Temporal dependent neuroprotection with propentofylline (HWA 285) in a temporary focal ischemia model

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Abstract

Propentofylline (HWA 285, 3-methyl-1-(5-oxo-hexyl)-7-propylxanthine) is an adenosine uptake and phosphodiesterase inhibitor that has been shown to be neuroprotective in both global and permanent focal ischemia animal models. However, to date, the efficacy of propentofylline has never been examined in an animal model of temporary focal ischemia or the 'therapeutic window' systematically examined in a focal ischemia model. The present experiments were designed to investigate these. Temporary (3 h) middle cerebral artery occlusion was accomplished by the monofilament method. Infarct volumes were determined at 24 h from 2,3,5-triphenyltetrazolieum chloride (TTC) stained coronal slices. Animals were dosed with vehicle or propentofylline at 3 mg/kg bolus and/or a 6 mg/kg per h infusion (24 h infusion) at 30 min, 1 h or 3 h post ischemia onset. Physiological monitoring on a subset of animals indicated no changes in mean arterial pressure, blood gases, blood pH, and glucose levels with either ischemia or drug treatment. Propentofylline treatment resulted in a statistically significant decrease in infarct volume when an infusion dose of 6 mg/kg per h was initiated at 30 min or when a bolus of 3 mg/kg plus an infusion dose was initiated at 1 h but not 3 h post ischemia. Therefore, propentofylline is neuroprotective in a model of temporary focal ischemia. This suggests that combination therapy with propentofylline might lead to clinical improvement beyond that which would occur with thrombolytics alone. The apparent short window of opportunity for effective dosing is consistent with the proposed mechanism of action for propentofylline. © 1998 Elsevier Science B.V.

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1. Introduction

Propentofylline (HWA 285, 3-methyl-1-(5-oxo-hexyl)-7-propylxanthine) has been reported to have a number of activities (for a review see Parkinson et al., 1994) that could theoretically lead to neuroprotection in animal models of ischemia as well as decreased infarct volume in human stroke. For instance, propentofylline has been found to be an adenosine uptake inhibitor, (Fredholm and Lindström, 1986; Ohkubo et al., 1991; Parkinson and Fred-

holm, 1991; Fredholm et al., 1992) and a weak adenosine A_1 autoreceptor antagonist (Parkinson and Fredholm, 1991; Fredholm et al., 1992) both of which act to increase levels of adenosine in the synaptic cleft. Since adenosine is known to regulate the release of the excitatory amino acid glutamate (for discussion see Rudolphi et al., 1992) it is not surprising to find that under ischemic conditions the levels of adenosine are higher and the levels of glutamate are lower in propentofylline treated animals than in vehicle controls (Andiné et al., 1990; Miyashita et al., 1992). Furthermore, propentofylline is known to non-competitively inhibit cAMP and cGMP phosphodiesterases (Nagata et al., 1985; Némoz et al., 1989; Murashima et al., 1990; Meskini et al., 1994). This action may well augment the neuroprotective adenosine actions of propentofylline and enhance propentofylline's inhibition of glutamatergic effects. Therefore, it is not surprising to find that there are several reports of neuroprotection with propentofylline in

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animal models of both global (DeLeo et al., 1987; DeLeo et al., 1988a; DeLeo et al., 1988b; Stanimirovic et al., 1994) and focal ischemia (Park and Rudolphi, 1994; Matsumoto et al., 1996).

Propentofylline is also known to increase the release of nerve growth factor (NGF) in vitro from astroglial cells (Shinoda et al., 1990) and has been found to partially prevent the age-associated decline in NGF with rats (Nabeshima et al., 1993; Nabeshima et al., 1994). In addition, propentofylline will inhibit the lipopolysaccharide stimulated release of tumor necrosis factor α (TNF-α) in vitro which may account for the decrease in microglial (DeLeo et al., 1987; McRae et al., 1994) and microglia-generated oxygen free radicals (Banati et al., 1994) under ischemic conditions. Either the NGF or TNF-α actions alone might explain the positive effects of propentofylline in forebrain lesion animal studies (Fuji et al., 1993) or the neuroprotective actions of propentofylline following global ischemia (DeLeo et al., 1987; DeLeo et al., 1988a; DeLeo et al., 1988b; Stanimirovic et al., 1994). Moreover, inflammation, microglial activation and loss of neurotropic factors have also been implicated in the mechanisms of neuronal death in focal ischemic stroke and would suggest possible efficacy in Alzheimer's disease and vascular dementia.

