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EFFECT OF HYPERBARIC OXYGENATION ON EXPERIMENTAL SCRAPIE IN MICE

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Summary. — The effect of hyperbaric oxygenation upon experimental infection of BALB/c mice with the scrapie agent was investigated by virological methods, histology and electron microscopy. A multiple exposure of scrapie-infected mice to hyberbaric oxygenation during the incubational period led to a certain aggravation of infection as evidenced by a greater accumulation of the agent in the central nervous system (CNS) and spleen, as well as by more pronounced ultramicroscopic changes in CNS. A single exposure to hypoxia failed to alter any manifestations of infection.

Key words: subacute transmissible spongiform encephalopathy, scrapie, central nervous system, hyperbaric oxygenation

Introduction

Infection of mice with the scrapie agent is a model to study the pathogenesis of slow infections in animals and man caused by the agents of subacute transmissible spongiform encephalopathy (STSE) (Kimberlin, 1976). The characteristic features of STSE are the absence of immune response or interferon syntesis and, consequently, a failure of factors modifying the immunological state affecting the infection process. Considering the 100% lethality of the diseases, this makes urgent to search for other ways altering the course of infection (Gibbs, Gajdusek, 1974; Kovanen *et al.*, 1980; Porter *et al.*, 1973; Worthington, 1972). The numerous attempts at treating experimental scrapie or Creutzfeldt-Jakob disease by various antiviral drugs produced mainly negative results, although some preparations did show a certain effect (Braham, 1971; Cohran, 1971; Sanders, Dunn, 1973; Kimberlin, Walker, 1979; Tateishi, 1981). It is well-known that oxygen concentration in the environment has a strong effect on the level of cellular metabolism notably in the cells of CNS. With this fact in mind, we attempted to affect the infection process by exposing scrapie-infected mice to hyperbaric oxygenation or hypoxia. In the available literature we found no such information as related to STSE, except for the observation of Chino (1981) on a similarity of ultrastructural changes in the CNS in Creutzfeldt-Jakob disease and hypoxia.

Materials and Methods

Animals. Two-three-week-old BALB/c mice were used.

Agent and infection. The scrapie agent, strain C-506, isolated at Dr. Gajdusek's laboratory from the brain of a scrapie-infected sheep by passages in mice, was obtained from Dr. Gibbs (The National Institute of Health, Bethesda, U.S.A). Titration of the agent was performed by means of intracerebral (i.c.) infection of mice, followed by recording the number of animals that developed the typical clinical pattern and histological lesions in the CNS. The agent titre was calculated according to Reed and Muench. The experimental animals were inoculated i.c. with the scrapie agent in the form ot a 10% brain suspension having the initial titre of 5.7 log LD_{50}/0.03 ml. The control animals were inoculated with a 10% suspension of normal mouse brain, while some of the mice were left intact. On the whole, 170 mice were used.

Hyperbaric oxygenation and hypoxia.[a]) One month after inoculation of the scrapie agent or of the normal brain suspension, the mice were subjected to hyperbaric oxygenation in a chamber filled with oxygen at the pressure of 3 kg . cm^{-2}. The compression time was 7 min, the exposure time 1 hr. In total, 6 exposures in the course of 3 weeks were performed.

The effect of hypoxia was investigated by a single exposure of the animals 24 hr after the inoculation. The animals were kept in an altitude chamber at an oxygen concentration of 15—16% for 18 hr. Immediately after finishing the hyperbaric oxygenation and hypoxia treatments, as well as in the course of infection, some animals were sacrificed, and their brains and spleens subjected to examination.

Histological examination. The brain of mice was fixed in 10% formalin, embedded into parafin, and the sections were stained with haematoxylin-eosine or by the Nissl's method.

Electron microscopy. Pieces of brain cortex and cerebellum were mixed in a solution of 20% glutaraldehyde in cacodylate buffer (pH 7.2), then in a 2% osmium tetroxide solution in the same buffer, dehydrated in alcohol solutions of increasing concentrations and in propylene oxide, and then embedded into epon-araldite. Ultrathin sections were made on LKB-4800 or Reichert ultramicrotomes, contrasted with uranyl acetate, and examined under a JEM-100 electron microscope at 80 kV. Semiquantitative assesment of the changes was made at the magnification of 10,000 x; at least 100 fields of view were examined. A detailed study was performed at the magnifications of 20,000—25,000 x.

