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Adenosine kinase inhibition protects brain against transient focal ischemia in rats

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Abstract

Endogenous adenosine released locally during cerebral ischemia is neuroprotective, and agents which decrease adenosine inactivation may potentiate its protective effects. The effects of 5′-deoxy-5-iodotubercidin (5′d-5IT), an inhibitor of the adenosine-catabolizing enzyme, adenosine kinase, were studied in male Wistar rats subjected to 2 h of transient middle cerebral artery occlusion. 5′d-5IT or the vehicle (10% DMSO in saline) was administered i.p. 30 min before, and 2 h and 6 h after the induction of middle cerebral artery occlusion. The infarct volume was determined using 2,3,5-triphenyltetrazolium chloride staining 48 h after middle cerebral artery occlusion. The infarct volume was significantly reduced in rats treated with 1.85 mg/kg × 3 (57% reduction, $P < 0.001$) or 1.0 mg/kg × 3 (34% reduction, $P < 0.05$), but not 0.3 mg/kg × 3 5′d-5IT compared to vehicle-treated rats. The reduction of infarct volume was accompanied by a significant improvement in behavioral measures of neurological deficit. These data further support a role of adenosine in neuroprotection and suggest that adenosine kinase inhibition may be a useful approach to the treatment of focal cerebral ischemia.

Keywords: Cerebral ischemia; Adenosine kinase inhibitor; Neuronal damage

1. Introduction

Nervous tissue damage secondary to ischemia results from a cascade of interconnected pathological processes, including changes in the regulation of cerebral blood flow as well as progressive alterations of ion homeostasis and cellular metabolism (Rothman and Olney, 1986; Choi, 1992; Siesjö, 1992). The endogenous regulatory systems influencing the physiological and biochemical processes may provide targets for pharmacological limitation of ischemic brain damage. The endogenous neuromodulator adenosine is one potential target. Adenosine has been characterized as a 'retaliatory metabolite' (Newby, 1984). Extracellular adenosine increases dramatically at the site of injury during ischemia and it exerts a neuroprotective effect via activation of adenosine receptors (Rudolphi et al., 1992). However, direct intravascular administration of adenosine may not be effective due to its extremely short biological half-life, in the range of 3–6 s (Paterson et al., 1987), and limited transendothelial passage of adenosine from blood to brain (Cornford and Oldendorf, 1975; Wu and Phillis, 1982; Beck et al., 1983). Agents capable of activating adenosine receptors via peripheral administration, thereby offering protection against ischemic cerebral damage, are classified into two categories: (1) direct-acting adenosine A_1 or A_{2A} receptor agonists (Block and Pulsinelli, 1987; Bielenberg, 1989; Rudolphi et al., 1992; Von Lubitz et al., 1994); or (2) adenosine-potentiating agents (Engler, 1987), which elevate endogenous adenosine levels by either inhibiting its degradation, such as by inhibiting the enzymes adenosine deaminase (Phillis et al., 1988), or adenosine kinase (Sciotti and Von Wylen, 1993), or by inhibiting adenosine transport (DeLeo et al., 1987). Because the side effects associated with adenosine agonists, such as hypotension and bradycardia (Sollevi, 1986),

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constitute barriers to be overcome before clinical application of this approach is possible, potentiating endogenous adenosine may represent an attractive alternative.

To date, most studies investigating neuroprotective effects against cerebral ischemia by activating adenosine receptors have focused on global cerebral ischemia (Block and Pulsinelli, 1987; DeLeo et al., 1987; Von Lubitz et al., 1988; Araki et al., 1989; Busto et al., 1989b; Helfman and Phillis, 1989; Phillis and O'Regan, 1989); focal cerebral ischemia has been the subject of less attention with fewer reports (Bielenberg, 1989; Roussel et al., 1991; Lin and Phillis, 1992; Park and Rudolphi, 1994). To our knowledge, no study has been reported on the effects of an adenosine kinase inhibitor in focal cerebral ischemia with recirculation. The present experiment was designed to determine if 5′-deoxy-5-iodotubercidin (5′d-5IT), an adenosine kinase inhibitor (Davies et al., 1984, 1986), reduces ischemic cell damage after 2 h of transient middle cerebral artery occlusion.