Clinically the effects with propentofylline have been consistent with the preclinical indications for efficacy in stroke, Alzheimer's disease and vascular dementia. For instance, in several clinical trials with dementia patients with Alzheimer's disease or vascular dementia, propentofylline demonstrated a significant improvement in several measure of cognitive function (Saletu et al., 1990; Mielke et al., 1996). In a limited placebo-controlled study with only 30 acute ischemic stroke patients, propentofylline was found to significantly increase the glucose utilization within the infarcted area at 2 weeks after ischemia and showed a trend towards an improved Barthel index score at 3 months (Huber et al., 1993). Therefore, with limited clinical studies in stroke patients and in more extensive trials in Alzheimer's disease and vascular dementia, propentofylline appears to show efficacy consistent with preclinical data.

There are several reports describing the neuroprotective effects of propentofylline given either pre- or post-ischemia in models of global ischemia (DeLeo et al., 1987; DeLeo et al., 1988a; DeLeo et al., 1988b; Stanimirovic et al., 1994). Similarly, propentofylline has been found to be effective in permanent focal ischemia models when given as a single i.p. dose pre-ischemia or as an i.v. infusion post-ischemia (Park and Rudolphi, 1994; Matsumoto et al., 1996). However, efficacy has not been investigated in a temporary middle cerebral artery occlusion animal model or the 'therapeutic window' examined in a model of focal ischemia. The present work was undertaken to examine the neuroprotective potential of propentofylline in temporary ischemia and the therapeutic window in focal ischemia.

2. Materials and methods

2.1. Surgical procedure

Adult male Wistar rats weighing 260–310 g (Charles River Laboratories, Wilmington, MA) were allowed free access to food and water throughout the experiment. Anesthesia was induced with 5% halothane and maintained with 1.5% halothane in a 70/30% nitrous oxide/oxygen mix. Rectal body temperature was monitored and maintained by use of a heating blanket. A permanent indwelling PE-50 catheter was implanted in the external jugular for i.v. drug delivery. In a subset of animals, a heparinized PE-50 catheter was permanently implanted in the femoral artery for physiological monitoring (mean arterial pressure, blood glucose, blood pCO_2, pO_2 and pH) just prior to ischemia onset and after 180 min of occlusion.

Middle cerebral artery occlusion was induced by the procedure of Zea Long et al. (1989) with minor modification as described by Belayev et al. (1996). Briefly, a 3.0 monofilament with a heat blunted tip was presoaked overnight in 0.1% poly-L-lysine and 1000 U/ml of heparin then dried at 60°C for 1 h. The left common carotid artery, external cerebral artery and internal cerebral artery were isolated and the monofilament introduced via the external cerebral artery up the internal cerebral artery 20–21 mm to occlude the origin of the middle cerebral artery. The wound was closed and the animals allowed to recover (over a 180-min period of occlusion) until reanesthetized for reperfusion such that there was 180 min of occlusion. Reperfusion was accomplished by pulling the monofilament back to the external/internal cerebral artery bifurcation.

2.2. Drug administration

Propentofylline was dissolved in normal saline. Vehicle treated animal received either 2 ml/kg normal saline as a bolus injection i.v. and/or 0.9 ml/h as a 24 h infusion. Propentofylline was injected as a bolus dose of 3 mg/kg and/or an infusion of 6 mg/kg per h for 24 h. Drug treatment was initiated either 30 min, 1 or 3 h after onset of ischemia.

2.3. Infarct determination and statistical analysis

Animals were sacrificed at 24 h and the brain quickly dissected. Animal brains were sliced into 2 mm coronal sections and stained with 2% 2,3,5-triphenyltetrazolieum chloride (TTC, Sigma) in normal saline at 39°C for 30 min then fixed in 10% buffered formalin as described by (Bederson et al., 1986). Within 1 week, each slice was photographed on 35 mm color slide film and the image later quantitated by computer-assisted image analysis using a Compix system (C Imaging 1280 system, Compix Image Systems, Mars, PA). The ipsilateral hemispheric area,

whole brain area and the area of infarct were found for each slice. Volumes were determined by calculating the area under the curve for the anterior–posterior levels verses the infarct or hemispheric areas measured from each of the six slices. Since this focal ischemia model does not involve a craniotomy, edema was indirectly determined by taking one-half of the difference between the ipsilateral and contralateral volumes. This method assumes that the contralateral side decreases in size in proportion to the amount the ipsilateral side increases in volume. Previous work from this laboratory has indicated that this more accurately estimates the degree of edema seen at 24 h after ischemia. 'Corrected Infarct' was then determined by subtracting edema from the infarct volume as measured for each animal. Where indicated, percent infarct is calculated as the uncorrected infarct volume as a percent of the ipsilateral hemispheric volume.

Separate vehicle control groups were run against each dosing times investigated. Statistical analysis indicate no differences between vehicle controls dosed 30 min to 3 h after ischemia, so the vehicle controls were combined and compared to the propentofylline-treated groups. Comparisons were made by an one-way or two-way analysis of variance (ANOVA) followed by Dunnett's analysis for individual drug treated groups. The physiological parameters were compared between treatment groups by a Student's t-test.