Results

Both the hyperbaric oxygenation and hypoxia experiments were arranged to compare 9 otherwise identical groups of animals: 3 groups were employed for each of the two treatments and the controls (scrapie-infected, normal brain-inoculated, and intact). Each of the 9 groups consisted of 18 to 20 animals. All animals were kept under observation until the development of clinical signs and death. Neither behavioural changes nor other pathological signs were detected in the animals during the hyperbaric oxygenation and hypoxia treatments or immediately thereafter. Histological examination of the CNS of animals sacrificed immediately after the treatments failed to reveal any significant difference between intact and scrapie-infected or normal brain-inoculated animals on the one hand, and infected or non-infected, subjected or not subjected to an altered oxygen concentration animals, on the other hand. Electron microscopy of the CNS preparations suggested that the observed variations were within the normal variability ranges and no gross ultrastructural changes caused by altered oxygen content were present in the brain cells.

a) We are indebted to Dr. D. B. Vandyshev (Department of Submarine Biomedicine, Institute for the Hygiene of Water Transportation, U.S.S.R. Ministry of Health, Moscow) for his assistance in the hyperbaric oxygenation and hypoxia experiments

Table 1. Scrapie agent in the brain and spleen of scrapie-infected BALB/c mice in hyperbaric oxygenation

Organ	Maitenance conditions	
	normal oxygen concentration	Hyperbaric oxygenation
Brain	5.14[a])	7.09
Spleen	4.90	6.50

[a]) The titre is expressed in log LD_{50}/0.03 ml

The first clinical signs of the disease (lowered mobility, flabbiness, ruffled--up appearance) showed up at the same time interval after inoculation (5.5 months), regardless of whether the animals were or were not subjected to hyperbaric oxygenation. However, the degree of clinical manifestation was somewhat greater in animals exposed to high oxygen concentration. In the next two weeks all animals showed the appropriate clinical signs and died. Yet the oxygenated animals accumulated greater amounts of the scrapie agent both in the brain and the spleen than the control ones (Table 1). Histological examination revealed a typical pattern of lesions in the CNS for this stage of infection, typical of clinical expression of STSE. At the same time, the magnitude of lesions as revealed by light microscopy was the same in the oxygenated and non-oxygenated groups. Electron microscopy of the CNS of mice sacrificed 5—6 months after inoculation revealed oedema of the brain parenchyma to which both the intracellular and extracellular oedemas contributed.

The cellular oedema was manifested by swelling of the cytoplasm of astroglia cells, of large dendrite profiles, and occasionally of neuron bodies. The extracellular oedema consisted of swelling of "acellular" neuropil

Table 2. Ultrastructural changes in the CNS of BALB/c mice infected with the scrapie agent and subjected to hyperbaric oxygenation

CNS lesion	Maitentenance conditions			
	normal oxygen concentration		hyperbaric oxygenation	
	onset of disease	peak of disease	onset of disease	peak of disease
Status spongiosus	±	±	±	++
Membrane formation in vacuoles	±	±	±	+
Swelling of synaptic endings	±	+	++	++

Notice: isolated (±), frequently seen (+), or multiple changes (++) for fields of view.

components, represented by the profiles of small branchings of axons, dendrites, and glial cell processes.

These changes, as well as changes in the ultrastructure of mitochondria, were nonspecific and occured in the CNS of both infected and mock infected animals inoculated with the normal brain suspension. Hyperbaric oxygenation did not affect these changes. Specific changes that took place only in the CNS of scrapie-infected animals were represented by vacuolization of the parenchyma, which determined the submicroscopic pattern of the status spongiosus, and the presence of typical membrane clusters in the vacuoles of affected parenchyma. Specific vacuolization that accompanied status spongiosus (Fig. 1) was ultrastructurally different from vacuolization due to nonspecific parenchymal oedema. (Fig. 2). The status spongiosus vacuoles were larger and more numerous, and besides, they often contained one or more daughter vacuoles having jagged, angular outlines. In the vacuole apertures scraps of twisted or concentrically located membrane formations could be detected (Fig. 3).

It should be also stressed that nonspecific vacuolization was seen primarily in the cellular elements of the parenchyma, while specific vacuolization affected "acellular" neuropil components. Specific microchanges also involved the swelling of pre- and post-synaptic terminals (Fig. 4). Signs of synapse destruction were observed, such as lowering the number of synaptic vesicles, alterations in their size, shape and location pattern. A comparison of ultrastructural CNS changes specific to scrapie in the mice that were and were not oxygenated indicated a higher magnitude of the effects in the former.

