Tacrine, a reversible acetylcholinesterase inhibitor, induces myopathy

Gayathri Jeyarasasingam, Michael Yeluashvili and Maryka Quik[CA]

The Parkinson's Institute, 1170 Morse Avenue, Sunnyvale, CA 94089, USA

[CA]Corresponding Author

Received 11 January 2000; accepted 2 February 2000

Acknowledgements: This work was supported by the Muscular Dystrophy Association No. 2421 and the California Tobacco Related Disease Research Program No. 7FT-0010.

Tacrine, an acetylcholinesterase (AChE) inhibitor that has been used in the treatment of Alzheimer's disease, increases available acetylcholine (Ach) levels in the synaptic cleft thereby enhancing the activity of cholinergic pathways. However, excessive stimulation of nicotinic receptors at the neuromuscular junction results in muscle deterioration. We tested whether reversible AChE inhibitors such as tacrine may induce similar effects. In the present study, tacrine administration (7.5 mg/kg twice daily) to rats produces a 20 and 30-fold increase in the number of degenerating cells in leg and diaphragm muscle, respectively, as compared to control. This myopathy is significantly decreased by co-administration of tacrine with the nitric oxide (NO) synthase inhibitor L-NAME. These results show that tacrine can induce myopathy which may be mediated by increased NO production. *NeuroReport* 11:1173–1176 © 2000 Lippincott Williams & Wilkins.

Key words: Muscle cell degeneration; Nicotinic receptors; Nitric oxide synthase; Tacrine

INTRODUCTION

Tacrine (1,2,3,4-tetrahydro-9-aminoacridine) is a reversible non-competitive acetylcholinesterase (AChE) inhibitor which, under the brand name Cognex, has been used in the treatment of Alzheimer's disease (AD). AD is a neurodegenerative disorder characterized by a deficit in acetylcholine (ACh) levels in the neocortex and hippocampus [1]. For this reason, AChE inhibitors have been investigated for their ability to raise ACh levels and thereby improve cognitive performance in lesioned animal models [2,3] and in AD patients [4]. Although AChE inhibitors have been reported to enhance certain aspects of learning and memory in AD patients [1,4] (but see also [5,6]), their administration also stimulates cholinergic activity in other regions of the body, resulting in detrimental side effects.

It is well established that administration of AChE inhibitors to rodents results in muscle degeneration. This has been demonstrated for a number of cholinesterase inhibitors including paraoxon, diisopropylfluorophosphate, neostigmine and physostigmine [7–9]. Such myopathy has been attributed to the ability of AChE inhibitors to increase ACh levels in the synaptic cleft at the neuromuscular junction resulting in the excessive stimulation of postsynaptic nicotinic receptors on muscle cells. This idea is supported by studies in which AChE inhibitor induced myopathy is prevented by prior nerve sectioning, co-administration of the nicotinic receptor antagonist *d*-tubocurarine, or depletion of ACh from the synaptic cleft with hemicholinium [7,10].

Calcium entry, resulting from nicotinic receptor stimulation, may play a role in agonist induced myopathy as evidenced from studies which show that carbachol-induced muscle degeneration in culture is prevented in the absence of calcium [8]. Enhanced receptor mediated calcium influx may result in the stimulation of numerous intracellular calcium dependent processes, including for example, the activation of nitric oxide synthase (NOS). Our experiments demonstrating that NOS inhibition prevents nicotine induced muscle cell degeneration in culture [11] supports such a hypothesis. Further evidence stems from our recent work which shows that myopathy induced by the irreversible AChE inhibitor paraoxon is also attenuated by NOS inhibitors [12].

Myopathy resulting from cholinesterase inhibition is a well established phenomenon; however, an effect of tacrine on muscle cell morphology has not yet been evaluated. The purpose of the present study was to determine whether tacrine, a reversible AChE inhibitor, resulted in muscle cell degeneration when administered to rats. Furthermore, we examined whether NOS inhibition can prevent tacrine mediated myopathy *in vivo.*

MATERIALS AND METHODS

Materials: Tacrine, nitro-L-arginine methyl ester (L-NAME) and haematoxylin and eosin were purchased from Sigma Chemical Co (St. Louis, MO). All other chemicals were purchased from standard commercial sources.