3. Results

In order to minimize the time of anesthesia, decrease surgical stress, and limit blood loss from sample withdraw, only a subset of animals were monitored for physiological variables. As can be seen in Table 1, there were no significant changes between the saline- and drug-treated groups pre- or post-ischemia. The data presented in Figs. 1 and 3 are uncorrected infarct volumes. However, data were analyzed either as 'corrected' infarct volume by subtracting out an estimate of edema or as uncorrected infarct volume (i.e., a direct calculation from measured areas in each slice). In both cases, the results were similar in that a
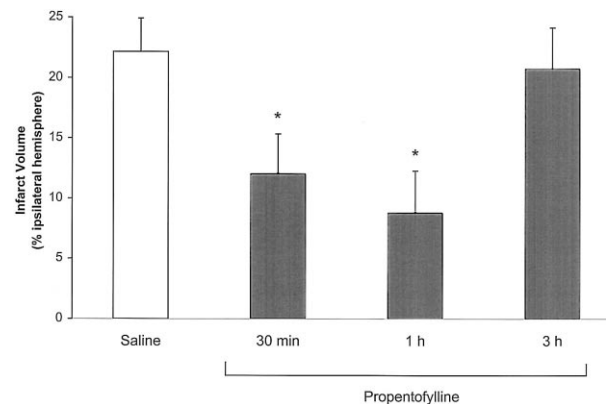

Fig. 1. Mean uncorrected infarct volumes as a percent of the ipsilateral hemisphere with standard error for each group ($n = 35$ for vehicle treated group and 12–14 in each drug-treated group). Animals were treated with either vehicle or propentofylline with 6 mg/kg per h i.v. infusion at 30 min, or 3 mg/kg bolus + 6 mg/kg per h infusion at 1 or 3 h post-ischemia. ∗ANOVA followed by contrast analysis post-hoc comparison indicated a significant ($P < 0.05$) decrease in infarct volume in the 30 min and 1 h propentofylline-treated groups.

significant decrease in infarct volume was seen when propentofylline treatment was initiated at 30 min or 1 h, but not 3 h after the onset of ischemia (Uncorrected infarct: control, $22.2 \pm 2.8\%$ ipsilateral hemisphere; 30 min, $12.1 \pm 3.3\%$; 1 h, $8.8 \pm 3.5\%$; 3 h, $20.8 \pm 3.4\%$. Corrected infarct: control, $17.2 \pm 2.8\%$ ipsilateral hemisphere; 30 min, $8.8 \pm 3.0\%$; 1 h, $5.9 \pm 3.0\%$; 3 h, $15.5 \pm 3.0\%$). Similarly, A small but significant decrease in edema was found when treatment started at 30 min and 1 h but not 3 h post-ischemia (control, $5.14 \pm 0.33\%$ ipsilateral hemisphere; 30 min, $3.66 \pm 0.35\%$; 1 h, $3.56 \pm 0.62\%$; 3 h, $5.42 \pm 0.57\%$). In an initial experiment it was found that an infusion only of 6 mg/kg per h initiated at 30 min post-ischemia gave a significant neuroprotective effect at 24 h. However, preliminary pharmacokinetic data (not shown) with propentofylline indicated that the blood levels would take some time to reach their maximum steady state levels. Therefore, in subsequent experiments (i.e., 1 and 3 h post-ischemia dosing) a bolus dose of 3 mg/kg was added to more rapidly achieve steady state blood levels.

Table 1
Physiological parameters: vehicle vs. propentofylline in ischemic animals

	Time after occlusion (min)	MAP (mmHg)	pH	pO$_2$	pCO$_2$	Glucose (mg/dl)	Temperature (°C)
Vehicle	0	76.4 ± 3.3	7.28 ± 0.05	132.8 ± 5.8	50.2 ± 3.7	134.5 ± 3.5	37.1 ± 0.1
	180	88.3 ± 4.5	7.33 ± 0.03	150.0 ± 7.3	43.7 ± 4.4	115.5 ± 7.2	38.4 ± 0.2
Propentofylline	0	77.2 ± 3.1	7.32 ± 0.02	120.3 ± 7.5	51.6 ± 2.0	134.5 ± 10.3	37.1 ± 0.1
	180	96.1 ± 4.3	7.34 ± 0.02	139.5 ± 16.0	42.6 ± 2.1	116.3 ± 6.8	38.4 ± 0.1