The relevant data are summarized in Table 2. Observations on scrapie-infected mice exposed to hypoxia failed to reveal any differences as compared to the non-exposed group in the time of appearance or in the degree of expression of the signs. Titration of the agent immediately after the hypoxia treatment, i.e. 48 hr after inoculation, indicated the presence in the brain (1.42 and 1.67 log LD_{50} per 0.03 ml) and spleen (3.37 and 2.67 log LD_{50} per 0.03 ml) of roughly the same amount of the agent in non-treated and hypoxia-treated animals, respectively. Histological studies on animals sacrificed at the height of clinical manifestation have likewise failed to demonstrate any difference in the expression of the STSE-associated changes between hypoxia-treated and untreated animals. A similar result has been obtained by electron microscopy.

Discussion

The STSE group includes the diseases of man (kuru and the Creutzfeldt-Jakob disease) and animals (scrapie and the transmissible encephalopathy of the mink) whose agents are so much different from the classical viruses in their nature and, perhaps, mode of reproduction that it was proposed to classify them into a special group of infectious agents termed prions (Prusiner, 1982). Studies of the STSE pathogenesis using the mouse scrapie as a model system has allowed to identify a number of features of the infection process relevant to its dependency on the route of administration and dose

of the agent, localization and manifestation of the pathohistological and ultrastructural changes in the CNS, and so on. Much less attention has been given to factors that modify the physiology of susceptible organs and tissues and thereby affecting reproduction of the scrapie agent. In the present work an attempt was made to evaluate the possible effect of hyperbaric oxygenation on infection process. It is concluded that a multiple exposure to elevated oxygen concentrations of mice in the incubation period contributes to a certain increase in accumulation of the agent in brain and spleen, as well as to more pronounced ultrastructural changes in the CNS. The histological picture of the CNS was not affected, though the level of the agent seemed to be high enough in the CNS of either mice group. A precise mechanism of this effect remains obscure. Our results suggest that hyperbaric oxygenation per se induced no histological or ultrastructural changes in the CNS even at remote times after the treatment. At the same time, it is well known that exposure of animals and man to elevated oxygen pressure brings about some changes in the cellular metabolism which are rapidly reversed upon returning to normal conditions (Haugaard, 1965). These changes involve inhibition of certain enzymes and suppression of cellular respiration and glycolysis (Dickens, 1946 a, b). The most sensitive to elevated oxygen concentrations are the SH-containing enzymes (Haugaard, 1968).

It is noteworthy that hyperbaric oxygenation changes the level of certain transmitters (Selivra, 1974; Wood, 1975) which have been shown to play a part in the scrapie pathogenesis (Rowwer *et al.*, 1981; Goudsmit *et al.*, 1981). The STSE are known to be accompanied by ultrastructural changes in synapses, which also are the main target of elevated oxygen effect (Selivra, 1974). Possible mechanisms of the stimulation of scrapie agent multiplication by hyperbaric oxygenation include the formation of lipid peroxides and free radicals, which results in a greater permeability of the cellular membrane (Wood, 1975; Zaltsman *et al.*, 1979). The complex effect of hyperbaric oxygenation on the cellular metabolism does not allow to associate the observed stimulation with any single factor, so that the stimulatory action may well be complex in nature. It is of interest that elevated oxygen concentration has not been shown to affect the replication of Sendai virus in vitro (Schift, Shefner, 1974). In view of the wide use of hyperbaric oxygenation therapy, a principal possibility of activation in the patients of slow STSE infections should be considered. Our results with hypoxia treatment do not allow a definite conclusion, although we were unable to demonstrate any effect on the infection process. This may be due to the fact that the treatment was applied only once, one day after inoculation. As follows from our data, hypoxia did not affect the results of titration of the agent in the brain and spleen of animals 48 hr after inoculation. The use of a more intensive hypoxia treatment might exert a detectable effect on the STSE process.

References

Braham, J. (1971): Jakob-Creutzfeldt Disease — treatment with amantadine. *Brit. Med. J.* **4**, 212.
Chino, F. (1981): Experimental transmission of Creutzfeldt-Jakob disease to guinea pigs. *Acta Path. Jap.* **31**, 963.

