CLINICAL RESEARCH

Postexposure immunoprophylaxis against B virus (Herpesvirus simiae) infection

E A BOULTER, H T ZWARTOUW, B THORNTON

Abstract

Local infiltration of antiserum into sites inoculated with B virus protected rabbits from an otherwise fatal encephalomyelitis. Treatment was effective when delayed for six hours but not after 24 hours. Homologous rabbit antisera were more effective than heterologous monkey antisera, and protection was unrelated to neutralisation titres. Protection apparently depended not on neutralisation of inoculated virus but on destruction of infected cells before they produced progeny virus. Normal human immunoglobulin able to neutralise B virus did not protect. Intravenously administered antibody was effective only if large doses were given.

The findings suggest that persons bitten or scratched by monkeys latently infected with B virus may be treated successfully by immunoprophylaxis with specific antibody. Stocks of human or of more readily available simian antisera should be held in laboratories where such animals are used.

Introduction

Monkeys with antibodies to monkey B virus (*Herpesvirus simiae*) may be latently infected with this virus,[1-3] and the possibility of their saliva containing infectious virus cannot be excluded. Bites or scratches from such monkeys thus entail a risk of infection with B virus and pose problems in management. Klenerman *et al*[1] outlined a procedure for treating animal bites potentially infected with neurotropic viruses. They recommended that local treatment should be followed by specific prophylaxis with vaccine and immune serum whenever possible. At that time specific prophylaxis against B virus infection was not available, but two measures can now be considered for wounds likely to be contaminated with B virus—chemoprophylaxis with an antiviral drug, and immunoprophylaxis with specific antibody.

Infecting rabbits with B virus causes a fatal encephalomyelitis that closely mimics the disease in man and provides a model in which prophylactic measures for man may be assessed. Though acyclovir proved an effective chemoprophylactic agent in this model, it needs to be given intravenously every six hours for at least 14 days.[5] This entails admitting the patient to hospital, however, so that until an oral form of the drug becomes available its use is likely to be confined to patients with a definite danger of being infected.

Infiltration of immune serum or globulin into wounds infected with B virus confers some protection on experimentally infected rabbits.[6 7] This procedure could be used to treat monkey bites where the risk of infection is not sufficient to justify admitting the patient to hospital for parenteral treatment with an antiviral agent such as acyclovir. It could also be a useful adjunct to chemoprophylaxis when such treatment is used. We describe experiments that confirm the value of immunoprophylaxis in experimentally infected rabbits.

Materials and methods

A strain of B virus isolated from the tongue of a cynomolgus monkey[8] was grown in Vero cells using medium 199 containing 50 000 units gentamicin and 14 mmol HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonate) per l and 5% fetal calf serum. The virus was titrated in microplates, using 10 wells for each dilution. The microplates were examined three days later for viral cytopathic effects: a tissue-culture dose (TCD_{50}) was defined as the amount of virus producing cytopathic effects in 50% of inoculated wells.

Antisera were produced by inoculating naturally immune rhesus or cynomolgus monkeys, or rabbits that had survived experimental infection, either intravenously with large doses of B virus or intramuscularly with virus mixed with Freund's complete adjuvant. Human immunoglobulin was obtained from the Public Health Laboratory Service. Antibody titres were measured in a microneutralisation test. Serial twofold dilutions of sera were prepared, in 50 μl volumes, in 96-well microplates, and 50 μl of virus containing 30-300 TCD_{50} and 2·5-5·0 haemolytic units of guinea-pig complement were added. After two hours at room temperature 100 μl of Vero cell suspension (250×10^6 cells/l) was added to each well. The microplates

Microbiological Research Establishment, Porton Down, Salisbury, Wilts

E A BOULTER, MB, BS, medical officer, research (now at the Chemical Defence Establishment, Porton Down)

H T ZWARTOUW, PHD, principal scientific officer (now at the Chemical Defence Establishment, Porton Down)

B THORNTON, PHD, senior microbiologist (now at the PHLS Centre for Applied Microbiology and Research, Porton Down)

were read after three days of incubation and the neutralising titre expressed as the reciprocal of the last dilution of serum that completely inhibited viral cytopathic effect.