Vehicle or propentofylline (3 mg/kg + 6 mg/kg per h for 24 h) was injected i.v. 1 h after the onset of ischemia. Physiological parameters were measured at the time of ischemia onset (0 min) and just prior to reperfusion (180 min). No statistically significant differences between vehicle and drug treatment were seen at either 0 or 180 min for any of the physiological parameters ($n = 4$/group, $P > 0.05$, Student's t-test).
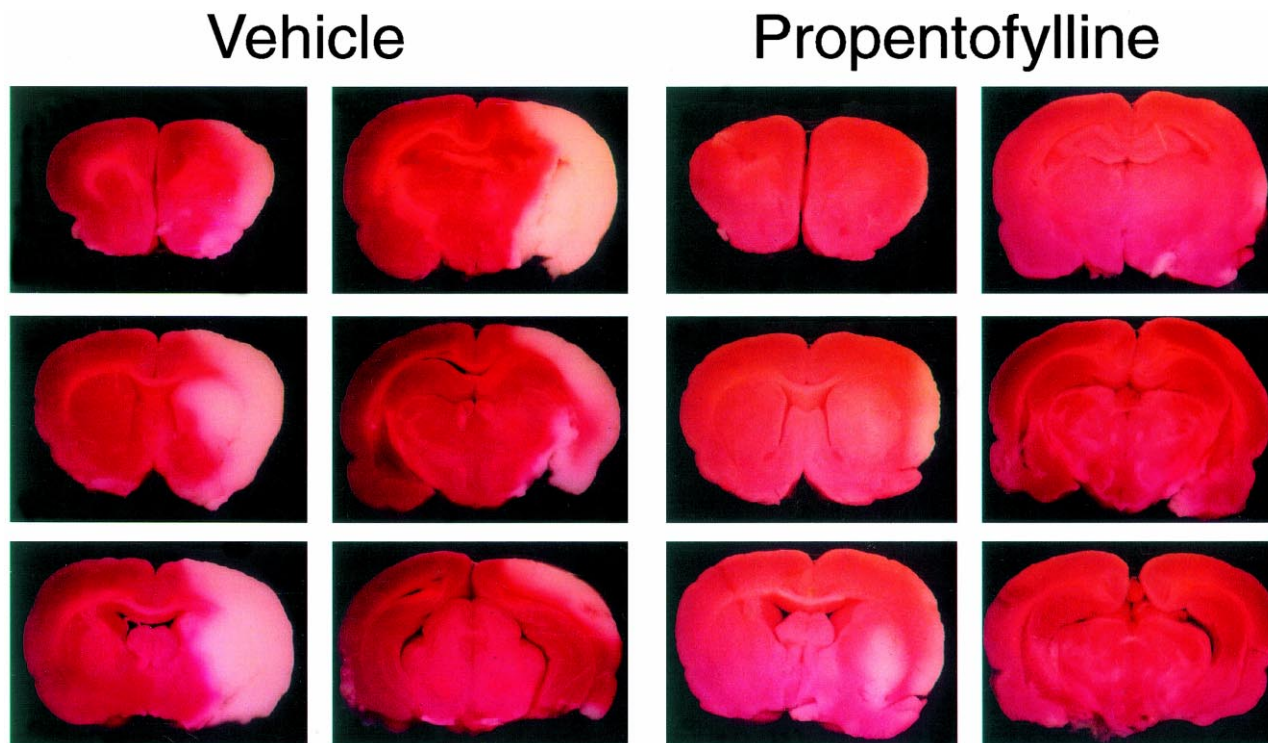
Vehicle

Propentofylline

Fig. 2. TTC-stained coronal slices of rat brain from a representative vehicle and propentofylline-treated (30 min post-ischemia group) animal. Note that neuroprotection with propentofylline is evident in both the cortical and subcortical regions of the area at risk.

As seen in Fig. 1, animals treated with propentofylline at 30 min or 1 h post-ischemia had an average infarct volume that was significantly less than vehicle-treated animals. This is clear by visually comparing the degree of pan-necrosis as evidenced by TTC staining in a typical vehicle-treated vs. a 30 min propentofylline-treated animal (Fig. 2). In contrast, animals treated with propentofylline at 3 h after the onset of ischemia had a similar average infarct volume to the vehicle-treated group. Neuroprotec-

tion was seen in several coronal sections examined (Fig. 3) and in both the cortical and subcortical regions (Fig. 2).

4. Discussion

It is clear from the current results that propentofylline is neuroprotective in an acute temporary focal ischemia model of stroke. This has special significance given the recent positive clinical results with thrombolytic therapies such as recombinant tissue plasminogen activator (Brott et al., 1995), and the fact that a certain percentage of focal ischemic patients will undergo spontaneous reperfusion of the infarcted area without pharmacological treatment (Minematsu, 1994). Given the 3 h temporary occlusion method utilized here and the 3 h treatment window for recombinant tissue plasminogen activator (Adams et al., 1996), it is suggested from the present results that even in patients where reperfusion is established, a significant pan-necrosis of effected area may occur. Furthermore, the apparent ability of propentofylline to limit the neuronal death that occurs even with reperfusion, indicates that the infarct is amenable to pharmacological intervention. The obvious implication is that safe neuroprotective treatments in combination with thrombolytics may provide a more beneficial outcome to patients than thrombolytic therapy alone.
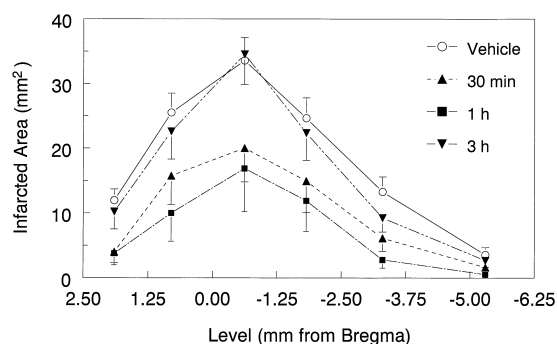

