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INFLUENCE OF AMINASINE ON EXPERIMENTAL SCRAPIE IN MICE

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Summary. — The influence of long-term treatment with aminasine — an inhibitor of monoamine oxidase — on duration of the incubation period, morbidity and on accumulation of the agent in the brain was followed in experimental scrapie of mice. Aminasine administration after intracerebral (i.c.) but not after intraperitoneal (i.p.) inoculation of the agent markedly prolonged the incubation period and slightly reduced the morbidity. The accumulation of the scrapie agent and the degree of histological lesions in the CNS detected 6 months post-inoculation (p.i.) were equal in both groups compared. The significance of these results is discussed from the viewpoint of the role of biogenic monoamines in the pathogenesis of scrapie.

Key words: biogenic monoamines; aminasine; scrapie; pathogenesis

Introduction

At present considerable information has accumulated suggesting the role of biogenic monoamines (BMA) in the pathogenesis of acute, chronic and latent virus infections (Popenenkova and Maslennikov, 1977). Much less is known on possible significance and place of BMA in the pathogenesis of slow infections belonging to subacute transmissible spongiform encephalopathies (STSE) caused by filterable agents essentially different from classical viruses (Gajdusek, 1978; Prusiner, 1982). The level of BMA is reduced in the brain of patiens with Jakob-Creutzfeld-disease (Brun *et al.*, 1971). Therefore, we attempted to find out whether aminasine depressing the activity of monoamine oxidase (MAO) would increase the activity of BMA in scrapie, an experimental model of STSE.

Materials and Methods

Animals. We used 2 to 3-week-old BALB/c mice.

The agent and inoculation conditions. Scrapie agent, strain C-506 was isolated from the brain of sheep suffering from scrapie (kindly provided by Dr. Gibbs, NIH, Bethesda, Md., U.S.A.) and passaged in mice. The agent was titrated by i.c. inoculation of mice, the titre being calculated according to Reed and Muench (1938). Experimental animals were inoculated i.c. (0.03 ml) or i.p. (0.2 ml) with 10% suspension of the scrapie-containing brain with the initial titre of 6.37 log

LD_{50}/0.03 ml. The control mice were inoculated with 10% suspension of normal mouse brain.

Aminasine. The drug produced by Lvov's Chemical Pharmaceutical Factory (U.S.S.R.) was injected subcutaneously (0.25% solution in a vol of 0.2 ml per mouse) 3 days before inoculation, then one day before inoculation (0.025% solution) and finally, once weekly for 5 months p.i.

Histological examination. The brains of mice were fixed in 10% formalin; paraffin blocks were cut and stained with haematoxylin and eosin or according to Nissl.

Results

The possible influence of aminasine on the process of scrapie infection was studied in different experimental variants including combination of i.c. or i.p. inoculated mice receiving aminasine and the corresponding controls not treated with aminasine. Each animal group consisted of 20—30 mice, in which the duration of incubation period, the level of sickness rate (taking into consideration the marked clinical manifestations) and the titre of the agent in CNS were determined. Injection of normal mice brain — alone or in combination with aminasine — did not result into appearance of any signs of the disease.

The scheme and the results of the experiment are shown in Table 1, from which follows that in mice inoculated with the scrapie agent by i.c. or i.p. routes, the disease with typical clinical symptoms of scrapie developed in 100% of animals by 150 and 210 days p.i. The combination of i.c. inoculation of the scrapie agent with aminasine treatment prolonged the incubation period by 30 days and lead to certain reduction of morbidity. At the same time aminasine treatment of mice inoculated by i.p. route neither changed the duration of incubation period nor the morbidity (observation time 9 months). Accumulation of the agent in mouse brain was determined 6 months p.i., i.e. in the period when either aminasine-treated or non-treated animals developed clinical symptoms. By this time the titre of the agent in brain was sufficiently high and practically did not differ in both groups compared. Histological examination of the brain of scrapie-infected mice either treated or non-treated with aminasine was performed 6 months p.i., it showed the existence of spongy oedema in the gray substance, pycnosis and vacuolization of neurons. The extent of histological changes was equal in both animal groups.

Table 1. Effect of aminasine in scrapie

Group	Inoculation route	Aminasine	No. of mice	Incubation period (days)	Morbidity (%)	Titre of the scrapie agent in CNS
Controls	i.c.	none	20			
	i.c.	yes	20			
Infected	i.c.	none	30	150	100	9.07*
	i.c.	yes	30	180	87	8.97*
	i.p.	none	30	210	100	n/e
	i.p.	yes	24	210	100	n/e

* Titre of scrapie agent was determined 6 months post-inoculation when clinical symptoms had developed (expressed in log LD_{50}/g); n/e = not examined.

Discussion

It is known that BMA provide mediator functions in the CNS. That is why quantitative changes of BMA are detected in brain in a number of diseases of CNS especially in parkinsonism (Brody *et al.*, 1970). Analysing the possible reasons of BMA reduction in parkinsonism, the increase of MAO activity seems striking, as indicated by the effect of MAO inhibitors on clinical symptoms (Ganev *et al.*, 1974). Based on the data of BMA reduction in CNS at STSE, we attempted to influence the animals inoculated with the scrapie agent by long-term treatment with aminasine, a drug decreasing MAO level in the brain (Chikvaidze *et al.*, 1967).

There was shown, that a 5 months treatment of scrapie-infected mice with aminasine resulted in marked extension of incubation period and some reduction of morbidity when the agent was injected directly into the brain, but not when it had been administered by i.p. route. It is known that the scrapie agent injected by subcutaneous or i.p. routes gets access to the brain after its accumulation in the spleen not being detected within brain for the first 1.5—2 months (Zlotnik, 1970; (Kimberlin and Walker, 1980). In addition, it was experimentally proved that the scrapie agent may reach the CNS by neural route (Roikhel *et al.*, 1983). According to our results, it can be suggested, that the interaction of the scrapie agent with the BMA system, altered due to aminasine treatment, depends not only on the stage of the disease, but also on the way by which the agent gets into the brain. Apparently, the scrapie agent, having passed a definite cycle of accumulation in the spleen before entering the brain, causes the usual course of the disease, independently of the BMA level in the CNS, whereas after direct i.c. inoculation the pathogenetic process is modified by the influence of the increased BMA level due to aminasine administration.

The results obtained do not allow to determine whether the shortened incubation period and reduced morbidity are due to a possible reduction of the agent in the CNS or a result of reparation of mediator functions in CNS injured during infection. At the same time the effect of aminasine allows to suggest that the change of BMA level in the CNS was not a secondary reaction, but plays a definite role in the pathogenesis of serapie. This is in accordance with the data on reduction of the level of the BMA substance serotonine in scrapie and Creutzfeldt-Jacob disease and with changes of clinical symptoms in scrapie-infected animals receiving serotonergic drugs; serotonine level in the CNS of scrapie-infected mice was by 20% lower than in controls (Goudsmit *et al.*, 1981; Rohwer *et al.*, 1981; Nyberg *et al.*, 1982). Bert *et al.*, (1977) point out the patricipation of the monoaminergic system in experimental kuru.

Obviously, pathogenetic role of the BMA system in STSE is an another sign linking these diseases with the infections caused by conventional viruses. Analysis of the role of BMA in acute and chronic influenza with the use of MAO inhibitors, showed the existing correlation between the BMA level and the development of infection. It allows to suggest that BMA could

function as nonspecific factor in pathogenesis of covert virus infections (Timakov *et al.*, 1975; Popenenkova *et al.*, 1977).

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