typical cytopathogenic changes. By plotting time versus the logarithm of percentage of cytopathogenic changes a straight line was usually obtained. Degree of protection was calculated from the equation:

$$\text{percentage protection} = 100\,\frac{(VC - T)}{VC}$$

where VC stands for percentage infected cells in virus control tubes and T per cent infected cells in tubes containing test substance. VC was always close to 100 (95 ± 5).

For the animal experiments male 10–12 g mice (2–3 weeks old) of the NMRI strain were used. Intranasal influenza infection (0·03 ml of virus suspension) of anesthetized (ether) animals was applied. Usually a LD_{90} dose of virus was inoculated. Not less than 10 mice were used for each concentration of substance to be tested. Intracerebral

TABLE 1. ANTIVIRAL EFFECT OF γG AGAINST INFLUENZA A2/STOCKHOLM/10/63

μg γG/ml mixed with virus	% Survivors with γG (*P < 0·05; †P < 0·01)	% Survivors, control group
20	0	7
40	0	7
60	0	7
80	40*	7
100	60†	7
100	60†	0
200	100†	50
200	100†	7
500	100†	50
1000	100†	7
1000	100†	7
1000	100†	13
5000	100†	7
10,000	100†	7

Virus suspension and substance mixed before infection.

TABLE 2. THERAPEUTIC AND PROPHYLACTIC EFFECTS OF γG AGAINST INFLUENZA A2/STOCKHOLM/10/63 IN MICE

Administered dose, mg/mouse (administration route)	Time of administration relative to time of virus infection (hr)	% Survivors in treated group (*P < 0·05; †P < 0·01)	% Survivors in control group
0·5 (i.n.)	−96	10	0
0·5 (i.n.)	−72	33*	0
0·5 (i.n.)	−48	90†	0
0·5 (i.n.)	−48	60†	0
0·5 (i.n.)	−41	90†	0
0·5 (i.n.)	−24	70†	0
0·5 (i.n.)	−24	50†	0
0·5 (i.n.)	−17	80†	0
0·5 (i.n.)	− 6	90†	0
0·5 (i.n.)	− 1	90†	0
0·02 (i.n.)	−0·25	0	7
0·03 (i.n.)	−0·25	70	7
0·04 (i.n.)	−0·25	0	7
0·06 (i.n.)	−0·25	0	7
0·08 (i.n.)	−0·25	30	7
0·10 (i.n.)	−0·25	20	0
0·1 (i.n.)	−0·25	70*	27
0·2 (i.n.)	−0·25	80†	27
0·3 (i.n.)	−0·25	60	27
0·4 (i.n.)	−0·25	90†	27
0·5 (i.n.)	−0·25	70*	27
0·5 (i.n.)	−0·25	80†	20
0·5 (i.n.)	−0·25	80†	0
0·5 (i.n.)	−0·25	100†	30
0·5 (i.n.)	−0·25	70†	13
0·5 (i.n.)	0	100†	7
0·3 (i.n.)	+0·25	10	0
0·5 (i.n.)	+0·25	90†	0
0·5 (i.n.)	+0·25	60†	7
0·5 (i.n.)	+0·25	30	20
0·5 (i.n.)	+0·5	50*	7
0·5 (i.n.)	+ 1	50†	0
0·5 (i.n.)	+ 1	80†	7
0·5 (i.n.)	+ 2	90†	7
0·5 (i.n.)	+ 3	80†	0
0·5 (i.n.)	+ 3	70†	7
0·5 (i.n.)	+ 4	60*	10
0·5 (i.n.)	+ 4	20	7
0·5 (i.n.)	+ 6	40	10
0·5 (i.n.)	+ 6	70†	0
0·5 (i.n.)	+18	45†	0
0·5 (i.n.)	+24	10	0
0·5 (i.p.)	−0·25	0	7
1·0 (i.p.)	−0·25	20	7
1·0 (i.p.)	−0·25	10	7

injection of Herpes simplex virus into mice (8–10 g) was carried out by introducing the syringe about 3 mm above the eye to a depth of about 3 mm. The injected volume was 0·03 ml. The control animals tolerated one such injection of saline above each eye.

In the animal experiments usually 15 infected control mice were used in each experiment. They were treated with the solvent used in the γG test (usually PBS).

RESULTS

Effects against influenza A2/Stockholm/10/63 in mice. γG has been shown to have excellent protective effect in mice against this virus. In titration experiments where γG of different concentrations and virus were mixed before intranasal infection, the number of surviving animals was optimal with a concentration of about 200 μg/ml of γG (Table 1).

Other experiments, where γG and virus was administrated at time intervals (Table 2) revealed three important facts: (a) the route of administration of substance is of extreme importance; it is clearly demonstrated than when intraperitoneally administered, γG is considerably less effective than when given intranasally; (b) γG can be shown to exert a certain therapeutic effect, which can be demonstrated up to about 20 hr post

TABLE 3. EFFECT OF γG ON VARIOUS INFLUENZA A2 STRAINS

Influenza A2 strain	% Survivors in treated group (*P < 0·05; †P < 0·01)	% Survivors in control group
Taiwan 64	90†	10
England 64	100	70
Singapore 1/57 Frankfurt	90†	0
Singapore 1/57 DM	100†	40
Japan 305/57	100†	0

Treatment: 0·5 mg/mouse i.n. 15 min before i.n. infection.

