

GTS-21, a Nicotinic Agonist, Attenuates Multiple Infarctions and Cognitive Deficit Caused by Permanent Occlusion of Bilateral Common Carotid Arteries in Rats

Masato Nanri^{1,2,*}, Hidekazu Miyake¹, Yukihiisa Murakami², Kinzo Matsumoto² and Hiroshi Watanabe²

¹Section of Pharmacology Research Laboratory, Taiho Pharmaceutical Co., Ltd.,

224-2 Ebisuno, Hiraishi, Kawauchi-cho, Tokushima 771-0132, Japan

²Department of Pharmacology, Research Institute for Wakan-Yaku (Oriental Medicines),

Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan

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ABSTRACT—We examined the effects of GTS-21 [3-(2,4-dimethoxybenzylidene)-anabaseine dihydrochloride], a nicotinic agonist, on histopathological changes of the brain and radial maze learning performance in rats with permanent occlusion of the bilateral common carotid arteries (2VO) and elucidated whether this compound has a protective effect against the neuronal degeneration and spatial cognitive deficit caused by chronic ischemia. Rats were administered GTS-21 (1 and 10 mg/kg, p.o.) or vehicle 24 hr and 30 min before the 2VO operation and then once daily for 2 months after the operation. The 2VO rats given vehicle had multiple infarctions in the cerebral cortex, hippocampus and striatum and rarefaction in the white matter at 2 months after the operation, although the number and distribution of infarctions varied among individual animals. In addition, the 2VO rats given vehicle showed a higher rate of errors in the acquisition trials of the 8-arm radial maze task than sham-operated controls. However, 2VO rats treated with GTS-21 (1 and 10 mg/kg, p.o.) showed significantly decreased neuropathological changes and less errors in the acquisition trials compared to the vehicle-treated 2VO rats. These results indicate that GTS-21 attenuates impairment of spatial cognitive deficit and progressive neuronal degeneration induced by 2VO and suggest that this compound is beneficial for the treatment of neurodegenerative diseases following chronic cerebral hypoperfusion.

Keywords: GTS-21, Nicotinic agonist, Cerebral ischemia, Multiple infarction, Hypoperfusion

There is evidence demonstrating the dysfunction of central cholinergic systems in Alzheimer's disease (AD) patients (1). Newhouse et al. (2) and Sahakian et al. (3) have reported that the administration of (–)-nicotine improves attention and information processing in AD patients, suggesting the involvement of central nicotinic acetylcholine receptors (nAChR) in the impaired cognitive function and pathophysiology in AD. These findings raise the possibility that stimulation of central nAChR may alleviate the cognitive deficits induced by dysfunction of central cholinergic systems. Disturbances in the central cholinergic systems also have been demonstrated in patients with cerebral vascular dementia (4, 5). Thus, it is conceivable that stimulation of central nAChR may improve the cognitive dysfunction and neuropathology

in patients suffering from cerebral vascular dementia. However, the therapeutic potential of (–)-nicotine for dementia is very limited because of its side effects and the lack of an effective administration route.

Permanent occlusion of the bilateral common carotid arteries (2VO) in rats is a chronic cerebral hypoperfusion model, and this model has been reported to exhibit impairment of learning and memory and circadian rhythm (6–8). In addition, it has been suggested that the impairment of cognitive function in 2VO rats is associated with neuronal degeneration such as multi-infarction and rarefaction and a reduction in the level of acetylcholine in the brain and that the impairment of cognitive function and pathophysiology observed in this model is similar to the pathosis in AD and cerebrovascular disease (9–11).

GTS-21 [3-(2,4-dimethoxybenzylidene)-anabaseine dihydrochloride] is a newly synthesized nAChR agonist

* To whom correspondence should be addressed⁽¹⁾.

with fourfold higher affinity than nicotine for the nAChR consisting of $\alpha 7$ -subunits (12). GTS-21, unlike nicotine, does not show undesirable effects (13). In our previous study, the peroral administration of GTS-21 had a protective effect against neuronal death in the hippocampal CA1 area caused by transient ischemia and neuronal cell loss in the parietal cortex caused by nucleus basalis magnocellularis (nBM) lesion (14, 15). From these findings, it could be expected that GTS-21 exerts a protective action against the 2VO-induced neuronal degeneration by stimulating nAChR in the brain and thereby improves the impaired learning behavior in the 2VO rats. Therefore, the present study had the following aims: i) to quantify infarctions and rarefaction following 2VO by means of histopathological examination of coronal section at six selected coordinates and ii) to assess the effects of sub-chronic treatment with GTS-21 before and after permanent 2VO on the learning behavior and neuropathological changes in rats.

