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ANTIVIRAL EFFECT OF HUMAN GAMMA GLOBULIN IN MICE. COMPARISON BETWEEN THE EFFICACY OF LOCAL AND SYSTEMIC ADMINISTRATION

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Abstract—In order to obtain a protective antiviral effect of human gamma globulin administered systemically to mice, large amounts of antibody are needed. Mice intranasally infected with influenza virus must receive 2000–3000 mg/kg of gamma globulin intraperitoneally or intravenously in order to survive. However, the same survival rates are obtained with only 50 mg/kg if antibody is given locally (intranasally). The situation is similar with herpes simplex viruses. Fifty mg/kg of gamma globulin has a pronounced effect if administered locally (intracerebrally) but at least 850 mg/kg are needed with intraperitoneal or intravenous treatment.

THE ANTIVIRAL effects of human gamma globulin in mice were studied initially by Heyl *et al.*[1] They measured the amount of neutralizing antibody in various commercial preparations by injecting mixtures of herpes simplex virus and gamma globulin intracerebrally. Local treatment of viral infections produced a high concentration of antibody, at least momentarily, in the infected area.[2] We therefore investigated the possible use of human globulin for local treatment of various virus infections in mice. The results[2] indicated that relatively small amounts of human gamma globulin, administered locally, had a pronounced early as well as late prophylactic effect against both influenza viruses and herpes simplex viruses types 1 and 2.

We have now extended the experiments to include systemic administration of human gamma globulin to mice to compare the efficacy of local and systemic administration of the antibody.

MATERIAL AND METHODS

Gamma globulin. The preparations used were commercially available 16·5 per cent solutions of gamma globulin (AB Kabi). Dilutions of the material, when necessary, were made in PBS immediately before use. The HSV and influenza A2/Stockholm/63 antibody titres in the preparations are reported in Tables 3 and 6.

Strains of viruses. These were obtained from the National Bacteriological Laboratory, Stockholm, Sweden.[2] HSV type 1 and type 2 were passaged intracerebrally in mice. Influenza virus A2/Stockholm/10/63 had been adapted to mice by intranasal infection followed by virus recovery from the lungs. Passages No. 35 and 36 were used.

Animals. Male mice (2–3 weeks old) of the NMRI-strain were used. Anesthesized (ether) animals (10–12 g) were intranasally infected with influenza virus (0·03 ml of virus containing one LD_{100}). Intracerebral injection of HSV into mice (8–10 g) was carried out by introducing the syringe about 3 mm above the eye to a depth of

approximately 3 mm. The injected volume was 0·03 ml containing *ca.* one LD_{90} of virus. The virus suspension was injected above one eye and gamma globulin above the other. Control animals received an injection of saline above each eye. Each test group contained ten animals. For each set of test groups there was one infected control group of fifteen animals, which usually was treated with phosphate-buffered saline.

RESULTS AND DISCUSSION

Effects against influenza A2 virus. Titration experiments revealed that about 0·4 μg of antibody were sufficient to neutralize one LD_{50}-dose of virus (Table 3) when virus and gamma globulin were mixed. An antiviral effect could be demonstrated by systemic administration of large amounts of gamma globulin. The smallest dose that could be shown to have an antiviral effect *in vivo* after intravenous treatment was 8·3 mg/animal (about 700 mg/kg). The smallest effective dose after intraperitoneal administration was in the order of 17 mg/animal (1400 mg/kg). (Tables 1–3.)

TABLE 1. EFFECT OF GAMMA GLOBULIN (16·5 PER CENT SOLUTION) IN MICE INFECTED INTRANASALLY WITH A2/STOCKHOLM/63

Intravenous treatment		
ml(mg)/animal	Time of administration relative to virus infection (hr)	Surviving animals in test group (%) (0% Surviving animals in the control groups)
0·10 (17)	−0·25	55†
0·003 (0·5)	+ 1	0
0·006 (1·0)	+ 1	0
0·01 (1·7)	+ 1	0
0·01 (1·7)	+ 1	30*
0·05 (8·3)	+ 1	40†
0·10 (17)	+ 1	73†
0·10 (17)	+ 1	54†
0·10 (17)	+ 3	40†
0·10 (17)	+ 3	30*
0·10 (17)	+ 6	30*
0·10 (17)	+20	10

* $P < 0·05$.
† $P < 0·01$.

In general, intravenous administration of gamma globulin shortly before (−15 min) or shortly after (+ 1 hr) intranasal infection had little effect on the survival rates. As expected, the efficiency of antibody decreased when the time between infection and treatment was increased. Moderate doses (17 mg/animal) had a protective effect when given about 6 hr after infection (Table 1). Larger doses (34 mg/animal) were effective when given intraperitoneally 48 hr after infection (Table 2). In Table 3 a comparison is made between the efficacy of local and systemic administration.