Fig. 3. Mean areas with standard error bars for each of the coronal slices vs. the anterior–posterior position of the slices for each treatment group. Two-way ANOVA followed by Dunnett's post hoc comparison indicated a significant effect of treatment vs. vehicle control with the 30 min and 1 h time points. Note that decreases in infarcted areas were seen across several sections, but are especially evident in sections 1 through 4.

It is also import to note that not all patients will reperfuse with thrombolytic treatment, nor will every patient meet the treatment criteria for thrombolytics. Therefore, it is reasonable to suggest that the future stroke population will remain a mixture of patients with permanent and temporary occlusion of the cerebral arteries. Given this, it remains important for any potential neuroprotective agent to show effectiveness in both permanent and temporary focal ischemia models. With this study and previous work (Park and Rudolphi, 1994; Matsumoto et al., 1996), it is clear that in animal models of focal ischemia, propentofylline is neuroprotective in both permanent and temporary ischemia.

Another interesting aspect of the current results is the apparent therapeutic window of between 1 to 3 h with propentofylline in this animal model of ischemia. As mentioned in the introduction, propentofylline has a number of mechanistic actions that could account for its neuroprotective effects. However, the experimental therapeutic window found here would tend to suggest that certain mechanism(s) are more important for the acute decrease in infarct volume seen with propentofylline. Specifically, it has been found that propentofylline has an adenosine uptake inhibition activity (Fredholm and Lindström, 1986; Ohkubo et al., 1991; Parkinson and Fredholm, 1991; Fredholm et al., 1992), as well as a weak A_1 receptor antagonism (Parkinson and Fredholm, 1991; Fredholm et al., 1992) plus cAMP and cGMP phosphodiesterase inhibition (Nagata et al., 1985; Némoz et al., 1989; Murashima et al., 1990; Meskini et al., 1994). All three of these actions could combine to give an increase in released adenosine and a synergistic postsynaptic adenosine receptor action. Since adenosine is reported to act as a inhibitory neurotransmitter for glutamate release, it is not surprising to see that under global ischemia conditions, propentofylline causes an increase in the release of adenosine and a decrease in glutamate release (Andiné et al., 1990; Miyashita et al., 1992). Given the relatively short therapeutic time window, decreasing excitatory amino acid release may account for the neuroprotective actions of propentofylline. In focal ischemia models, glutamate levels rise rapidly reaching a peak within 1 h (Busto et al., 1989; Hillered et al., 1989; Graham et al., 1990; Uchiyama-Tsuyuki et al., 1994; Chen et al., 1995; Herz et al., 1996). In contrast, TNF-α (another potential neuroprotective mechanism with propentofylline) does not peak for 6 to 12 h after onset of focal ischemia (Feuerstein et al., 1994; Liu et al., 1994). Furthermore, a recent study with 619C89, a glutamate release inhibitor, found decreases in infarct volume when treatment was initiated at 30 min or 1 h but not 2 h after the onset of ischemia (Leach et al., 1993). Therefore, a therapeutic window of 1 h with propentofylline is much more consistent with a neuroprotective adenosine enhancing and subsequent glutamatergic limiting mechanisms of propentofylline than any of the other potential actions of propentofylline.

It is also interesting to compare the present results with those of the one clinical trial with propentofylline (Huber et al., 1993). In this small trial with ischemic stroke patients, treatment was initiated within 48 h of symptoms onset with a mean dosing time of 25 ± 11 h. Two weeks after treatment significant increases in regional brain glucose metabolism were found within the infarct and the hemispheric gray matter although no changes in overt infarct volume were found. Given the present results where infarct volume is quantitated by the standard TTC staining methods and the prolonged initiation of treatment in the clinical study, it might be expected that no change in infarct volume would be seen in the clinical study. However, it is interesting to speculate that propentofylline-induced increases in glucose utilization might reflect some benefit even past a treatment time that can result in a decreased infarct volume either in the clinical setting or in preclinical animal models.