MATERIALS AND METHODS

Animals

Male Wistar rats (13-week-old; Japan SLC Inc., Shizuoka) were used. The animals were housed 3–4 per cage for at least 1 week before the experiment. The animals were housed in a room thermostatically maintained at $24 \pm 1^\circ\text{C}$ with constant humidity ($55 \pm 5\%$) and a 12-hr light-dark cycle (lights on 07:30–19:30) and allowed free access to food and water. During the radial maze task, the animals were maintained on a restricted feeding schedule designed to keep their body weight at approximately 85% of the free-feeding level.

Procedure

The surgery was performed as described previously (7). Briefly, rats were anesthetized with pentobarbital-Na (30 mg/kg, i.p.). The bilateral common carotid arteries were exposed and occluded with silk sutures simultaneously in the permanent 2VO group. As sham-operated controls, another group of animals received the same operation without ligation.

Eight-arm radial maze

The 8-arm radial maze consisted of an octagonal platform (30-cm-across) and eight arms (50×11.5 cm) radially extending from the platform. The maze was elevated 40 cm above the floor. Small cups (3 cm in diameter and 5 mm in depth) were mounted at the end of each arm as receptacles for reinforcer (45 mg pellet; Bio-Serv, Frenchtown, NJ, USA). Guillotine-type doors surrounded the platform and controlled access to each arm.

Eight-arm radial maze task learning

Each animal was handled for 5–10 min daily for 2 days and was allowed 5 days for adaptation to the maze. Thereafter, these animals received 2VO or sham operation. Maze learning was started 10 days after the surgical operation. Each rat was placed individually on the central platform with all guillotine doors closed and then all of the doors were opened simultaneously to allow the animal to access the arms freely. Entry into the arm that the rat had not previously visited was recorded as a correct choice, and re-entry was counted as an error. The number of errors and the number of initial correct choices (i.e., the number of correct responses before the first error) were used as indices of radial maze performance. The trial was judged when the rat had visited all eight arms or had spent 10 min on the maze. Each rat was tested once daily for 5 days. Data are shown as the average of two trials.

Histology

The histological experiments were performed at 2 months after 2VO operation. Under pentobarbital (50 mg/kg, i.p.) anesthesia, the rats were perfused intracardially with heparinized saline and then with 10% formalin solution. The whole brain was removed, and a tissue block containing the hippocampus area was dissected out and embedded in paraffin. Six coronal sections (5- μm -thick) were taken at the level from the striatum to the hippocampus (i.e., 1.7, 0.7, -0.4 , -1.8 , -3.14 and -4.3 mm from the bregma according to the atlas of Paxinos and Watson (16)) using a frozen-stage microtome and stained with 1% Cresyl violet. The number of infarctions in the cerebral cortex, striatum and hippocampus and the number of rarefactions in the white matter were counted by microscopic observation of each coronal section. One infarction or rarefaction area in a section was simply counted as one, no matter what the size of the area is, because the size of the damaged area in a section differed depending on the distance from the core of infarction or rarefaction. The total number of infarctions and rarefaction in each brain area was calculated from 6 sections as an index of 2VO-induced histological damage to evaluate the effect of GTS-21 on 2VO-induced neuropathological changes.

Drugs

GTS-21 was synthesized by Taiho Pharmaceutical Co., Ltd., as described previously (17). To examine the pre- and postoperative effects of GTS-21 on 2VO-induced neuronal and cognitive impairment, GTS-21 (1 and 10 mg/kg, p.o.) or distilled water was given to rats 24 hr and 30 min before 2VO operation and once daily over a 2-month period after 2VO operation. GTS-21 was given 30 min before the radial maze learning performance test.

Doses are expressed in terms of salts.

Statistics

Data obtained by behavioral and histological studies are expressed as means \pm S.E.M. and were analyzed by Dunnett's test and Wilcoxon test, respectively. Differences with $P < 0.05$ were considered significant.