2. Materials and methods

All experimental procedures have been approved by the Care of Experimental Animals Committee of Henry Ford Hospital.

Male Wistar rats ($n = 42$) weighing 270–300 g were employed in all our experiments. Nonfasted animals were anesthetized with 3.5% halothane, and maintained with 1.0–2.0% halothane in 70% N_2O and 30% O_2 using a face mask. Rectal temperature was maintained at 37°C throughout the surgical procedure using a feedback regulated water heating system. The right femoral artery was cannulated with medical grade silicone tubing (Technical Products, Decatur, GA, USA) for sampling of blood for blood gas measurement (pH, pO_2, pCO_2), and for monitoring blood pressure before and 15 min after initial administration of 5′d-5IT or vehicle.

Middle cerebral artery occlusion was induced as previously described (Koizumi et al., 1986; Chen et al., 1992). Briefly, the right common carotid artery, external carotid artery and internal carotid artery were exposed. A length of 4-0 monofilament nylon suture (18.5–19.5 mm), determined by the animal weight, with its tip rounded by heating near a flame, was advanced from the external carotid artery into the lumen of the internal carotid artery until it blocked the origin of the middle cerebral artery. Two hours after middle cerebral artery occlusion, animals were reanesthetized with halothane and reperfusion was performed by withdrawal of the suture until the tip cleared the lumen of the internal carotid artery.

Four randomly assigned populations of animals were tested. Animals received 5′d-5IT at a dose of 0.3 mg/kg i.p., 1.0 mg/kg i.p. or 1.85 mg/kg i.p., or the vehicle (10% dimethyl sulfoxide (DMSO) in normal saline, 5 ml/kg) i.p 30 min before, and 2 h and 6 h after middle cerebral artery occlusion. 5′d-5IT was synthesized at Abbott Laboratories.

All animals were weighed before surgery for middle cerebral artery occlusion and at 24 and 48 h after middle cerebral artery occlusion. Neurological abnormalities were also evaluated 24 and 48 h after middle cerebral artery occlusion using the scale (0–4) described by Zea Longa et al. (1989): 0, normal; 1, failure to extend the left forepaw; 2, circling to the left; 3, falling to the left; 4, did not walk spontaneously and exhibited a depressed level of consciousness. 48 h post middle cerebral artery occlusion, the animals were reanesthetized with ketamine (44 mg/kg) and xylazine (13 mg/kg). Transcardiac perfusions with heparinized saline were performed to remove the blood from cerebral vessels. Thereafter, the animals were decapitated, and the brains were quickly removed. Each brain was cut into 2-mm thick coronal sections (7 sections per brain) using a rat brain matrix and then stained for 30 min in a 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) at 37°C, and fixed by immersion in 10% buffered formalin solution. Each TTC stained section was photographed with a 35-mm camera mounted on an operating microscope within 2 days of TTC staining. The unstained area as well as the total right and left hemispheric area for each coronal section were measured using the Global Lab Image analysis system (Data Translation, Marlboro, MA, USA). Tissue volumes (mm^3) were determined by multiplying the appropriate areas by the section interval thickness. The indirect method for calculating lesion volume, in which the intact area of the ipsilateral hemisphere was subtracted from the area of the contralateral hemisphere, was used (Swanson et al., 1990). The lesion volume is presented as the percentage of lesion relative to the contralateral hemisphere.

Additional animals without middle cerebral artery occlusion were used to test whether administration of 5′d-5IT alters cerebral temperature. The doses and time for injections of 5′d-5IT or vehicle, anesthesia conditions at the times of injection are identical to the middle cerebral artery occlusion experiments. Thirty minutes before 5′d-5IT (for each dose group: $n = 4$) or vehicle ($n = 4$) injection, a microthermocouple (100 μm), placed into a 27 gauge needle, was inserted into the right caudoputamen through a 1 mm burr hole in the skull. The hole was drilled 4 mm lateral to the midline, 0.8 mm posterior to the bregma, and the microthermocouple was placed 5 mm in depth. Cranioplastic cement (Plastics One, Roanoke, VA, USA) was used to seal the burr hole and to fix the microthermocouple. The brain temperature was recorded before injection and every 15 min until 1 h and then every 30 min until 5.5 h and at 6.5, 8.5, 10.5, 12.5, 24.5 and 48.5 h after initial injections of 5′d-5IT or vehicle using a digital thermometer (Physitemp, Clifton, NJ, USA).

