Short communication

Effect of K⁺ channel blockers on the clinical course and histological features of experimental allergic encephalomyelitis


Introduction – Beneficial clinical effects of 4-aminopyridine (4-AP) in multiple sclerosis (MS) have been reported. The use of 4-AP in MS is based upon its ability to facilitate conduction in axons blocked by demyelination. This improvement is due to blocking of potassium (K⁺) channels in these fibres. Because K⁺ channels also play an important role in immune mechanisms successful treatment with K⁺ channel blockers in neuroimmunological diseases may have several causes. Therefore it seems important to study effects of K⁺ channel blockers in animal models of autoimmune disease. Material & methods – We studied the effects of 4-AP and quinidine on actively induced acute experimental allergic encephalomyelitis (EAE) in Lewis rats. Results – There was no effect on the incidence of the disease. The severity of the disease was also unchanged although the disease duration was slightly diminished in the treated groups. Immunohistological comparison between the animals of different groups showed no differences. Conclusion – We conclude that 4-AP and quinidine are not capable of significantly changing the clinical course of EAE.

4-aminopyridine (4-AP) can restore conduction in blocked demyelinated nerves in animals (1–3) by prolonging nerve action potentials. This increase in action potential is the result of the blocking of potassium (K⁺) channels (4). Because of its ability to improve nerve conduction 4-AP is a promising drug for symptomatic therapy in multiple sclerosis (MS). Beneficial effects of 4-AP on clinical signs in MS have been published (5–9).

K⁺ channels are not only important in the central nervous system (CNS) but also in the immune system. Blocking of K⁺ channels influences the function of T lymphocytes (10), B lymphocytes (11, 12) and natural killer cells (13). Major histocompatibility class II (Ia) antigen expression can be inhibited by potassium channel blockers (14). Thus, treatment with K⁺ channel blockers like 4-AP may also have immunomodulatory effects. In MS clinical effects of 4-AP might, therefore, be the result of both neurophysiological and immunological changes.

The only studies of 4-AP in experimental allergic encephalomyelitis (EAE), the most frequently used animal model of MS, that have been performed up to date focused on the acute electrophysiological effects (15, 16). Treatment was not prolonged and clinical and histological effects were not studied. In experimental allergic neuritis (EAN) the K⁺ channel blocker quinidine was successfully administered (17). It resulted in a significant reduction of neurological deficits and considerably less inflammatory infiltration in target tissue. The dominant effect of quinidine was attributed to a reduction of the demyelinating autoimmune process.

In this study the effects of both 4-AP and quinidine were tested in EAE.
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Material and methods

Induction of EAE and assessment of clinical signs

EAE was induced in male Lewis rats by a single intradermal injection of guinea pig spinal cord homogenate together with Freund's complete adjuvant containing 10 mg/ml of heat-inactivated *Mycobacterium tuberculosis H37Ra* (Difco).

Rats were weighed and examined daily for clinical signs of EAE. Clinical signs were scored on a scale from 0 to 4 as follows: 0 = no clinical signs, 1 = loss of tail tonus, 2 = incomplete paresis of hind limbs, 3 = complete paralysis of hind limbs often accompanied by incontinence, 4 = death due to EAE.

Treatment with 4-AP and quinidine

Oral treatment with 4-AP started on the day of induction of EAE and was continued until the day the rats were killed. Experiments were performed in 2 series of 3 groups. Each group consisted of 6 rats. 4-AP was dissolved in different concentrations for each group in the drinking water leading to a daily intake of respectively 1, 5, 10 and 15 mg per kg body weight.

Intraperitoneal (i.p.) treatment with quinidine was given in a separate experiment in 2 groups of 8 rats. The rats received 4 mg quinidine dissolved in 1 ml sterile saline. This treatment started simultaneously with the induction of EAE and was repeated daily until the end of the study. Control animals received 1 ml sterile saline i.p. daily on the same days.

Histological and immunohistochemical examination

At Day 24 after immunization the rats were killed and CNS tissues (cerebrum, medulla oblongata, cerebellum, optic nerve and thoraco-lumbar spinal cord) were obtained. In the experiment in which the rats received i.p. injections 2 animals of each group were killed at Day 11 after immunization. The technique we used is described elsewhere (18). The following monoclonal antibodies and antisera were used: mouse moabs to rat macrophages ED1, ED2, ED3 and ED8 obtained by the production of a cloned hybrid cell line (19, 20); mouse moab against rat Ia antigen (OX4), against rat T lymphocytes (W3/13), against CD4 antigen (W3/25) and against CD8 antigen (OX8) obtained from Serotec (Oxford, UK).