RESULTS

Histological observation

Figures 1 and 2 show the typical neuropathological changes observed in the cerebral cortex, striatum, hippocampus and white matter at 2 months after 2VO. The cortical histological changes were also evident in the parietal cortex area 1. Neuronal loss, shrinkage and dark staining of neurons and infarction were observed in the CA1 areas of the hippocampus in 2VO rats. Infarction was distributed in all regions of the striatum of 2VO rats. Most of the 2VO rats histologically examined in this study also exhibited rarefaction in the white matter of the corpus callosum and cingulum.

Infarctions in the cerebral cortex, striatum and hippocampus were observed in coronal sections obtained at 1.7 to -4.30 mm, 1.7 to -1.80 mm and -1.80 to -4.30 mm, respectively, from the bregma. Moreover, rarefaction of the white matter was observed in coronal sections 1.70 to -4.30 mm from the bregma. As shown in Fig. 1, GTS-21 at a dose of 10 mg/kg prevented the rarefaction in the white matter induced by the ischemia. To analyze quantitatively the effects of GTS-21 on this histological damage, we calculated the total numbers of histopathological changes (infarction and rarefaction) in these sections. As summarized in Table 1, GTS-21 (1 and 10 mg/kg/day, p.o.) administration that started from 1 day

before 2VO operation and continued for 2 months after the operation attenuated 2VO-induced rarefaction in the white matter and infarction in the cerebral cortex. Furthermore, GTS-21 significantly decreased the total number of histopathological changes in the 2VO rat brain. However, repeated treatment with 0.1 mg/kg/day GTS-21 had no effect on the total number of histopathological changes caused by 2VO (data not shown).

Effects of GTS-21 on the acquisition performance of 2VO rats in the radial maze task

Impairment of maze learning performance in 2VO rat was observed 10–35 days after the operation. The 2VO rats showed a higher error rate and less initial correct responses than the sham-operated control during a repeated training period. The repeated administration of GTS-21 (1 and 10 mg/kg/day, p.o.) before and after 2VO operation significantly decreased the number of errors and increased the number of initial correct responses (Fig. 3). However, no significant improvement of maze performance was observed in the 2VO rats given repeated administration of 0.1 mg/kg/day GTS-21 (data not shown).

DISCUSSION

In the previous study, the 2VO rats exhibited infarction in the cerebral cortex, striatum and hippocampus CA1 and rarefaction in the white matter but the extent of histological brain damage varied among individual animals (9). In the present study, we found by calculating the total number of infarctions and rarefaction from 6 coronal brain sections that 2VO rats exhibit constant histological damages in the brain. In addition, these lesions observed in the brains of 2VO rats seem quite different from those found in the acute ischemic model,

Table 1. Preventive effects of subchronic administration of GTS-21 on the number of histopathological changes following permanent 2VO in rats

Structures	Number of histopathological changes			
	Sham operation	2VO operation		
	Vehicle	Vehicle	GTS-21, 1 mg/kg	GTS-21, 10 mg/kg
Cerebral cortex	ND	3.6 ± 1.4	$0.7 \pm 0.6^*$	2.9 ± 1.4
White matter	ND	4.6 ± 1.2	$0.6 \pm 0.4^{**}$	$0.3 \pm 0.2^{**}$
Striatum	ND	2.1 ± 0.6	0.8 ± 0.6	0.8 ± 0.4
Hippocampus	ND	1.9 ± 0.7	1.6 ± 0.6	1.5 ± 0.7
Total	ND	12.2 ± 2.3	$3.7 \pm 1.4^{**}$	$5.5 \pm 1.6^*$

Rats were orally pretreated with GTS-21 (1 and 10 mg/kg) or distilled water from 24 hr and 30 min before the 2VO and once daily for 2 months. Each value is a mean \pm S.E.M. and represents the total number of histopathological changes detected in 6 coronal sections. $^*P < 0.05$, $^{**}P < 0.01$, compared with the 2VO vehicle group (Wilcoxon test). N=10. ND, not detected.