For parametric variables, a one way ANOVA was applied to determine the statistical significance of differences among groups. If a significant difference was de-

Table 1
Physiological parameters

Parameter	Vehicle ($n=11$)	5′d-5IT 1.85 mg/kg ($n=10$)	5′d-5IT 1.0 mg/kg ($n=10$)	5′d-5IT 0.3 mg/kg ($n=10$)
Blood gas				
Pre-injection				
pH	7.43±0.01	7.42±0.01	7.36±0.02	7.38±0.01
P_{CO2}(mmHg)	39.1±1.2	39.8±3.1	46.0±0.9	46.6±1.1
P_{O2}(mmHg)	125.8±3.7	134.0±7.6	113.3±2.4	119.7±5.5
Mean blood pressure (mmHg)				
Pre-injection	84±1	81±2	87±3	90±3
15-min post-injection	84±1	30±2 [a]	44±3 [a]	80±7

Values are means±S.E.M. [a] $P < 0.001$ compared with pre-injection.

tected, then two sample *t*-tests with Bonferroni correction were performed to evaluate differences between vehicle-treated and 5′d-5IT-treated groups. Paired *t*-tests were performed on physiological parameters before and after administration of 5′d-5IT and vehicle within each group. Values presented in this study are means ± S.E.M. A probability value less than 0.05 was considered significant.

3. Results

The physiological variables before and after initial injections of 5′d-5IT and vehicle are shown in Table 1. Statistically significant hypotension was observed after injections of 1.85 mg/kg or 1.0 mg/kg 5′d-5IT, while blood pressures were within normal range for rats after injection of 0.3 mg/kg 5′d-5IT or vehicle. For all groups, blood gas values measured before injection of 5′d-5IT and vehicle were within normal ranges.

As shown in Fig. 1, the percentage infarct volume was significantly decreased in both 1.85 mg/kg (57% reduction) and 1.0 mg/kg (34% reduction) 5′d-5IT-treated groups compared with the vehicle-treated group. No beneficial effect was observed in the 0.3 mg/kg 5′d-5IT group. Fig. 2 shows the distribution of percent area of infarct in each of the seven brain sections from all the four groups illustrating a relatively uniform reduction in the ischemic lesion in nearly all (5/7) coronal sections with the 1.85 mg/kg 5′d-5IT treatment.

Table 2 presents results of body weight loss and the neurological deficit score 24 and 48 h after onset of ischemia in each group. Both the 1.85 mg/kg and 1.0 mg/kg 5′d-5IT-treated animals exhibited a significantly improved physiological response as reflected by a reduced body weight loss and improved neurological function (lower score) compared with the vehicle-treated group. No significant effect was seen on either parameter in the 0.3 mg/kg 5′d-5IT- or vehicle-treated groups.

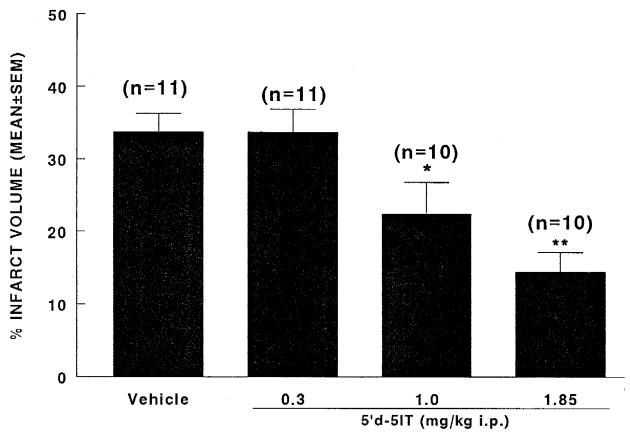

Fig. 1. Bar graph shows lesion volumes presented as a % of lesion relative to contralateral hemisphere in 5′d-5IT- and vehicle-treated groups. Values are means±S.E.M. * $P < 0.05$, ** $P < 0.001$ versus vehicle-treated group.