Half-lop albino rabbits were inoculated with appropriately diluted virus subcutaneously in the right thigh. The inoculation site was marked, and subsequently about 1·5 ml of antibody was injected at this site; the first 0·5 ml was inoculated intradermally and the remainder subcutaneouly. The rabbits were then observed for signs of disease and death.

All manipulations of B virus under in-vitro conditions were carried out in a class 3 safety cabinet. Experimentally inoculated animals were held under category A conditions.

Results

Table I summarises the results of experiments in which serum or immunoglobulin was infiltrated into the site of B virus inoculation in rabbits. The best results were obtained with homologous (rabbit) antisera, whose neutralisation titres ranged from 64 to 1300. With low doses (4-102 TCD_{50}) of virus, treatment wihin 10 minutes of infection reduced the death rate from 91% to 7%: similar results were obtained when treatment was delayed for six hours. Treatment at 24 hours resulted in a death rate of 60%; with the small numbers of animals this was not significantly different from the death rate in controls. The amount of virus likely to be implanted by a monkey bite is not known, but it could be more than the maximum dose of 102 TCD_{50} used in these experiments; further experiments were therefore carried out with doses of 10^3-10^5 TCD_{50}. Though the death rate was higher under these severe conditions, local immunoprophylaxis still gave a very high degree of protection. Interestingly, as in the experiments with low doses of virus, treatment at six hours was as effective as that given within 10 minutes of infection.

TABLE I—*Local immunoprophylaxis against experimental B virus infection in rabbits*

Source of antiserum	Virus dose (TCD_{50})	Time treated after infection	No dying/No infected (%) Untreated controls	Treated rabbits
Rabbit	Low dose (4–102)	10 min	32/35 (91)	3/46 (7)
		6 h	11/14 (79)	1/15 (7)
		24 h	9/10 (90)	6/10 (60)
	High dose (10^3–10^5)	10 min	12/14 (86)	7/29 (24)
		6h	4/5 (80)	2/10 (20)
Monkey	Low dose (22–50)	10 min	12/13 (92)	3/17 (18)
	High dose (10^3–10^5)	10 min	13/15 (87)	18/19 (95)
		6 h	4/5 (80)	6/9 (67)
Human gammaglobulin	Low dose (40)	10 min	3/4 (75)	2/4 (50)
	High dose (4×10^3)	10 min	ND	3/3 (100)

ND = Not done.

TABLE II—*Comparison of homologous (rabbit) and heterologous (monkey) antisera in immunoprophylaxis against B virus infection in rabbits*

Virus dose (TCD_{50})	Antibody titre	Source of antiserum	No dying/No infected
21	32	Rabbit 53	0/5
	16	Monkey pool	0/5
	128	Rabbit 95	0/5
	128	Monkey 64	0/5
		None	5/5
10^3	32	Rabbit 53	1·4
	16	Monkey pool	5·5
	128	Rabbit 95	0·5
	128	Monkey 64	3·4

Some human sera[9-12] and batches of human gammaglobulin will neutralise B virus as well as herpes simplex virus. These would be ideal for immunoprophylaxis in man if they were protective, but human immunoglobulin failed to protect against only 40 TCD_{50} of B virus (table I). This preparation had a neutralisation titre of 1 in 16, which was similar to the monkey and rabbit sera that fully protected against a similar dose of B virus (table II).

Table III shows the results of three experiments in which rabbit antiserum was inoculated intravenously instead of locally. This route of administration was effective only when larger volumes of serum were used.

TABLE III—*Effect of intravenous inoculation of antiserum on B virus infection in rabbits*

Experiment No	Virus dose (TCD_{50})	Serum volume (ml)	No dying/No inoculated Untreated controls	Treated rabbits
1	17	1·5	4/5	3/5
2	390	1·0	2/2	2/2
		2·5	2/2	0/2
		5·0	2/2	1/2
3	102	5·0	3/3	0/3

Discussion

Our findings are in general agreement with two earlier reports,[6 7] and confirm Buthala's[7] observation that homologous antibody was more effective than antibody from other animal species. Though Buthala noted some protection when treatment was delayed for six hours, this was mostly shown by increased survival time rather than reduced mortality. We cannot explain this discrepancy from our results, where treatment at six hours was as effective as that given 10 minutes after infection.