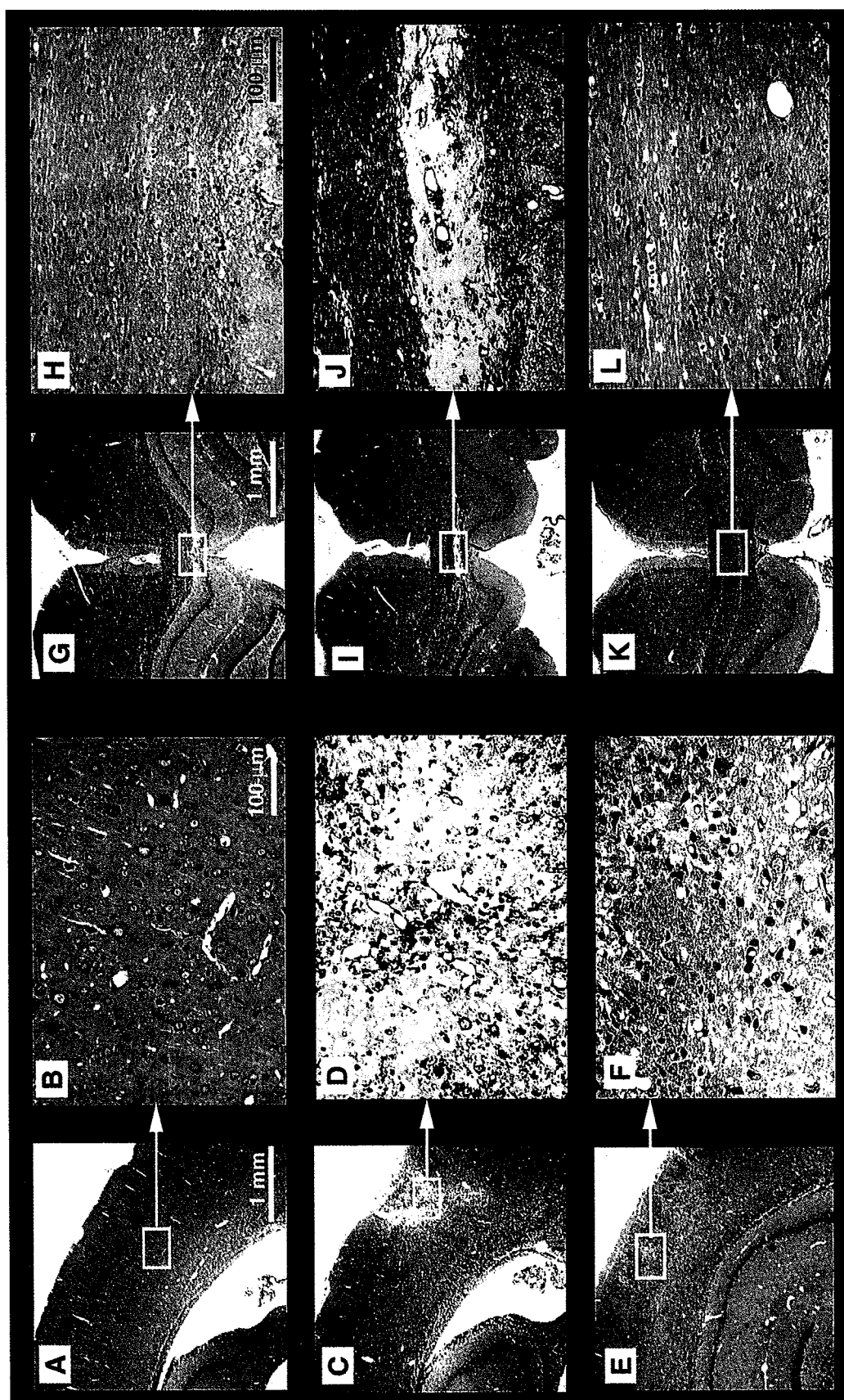


Fig. 1. Histopathological changes in the cerebral cortex and white matter following 2VO operation. A and B: the cerebral cortex of the vehicle-sham operation group, C and D: vehicle-2VO operation control group, E and F: GTS-21 (10 mg/kg)-treated group, G and H: the white matter of vehicle-sham operation group, I and J: vehicle-2VO operation control group, K and L: GTS-21 (10 mg/kg)-treated group. GTS-21 was given to rats 24 hr and 30 min before 2VO operation and once daily over a 2-month period after the operation.