The obvious danger here is making direct comparisons between a therapeutic window in humans and those found experimentally in animal models of ischemia, especially rodent models. It is tempting to point to the similarity between the recombinant tissue plasminogen activator trial results, indicating a 3 h treatment window, and the fact that reperfusion within 3 h in rodent focal ischemia models provides some degree of neuroprotection (Hsien and Hsu, personal communication; Memezawa et al., 1992; Garcia et al., 1993; Garcia et al., 1995) and conclude that a 1 h treatment window in a rodent focal ischemia model means a 1 h treatment window in stroke patients. However, a 3 h reperfusion window may not be true of all species. For instance, Young et al. (1997) recently reported that in a baboon model of focal ischemia, a 6 h ischemic event followed by reperfusion resulted in a significantly smaller infarct than permanent occlusion of the vessel, results that have been suggested from previous studies (Nehls et al., 1987; Hadley et al., 1989). Therefore, the 'therapeutic window' for reperfusion in non-human primates may be significantly longer than 3 h. This suggests that if the 3 h window with recombinant tissue plasminogen activator holds true in ongoing and subsequent clinical trials, then possibly treatment with recombinant tissue plasminogen activator is limited not by the neuroprotective limits of reperfusion per say but by an undesirable effect of the thrombolytic treatment itself. Certainly, more animal and clinical work is necessary before the relationship between animal models and human stroke can be fully elucidated. In any case, until at least one neuroprotective compound's therapeutic window is fully characterized both in animal models of focal ischemia and in clinical trials, it is difficult to make direct conclusions about the relative predictability of the type of data presented here to clinical treatment.

In summary, the current report extends previous work to show that propentofylline is neuroprotective in a model of temporary focal ischemia as well as permanent focal ischemia. Also, it was found that this neuroprotection

persisted when dosing was delayed by 1 but not 3 h after the onset of ischemia. Together, these data would suggest that propentofylline would be efficacious in human stroke either with or without combination thrombolytic therapy. Furthermore, the data suggests that there is a therapeutic window of at least 1 h if not more to allow treatment to commence. Efficacy in temporary focal ischemia with a relatively short therapeutic window is consistent with the proposed adenosine/phosphodiesterase mechanism for propentofylline.

References

Adams, H., Brott, T., Furlan, A., Gomez, C., Grotta, J., Helgason, C., Kwiatkowski, T., Lyden, P., Marler, J., Torner, J., Feinberg, W.M.M., Thies, W. et al., 1996. Guidelines for thrombolytic therapy for acute stroke: A supplement to the guidelines for the management of patients with acute ischemic stroke. Stroke 27, 1711–1718.

Andiné, P., Rudolphi, K., Fredholm, B., Hagberg, H., 1990. Effect of propentofylline (HWA 285) on extracellular purines and excitatory amino acids in CA1 of rat hippocampus during transient ischaemia. Br. J. Pharmacol. 100, 814–818.

Banati, R., Schubert, P., Rothe, G., Gehrmann, R., Rudolphi, K., Valet, G., Kreutzberg, G., 1994. Modulation of intracellular formation of reactive oxygen intermediates in peritoneal macrophages and microglia/brain macrophages by propentofylline. J. Cereb. Blood Flow Metab. 14, 145–149.

Bederson, J., Pitts, L., Germano, S., Nishimura, M., RL, D., Bartkowski, H., 1986. Evaluation of 2,3,5-triphenyltetrazolium chloride as a stain for detection and quantification of experimental cerebral infarction in rats. Stroke 17, 1304–1308.

Belayev, L., Alonso, O., Busto, R., Zhao, W., Ginsberg, M., 1996. Middle cerebral artery occlusion in the rat by intraluminal suture. Neurological and pathological evaluation of an improved model. Stroke 27, 1616–1623.

Brott, T., Broderick, J., Kothari, R., O'Donoghue, M., Barsan, W., Tomsick, T., Spilker, J., Miller, R., Sauerbeck, L., Lyden, P., Rapp, K., Babcock, T., Daum, P., Persona, D., Brody, M., Jackson, C., Lewis, S., Liss, J., Mahdavi, Z.J.R. et al., 1995. Tissue plasminogen activator for acute ischemic stroke. New Engl. J. Med. 333, 1405–1581.

Busto, R., Globus, M.-T., Dietrich, W., Martinez, E., Valdés, I., Ginsberg, M., 1989. Effect of mild hypothermia on ischemia-induced release of neurotransmitters and free fatty acids in rat brain. Stroke 20, 904–910.

Chen, J., Graham, S., Simon, R., 1995. A comparison of the effects of a sodium channel blocker and an NMDA antagonist upon extracellular glutamate in rat focal cerebral ischemia. Brain Res. 699, 121–124.

DeLeo, J., Schubert, P., Kreutzberg, G., 1988a. Protection against ischemic brain damage using propentofylline in gerbils. Stroke 19, 1535–1539.