Tissue preparation: All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee. Adult male Sprague–Dawley rats weighing 180–200 g were used. Tacrine was injected i.p. two or three times daily at doses of 3, 5, 7.5 or 10 mg/kg each for 4 days. L-NAME (5–50 mg/kg) was injected i.p. 1 h before and 6 h after each tacrine injection. The dose range for tacrine and L-NAME used in these experiments are within those reported in the literature [13,14,15]. Animals were euthanized with CO_2 on day 5 and diaphragm and leg muscles including soleus, gastrocnemius and plantaris were dissected. Muscle tissue was washed in 0.9% saline, mounted in histoprep mounting media and snap frozen in liquid N_2-cooled isopentane. Muscle tissue was then sectioned at 10 μm on a cryostat. Sections were fixed in 100% ethanol for 10 min, and washed in running tap water for 5 min. After incubation in Harris haematoxylin for 15–20 min, the sections were washed in tap water, incubated in acid alcohol, rinsed in tap water, followed by incubation in ammonia water. Sections were rinsed again before staining in eosin for 5 min, washed and dehydrated in graded series of ethanol, cleared in xylene and coverslipped using Depex.

Cell counts: A minimum of 10 grids from each tissue section was counted at a final magnification of ×100. A cell was considered degenerating if it had a centralized nuclei, macrophage infiltration, or eosinophilia [7]. The number of degenerating profiles is expressed as number of lesions/1000 total cells.

Data analysis: All values are expressed as the mean ± s.e.m. Results were analyzed using an analysis of variance (ANOVA) followed by a Fischer's PLSD test.

RESULTS

Tacrine treatment induces muscle cell degeneration: Adult rats were injected with either 5, 7.5 or 10 mg/kg of the AChE inhibitor tacrine twice daily or 3, 5 or 7.5 mg/kg tacrine three times daily for 4 days. Degeneration fibers from leg and diaphragm muscle sections were identified based on the presence of centralized nuclei, eosinophilia and macrophage infiltration [7]. There was a significant increase in the number of degenerating muscle cells in the leg and diaphragm at doses of 7.5 and 10 mg/kg when tacrine was injected twice daily compared with controls (Fig. 1, Fig. 2). When tacrine was injected three times daily at lower doses there was increased degeneration that reached significance at doses of 5 and 7.5 mg/kg for leg