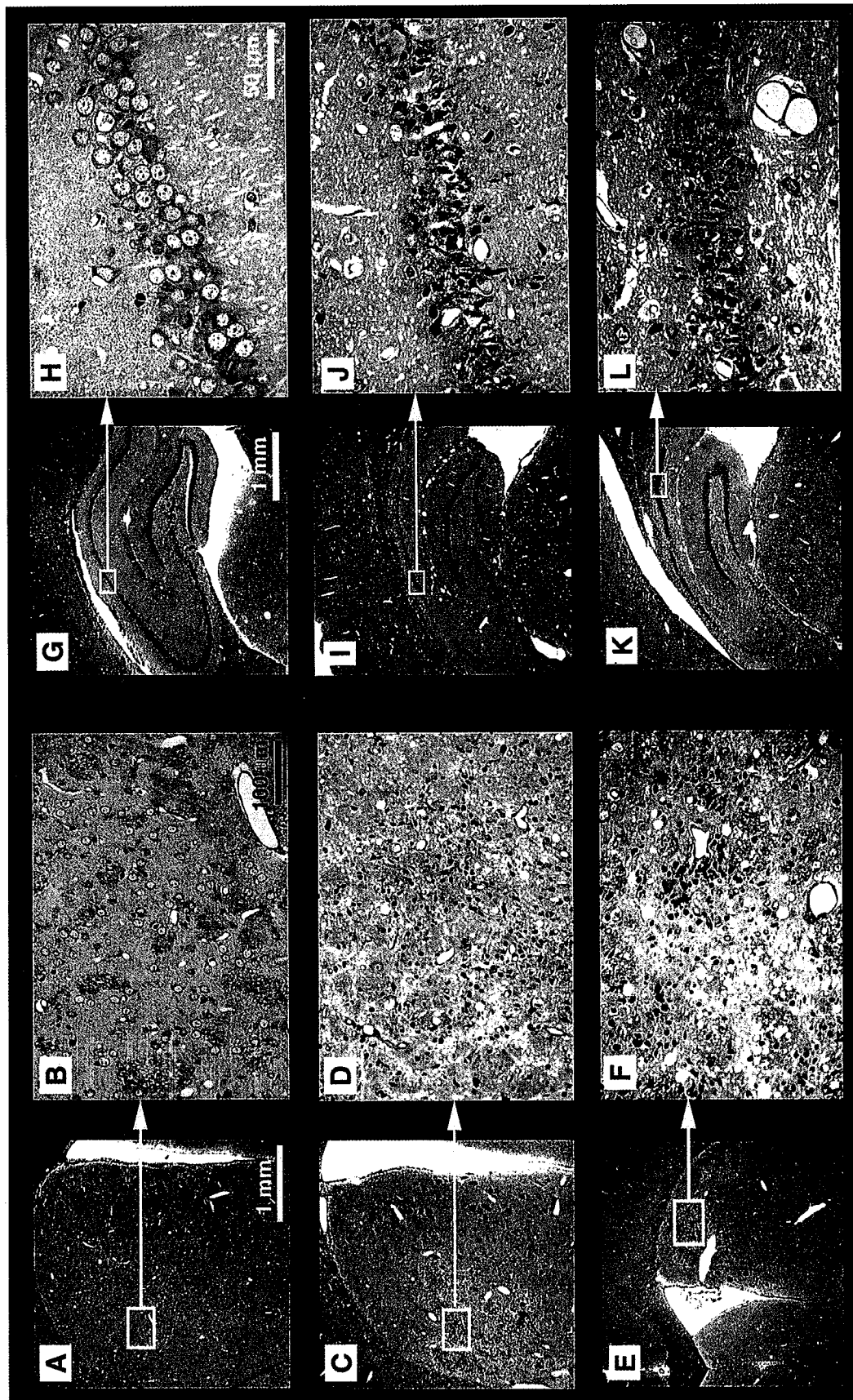


Fig. 2. Histopathological changes in the striatum and the hippocampus following 2VO operation. A and B: the striatum of the vehicle-sham operation group, C and D: vehicle-2VO operation control group, E and F: GTS-21 (10 mg/kg)-treated group, G and H: the hippocampus of the vehicle-sham operation group, I and J: vehicle-2VO operation control group, K and L: GTS-21 (10 mg/kg)-treated group. GTS-21 was given to rats 24 hr and 30 min before 2VO operation and once daily over a 2-month period after the operation.