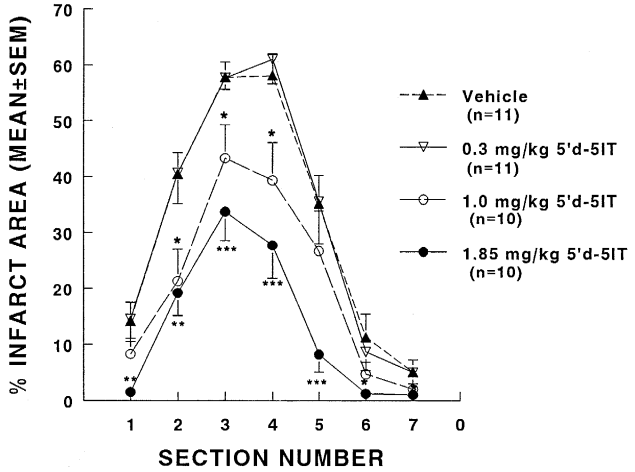

Fig. 2. Line graph shows the percent area of infarction relative to the area of the contralateral hemisphere in each of seven forebrain sections in 5′d-5IT- and vehicle-treated groups. Values are means±S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus vehicle-treated group.

Table 2
Animal body weight loss and the neurological deficit (score) after MCAO

Group	Vehicle ($n=11$)	5′d-5IT 1.85 mg/kg ($n=10$)	5′d-5IT 1.0 mg/kg ($n=10$)	5′d-5IT 0.3 mg/kg ($n=11$)
Body weight (g)				
Pre-MCAO	280±2	280±2	283±3	286±2
Post-MCAO				
24 h	239±2	251±2 [b]	248±5	243±4
48 h	221±3	241±2 [b]	238±7 [a]	224±5
Neurological score				
Post MCAO				
1.5 h	2±0	2.0±0	2.0±0	2.0±0
24 h	2±0	1.2±0.1 [b]	1.7±0.2	1.9±0.1
48 h	2±0	1.2±0.1 [b]	1.5±0.1 [a]	1.9±0.1

Values are means±S.E.M. [a] $P < 0.01$, [b] $P < 0.001$ versus vehicle-treated group. See Section 2 for scoring system details. Maximum possible score = 4.

Table 3
The temporal profile of brain temperatures (°C) before and after administration of various doses of 5′d-5IT and vehicle

