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Can human polyclonal immunoglobulin raise the threshold for convulsions in rats?

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In the present preliminary study we explored the possibility of an anticonvulsant effect of normal human immunoglobulin in an animal epilepsy model based on direct cortical stimulation in freely moving rats. After human immunoglobulin administration a significant and prolonged elevation of the threshold for convulsions was measured in 12% (6/49) of the total group of outbred Wistar rats. In the subgroup of more than seven months old Wistar rats this was 67% (6/9). When a threshold increasing effect of immunoglobulin occurred, it was detectable within 0.5–1 hour after administration, reached its maximum after approximately two hours and continued for at least 40 hours.

Key words: immunoglobulin; treatment; threshold for seizures; epilepsy; rats.

INTRODUCTION

Normal human immunoglobulin (IVIg) initially used for substitution of immunodeficiency syndromes has recently been reported to be effective in an increasing amount of diseases thought to be produced by immunopathology[1,2]. Although epilepsy is one of the oldest applications of IVIg in neurological diseases[3], and although positive results have been published, IVIg treatment of intractable epilepsies, nevertheless, remains controversial[4]: on the one side the various studies on IVIg in human epilepsy could not provide an indication for an immunological mechanism of action of IVIg[4], on the other side it has been stated without published data that IVIg lacks an anticonvulsant effect[5]. In the present study we explored the possibility of an anticonvulsant effect of IVIg in an animal epilepsy model based on direct cortical stimulation in freely moving rats[6,7]. In the subgroup in which IVIg was ineffective we investigated whether this was due to an inability of IVIg to cross the blood–brain barrier.

MATERIALS AND METHODS

Female (outbred) Wistar rats of 175–200 g were purchased from Harlan/CPB in Zeist, The Netherlands. They were housed individually in plastic cages, at constant temperature (21°C), and in controlled lighting (light period 7.00 a.m. to 7.00 p.m.). Food (standard laboratory rat, mouse, and hamster diets, R.M.H.-T.M., Hope Farms, Woerden, The Netherlands) and water were supplied ad libitum, except during actual testing. Forty-nine rats were used conforming to institutional policies and guidelines.

Human IVIg was produced from the plasma of more than 3000 Dutch blood donors by ethanol fractionation, treated at pH 4 with a low concentration of pepsin (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, NL). IVIg dissolved in sterile water without solvents contains 99% IgG, 1% IgA and traces of IgM. Its osmolarity is equivalent to that of plasma, with a sodium concentration between 25 and 50 mmol/l, and a glucose concentration between 160 and

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300 mmol/l. No mannitol or glycine is added in this IVIg preparation. IVIg was administered in a comparable dosage as in humans (400–1000–1500 mg/kg body/weight), but by a different route, i.e. by a single intraperitoneal (i.p.) dose.

The anticonvulsant efficacy was tested and quantified using direct cortical stimulation with a ramp-shaped pulse train as described in detail elsewhere[6–8]. The threshold for localized seizure activity (TLS) has been used to quantify anticonvulsant effects. The TLS was defined as the current at which the first mild clonic forepaw movements appeared. TLS stimulation was stopped at this point and further convulsive activity was then immediately aborted. In this way a postictal threshold increase was avoided. Before IVIg administration, the TLS was determined five times at five minute intervals, to determine the average baseline value. Shortly after i.p. IVIg injection, TLS determination was performed, and during three days the TLS was measured at regular intervals. TLS changes were expressed as elevation over the baseline value in μA. All test sessions were recorded on video. Before IVIg experiments were carried out, the animals were tested twice daily for two weeks to stabilize the threshold[7]. The effect of IVIg on TLS was tested in more than seven months old rats ($n = 9$), and in younger rats (3–4.5 months, $n = 40$). Some rats of the younger age group ($n = 10$) were treated previously with carbamazepine (40 mg/kg), others ($n = 30$) were drug-naïve.

In addition, we investigated ($n = 3$) whether IVIg was ineffective due to an inability to cross the blood–brain barrier: two days after IVIg administration rates were killed, and brains were freshly fixed in formaldehyde-sublimate at 4°C for 24 hours. After dehydration tissue was embedded in paraplast and four micron sections were cut using a Jung 2050 microtome. Dewaxed poly-L-lysine mounted sections were incubated with human IgG antiserum (gamma chain specific, Dakopatts) for 48 hours at 4°C in a dilution of 1/250. Immunohistochemical staining was performed using the avidin-biotin-peroxidase and the avidin-biotin-alkaline-phosphatase complex technique (ABC) according to the manufacturer instructions (Vector). All antibody dilutions were made in 0.05 M phosphate buffered saline (PBS) to which 0.1% Triton-X-100 was added. As negative controls we used rats ($n = 3$) that also had experienced seizures due to cortical stimulation but without administration of human IVIg.