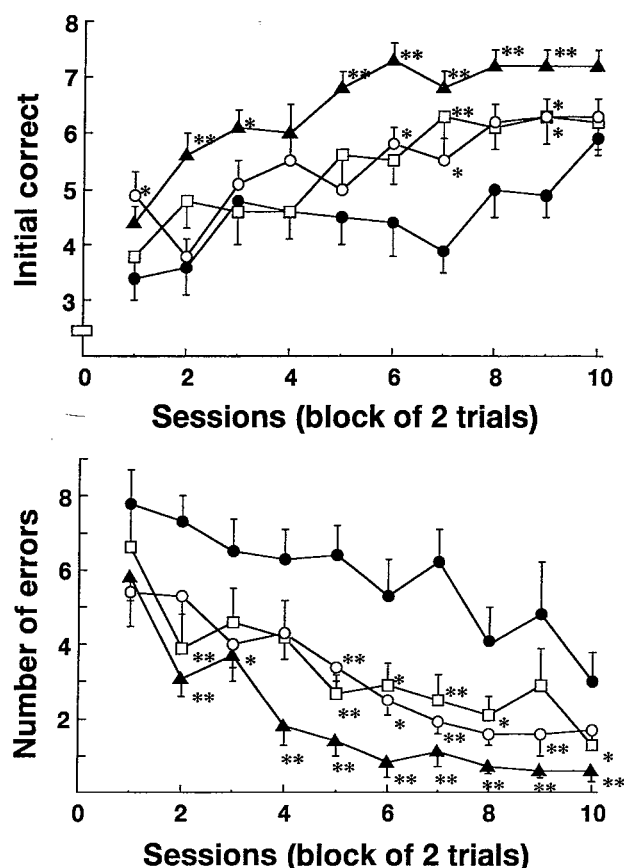


Fig. 3. Effects of subchronic administration of GTS-21 (1, 10 mg/kg/day) on the permanent 2VO-induced disruption of the radial maze learning performance. The learning task was started 10 days after 2VO. GTS-21 (1, 10 mg/kg) or distilled water was given p.o. 24 hr and 30 min before 2VO operation, and once daily over a 2-month period after the operation. The number of animals in each group was 10. * $P < 0.05$ and ** $P < 0.01$ vs vehicle-treated 2VO group (Dunnett's test). ▲, Sham operation + vehicle; ●, 2VO operation + vehicle; □, 2VO operation + GTS-21 (1 mg/kg); ○, 2VO operation + GTS-21 (10 mg/kg).

since transient occlusion of bilateral carotid arteries in gerbils is known to mainly cause neuronal cell death in the hippocampal area (18).

The pathological changes occurred following 2VO were quite similar to those observed in patients suffering from multi-infarct dementia, Binswanger's and Alzheimer's disease, and have been considered to be associated with various forms of cognitive dysfunction (11, 19, 20). In the present behavioral experiments, learning performance in the radial maze task was severely impaired in the 2VO rats. The memory used in this task is generally considered to be the working memory (6). This type of memory is analogous to recent memory in humans (21) and is known to be more severely impaired than remote memory in human dementia (22). Taken together, the present results suggested that the 2VO rat is a useful model in which to

investigate the pathophysiology of human dementia and to elucidate the therapeutic potential of drugs for this disease, especially cerebrovascular disease.

In the present study, we found that when peroral administration of GTS-21, a novel nAChR agonist, was started 1 day before 2VO operation and continued for 2 months after the operation, this compound significantly attenuated 2VO-induced histological lesions in the brain and improved radial maze performance. Ni et al. (9) reported that the 2VO rats exhibited a decrease in acetylcholine contents in the brain. In addition, 2VO operation has been shown to decrease acetylcholine receptor binding and choline acetyltransferase activity in the cortex, hippocampus and striatum (23, 24). Therefore, the protective action of GTS-21 was associated with enhancing the function of the nAChR. Akaike et al. (25) and Kaneko et al. (26) have demonstrated that GTS-21 suppressed glutamate-induced neurotoxicity in cultured cortical neurons and that this effect was blocked by α -bungarotoxin, an $\alpha 7$ -selective antagonist. Thus, a plausible explanation for the protective action of GTS-21 observed here in 2VO rats is that GTS-21 stimulation of $\alpha 7$ nicotine receptors may directly suppress 2VO-induced neuronal damage in the brain, although it is unclear to what extent glutamatergic systems contribute to chronic cerebral hypoperfusion-induced neuronal damage in rats.

