GTS-21, a Nicotinic Agonist, Protects against Neocortical Neuronal Cell Loss Induced by the Nucleus Basalis Magnocellularis Lesion in Rats

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ABSTRACT—Effect of subchronically administered GTS-21 [3-(2,4-dimethoxybenzylidene)-anabaseine dihydrochloride], a selective nicotinic agonist, on neuronal cell loss caused by nucleus basalis magnocellularis (nBM) lesion was studied in rats. After 2 weeks of bilateral nBM excitotoxic lesion, GTS-21 was orally administered once daily for 20 weeks. Neuronal cell loss was observed in layers II-III of the parietal cortex in the lesioned control rats. GTS-21 significantly attenuated the neuron loss in these layers. These results suggest that GTS-21 exhibits a protective action against the neuronal cell death in the parietal cortex and may have a beneficial effect on neurodegenerative disorders such as an Alzheimer-type disease.

Keywords: GTS-21, Nicotinic agonist, Neuronal cell loss

Dysfunction of central cholinergic systems has been demonstrated in the brain of Alzheimer's disease (AD) patients. Whitehouse et al. (1) have found neuronal cell loss and cytopathological abnormalities in the nucleus basalis magnocellularis (nBM) in AD patients. These authors also have demonstrated a strong correlation between the frequency of neuritic plaques in the cerebral cortex and the neuronal loss in the nBM. Moreover, the decrease of nicotinic receptors in the frontal cortex has been shown in AD patients (2). These findings suggest that the nicotinic receptor system plays an important role in cognitive and neurophysiological function.

The recent report by Newhouse et al. (3) has demonstrated that the intravenous administration of nicotine partially improves cognitive performance in AD patients, suggesting the role of central nicotinic acetylcholine receptors in cognitive and pathophysiological changes in AD. Moreover, their findings give rise to the possibility that stimulation of the remaining nicotinic receptors can alleviate the cognitive deficits induced by dysfunction of central cholinergic systems.

GTS-21 [3-(2,4-dimethoxybenzylidene)-anabaseine dihydrochloride], a selective nicotinic agonist, reportedly improves the learning performance in nucleus basalis-lesioned rats (4). Moreover, a recent study indicates that nicotinic drugs exert a protective activity against fimbrial transection-induced neuronal cell loss when administered systemically 1 hr before and every 12 hr after the transection (5).

In rodents, an injection of ibotenic acid, an excitotoxin, into the nBM causes a decrease in the number of neuronal cells in the cerebral cortex and attenuates the cortical cholinergic activities in rats (6, 7). This nBM lesion is also known to impair memory-related behaviors. Thus, the nBM-lesioned rats provide an animal model with brain dysfunction similar to that observed in AD and have been used to elucidate the therapeutic potential of drugs in AD patients.

In the present study, to elucidate this possibility, we examined whether orally administered GTS-21 exerts a protective action against neocortical neuronal cell loss caused by bilateral nBM-lesion in rats.

Male Wistar rats (8-weeks-old; Clea Japan, Inc., Tokyo) were housed 3–4 per cage at least for 1 week before the start of the experiments. The housing was thermally maintained at 21 ± 1°C with a constant humidity (45–65%) and a 12-hr light-dark cycle (lights on 06:00–18:00); the animals were given free access to food and water.

In the experiments, the animals (9–10-weeks-old) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and then fixed on a stereotaxic apparatus. Five
Fig. 1. Effects of GTS-21 and nicotine on the histological changes in layers II-III of the parietal cortex area 2 of nBM-lesioned rats. At 22 weeks after lesioning, histological observation was performed as described in the text. A and B: Sham-operated group, C and D: nBM-lesioned control group, E and F: GTS-21-treated group, G and H: Nicotine-treated group. Scale bar represents 1 mm in A, C, E and G and 20 μm in B, D, F and H. Arrows in D show shrunken and strongly stained cells.
micrograms of ibotenic acid (Sigma Chem., St. Louis, MO, USA) in 0.5 μl phosphate-buffered saline (PBS, pH 7.4) was injected bilaterally into the nBM (anterior, 7.2 mm; lateral, 2.5 mm; and ventral, 7.0 mm) according to the atlas of Paxinos and Watson (8). Rats that received only needle insertion into the nBM were used as the sham-operated control group.