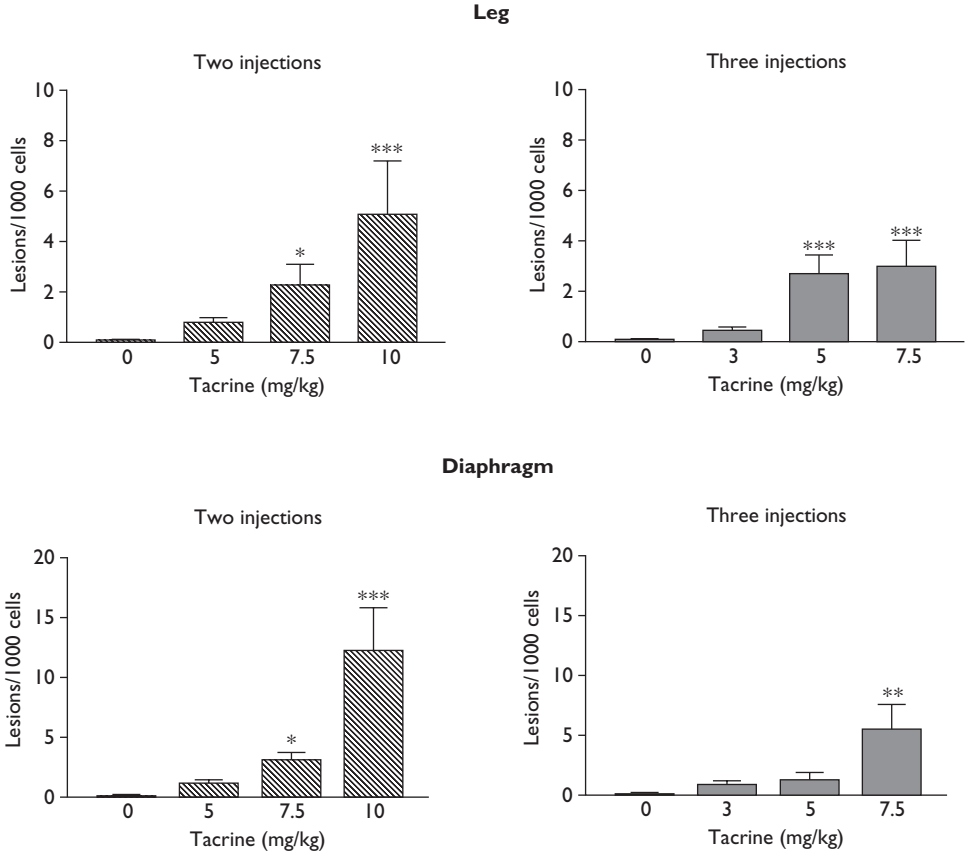

Fig. 1. The effect of varying dose and number of injections of tacrine on muscle tissue degeneration. Rats were injected with either 5, 7.5 or 10 mg/kg tacrine twice a day or 3, 5 or 7.5 mg/kg tacrine three times a day for 4 days. The number of degenerating fibers/1000 cells was counted. The results are the mean ± s.e.m. of 4–13 animals. Significance of difference from control: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