When GTS-21 administration was started 7 days after 2VO, it failed to improve learning behavior or neuropathological changes in 2VO rats (data not shown). The precise reason for the differences in the neuroprotective effect between the two treatment schedules remains unclear, but it may be due to the time course of appearance of 2VO-induced neuropathological changes. Wakita et al. (27) showed that activation of microglia, an early mediator of rarefaction, occurs in the white matter within a day after 2VO operation. Thus, treatment with GTS-21 within this short period may be required to exert the protective effect against ischemic damages occurring in the acute phase.

A number of studies have demonstrated an important role of the hippocampus in spatial cognitive performance in rodents (28). However, in the present and previous studies, impairment of learning performance in 2VO rats did not necessarily appear in parallel with the hippocampal histological damage in these animals. In addition, pretreatment of 2VO animals with GTS-21 significantly improved the spatial cognitive impairment, but not the hippocampal tissue damage in these animals. The reason for this discrepancy remains unclear, but it can be speculated that the damage of other brain areas such as the white matter of the corpus callosum and cingulum may also contribute to the spatial cognitive impairment, since the relationship between white matter

lesions and cognitive impairment were found in normal aged people (18).

In conclusion, the 2VO rats exhibited neurodegeneration and spatial cognitive deficit. GTS-21 administration exerted a protective effect against the neuropathological changes and improved spatial cognitive impairment in 2VO rats. These results suggest that GTS-21 may be useful for treatment of dementia attributable to chronic cerebral hypoperfusion, as well as AD.