GTS-21 (10 mg/kg), (−)-nicotine (3 mg/kg) or distilled water was orally given once daily over a 20-week period from 2 weeks after the operation. The doses of GTS-21 and nicotine were chosen based on the data reported by Meyer et al. (4) that both drugs enhanced cognition in nBM-lesioned rats. No undesirable effect was observed following daily administration of GTS-21 at 10 mg/kg, p.o. (M. Nanri et al., unpublished data). At 22 weeks after ibotenic acid treatment, the rats were anesthetized with pentobarbital sodium and perfused with 4% PBS paraformaldehyde solution through the left cardiac ventricle. The brain was removed, and a tissue block including the nBM area was dissected out and embedded in paraffin. Tissue slices (5-μm-thick) were prepared and stained by Nissl's method. Three different brain regions (frontal cortex areas 1 and 2, parietal cortex area 1 and parietal cortex area 2) in a slice were selected and histologically analyzed. Neuronal cells in layers II and III of the cerebral cortex in each hemisphere were counted using an image analysis system (Color Image Analyzer, Image Command 5098; Olympus, Tokyo), and the neuronal densities (neurons/0.09 mm²) were calculated. Only the neuron with a distinct nucleus was counted as an undamaged cell.

GTS-21 was synthesized in our laboratory as described previously (9). (−)-Nicotine was administered as (−)-nicotine dihydrate (Sigma Chem.) at the dose (8.6 mg/kg) equivalent to 3 mg/kg (−)-nicotine.

Data were expressed as the means±S.E.M. and analyzed with Tukey-Kramer's multiple comparisons test. Differences of P<0.05 were considered significant.

Microscopic observation showed that neuronal cells were quite visible and well-preserved in the cerebral cortex in the sham-operated rats. Most cells in layers II-III of the cerebral cortex exhibited shrinkage at 22 weeks after bilateral injection of ibotenic acid to the nBM and were strongly stained (Fig. 1). An increase in glial cells and a decrease in the soma size of neurons were observed in parietal cortex area 2. However, changes in the soma size of neurons were not observed in other cortical areas. The numbers of glial cells in the GTS-21-treated and nicotine-treated nBM-lesioned animals were less than those in the vehicle-treated nBM-lesioned animals.

As summarized in Table 1, nBM lesion significantly decreased the neuronal cell density in parietal cortex areas 1 and 2 by 17.8% and 20.2% compared to the same areas of the sham-operated controls, respectively. In contrast, nBM lesion did not change the neuronal cell density in frontal cortex areas 1 and 2. The daily administration of GTS-21 for 20 weeks significantly prevented the reduction of neuronal cell density in parietal cortex area 2 caused by nBM lesion. GTS-21 administration also tended to inhibit the neuronal cell loss in parietal cortex area 1 and frontal cortex areas 1 and 2. Nicotine slightly attenuated the decrease in neuronal cell density in these three areas of the cerebral cortex, but was not significantly.

The present results demonstrate that GTS-21 exerts a protective activity against nBM lesion-induced neuronal cell loss in the cerebral cortex in rats and suggest that this compound may have a beneficial effect on cognitive impairment caused by neuropathological changes in central cholinergic systems.

The nBM lesion caused by ibotenic acid reportedly reduces choline acetyltransferase activity in the frontoparietal cortex in rats (10). This lesion also causes neuronal cell loss in the frontoparietal cortex (7, 10). In the present study, the neuronal cell density in the cerebral