RESULTS

After human immunoglobulin administration (500 mg/kg; Table 1) a significant and longlasting increase in TLS was measured in 12% (6/49) of the total group of outbred Wistar rats. In the subgroup of more than seven months old Wistar rats this was 67% (6/9), in the other subgroup this was 0% (0/40) (Fisher exact test ($P < 0.0001$), Table 1). Figure 1 depicts the typical time-effect relationship in an animal showing an increase in TLS. When a TLS increasing effect of IVIg occurred, it was detectable within 0.5–1 hour after administration, reached its maximum after approximately two hours and continued for at least 40 hours.

In the subgroup ($n = 40$) of 3–4.5 months old rats, neither three different doses of IVIg (400, 1000, 1500 mg/kg), nor pretreatment with carbamazepine (40 mg/kg) showed any effect on TLS.

In control rats (with cortical stimulation, without IVIg treatment) immunohistochemical staining for human IgG was negative (Fig. 2a), whereas in all (non-responding) rats ($n = 3$) killed two days after IVIg administration, staining for human IgG was present in brain capillaries, chorioid plexus, and in brain tissue around blood vessels (Fig. 2b). Some neuronal and glial cells showed immunohistochemical staining. Thus, the lack of a threshold increasing effect by IVIg was not due to an inability of IgG to cross the blood–brain barrier.

DISCUSSION

These preliminary investigations indicate that human IVIg has the potential to raise the threshold for convulsions in rats. Already within the first hour after i.p. administration a significant and prolonged anticonvulsant effect occurred in 12% of all tested outbred Wistar rats. In the subgroup of more-than-seven-months-old Wistar rats this percentage was 67%, which suggests an age-related effect of the IVIg response. An age-related effect of epilepsy itself has been reported previously: seizure intensity increased with increasing age in genetically epilepsy-prone rats[9]. The present study confirmed the results of the only other study on administration of human IVIg in an animal epilepsy model (kindled cats)[10]. In the latter study and in our study a similar time-effect relationship and a similar immunohistochemical staining pattern for human IgG were

Table 1: The effect of human IVIg on the convulsive threshold in rats*

Animals		Immunoglobulin	Results
Number	Age (months)	Dose (mg/kg)	Increase of TLS**
$n = 9$	>7	500	6 of 9 animals, 200 μA (Avg. baseline 400 μA)
$n = 40$	3–4.5	400, 1000 or 1500	0 of 40 animals

* IVIg, Human immunoglobulin; TLS, threshold for localized seizures.
** $P < 0.0001$ by Fisher Exact test.

seen: IgG penetrated the blood–brain barrier, could be found around the blood vessels, and was even taken up by some neuronal and glial cells in the cerebral cortex and deep structures of the CNS. These findings might be compatible with reports on blood–brain barrier permeability in various types of experimental epilepsies[11], the existence of IgG Fc receptors in adult rat brain and in normal human brains[12,13], and immunoglobulin affinity of neuronal and glial cells[14,15].

The present experimental data are in accordance with the results of clinical studies on IVIg in patients with epilepsy. Of the several hundreds of IVIg treated patients with otherwise intractable epilepsies, overall some 23% showed total cessation of seizures[4]. The variability of IVIg treatment effect in human epilepsy is still unexplained. It has been attributed to differences in IVIg preparations, types of epilepsy and seizures, duration of epilepsy, immunological abnormalities, or study design[4]. We cannot exclude that on the clinical level these factors contribute to the variable responses, but as can be concluded from the present study, in the more controllable animal-model a remarkable age-related variability in treatment-response was found. Another contributing factor for the variability in IVIg response might be genetic heterogeneity in both humans with epilepsy and outbred Wistar rats.