REFERENCES

- Gottfries CG, Adolfsson R, Aquilonius SM, Carlsson A, Eckernas SA, Nordberg A, Orelund L, Svennerholm L, Wiberg A and Winblad B: Biochemical changes in dementia disorders of Alzheimer type (AD/SDAT). *Neurobiol Aging* **4**, 261–271 (1983)
- Newhouse PA, Sunderland T, Tariot PN, Blumhardt CL, Weingartner H, Mellow A and Murphy DL: Intravenous nicotine in Alzheimer's disease: a pilot study. *Psychopharmacology (Berl)* **95**, 171–175 (1988)
- Sahakian B, Jones G, Levy R, Gray J and Warburton D: The effects of nicotine on attention, information processing, and short-term memory in patients with dementia of the Alzheimer type. *Br J Psychiatry* **154**, 797–800 (1989)
- Perry EK, Perry RH, Blessed G and Tomlinson BE: Necropsy evidence of central cholinergic deficits in senile dementia. *Lancet* **1**, 189 (1977)
- Wallin A, Alafuzoff I, Carlsson A, Eckernas SA, Gottfries CG, Karlsson I, Svennerholm L and Winblad B: Neurotransmitter deficits in a non-multi-infarct category of vascular dementia. *Acta Neurol Scand* **79**, 397–406 (1989)
- Murakami Y, Tanaka E, Sakai Y, Matsumoto K, Li H-B and Watanabe H: Tacrine improves working memory deficit caused by permanent occlusion of bilateral common carotid arteries in rats. *Jpn J Pharmacol* **75**, 443–446 (1997)
- Ni J-W, Ohta H, Matsumoto K and Watanabe H: Progressive cognitive impairment following chronic cerebral hypoperfusion induced by permanent occlusion of bilateral carotid arteries in rats. *Brain Res* **653**, 231–236 (1994)
- Ohta H, Nishikawa H, Kimura H, Anayama H and Miyamoto M: Chronic cerebral hypoperfusion by permanent internal carotid ligation produces learning impairment without brain damage in rats. *Neuroscience* **79**, 1039–1050 (1997)
- Ni J-W, Matsumoto K, Li H-B, Murakami Y and Watanabe H: Neuronal damage and decrease of central acetylcholine level following permanent occlusion bilateral common carotid arteries in rat. *Brain Res* **673**, 290–296 (1995)
- Bartus RT, Dean RL, Beer B and Lippa AS: The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217**, 408–417 (1982)
- Ferrer I, Bella R, Serrano MT, Marti E and Guionnet N: Arteriosclerotic leucoencephalopathy in elderly and its relation to white matter lesions in Binswanger's disease, multi-infarct encephalopathy and Alzheimer's disease. *J Neurol Sci* **98**, 37–50 (1990)
- de Fiebre CM, Meyer EM, Henry JC, Muraskin SI, Kem WR and Papke RL: Characterization of a series of anabaseine-derived compounds reveals that the 3-(4)-dimethylaminocinnamylidene derivative is a selective agonist at neuronal nicotinic $\alpha 7 / ^{125} \text{I} - \alpha$ -bungarotoxin receptor subtypes. *Mol Pharmacol* **47**, 164–171 (1995)
- Nanri M, Kasahara N, Yamamoto J, Miyake H and Watanabe H: A comparative study on the effects of nicotine and GTS-21, a new nicotinic agonist, on the locomotor activity and brain monoamine level. *Jpn J Pharmacol* **78**, 385–389 (1998)
- Nanri M, Kasahara N, Yamamoto J, Miyake H and Watanabe H: GTS-21, a nicotinic agonist, protects against neocortical neuronal cell loss induced by the nucleus basalis magnocellularis lesion in rats. *Jpn J Pharmacol* **74**, 285–289 (1997)
- Nanri M, Yamamoto J, Miyake H and Watanabe H: Protective effect of GTS-21, a novel nicotinic receptor agonist, on delayed neuronal death induced by ischemia in gerbils. *Jpn J Pharmacol* **76**, 23–29 (1998)
- Paxinos G and Watson C: *The Rat Brain in Stereotaxic Coordinates*, Second Ed, Academic Press Inc, New York (1986)
- Zoltewicz JA, Prokai-Tatrai K, Bloom LB and Kem WR: Long range transmission of polar effects in cholinergic 3-arylidene anabaseines. Conformations calculated by molecular modeling. *Heterocycles* **35**, 171–179 (1993)
- Kirino T: Delayed neuronal death in the gerbil hippocampus following ischemia. *Brain Res* **239**, 57–69 (1982)
- Brun A and Englund E: A white matter disorder in dementia of the Alzheimer type: a pathoanatomical study. *Ann Neurol* **19**, 253–262 (1986)
- Leuchter AF, Dunkin JJ, Lufkin RB, Anzai Y, Cook IA and Newton TF: Effect of white matter disease of functional connections in the aging brain. *J Neurol Neurosurg Psychiatry* **57**, 1347–1354 (1994)
- Levin ED: Psychopharmacological effects in the radial-arm maze. *Neurosci Behav Rev* **12**, 169–175 (1988)
- Chui HC: Dementia. A review emphasizing clinicopathologic correlation and brain-behavior relationships. *Arch Neurol* **46**, 806–814 (1989)
- Egashira T, Takayama F and Yamanaka Y: Effects of Bifemelane on muscarinic receptors and choline acetyltransferase in the brains of aged rats following chronic cerebral hypoperfusion induced by permanent occlusion on bilateral carotid arteries. *Jpn J Pharmacol* **72**, 57–65 (1996)
- Tanaka K, Ogawa N, Asanuma M, Kondo Y and Nomura M: Relationship between cholinergic dysfunction and discrimination learning disabilities in Wistar rats following chronic cerebral hypoperfusion. *Brain Res* **729**, 55–65 (1996)
- Akaike A, Tamura Y, Yokota T, Shimohama S and Kimura J: Nicotine-induced protection of cultured cortical neurons against *N*-methyl-D-aspartate receptor-mediated glutamate cytotoxicity. *Brain Res* **644**, 181–187 (1994)
- Kaneko S, Maeda T, Kaneko S, Akaike A, Shimohama S and Kimura J: Protective effects of $\alpha 7$ neuronal nicotinic receptor agonist on glutamate-induced neurotoxicity in cultured cortical neurons. *Jpn J Pharmacol* **71**, Suppl I, 171P (1996)
- Wakita H, Tomimoto H, Akiguchi I and Kimura J: Glial activation and white matter changes in the rat brain induced by chronic cerebral hypoperfusion: an immunohistochemical study. *Acta Neuropathol* **87**, 484–492 (1994)
- Breteler MMB, van Swieten JC, Bots ML, Grobbee DE, Claus JJ, van den Hout JHW, van Harskamp F, Tanghe HLJ, de Jong PTVM, van Gijn J and Hofman A: Cerebral white matter lesions, vascular risk factors, and cognitive function in a population-based study: the Rotterdam Study. *Neurology* **44**, 1246–1252 (1994)