Group	5′d-5IT (i.p.) 1.85 mg/kg ($n = 4$)	5′d-5IT (i.p.) 1.0 mg/kg ($n = 4$)	5′d-5IT (i.p.) 0.3 mg/kg ($n = 4$)	Vehicle ($n = 4$)
Preinjection	37.1 ± 0.1	37.0 ± 0.0	37.1 ± 0.1	37.1 ± 0.1
First injection				
0.25 h	36.7 ± 0.2	36.9 ± 0.1	37.0 ± 0.1	37.1 ± 0.1
0.5 h	35.8 ± 0.2 [a,d]	36.2 ± 0.2 [a,d]	37.0 ± 0.1 [f]	37.3 ± 0.1 [f]
0.75 h	35.1 ± 0.1 [b,d,f]	36.1 ± 0.2 [a,d]	36.8 ± 0.2 [c,e]	37.3 ± 0.1 [f]
1 h	35.5 ± 0.2 [d,f]	36.4 ± 0.1 [b,d]	36.9 ± 0.1 [c,e]	37.4 ± 0.1 [f]
1.5 h	36.3 ± 0.3 [a,c]	36.8 ± 0.1 [c]	37.0 ± 0.1	37.3 ± 0.2 [e]
2 h	37.0 ± 0.1	37.0 ± 0.1 [c]	37.1 ± 0.1	37.3 ± 0.1 [e]
2.5 h (2nd inj)	37.1 ± 0.0 [c]	37.1 ± 0.1	37.3 ± 0.1	7.3 ± 0.1 [a]
3 h	35.5 ± 0.1 [b,d,f]	36.1 ± 0.1 [b,d]	37.0 ± 0.1 [c,f]	37.4 ± 0.1 [f]
3.5 h	35.0 ± 0.1 [b,d]	35.0 ± 0.0 [b,d]	36.9 ± 0.0 [d,f]	37.6 ± 0.1 [a,f]
4 h	34.9 ± 0.1 [b,d,f]	35.7 ± 0.1 [b,d]	36.8 ± 0.1 [a,d,f]	37.4 ± 0.0 [a,f]
4.5 h	35.2 ± 0.2 [b,d]	35.8 ± 0.1 [b,d]	37.0 ± 0.1 [d,f]	37.3 ± 0.0 [f]
5 h	35.6 ± 0.2 [b,d]	35.9 ± 0.1 [b,d]	37.1 ± 0.0 [c,f]	37.3 ± 0.0 [f]
5.5 h	36.0 ± 0.1 [b,d]	36.2 ± 0.1 [b,d]	37.2 ± 0.1 [f]	37.2 ± 0.0 [f]
6.5 h (3rd inj)	36.2 ± 0.1 [b,d]	36.3 ± 0.0 [b,d]	37.4 ± 0.1 [f]	37.1 ± 0.1 [f]
8.5 h	33.9 ± 0.3 [b,d]	33.9 ± 0.1 [b,d]	36.6 ± 0.1 [d,f]	37.3 ± 0.1 [f]
10.5 h	34.3 ± 0.3 [b,d]	35.0 ± 0.5 [a,d]	36.9 ± 0.0 [d,f]	37.4 ± 0.0 [a,f]
12.5 h	35.6 ± 0.2 [b,d]	36.0 ± 0.1 [b,d]	37.2 ± 0.1 [f]	37.5 ± 0.0 [b,f]
24.5 h	37.8 ± 0.1 [b,d,e]	37.6 ± 0.1 [a,d]	37.2 ± 0.0 [f]	37.1 ± 0.0 [f]
36.5 h	38.0 ± 0.1 [b,d]	37.8 ± 0.1 [b,c]	37.5 ± 0.0 [b,e]	37.5 ± 0.1 [b,e]
48.5 h	37.4 ± 0.0 [a,d,e]	37.2 ± 0.0	37.2 ± 0.0	37.1 ± 0.1

Values are means ± S.E.M. [a] $P < 0.05$, [b] $P < 0.01$ compared to the values preinjection; [c] $P < 0.05$, [d] $P < 0.01$ compared to the value of vehicle-treated group; [e] $P < 0.05$, [f] $P < 0.01$ compared to the value of 1.0 mg/kg 5′d-5IT group.

Table 3 shows the temporal profile of brain temperature before and after consecutive administrations of various doses of 5′d-5IT and vehicle. Brain temperatures fluctuated along with each injection of 5′d-5IT. A dose-dependent hypothermia followed by a rebound hyperthermia was observed, with maximal hypothermia at 8.5 h after initial injection of 1.85 mg/kg or 1.0 mg/kg 5′d-5IT. A slight rise of brain temperatures immediately after vehicle injection was detected.

4. Discussion

The present results demonstrate that administration of the adenosine kinase inhibitor, 5′d-5IT is neuroprotective in a dose-dependent fashion in transient focal cerebral ischemia in the rat when treatment is initiated 30 min before the onset of ischemia. Significant reductions in ischemic cell damage and neurological deficit were observed and physiological function was improved as reflected by a reduction in body weight loss in animals treated with 1.85 mg/kg or 1.0 mg/kg of 5′d-5IT, while treatment with 0.3 mg/kg 5′d-5IT failed to provide benefits to the ischemic animal. The finding that the adenosine kinase inhibitor offers neuroprotection against focal ischemia is consistent with the results of experiments evaluating the neuroprotective activity of other adenosine-potentiating agents such as HWA 285, an inhibitor of adenosine transport (Park and Rudolphi, 1994), and deoxycoformycin, an inhibitor of adenosine deaminase (Lin and Phillis, 1992), both of which attenuate focal cerebral infarct damage after permanent middle cerebral artery occlusion in rats.