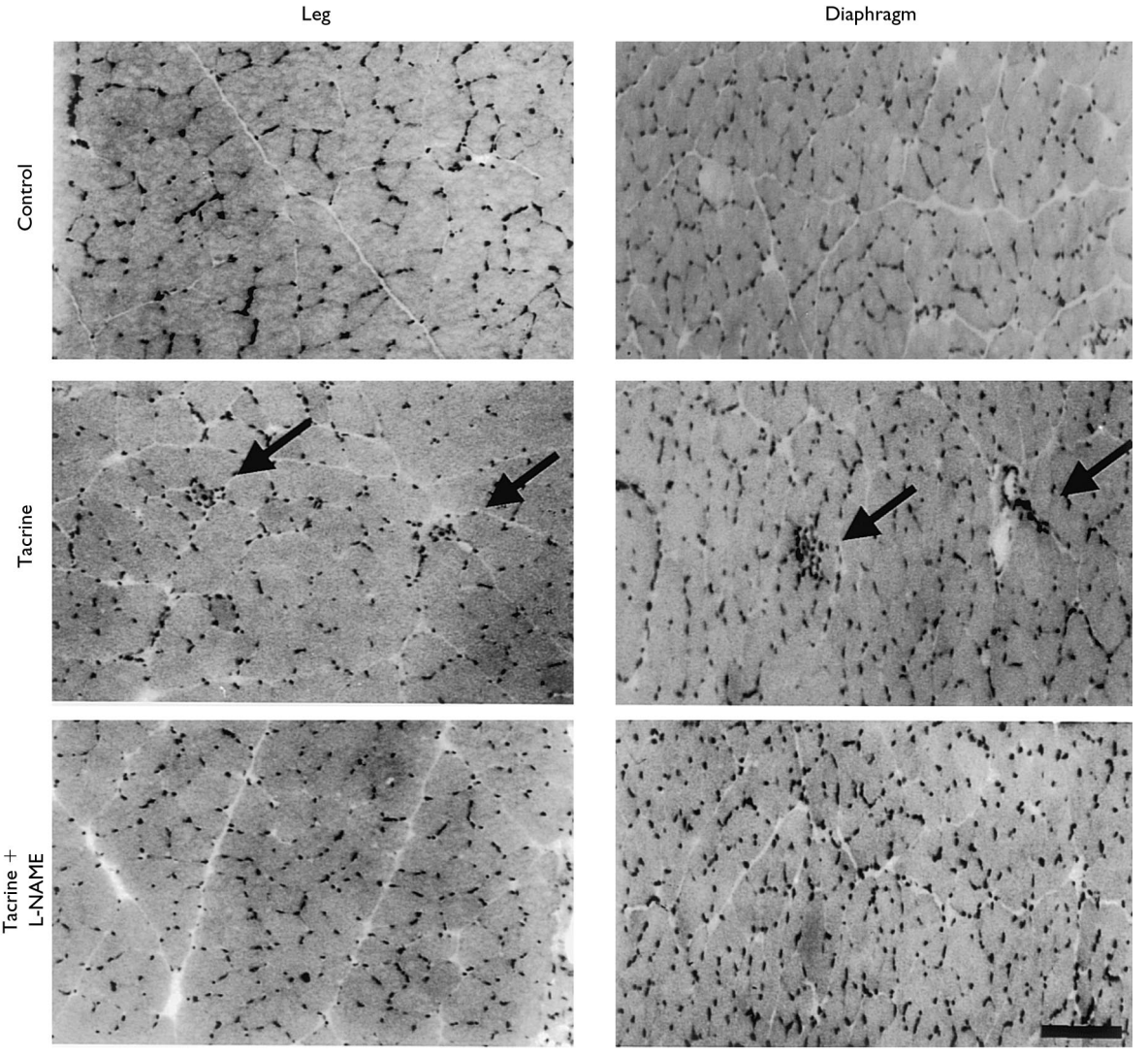

Fig. 2. Photomicrographs of leg and diaphragm muscle sections treated with vehicle (control), tacrine (7.5 mg/kg twice daily), and tacrine + L-NAME (20 mg/kg for leg and 50 mg/kg for diaphragm). Arrows indicate degenerating fibers. Bar = 100 μm.

and at a dose of 7.5 mg/kg for diaphragm (Fig. 1). It is interesting to note that the myopathy was generally greater in the diaphragm than in the leg. This is similar to previous reports in the literature [7,12]. Because rats show signs of excess cholinergic stimulation with doses of 10 mg/kg tacrine, we used an injection regimen involving 7.5 mg/kg of the drug.

L-NAME prevents tacrine induced degeneration: To determine whether NO mediates tacrine induced myopathy, rats were treated with the reversible NOS inhibitor L-NAME 1 h prior to and 6 h after tacrine treatment. Doses of 5, 10 and 20 mg/kg L-NAME significantly attenuated tacrine-induced muscle degeneration in leg sections (Fig. 2, Fig. 3). In contrast, 50 mg/kg L-NAME significantly reduced tacrine-induced muscle fiber degeneration in sections from diaphragm (Fig. 2, Fig. 3). Furthermore, the number of degenerating muscle cells observed with the combination of tacrine and 5 or 20 mg/kg L-NAME was no longer significantly different from control. L-NAME injections alone showed no effect on muscle cell morphology compared with controls (Fig. 3).

DISCUSSION

The present study demonstrates that tacrine induces muscle cell degeneration in rats. As has been postulated for other AChE inhibitors, tacrine induced myopathy could result from increased ACh levels at the neuromuscular junction resulting in the excessive stimulation of nicotinic acetylcholine receptors on muscle cells [7,10]. For example, tacrine has been shown to augment the response of muscles to nerve stimulation *in vitro* by increasing the frequency of miniature end plate potentials reflecting an increase in ACh levels at the postsynaptic receptor [16]. Although the long-term effects of tacrine on muscle viability were not assessed, this study supports the idea that muscle degeneration after tacrine administration could

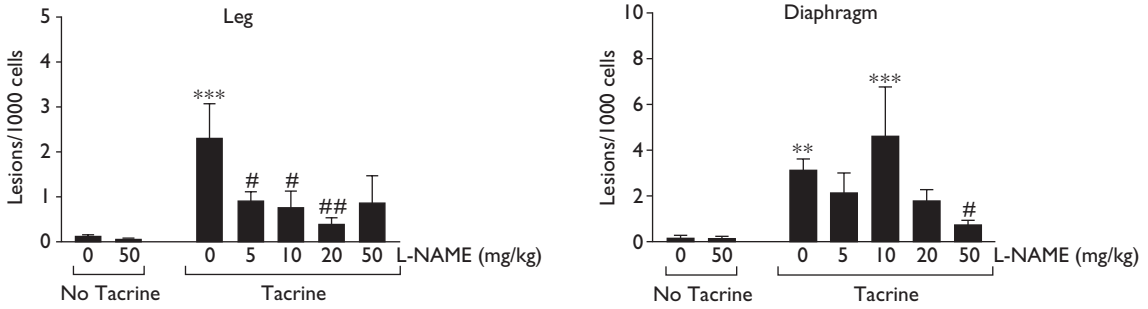

Fig. 3. The effect of L-NAME on tacrine-induced muscle degeneration. Rats were injected with the indicated doses of L-NAME or saline 1 h prior to and 6 h after tacrine or saline treatment. The results are expressed as the mean ± s.e.m. of 4–13 animals. Significance of difference from control: ** $p < 0.01$; *** $p < 0.001$. Significance of difference from tacrine alone: # $p < 0.05$; ## $p < 0.01$.

result from the excessive stimulation of acetylcholine receptors.