Results

The clinical data of the experiments are summarized in Table 1. Oral treatment with 4-AP was well tolerated and had no effect on the occurrence of clinical signs nor on the severity. Because 2 of 6 animals treated with the highest dose 4-AP did not develop clinical signs it seems as if this treatment is to some extend effective, however in that experiment also 2 of 6 control animals failed to develop clinical symptoms. Treatment with 4-AP did have an effect on the duration of the symptoms. Overall, the mean disease duration in the control animals was 7.7 days and in the treated animals 5.6 days (difference 2.1 day; 99% confidence interval 0.6–3.6 days). Treatment with i.p. quinidine had no effect on the clinical course of EAE in regard to the severity and the duration.

Histological and immunohistochemical examination of the CNS tissues did not reveal differences between the different groups. There was no signs of reduced inflammatory reaction in the 4-AP treated animals, nor of reduced Ia expression. Also the CNS tissues of the rats treated with quinidine that were sacrificed at the day of expected onset of the disease showed no difference compared to the tissues obtained from control animals on the same day.

Discussion

Theoretically, 4-AP is able to influence immune mechanisms because of its potential effect on potassium channels. In EAN another potassium channel blocker, quinidine, indeed reduced the inflammatory reaction and thereby the severity of clinical symptoms (17). We used the same therapeutic regimen in our EAE model but no effects were observed. There was no effect on the clinical symptoms. The immunohistological features both at the start of the inflammatory reaction in the CNS and at the end of the disease in the treated animals were identical with those in the control animals.

Oral treatment with 4-AP in different dosages did not change the incidence or the severity of EAE. However the duration of the symptoms was reduced. At the end of an attack the clinical symptoms in

Table 1. Effect of 4-aminopyridine (4-AP) and quinidine on the clinical symptoms of EAE.

<table>
<thead>
<tr>
<th>Oral treatment</th>
<th>n</th>
<th>Incidence EAE</th>
<th>Max severity ≥2</th>
<th>Mean disease duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>first experiment</td>
<td></td>
<td></td>
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<tr>
<td>4-AP 1 mg*</td>
<td>6</td>
<td>6/6</td>
<td>2/6</td>
<td>6.7 days</td>
</tr>
<tr>
<td>4-AP 5 mg*</td>
<td>6</td>
<td>6/6</td>
<td>2/6</td>
<td>6.3 days</td>
</tr>
<tr>
<td>controls</td>
<td>6</td>
<td>6/6</td>
<td>2/6</td>
<td>8.3 days**</td>
</tr>
<tr>
<td>second experiment</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>4-AP 10 mg*</td>
<td>6</td>
<td>5/6</td>
<td>2/5</td>
<td>4.2 days</td>
</tr>
<tr>
<td>4-AP 15 mg*</td>
<td>6</td>
<td>4/6</td>
<td>2/4</td>
<td>4.8 days</td>
</tr>
<tr>
<td>controls</td>
<td>6</td>
<td>4/6</td>
<td>2/4</td>
<td>5.8 days</td>
</tr>
<tr>
<td>i.p. treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>quinidine 4 mg</td>
<td>8</td>
<td>6/6***</td>
<td>2/6</td>
<td>4.5 days</td>
</tr>
<tr>
<td>controls</td>
<td>8</td>
<td>6/6***</td>
<td>2/6</td>
<td>5.0 days</td>
</tr>
</tbody>
</table>

* daily dose per kg body weight
** significant p<0.05, Wilcoxon rank sum test
*** per group 2 animals were killed before onset of symptoms
EAE consist mainly of a (partial) loss of tail tonus. Although we did not perform neurophysiological studies in our rats we assume that the reduction of the duration of symptoms might be due to improvement of nerve conduction resulting in an increased tail tonus. There were no signs of immunomodulatory effects of 4-AP that could account for the observed reduction of the duration of symptoms.

In summary, we did not find evidence for the inhibition of the immune response in EAE by 4-AP or quinidine. The only effect we observed was a reduction of the disease duration probably due to improvement of nerve conduction. The dosages of 4-AP we used are much higher than those used in humans. Based on this study it is unlikely to expect important immunomodulatory effects to occur in MS patients to whom 4-AP is given. This is compatible with the clinical experience with 4-AP that supports predominant effects on nerve conduction as the main effector mechanism.

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References