Local infiltration is much more effective than giving antiserum intravenously or gammaglobulin intramuscularly. McLeod *et al*[6] found that 5 ml of monkey gammaglobulin inoculated intramuscularly was of little value. Buthala[7] noted that 10 ml of rabbit serum intravenously protected four out of four rabbits, but doses of 5 ml and 2 ml gave progressively decreasing survival rates. Taken with our results, these findings suggest that a 60 kg man would require 200-240 ml of antiserum intravenously to give the same protection provided by 1·0-1·5 ml instilled locally.

The mechanism of immunoprophylaxis against B virus infection is not clear. It is unlikely to be simple neutralisation of inoculated virus, firstly, because of the lack of correlation between protection and neutralisation titre (table II) and, secondly, because antiserum given at six hours, when the viral inocula should have penetrated cells and become inaccessible to neutralising antibody, was as effective as that given 10 minutes after infection. Possibly infected cells are destroyed before progeny virus is produced, either by antibody-dependent, cell-mediated or antibody-dependent, complement-mediated cell lysis. Until the mechanism is identified animal experiments will remain the only way of proving the protective potency of an antiserum or immunoglobulin.

One objective of this study was to assess the potential value of immunoprophylaxis for monkey bites in man. Since in rabbits homologous antisera were more effective than others, for man either human or simian sera should be used. Human antiserum to B virus would be ideal but is in very short supply. Human immunoglobulin is more readily available and generally contains antibody to herpes simplex virus that neutralises B virus in a heterotypic cross-reaction. Though this has been recommended for accidental exposure to B virus,[9-11] our results and those of McLeod *et al*[6] suggest that it would be ineffective; on the occasions when it has been used no apparent protection was observed.[12-15]

Man and monkeys are sufficiently closely related to make simian antisera an acceptable alternative to human antisera. Doses of 15-20 ml of monkey gammaglobulin have been given to three people without untoward effect[6]; one of them had received five injections of 0·1-0·3 ml two months before. The rate of

antibody decline in this subject was similar to that of maternal antibody in infants. We have infiltrated monkey serum into the distal phalanx of the thumb of a person bitten by a monkey from a colony in which B virus was known to have been circulating. This was done under ring block local anaesthesia; the only reaction was severe pain during the night after the anaesthesia had worn off. Though this was not unexpected with such a sensitive area as the pulp of the thumb, it makes the procedure less than ideal for wounds in these areas unless the risk of infection is high—as it was on that occasion.

If immunoprophylaxis is to be used for monkey bites or similar trauma it is essential that antisera or immunoglobulin should be held in laboratories where monkeys are used. Stocks of monkey sera are held by us at Porton Down and at the National Institute for Biological Standards and Control, London. We hope that these stocks will be converted into immunoglobulin and made available as freeze-dried preparations.

We acknowledge the financial help from the Medical Research Council and the provision of laboratory facilities by the PHLS Centre for Applied Microbiology and Research. Our thanks are due to Miss B Burrell, Mrs G Green, and Mrs P Plank for technical work.

Requests for reprints should be sent to Dr B Thornton.

References

[1] Boulter EA. The isolation of monkey B virus (Herpesvirus simiae) from the trigeminal ganglia of a healthy seropositive rhesus monkey. *J Biol Stand* 1975;**3**:279-80.

[2] Boulter EA, Grant DP. Latent infection of monkeys with B virus and prophylactic studies in a rabbit model of this disease. *J Antimicrob Chemother* 1977;**3**,suppl A:107-13.

[3] Vizoso AD. Recovery of Herpes simiae (B virus) from both primary and latent infections in rhesus monkeys. *Br J Exp Pathol* 1975;**56**:485-8.

[4] Klenerman L, Coid CR, Aoki FY. Treatment of wounds from animals suspected of carrying neurotropic viruses. *Br Med J* 1975;iii:740-1.

[5] Boulter EA, Thornton B, Bauer DJ, Bye A. Successful treatment of experimental B virus (Herpesvirus simiae) infection with acyclovir. *Br Med J* 1980;**280**:681-3.