TABLE 4. EFFECT OF γG ON AMNION AV3 CELLS INFECTED WITH HERPES SIMPLEX

Treatment	Time of treatment relative to time of infection (hr)	Concentration of γG (μg/ml)	Protective effect (%)
Prophylactic and therapeutic	See experimental section	5	58* ± 12
		10	80* ± 5
		50	99* ± 1
Therapeutic	+1	50	77† ± 22
	+3	50	90† ± 8
	+5	50	93† ± 7

* Average of two experiments (± max. dev.).
† Average of three experiments (± max. dev.).

infection with the applied doses; (c) the excellent prophylactic effect of γG lasts for at least 48 hr, indicating a strong affinity between γG and epithelial cells.

It is noteworthy that the γG used in these investigations, which was taken from batches prepared during 1969, was very effective against a number of "old" influenza strains (Table 3).

Effects against herpes simplex virus. γG has a pronounced antiviral effect against herpes simplex virus both in tissue culture and in *in vivo* experiments with mice.

TABLE 5. EFFECT OF INTRACEREBRALLY OR INTRAPERITONEALLY ADMINISTERED γG ON MICE INFECTED WITH HERPES SIMPLEX VIRUS

Administered dose (mg/mouse)	Time of administration relative to time of virus infection (hr)	% Survivors in treated group (*P < 0·05; †P < 0·01)	% Survivors in control group
0·5 (i.c.)	−48	30	20
0·5 (i.c.)	−24	80†	20
0·5 (i.c.)	−17	70†	20
0·5 (i.c.)	− 6	70*	20
0·5 (i.c.)	− 6	80*	33
0·5 (i.c.)	− 5	70	33
0·5 (i.c.)	− 4	80*	33
0·5 (i.c.)	− 3	30	33
0·5 (i.c.)	− 2	60	33
0·5 (i.c.)	− 1	90†	33
0·03 (i.c.)	−0·25	0	20
0·15 (i.c.)	−0·25	40	20
0·30 (i.c.)	−0·25	45	20
0·45 (i.c.)	−0·25	70†	0
0·03 (i.c.)	0	40‡	13
0·45 (i.c.)	0	90†‡	0
0·50 (i.c.)	0	50†§	33
0·03 (i.c.)	+0·25	30	20
0·15 (i.c.)	+0·25	50	20
0·30 (i.c.)	+0·25	90†	20
0·45 (i.c.)	+0·25	70†	0
0·5 (i.c.)	+ 1	67	33
0·5 (i.c.)	+ 3	70	33
0·5 (i.c.)	+ 6	90†	33
0·5 (i.c.)	+ 6	33	10
0·5 (i.c.)	+19	60	20
0·5 (i.c.)	+19	90†	33
0·5 (i.c.)	+24	40	20
0·5 (i.c.)	+24	90†	33
0·5 (i.c.)	+36	60	29
0·5 (i.c.)	+48	78*	29
0·5 (i.c.)	+72	50	27
0·5 (i.c.)	+96	25	27
1·0 (i.p.)	−0·25	11	7
2·0 (i.p.)	−0·25	30	7

‡ γG and virus suspension mixed before administration.
§ γG and virus suspension injected separately above each eye.

Complete protection of human amnionic AV3 cells was obtained with a concentration of 50 μg/ml (Table 4). A noticeable therapeutic effect of γG was also observed in these cells. For instance, when infected cells were treated with γG in a concentration of 50 μg/ml 5 hr after infection this concentration was able to protect the cells almost completely from detectable virus growth (Table 5).

For the *in vivo* investigations mice were infected intracerebrally with herpes simplex virus. In some experiments virus and substance were mixed before the animals were infected. In other experiments virus and γG were separately administered to the mice in different areas of the brain. In both series of experiments the protective effect of γG was good. 0·5 mg/mouse was a highly effective dose. As is seen from Table 4 the therapeutic effect of γG could be demonstrated at least as late as 48 hr post infection.

TABLE 6. EFFECT OF γG ON AMNION AV3 CELLS INFECTED WITH RHINOVIRUS 33342 AND ADENOVIRUS 3

Treatment	Time of treatment relative to time of infection (hr)	Concentration of γG (μg/ml)	Virus used	Protective effect (%)
Prophylactic and therapeutic	See experimental section	5	Rhino	33* ± 3
,, ,,	,, ,,	10	,,	42* ± 7
,, ,,	,, ,,	50	,,	89* ± 1
,, ,,	,, ,,	5	Adeno	31† ± 43
,, ,,	,, ,,	10	,,	49† ± 30
,, ,,	,, ,,	50	,,	84† ± 23
Therapeutic	+ 1	50	Adeno	58
,,	+ 3	50	,,	32
,,	+ 5	50	,,	28

* Average of two experiments (± max. dev.).
† Average of three experiments (± max. dev.).

Effects against rhino and adeno viruses. The antiviral effect of γG on rhino and adenoviruses has been investigated in a tissue culture system using human amnion AV3 cells. With both viruses a concentration of 50 μg/ml of γG was sufficient to bring about 80–90 per cent protection of the cells (Table 6). A slight therapeutic effect was noted with adenovirus.

DISCUSSION

Only a few batches of γG were used during these experiments and no attempt was made to look for differences in antibody titres between different batches. However, it seems quite clear from the results of Magnusson and Kallings,[6] who compared titres in ten different batches of γG, and also from the results of Cabasso *et al.*,[4] who tested twenty-three lots, that the variations in titres between individual batches is usually small. It therefore seems that γG is a suitable internal reference antiviral substance, (even when obtained from relatively small donor pools) which can be used with a number of different viruses, for which effective, nontoxic reference substances today are lacking. The prophylactic and therapeutic effects of human γG reported here are intriguing and warrant further studies.

It should be pointed out that the results in this article do not prove that the observed antiviral effect of γG is directly attributable to antibody. For instance, contaminants from serum may contribute. This question is under investigation.

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