DeLeo, J., Schubert, P., Kruetzberg, G., 1988b. Propentofylline (HWA 285) protects hippocampal neurons of Mongolian gerbils against ischemic damage in the presence of an adenosine antagonist. Neurosci. Lett. 84, 307–311.

DeLeo, J., Toth, L., Schubert, P., Rudolphi, K., Kreutzberg, G., 1987. Ischemia-induced neuronal death, calcium accumulation and glial response in the hippocampus of the Mongolian gerbil and protection by propentofylline (HWA 285). J. Cereb. Blood Flow Metab. 4, 745–751.

Feuerstein, G.-Z., Liu, T., Barone, F., 1994. Cytokines, inflammation, and brain injury: Role of tumor necrosis factor-α. Cerebrovasc. Brain Metab. Rev. 6, 341–359.

Fredholm, B., Fastbom, J., Kvanta, A., Gerwins, P., Parkinson, F., 1992. Further evidence that propentofylline (HWA 285) influences both adenosine receptors and adenosine transport. Fundam. Clin. Pharmacol. 6, 99–111.

Fredholm, B., Lindström, K., 1986. The xanthine derivative 1-(5'-oxohexyl)-3-methly-7-propyl xanthine (HWA 285) enhances the actions of adenosine. Acta Pharmacol. Toxicol. 58, 187–192.

Fuji, K., Hiramatsu, M., Kameyama, T., Nadeshima, T., 1993. Effects of repeated administration of propentofylline on memory impairment produced by basal forebrain lesion in rats. Eur. J. Pharmcol. 236, 411–417.

Garcia, J., Liu, K.-F., Ho, K.-L., 1995. Neuronal necrosis after middle cerebral artery occlusion in Wistar rats progresses at different time intervals in the caudoputamen and the cortex. Stroke 26, 636–643.

Garcia, J., Yoshida, Y., Chen, H., Li, Y., Zhang, Z., Lian, J., Chen, S., Chopp, M., 1993. Progression from ischemic injury to infarct following middle cerebral artery occlusion in the rat. Am. J. Pathol. 142, 623–635.

Graham, S., Shiraishi, K., Panter, S., Simon, R., Faden, A., 1990. Changes in extracellular amino acid neurotransmitters produced by focal cerebral ischemia. Neurosci. Lett. 110, 124–130.

Hadley, M., Zabramski, J., Spetzler, R., Rigamonti, D., Fifield, M., Johnson, P., 1989. The efficacy of intravenous nimodipine in the treatment of focal cerebral ischemia in a primate model. Neurosurgery 25, 63–70.

Herz, R., Gaillard, P., de Wildt, D., Verstegg, D., 1996. Differences in striatal extracellular amino acid concentrations between Wistar and Fischer 344 rats after middle cerebral artery occlusion. Brain Res. 715, 163–171.

Hillered, L., Hallström, A., Segersvärd, S., Persson, L., Ungerstedt, U., 1989. Dynamics of extracellular metabolites in the striatum after middle cerebral artery occlusion in the rat monitored by intracerebral microdialysis. J. Cereb. Blood Flow Metab. 9, 607–616.

Huber, M., Kittner, B., Hojer, C., Fink, G., Neveling, M., Heiss, W.-D., 1993. Effect of propentofylline on regional cerebral glucose metabolism in acute ischemic stroke. J. Cereb. Blood Flow Metab. 13, 526–530.

Leach, M., Swan, J., Eisenthal, D., Dopson, D., Nobbs, M., 1993. BW619C89, a glutamate release inhibitor, protects against focal cerebral ischemic damage. Stroke 24, 1063–1067.

Liu, T., Clark, R., McConnell, P., Young, P., White, R., Barone, F., Feuerstein, G., 1994. Tumor necrosis factor in ischemic neurons. Stroke 25, 1481–1488.

Matsumoto, K., Sakaki, T., Kohmura, E., Hayakawa, T., Yamada, K., 1996. Amelioration of ischemic brain damage by the preischemic administration of propentofylline (HWA 285) in a rat focal ischemia. Brain Res. 723, 228–230.

McRae, A., Rudolphi, K., Schubert, P., 1994. Propentofylline depresses amyloid and Alzheimer's CSF microglial antigens after ischaemia. NeuroReport 5, 1193–1196.

Memezawa, H., Smith, M.-L., Siesjö, B., 1992. Penumbral tissue salvaged by reperfusion following middle cerebral artery occlusion in rats. Stroke 23, 552–559.

Meskini, N., Mémoz, G., Okyayuz-Baklouti, I., Lagard, M., Prigent, A., 1994. Phosphodiesterase inhibitory profile of some related xanthine derivatives pharmacologically active on the peripheral microcirculation. Biochem. Pharmacol. 47, 781–788.