In order to neutralize most of the infectious virus particles it is evident that treatment at the site of infection requires much less antibody per animal than treatment by

TABLE 2. EFFECT OF GAMMA GLOBULIN (16·5 PER CENT SOLUTION) IN MICE INFECTED INTRANASALLY WITH A2/STOCKHOLM/63

Intraperitoneal treatment		
ml (mg)/animal	Time of administration relative to virus infection (hr)	Surviving animals in test group (%) (0% Surviving animals in the control groups)
0·10 (17)	−0·25	50†
0·20 (34)	−0·25	80†
0·01 (1·7)	+0·25	0
0·05 (8·3)	+0·25	10
0·10 (17)	+0·25	50†
0·20 (34)	+ 1	60†
0·20 (34)	+ 3	70†
0·20 (34)	+ 6	90†
0·01 (1.7)	+20	0
0·05 (8·3)	+20	0
0·05 (8.3)	+20	0
0·10 (17)	+20	30*
0·15 (25)	+20	40†
0·20 (34)	+20	30*
0·20 (34)	+20	40†
0·20 (34)	+24	90†
0·20 (34)	+48	40†
0·20 (34)	+72	10

* $P < 0·05$.
† $P < 0·01$.

TABLE 3. EFFICIENCY OF HUMAN GAMMA GLOBULIN ADMINISTERED BY DIFFERENT ROUTES IN MICE INTRANASALLY INFECTED WITH INFLUENZA A2/STOCKHOLM/63

Mode of administration	Minimum active dose (mg/kg)	Amount of gamma globulin needed to neutralize most virus (16 LD_{50} doses)	
		(mg/kg)	(μg/mouse LD_{50} dose)
At site of infection (gamma globulin and virus mixed)	0·2	0·6	0·4
At site of infection* (gamma globulin administered separately)	8	50	30
Intravenous*	700	≥2000	≥1000
Intraperitoneal*	1400	3300	2000

* Gamma globulin was given separately, but within 1 hr of virus infection.

systemic administration. The high inefficiency of intravenous administration is exemplified by the following. If we assume that an effective dose of heterologous gamma globulin (17 mg/animal) is not rapidly cleared by phagocytosis but is evenly distributed in the blood stream, the resulting concentration will be about 17,000 μg/ml. Only about 6 μg were needed to neutralize the virus present (Table 3).

There are some reports on the local treatment of upper respiratory tract illnesses in man with gamma globulin. Buthala *et al.*[3] have summarized the clinical experiments made up to 1970. These authors found that local therapy can influence the amount of nasal secretion and detectable virus exudates. More recently Fruchtman *et al.*[4] published the results of a double blind controlled study where commercial gamma globulin or placebo was administered by aerosol to volunteers. Depending on the method used to evaluate the protective effect, the gamma globulin group had 44–79 per cent lower incidence of influenza. However, these results were not statistically significant. The quantity used in these studies was of the order of 3 ml (*ca.*500 mg)/day and person. The amount given was thus 1000-times that needed in mice.

It is, however, doubtful whether the model infection of mice with influenza virus is comparable to an upper respiratory tract infection by the same virus in man. The mice develop severe lung consolidation which appears to be responsible for the fatal outcome of the infection. It is possible that intranasal treatment of mice with gamma globulin also involves local treatment of the lungs with antibody, since mice increase their respiration under anesthesia and therefore are likely to inhale part of the antibody into the lungs. The "local" experiments may be regarded as local treatment of the

TABLE 4. EFFECT OF GAMMA GLOBULIN (16·5 PER CENT SOLUTION) IN MICE INFECTED INTRACEREBRALLY WITH HSV 1

Intravenous treatment			
	Time of administration relative to virus infection (hr)	Surviving animals in	
ml(mg)/animal		Test group (%)	Control group (%)
0·10 (17)	− 0·25	73†	7
0·003 (0·5)	+ 1	44	20
0·006 (1·0)	+ 1	30	20
0·01 (1·7)	+ 1	60*	20
0·01 (1·7)	+ 1	80*	30
0·05 (8·3)	+ 1	60*	30
0·05 (8·3)	+ 1	78*	20
0·10 (17)	+ 1	60*	30
0·10 (17)	+ 1	62†	7
0·10 (17)	+ 3	64†	7
0·10 (17)	+ 3	100†	7
0·10 (17)	+ 6	92†	7
0·10 (17)	+ 20	100†	7
0·10 (17)	+ 24	30	7
0·10 (17)	+ 48	27	7
0·10 (17)	+ 72	9	7
0·10 (17)	+106	0	7

* $P < 0{\cdot}05$,
† $P < 0{\cdot}01$.

lungs rather than local treatment of the upper respiratory tract. This possibility needs further investigation.