In conclusion, we can not confirm that IVIg lacks an anticonvulsant effect[5]. On the contrary, this preliminary study suggests the potential of a significant and longlasting anticonvulsant effect of IVIg by increasing the threshold for convulsions. However, future studies are needed and should investigate the effect of albumin (as a control) on TLS in more than seven-month-old animals, the repetitive IVIg administration in IVIg-responding

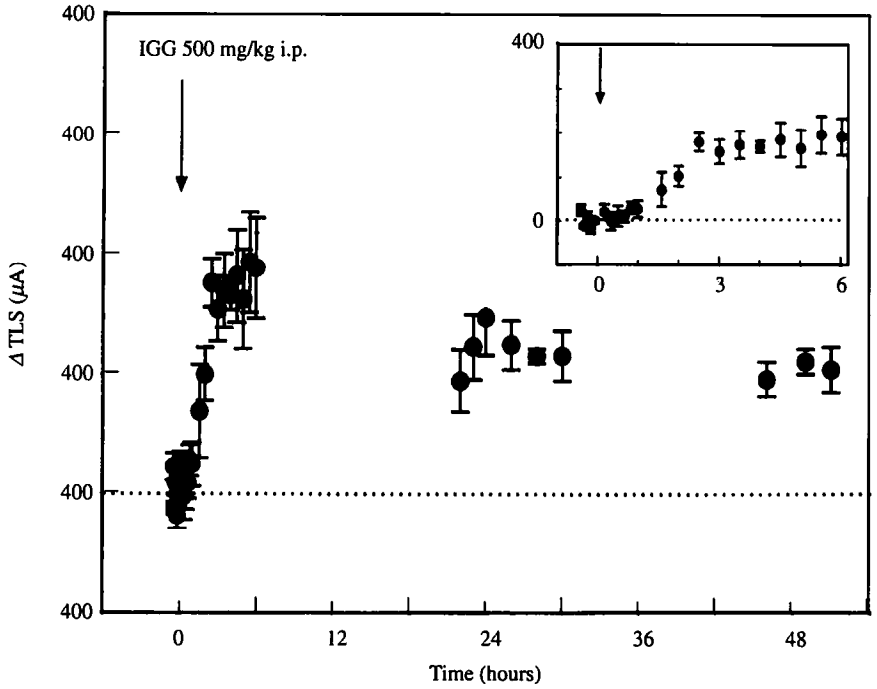

Fig. 1: The effect of 500 mg/kg human IVIg on the threshold for localized seizures (TLS) in eight months old rats who had previously experienced generalized seizures and had been treated with carbamazepine.

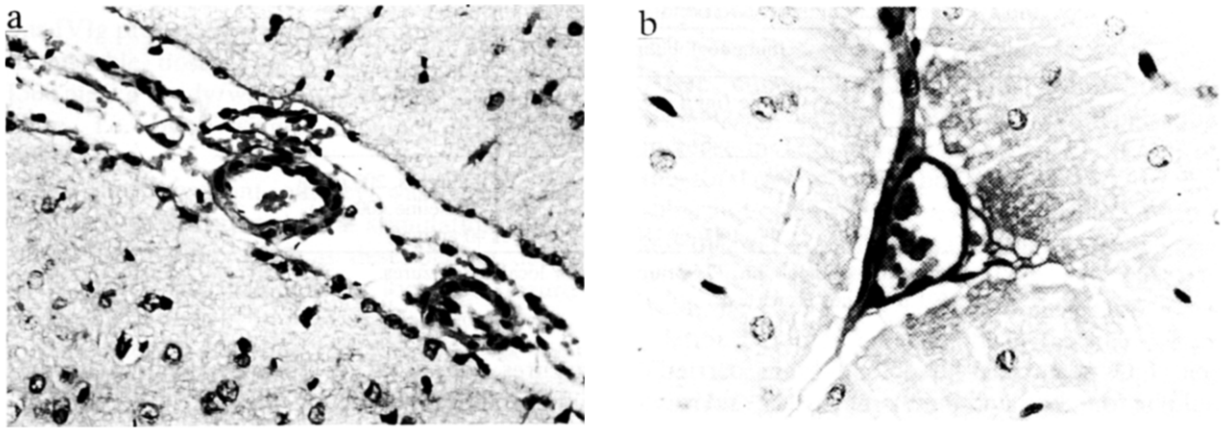

Fig. 2: (a) Immunohistochemical staining for human IgG was negative in control rats (with cortical stimulation, without IVIg treatment). (b) Immunohistochemical staining for human IgG in rats treated with a single dose of IVIg was present in and around cerebral bloodvessels.

animals, and the capability of IgG to cross the blood-brain barrier in IVIg-responding and non-responding animals.

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