Cohran, K. W. (1971): Failure of adenine arabinosine to modify scrapie in mice. *J. gen. Virol.* **13**, 352.
Dickens, F. (1946*a*): The tocic effect of oxygen on brain metabolism and on tissue enzymes. I. Brain metabolism. *Biochem. J.* **40**, 145.
Dickens, F. (1946*b*): The toxic effect of oxygen on brain metabolism on tissue enzymes, II. Tissue enzymes. *Biochem. J.* **40**, 171.
Gibbs, C. J., and Gajdusek, D. C. (1974): Cell-virus interactions in slow infections of the central nervous system, p. 1025. In F. O. Schmitt and F. G. Worden (Eds): *The Neurosciences*, New York.
Goudsmith, J., Rohwer, R. G., Silbergels, E. K., and Gajdusek, D. C. (1981): Hypersensitivity to central serotonin receptor activation in sccapie-infected hamsters and the effect of serotonergic drugs on scrapie symptoms. *Brain Res.* **220**, 372.
Haugaard, N. (1965): Poisoning of cellular reactions by oxygen. *Ann. N. Y. Acad. Sci.* **117**, 736.
Haugaard, N. (1968): Cellular mechanisms of oxygen toxicity. *Physiol. Rev.* **48**, 311.
Kimberlin, R. H. (1976): Experimental scrapie in the mouse: a review of an important model disease. *Sci. Progr.* **63**, 252, 461.
Kimberlin, R. H., and Walker, C. A. (1979): Antivital compound effective against experimental scrapie. *Lancet* **11**, 591.
Kovanen, J., Haltia, M., and Cantell, K K (1980): Failure of interfer onto modify Creutzfeldt-Jakob disease. *Brit. Med. J.* **280**, 902.
Porter, D. D., Porser, H. G., an Gox, N. A. (1973): Failure to demonstrate a humoral response to scrapie infection in mice *J. Immunol.* **111**, 1407.
Prusiner, S. B. (1982): Novel proteinaceous infectious particles cause scrapie. *Science* **216**, 136.
Rohwer, R. G., Neckers, L. M., Trepel, J. B., Gajdusek, D. C., and Nyatt, R. J. (1981): Serotonin concentrations in brain and blood of scrapie-infected and normal hamsters and mice. *Brain Res.* **220**, 367.
Sanders, W., and Dunn, T. (1973): Creutzfeldt-Jakob disease treated with amantadine. *J. Neurol. Neurosurg. Psychiatry* **36**, 581.
Schift, L. J., and Shefner, A. M. (1974): Influence of oxygen and culture media on maintenance of whole hamster trachea organ culture and replication of Sendai virus, p. 250, Abstr. Annual Meet. Amer. Soc. Microb.
Selivra, A. I. (1974): The central nervous system functions under conditions of hyperbaric oxygenation, pp. 14—63. In G. A. Stepanski (Ed.): *Physiology of Man and Animals.* Meditsina, Moscow.
Tateishi, J. (1981): Antibodies and antivirals do not modify experimentally induced Creutzfeldt--Jakob disease. *J. Neurol. Neurosurg. Psychiat.* **44**, 723.
Wood, J. D. (1975): Oxygen toxcity, p. 166. In P. B. Bennet and D. H. Elliot (Eds): *Medicine od diving and compressed Air Work*, London.
Worthington, M. (1972): Interferon system in mice infected with the scrapie agent. *Infect. Immun.* **6**, 643.
Zaltsman, G. P., Kuchuk, G. A., and Gurgenidze, A. G. (1979): *The Bases of Hyperbaric Physiology.* Meditsina, Moscow.

Description of Electron Micrographs (Plate XLVII):

Fig. 1. Specific vacuolization (status spongiosus) in the neuropil of cerebral cortex of a mouse infected with the scrapie agent. The arrow shows daughter vacuoles. Magn. 8 400x.
Fig. 2. Nonspecific vacuolization of a Purkinje's cell dendrite from the cerebellum of a mouse inoculated with normal mouse brain and subjected to hypoxia. Magn. 28,000x.
Fig. 3. A vacuole typical of the status spongiosus and containning membraneous formations in the cerebellum of a scrapie-infected mouse. Magn. 14,000x.
Fig. 4. Swelling of the post-synaptic terminal in the brain cortex of a scrapie-infected mouse. Magn. 28,000x.