Effects against herpes simplex virus (HSV) types 1 *and* 2. It was shown earlier[2] in tissue culture experiments (AV3 cells infected with the same amount of virus as in the mice experiments) that about 2 μg of gamma globulin was sufficient to neutralize one LD_{50}-dose of virus. In mice, 3–6 μg of gamma globulin were needed when virus and antibody were mixed before infection (Table 6).

An antiviral effect against HSV 1 and HSV 2 could be demonstrated by systemic administration of large doses of gamma globulin to mice. The smallest effective amount was about the same for both HSV 1 and HSV 2, namely 1·7 mg/animal (170 mg/kg) regardless of whether the gamma globulin was administered intravenously or intraperitoneally (Tables 4–7).

TABLE 5. EFFECT OF GAMMA GLOBULIN (16·5 PER CENT SOLUTION) IN MICE INFECTED INTRACEREBRALLY WITH HSV 1

Intraperitoneal treatment			
	Time of administration relative to virus infection (hr)	Surviving animals in	
ml (mg)/animal		Test group (%)	Control group (%)
0·10 (17)	− 0·25	60*	13
0·20 (34)	− 0·25	80†	13
0·01 (1·7)	+ 0·25	50†	0
0·05 (8·3)	+ 0·25	90†	0
0·10 (17)	+ 0·25	90†	0
0·10 (17)	+ 0·25	60*	13
0·20 (34)	+ 0·25	70†	13
0·20 (34)	+ 1	70†	7
0·20 (34)	+ 2	80†	7
0·20 (34)	+ 3	60*	20
0·20 (34)	+ 3	50*	7
0·20 (34)	+ 4	50*	7
0·20 (34)	+ 5	70†	7
0·20 (34)	+ 6	40*	7
0·01 (1·7)	+20	10	7
0·05 (8·3)	+20	40*	7
0·05 (8·3)	+20	70†	27
0·10 (17)	+20	60†	7
0·15 (25)	+20	60†	7
0·15 (25)	+20	90†	27
0·20 (34)	+20	90†	7
0·20 (34)	+20	70*	20
0·20 (34)	+48	50	20
0·20 (34)	+72	20	20

* $P < 0·05$.
† $P < 0·01$.

TABLE 6. EFFICIENCY OF HUMAN GAMMA GLOBULIN IN MICE INTRACEREBRALLY INFECTED WITH HSV TYPE 1

Mode of administration	Minimum active dose (mg/kg)	Amount of gamma globulin needed to neutralize most virus (5 LD_{50}-doses)	
		(mg/kg)	(μg/mouse LD_{50}-dose)
Tissue culture* (AV 3 cells)			2
At site of infection (gamma globulin and virus mixed)	0·6	1·5–3	3–6
At site of infection* (gamma globulin administered separately)	30	50	100
Intravenous*	170	850	1700
Intraperitoneal*	170	850	1700

*Gamma globulin was given in close connection but separately from virus. ($\pm$1 hr.)

TABLE 7. EFFECT OF GAMMA GLOBULIN (16·5 PER CENT SOLUTION) IN MICE INFECTED INTRACEREBRALLY WITH HSV 2

ml (mg)/animal	Time of administration (hr)	Surviving animals in Test group (%)	Surviving animals in Control group (%)
Intravenous treatment			
0·10 (17)	− 0·25	80†	0
0·10 (17)	+ 1	55†	0
0·10 (17)	+ 3	64†	0
0·10 (17)	+ 6	73†	0
0·10 (17)	+20	73†	0
0·10 (17)	+48	45†	0
Intraperitoneal treatment			
0·2 (34)	+ 1	80†	20
0·2 (34)	+ 2	40	20
0·2 (34)	+ 3	80†	20
0·2 (34)	+ 4	30	20
0·2 (34)	+ 5	60*	20
0·2 (34)	+ 6	60*	20
0·2 (34)	+ 6	80*	40
0·2 (34)	+20	100†	20
0·2 (34)	+24	30	40
0·2 (34)	+48	90*	40
0·2 (34)	+72	20	40

* $P < 0{\cdot}05$.
† $P < 0{\cdot}01$.

The conclusion from these results is that even heterologous antibodies can relatively easily enter the nervous system and exert an antiviral effect. The antiviral effect of gamma globulin administration could be seen until about 20 hr post infection.

When gamma globulin and virus were injected separately into the mouse brain (one injection above each eye) about 100 μg per LD_{50}-dose of virus sufficed to neutralize most virus. This indicates an effective distribution of the antibody in the mouse brain.

In summary these investigations have shown that: (1) local treatment of the virus infections studied is much more efficient than systemic (i.p. or i.v.) treatment; (2) certain human antibodies are able to penetrate from the blood into the nervous system of mice.

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