Acta Paediatrica Academiae Scientiarum Hungaricae Vol. 9 (3—4), pp. 213—217 (1968)

Further Experiments with Alcohol-Treated Hepatitogenic Serum

By

J. Csapó, J. Budai, G. Nyerges, P. Richter and I. Tóth

László Central Hospital for Infectious Diseases, Budapest, and Institute for Serobacteriological Production and Research "Human" Budapest

(Received December 10, 1967)

Our experiments aimed at the immunoprophylaxis of infectious hepatitis had started in 1959. We have supposed that the prophylactic effect of the commercial gamma globulin preparations is composed of two factors, *viz.*, a passive protection of short duration and an active protection lasting several months, the latter being due to a hepatitis virus contamination originating from virus-carrying symptomless donors. We prefer this hypothesis to Stokes's well-known interpretation [1] suggesting that subclinical infections occurring during the period of passive protection are responsible for the prolonged immunity.

To check our hypothesis, we prepared gamma globulin from blood collected from adult patients with infectious hepatitis and injected it into mentally retarded children. The preparation proved to be hepatitogenic, suggesting that the virus may survive Cohn's procedure. Considering that symptomless carriers cannot be excluded from donorship, it is evident that even commercial gamma globulin preparations may contain small quantities of infectious hepatitis virus and thus give rise to an active immunity under the protection of the passively introduced antibody [2].

In previous experiments we examined the effect of alcohol on infectious hepatitis virus. Forty-hour treatment with 21 % alcohol, the method employed in the production of commercial gamma globulin, was insufficient to kill the virus. Raising the alcohol concentration and the time of treatment step by step we found that 14 days in 40 % alcohol was the limit of treatment which saved an extremely weak hepatitogenic effect. Parenteral administration of the preparation was followed by a prolonged (45—80 day) incubation period. The course of the illness caused by this virus was mild, indicated sometimes only by laboratory tests carried out at short intervals. The inoculated children remained in the community except those who had become ill. These were hospitalized. According to epidemiological observations contact infections did not occur. It may be supposed that either no virus was excreted with the faeces or, due to the alcohol treatment, virus devoid of oral invasiveness was excreted.

Our experiments allowed to assume that some of the cases of post-transfusion hepatitis are caused by the infectious hepatitis virus and in these cases due to the large amount of antibody, the incubation period is prolonged like that of serum hepatitis.

The present investigations had two aims.

(i) A serum pool obtained from children with infectious hepatitis was subjected to the most intensive treatment that had still saved some virus in the adult serum pool, in order to establish whether any part of the virus present in children's sera will resist this procedure.

(ii) Alcohol-treated serum was administered by the oral route to test, whether such administration of a slightly hepatitogenic preparation was followed by illness. The immunity of the orally treated children was challenged later. We supposed that, like in the case of poliomyelitis, the local resistance of the intestinal mucosa may play some role in the immunity following the natural oral infection with infectious hepatitis virus. If so, oral immunization with the alcohol-treated infectious hepatitis agent would be the most hopeful method of immunization against infectious hepatitis.

Materials and Methods

Blood was taken on the first day of manifest illness from 50 children suffering from infectious hepatitis. The sera were pooled, lyophilized, and stored at — 10 °C. For alcohol treatment an amount of serum necessary for one experiment was re-dissolved and lyophilized again after alcohol treatment. For parenteral injection, a solution containing 1% serum protein was prepared, whereas for oral administration the dry lyophilized preparation, 0.1 g per child, was used.

Like in the previous experiments, mentally severely retarded children with a negative hepatitis history were inoculated. Hepatitis had not occurred in the institution for years. Liver function tests were carried out from the 50th day after intramuscular inoculation on every 10th day, on five occasions altogether. After oral administration the laboratory control was started on the 25th day, repeated on every 5th day till the 50th day and then 5 times at 10-day intervals. Serum bilirubin, SGPT, SGOT and gold sol values were determined and the thymol reaction was performed.

Results

I. First, the hepatitogenic effect of the children's serum pool had to be checked. A sample was treated with 40% alcohol for 14 days at — 5° C. With this preparation 36 children were inoculated intramuscularly (Group I). The individual dose contained 0.01 g serum protein. All the children remained healthy.

For further experiments a sample of the lyophilized serum was treated with 40% alcohol for 12 days and the introduced quantity of serum protein was raised first to 0.03 g (Group II, 24 children) and then to 0.04 (Group III, 12 children). No illness occurred.