[6] McLeod DRE, Shimada FT, Walcroft MJ. Experimental immunization against B virus. *Ann NY Acad Sci* 1960;**85**:980-9.

[7] Buthala DA. Hyperimmunized horse anti-B virus globulin: preparation and effectiveness. *I Infect Dis* 1962;**111**:101-6.

[8] Vizoso AD. Latency of Herpes simiae (B virus) in rabbits. *Br J Exp Pathol* 1975;**56**:489-94.

[9] Melnick JL, Banker DD. Isolation of B virus (herpes group) from the central nervous system of a rhesus monkey. *J Exp Med* 1954;**100**:181-94.

[10] Van Hoosier GL Jr, Melnick JL. Neutralizing antibodies in human sera to Herpesvirus simiae (B virus). *Tex Rep Biol Med* 1961;**19**:376-81.

[11] Cabasso VJ, Chappell WA, Avampato JE, Bittle JL. Correlation of B virus and herpes simplex virus antibodies in human sera. *J Lab Clin Med* 1967;**70**:170-8.

[12] Breen GE, Lamb SG, Otaki AT. Monkey-bite encephalomyelitis. Report of a case—with recovery. *Br Med J* 1958;ii:22-3.

[13] Hummeler K, Davidson WL, Henle W, La Boccetta AC, Ruch HG. Encephalomyelitis due to infection with Herpesvirus simiae (herpes B virus). A report of two fatal, laboratory-acquired cases. *N Engl J Med* 1959;**261**:64-8.

[14] Love FM, Jungherr E. Occupational infection with virus B of monkeys. *JAMA* 1962;**179**:160-2.

[15] Fierer J, Bazeley P, Braude AI. Herpes B virus encephalomyelitis presenting as ophthalmic zoster. *Ann Intern Med* 1973;**79**:225-8.

(*Accepted 22 September 1981*)

Smoking and drinking by middle-aged British men: effects of social class and town of residence

RICHARD O CUMMINS, A G SHAPER, MARY WALKER, C J WALE

Abstract

In 7735 men aged 40-59, selected at random from general practices in 24 towns throughout Britain, pronounced differences were noted in the prevalences of smoking and drinking between the social classes. Social class differences also existed for frequency and quantity of drinking, type of beverage, and several aspects of smoking behaviour. Increasing amounts of smoking were associated with higher prevalences of moderate to heavy drinking, particularly in daily rather than weekend drinkers. Between drinking groups, however, the relation with smoking was more U-shaped, with light and heavy drinkers smoking more than moderate drinkers. The lowest rates of moderate to heavy smoking were observed in frequent light drinkers, particularly in the non-manual workers. The proportion of moderate to heavy drinkers was no higher among ex-cigarette smokers than among current smokers. When the data were examined by town of residence social class differences persisted. Controlling for social class still showed pronounced differences between towns in both smoking and drinking behaviour.

These data confirm that town of residence and social class have independent effects on smoking and drinking. The established regional and social class differences in cardiovascular disease may be due in part to the independent influences of town and social class on smoking and drinking behaviour.

Department of Clinical Epidemiology and General Practice, Royal Free Hospital School of Medicine, London NW3 2QG

RICHARD O CUMMINS, MD, MPH, Milbank Memorial Fund scholar and assistant professor of medicine, University of Washington, Seattle, Washington (on leave)

A G SHAPER, FRCP, FRCPATH, professor of clinical epidemiology

MARY WALKER, SRN, SCM, research administrator

C J WALE, statistical technician

Introduction

The pronounced regional differences in mortality from cardiovascular disease in Britain have interested observers for decades and led to several studies.[1 2] The British Regional Heart Study is a further effort to investigate the problem.[3 4] Researchers have noted associations between cardiovascular diseases and cigarette smoking and, to a less extent, drinking alcohol.[5 6] Cigarette smoking has been positively associated with the prevalence and incidence of ischaemic heart disease,[7] light drinking has been associated with lower blood pressure[8] and less cardiovascular disease,[9 10] and heavy drinking has been associated with both higher blood pressure[4 8 11] and more ischaemic heart disease.[12 13] Probably at least part of the well-known regional variation in mortality from cardiovascular disease may be explained by regional variations in these two risk factors of smoking and drinking.