Mielke, R., Kittner, B., Ghaemi, M., Kessler, J., Szelies, B., Harholz, K., Heiss, W.-D., 1996. Propentofylline improves regional cerebral glucose metabolism and neuropsychologic performance in vascular dementia. J. Neurol. Sci. 141, 59–64.

Minematsu, K., 1994. Cardioembolic Stroke. In: Fisher, M. (Ed.) Cerebrovascular Disorders. Wolfe, St. Louis, MO, pp. 7/1–22.

Miyashita, K., Nakajima, T., Ishikawa, A., Miyatake, T., 1992. An adenosine uptake blocker, propentofylline, reduces glutamate release in gerbil hippocampus following transient forebrain ischemia. Neurochem. Res. 17, 147–150.

Murashima, S., Tanaka, T., Hockman, S., Manganiello, V., 1990. Characterization of particulate cyclic nucleotide phosphodiesterases from bovine brain: Purifications of a distinct cGMP-stimulated isoenzyme. Biochemistry 29, 5285–5292.

Nabeshima, T., Nitta, A., Fuji, K., T, K., Hasegawa, T., 1994. Oral administration of NGF synthesis stimulators recovers reduced brain NGF content in aged rats and cognitive dysfunction in basal-forebrain-lesioned rats. Gerontology 40, 46–56.

Nabeshima, T., Nitta, A., Hasegawa, T., 1993. Impairment of learning and memory and the accessory symptom in aged rat as senile dementia model: 3. Oral administration of propentofylline produces recovery of reduced NGF content in the brain of aged rats. Jpn. J. Psychopharmacol. 13, 89–95.

Nagata, K., Ogawa, T., Omosu, M., Fujimoto, K., Hayashi, S., 1985. In vitro and in vivo inhibitory effects of propentofylline on cyclic AMP phosphodiesterase activity. Arzneim.-Forsch. Drug Res. 35, 1034–1036.

Nehls, D., Todd, M., Spetzler, R., Drummond, J., Thompson, R., Johnson, P., 1987. A comparison of the cerebral protective effects of isoflurane and barbiturates during temporary focal ischemia in primates. Anesthesiology 66, 453–464.

Némoz, G., Moueqqit, M., Prigent, A., Pachéco, H., 1989. Isolation of similar rolipram-inhibitable cyclic-AMP-specific phosphodiesterases from rat brain and heart. Eur. J. Biochem. 184, 511–520.

Ohkubo, T., Mitsumoto, Y., Mohri, T., 1991. Characterization of the uptake of adenosine by cultured rat hippocampal cells and inhibition of the uptake by xanthine derivatives. Neurosci. Lett. 133, 275–278.

Park, C., Rudolphi, K., 1994. Antiischemic effects of propentofylline (HWA 285) against focal cerebral infarction in rats. Neurosci. Lett. 178, 235–238.

Parkinson, F., Fredholm, B., 1991. Effects of propentofylline on adenosine A_1 and A_2 receptors and nitrobenzylthioinosine-sensitive nucleoside transporters: Quantitative autoradiographic analysis. Eur. J. Pharmcol. 202, 361–366.

Parkinson, F., Rudolphi, K., Fredholm, B., 1994. Propentofylline: A nucleoside transport inhibitor with neuroprotective effects in cerebral ischemia. Gen. Pharmacol. 25, 1053–1058.

Rudolphi, K., Schubert, P., Parkinson, F., Fredholm, B., 1992. Neuroprotective role of adenosine in cerebral ischaemia. Trends Pharmacol. Sci. 13, 439–445.

Saletu, B., Möller, H., Grünberger, J., Deutsch, H., Rössner, M., 1990. Propentofylline in adult-onset cognitive disorders: Double-blind, placebo-controlled, clinical psychometric and brain mapping studies. Pharmacopsychiatry 24, 173–184.

Shinoda, I., Furukawa, Y., Furukawa, S., 1990. Stimulation of nerve growth factor synthesis/secretion by propentofylline in cultured mouse astroglial cells. Biochem. Pharmacol. 39, 1813–1816.

Stanimirovic, D., Micic, D., Markovic, M., Spatz, M., Mrsulja, B., 1994. 'Therapeutic window' for multiple drug treatment of experimental cerebral ischemia in gerbils. Neurochem. Res. 19, 189–194.

Uchiyama-Tsuyuki, Y., Araki, H., Yae, T., Otomo, S., 1994. Changes in the extracellular concentrations of amino acids in the rat striatum during transient focal cerebral ischemia. J. Neurochem. 62, 1074–1078.

Young, A., Touzani, O., Derlon, J.-M., Sette, G., MacKenzie, E., Baron, J.-C., 1997. Early reperfusion in the anesthetized baboon reduces brain damage following middle cerebral artery occlusion. Stroke 28, 632–638.

Zea Long, E., Weinstein, P., Carlson, S., Cummins, R., 1989. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 20, 84–89.