We therefore returned to the procedure applied in the preparation of commercial gamma globulin, with the only difference that the alcohol treatment lasted 70 hours instead of

TABLE I

Hepatitogenic effect of pooled serum after pretreatment with alcohol

Group	Time of inoculation	No. of children	Inoculum (g serum protein)	Alcohol concentration	Length of treatment	Illness
I	May, 1963	36	0.01	40%	14 days	—
II	April, 1964	24	0.03	40%	12 days	—
III	June, 1964	12	0.04	40%	12 days	—
IV	June, 1964	15	0.04	21%	70 hours	2*

*Incubation period: 82 and 83 days.

TABLE II

Data of oral immunization

Group	Number of children	Oral immunization*		Intramuscular challenger*		Illness after	
		time	dose (g protein)	time	dose (g protein)	immunization	challenge
V	24	October, 1964—June, 1965	0.1	November, 1965	0.04	No	No
VI	24	—	—	November, 1965	0.04	—	No

*The pooled serum was treated with 21% alcohol for 70 hours before use for immunization or challenge

40 hours. The alcohol concentration was 21% and 0.04 g dissolved serum protein was inoculated intramuscularly into each of 15 children (Group IV). Two of these became ill with mild hepatitis on the 82nd and 83rd day, respectively. In the first case the serum bilirubin level rose to 2.5 mg per 100 ml and the colloid tests and transaminase values were moderately positive. In the second case nonicteric hepatitis developed, indicated only by the elevated SGPT and SGOT values and an increased urobilinogenuria. The children were isolated in hospital, where they completely recovered in three weeks.

II. According to the experiments under I the material treated with 21% alcohol for 70 hours had proved to contain virus. Thus, we tried to immunize 24 children (Group V) orally with a product prepared from the serum pool by the same alcohol treatment. The oral dose was 0.1 g of the lyophilized powder. Since it was certain that the preparation contained virus, only three children were fed simultaneously and then isolated for 50 days. Thus, the vaccination of

the 24 children lasted 8 months. None of the children became ill and signs suggestive of hepatic injury could not be detected within 100 days post-inoculation.

Five to 13 months after oral vaccination the children were challenged intramuscularly with a preparation made from the serum pool by the method applied in the preparation of the oral vaccine. The individual dose contained 0.04 g of serum protein. As a control group, 24 children (Group VI) kept in another institution were challenged. No sign of hepatitis could be observed in either of the two groups.

Discussion

The results of the first part of the present experiments suggest that the hepatitogenicity of the applied serum pool was considerably different from that of the serum pool used previously. The same alcohol treatment (40 % for 14 days) which weakened but did not completely destroy the hepatitogenicity of the earlier pool appeared to kill the pathogen in the present pool. We had to reduce the alcohol concentration and shorten the alcohol treatment to prevent total inactivation. The discrepancy between the previous and present experiments might be explained either by qualitative or quantitative differences in the virus contained in the two serum pools.

In the second part of the experiments both the oral vaccine and the challenge virus were subjected to the same procedure (21 % alcohol for 70 hours) that had resulted in a slightly hepatitogenic preparation in the first part of experiments. The fact that the challenge 5—13 months later was followed by hepatitis in none of the vaccinated children might be explained (i) by a good immune status due to the vaccination or (ii) by assuming that the lyophilized virus had lost its infectivity during the 18-month storage at —10° C. Since the control children, too, remained healthy after challenge, the second explanation is preferable.

Summary

Mentally retarded children have been inoculated intramuscularly with alcohol-pre-treated hepatitogenic serum, varying the period of time of alcohol treatment and the alcohol concentration.

Treatment with 40 % alcohol for 12 days appeared to destroy the hepatitogenicity of the serum completely. In a previous experiment some virus survived even a 14-day treatment. Treatment with 21 % alcohol for 70 hours resulted in a preparation with rather weak hepatitogenic effect. Mild hepatitis was observed in 2 of the 15 children inoculated intramuscularly with that preparation. The lyophilized powder of an identically pre-treated preparation (21 % alcohol, for 70 hours) was administered orally to 24 children, 0.1 g per child. The children remained healthy. These and 24 control children were challenged 5—13 months later, intramuscularly, with a prepara-

tion obtained by the same treatment from the same hepatitogenic serum. The challenge was followed by no sign of hepatitis, suggesting that the serum had become non-infective during the 18-month interval.

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Prof. J. Csapó
Gyáli út 5
Budapest IX., Hungary