In the present study we demonstrate that tacrine-induced myopathy is decreased by co-administration with the NOS inhibitor L-NAME. This finding is consistent with our previous work showing that NOS inhibition prevents nicotine induced muscle cell death in culture [11]. NOS has been localized to muscle cells, where it contributes to muscle function [17]. Although nitric oxide (NO) may be essential for normal muscle activity, it is plausible that excessive receptor stimulation increases NO production to pathological levels.

Although the present work is the first to show that tacrine administration may damage skeletal muscle cells through the activation of NOS, there is precedence for a similar action of tacrine in the brain. Tacrine in combination with lithium chloride induces hippocampal seizures and subsequent brain damage through the activation of muscarinic receptors [18]. In this model, tacrine administration increases hippocampal NOS mRNA levels and enzyme activity [18]. Moreover, administration of L-NAME prevents such an increase in mRNA levels and the resulting seizure activity [15]. These studies suggest that the neurotoxic actions of tacrine result from an increase in ACh levels resulting in the over-stimulation of muscarinic receptors. The subsequent rise in intracellular calcium may result in enhanced NOS activity with an increased production of NO [18]. Similarly, in the present study, tacrine may result in muscle degeneration by raising ACh levels to over-stimulate nicotinic receptors resulting in enhanced calcium influx and a pathological increase in NOS activity.

Tacrine administration to AD patients results in a variety of adverse responses, most notably hepatotoxicity. In addition, cholinergic side effects have been noted such as nausea, vomiting, diarrhoea and abdominal pain [4,5]. Although fatigue, a symptom associated with myopathy, has not been reported, the mean age of the patients in these studies is ~70 years. It is possible that, following drug treatment, an enhanced difficulty in movement over that normally reported for this age group was overlooked because of the other problems associated with AD.

Because of the liver toxicity observed with tacrine, the identification of other AChE inhibitors with fewer adverse side effects continues to be an active area of research in the search for therapeutic drugs to combat AD. Our work demonstrating tacrine induced muscle cell degeneration, therefore, highlights a potential risk associated with the use of AChE inhibitors that must be explored if these drugs are used in man.

CONCLUSION

The present study shows that tacrine administration induces muscle cell degeneration in rodents. Moreover, NOS inhibition decreases tacrine induced myopathy. The current work suggests that tacrine or other reversible AChE inhibitors, used therapeutically to counteract the cognitive declines observed in AD, should be evaluated for potential degenerative effects on muscle tissue.

REFERENCES

1. Giacobini E. *Neurochem Int* **32**, 413–419 (1998).
2. Flood JF and Cherkin A. *Neurobiol Aging* **9**, 5–8 (1988).
3. Mohammed AH. *Acta Neurol Scand Suppl* **149**, 13–15 (1993).
4. Knapp MJ, Knopman DS, Solomon PR *et al. JAMA* **271**, 985–991 (1994).
5. Gauthier S, Bouchard R, Lamontagne A *et al. N Engl J Med* **322**, 1272–1276 (1990).
6. Maltby N, Broe GA, Creasey H *et al. BMJ* **308**, 879–883 (1994).
7. Ariens AT, Meeter E, Wolthuis OL *et al. Experientia* **25**, 57–59 (1969).
8. Leonard JP and Salpeter MM. *J Cell Biol* **82**, 811–819 (1979).
9. Wecker L, Kiarta T and Dettbam WD. *J Pharm Exp Ther* **206**, 97–104 (1978).
10. Fenichel GM, Kibler WB, Olson WH *et al. Neurology* **22**, 1026–1033 (1972).
11. El-Dada MD and Quik M. *J Pharmacol Exp Ther* **281**, 1463–1470 (1997).
12. Jeyarasasingam G, Yeluashvili M and Quik M. *Soc Neurosci Abstr* **25**, 1492 (1999).
13. Taraska T and Finnegan KT. *J Pharm Exp Ther* **280**, 941–947 (1997).
14. Jones PA, Smith RA and Stone TW. *Neurosci Lett* **249**, 75–78 (1998).
15. Bagetta G, Iannone M, Scorsa AM *et al. Eur J Pharm* **213**, 301–304 (1992).
16. Braga MFM, Harvey AL and Rowan EG. *Br J Pharm* **102**, 909–915 (1991).
17. Kobzik L, Reid MB, Bredt DS *et al. Nature* **372**, 546–548 (1994).
18. Bagetta G, Corasaniti MT, Melino G *et al. Biochem Biophys Res Commun* **197**, 1132–1139 (1993).