<table>
<thead>
<tr>
<th>Drug</th>
<th>Animal No.</th>
<th>Cortical area</th>
<th>Neuronal density (neurons/0.09 mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Frontal cortex areas 1 and 2</td>
<td>Parietal cortex area 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham operation</td>
<td>11</td>
<td>118.5±7.2 (100.0)</td>
<td>128.4±6.3 (100.0)</td>
</tr>
<tr>
<td>Lesioned control</td>
<td>10</td>
<td>110.9±4.0 (93.6)</td>
<td>106.3±4.3 (82.8)</td>
</tr>
<tr>
<td>GTS-21 10 mg/kg</td>
<td>10</td>
<td>114.9±4.5 (97.0)</td>
<td>111.4±5.0 (86.8)</td>
</tr>
<tr>
<td>Nicotine 3 mg/kg</td>
<td>10</td>
<td>118.8±7.4 (100.3)</td>
<td>118.5±6.5 (92.3)</td>
</tr>
</tbody>
</table>

Lesioned control and sham operation rats were given distilled water instead of drug. Each value is the mean±S.E.M. of 10−11 animals/group. *P<0.05, compared with the lesioned control group. Values in parentheses represent percentages relative to sham-operated controls.
cortex was decreased by nBM lesion with the following order of decreasing degree: parietal cortex area 2 ≥ parietal cortex area 1 > frontal cortex areas 1 and 2. These findings agree with the data reported by Roßner et al. (6). These authors prepared brain coronal sections at −3.3 mm distance from the bregma and found that ibotenic acid-induced nBM lesion produced a decrease in acetylcholinesterase in parietal cortex areas 1 and 2, but not in frontal cortex area 1 or 2. Moreover, Wellman and Sengelaub (7) have shown atrophy in the cortical laminae in the bilateral nBM-lesioned rats and indicated that this histological change is predominantly due to a decrease in soma size of neuronal cells in this area. Taken together, the present results suggest that bilateral nBM lesion selectively causes neuronal damages in the parietal cortex, resulting in a decrease in neuronal cell density and morphological changes in neuronal cells in parietal cortex areas 1 and 2.

It is quite interesting that the GTS-21-treated nBM-lesioned animals exhibited a significantly higher density of neuronal cells in parietal cortex area 2 than the vehicle-treated nBM-lesioned rats. It has been reported that stimulation of nicotinic acetylcholine receptors but not muscarinic acetylcholine receptors attenuates the nBM lesion-induced decrease in neuronal cell density in frontal-parietal cortex layer II (11). The central nicotinic-cholinergic system is known to stimulate expression of nerve growth factor (NGF) (12). Furthermore, NGF synthesis stimulator has improving effects on memory impairment in basal forebrain-lesioned rats (13). Recently, Martin et al. (5) reported that GTS-21 exerts cytoprotective activity similar to that of NGF in vitro. Therefore, it is likely that the protective activity of GTS-21 against the nBM lesion-induced decrease in neuronal cell density in this study may be mediated by NGF or the NGF-like activity of GTS-21.

In this study, chronic administration of nicotine showed a tendency to suppress the neuronal cell damages induced by bilateral nBM lesion, but the effect was not significant. The exact reason for the difference in the neuroprotective activity between GTS-21 and nicotine remains unclear, but several factors may explain this difference. First, the dose of nicotine used in this study was not sufficient to produce a significant protective activity against nBM lesion-induced neuronal cell loss. Although chronic administration of nicotine reportedly produce undesirable side effects (14), the larger doses of nicotine will need to be examined. Secondly, the difference in the selectivity for the nicotinic receptor subtypes between GTS-21 and nicotine may be implicated in the lack of cytoprotective activity of nicotine in the present study. GTS-21 has been reported to have 4 times higher affinity than nicotine for the nicotinic receptor subtype that consists of α7-subunits (15). It could be speculated that the α7-subunit-composed nicotinic receptor subtype plays an dominant role in the cytoprotection caused by GTS-21.

Although a report demonstrated the beneficial effect of nicotine on the cognitive function of AD patients (3), the clinical use of nicotine for AD patients is limited because of its side effects and lack of effective mode of administration. The present findings that chronic oral administration of GTS-21 attenuated the neuronal cell loss in the cerebral cortex without producing undesirable side effects suggest that this compound may be useful for treatment of AD patients.

REFERENCES
12 Terry JAV and Clarke MSF: Nicotine stimulation of nerve