The mechanisms underlying the neuroprotection against cerebral ischemia by 5′d-5IT may be attributed to the reinforcement of the neuroprotective effects of endogenous adenosine. 5′d-5IT is a nanomolar inhibitor of adenosine kinase, which is at least four to five orders of magnitude less potent as an agonist at adenosine A_1, A_{2A} receptors, adenosine deaminase and the adenosine transporter (Davies et al., 1984, 1986; E. Kowaluk et al. unpublished results). Adenosine kinase avidly phosphorylates adenosine and contributes to its removal from the extracellular space (Arch and Newsholme, 1978). 5′d-5IT inhibits the cellular uptake of adenosine by inhibiting adenosine kinase (Davies et al., 1984), thus potentiating the ischemia-evoked local accumulation and neuroprotective effects of extracellular adenosine. Adenosine interacts with discrete cell-surface receptors to initiate a number of responses, the composite of which results in decreased neuronal activity while increasing local nutrient supply (Newby, 1984; Bruns, 1991). Presynaptically, activation of adenosine A_1 receptors attenuates the release of excitatory neurotransmitters, particularly glutamate (Corradetti et al., 1984; Fastbom and Fredholm, 1985; Burke and Nadler, 1988; Prince and Stevens, 1992). Glutamate-induced excitotoxicity is considered to be a fundamental mechanism underlying cerebral ischemic damage (Rothman and Olney, 1986; Choi, 1992). Adenosine also stabilizes membrane potential, act-

ing both pre- and postsynaptically (Schubert et al., 1985; Rudolphi et al., 1992). These effects are mediated by activation of A_1 receptors and subsequent modulation of potassium, calcium and/or chloride conductances. Adenosine may also hyperpolarize astrocyte cell membranes (Hosli et al., 1987), which may in turn limit extracellular glutamate and potassium release (Drejer et al., 1985; Kaupinnen et al., 1988). In addition, adenosine, acting at A_{2A} receptors elicits effects on free radical production, local inflammatory responses, platelet aggregation and local cerebral blood flow, which contribute further to its neuroprotective potential (Miller and Hsu, 1992; Cronstein, 1994).

As previously reported (Davies et al., 1984, 1986), 5′d-5IT evokes hypothermia and hypotension. Mild hypothermia (2–3°C below normal) is a potent cerebroprotectant even 30 min postreperfusion (Busto et al., 1987, 1989a,b; Minamisawa et al., 1990; Welsh et al., 1990) and the neuroprotective effect of an adenosine agonist was eliminated when body temperature was maintained normothermic in the global ischemia model (Miller and Hsu, 1992). Additional experiments in which the brain is fixed at a temperature of 37°C may be required in order to determine the contribution of 5′d-5IT induced hypothermia to the neuroprotection after focal cerebral ischemia. Thus, the possibility that hypothermia contributed to the beneficial effects provided by 5′d-5IT cannot be excluded. However, the maximal hypothermia for both the 1.85 mg/kg and 1.0 mg/kg 5′d-5IT groups was identical, i.e., 33.9°C occurring at 8.5 h after the initial injection of 5′d-5IT and the only significant difference of the degree of hypothermia (≤ 1.0°C) occurred at selected time points within 4 h after initial injection. The small differences in the minor level of hypothermia detected in the 1.85 mg/kg group compared to the 1.0 mg/kg 5′d-5IT group may not account completely for the great difference in reduction of ischemic cell damage (57% versus 34%), although the difference is not statistically significant ($P = 0.13$) due to the small sample size ($n = 10$, for each group). Moreover, the extent of neuroprotection achieved with 1.85 mg/kg 5′d-5IT groups (57%) in focal cerebral ischemia has never been detected for such mild post ischemia hypothermic conditions, suggesting that hypothermia may only in part contributes to the neuroprotection by 5′d-5IT. A caveat to the above discussion, is that the assumption is made that the temperature perturbations induced by 5′d-5IT are the same in ischemic and non ischemic animals. Although the experimental protocols were identical for the middle cerebral artery occlusion and non middle cerebral artery occlusion groups, the effect of 5′d-5IT may differ in ischemic and non ischemic animals.

Cerebral vascular autoregulation can keep cerebral blood flow relatively constant when the blood pressure is within the range of 60–150 mmHg in rat (Hernandez et al., 1978). The 30–44 mmHg decrease in blood pressure after initial injections of 1.85 mg/kg and 1.0 mg/kg 5′d-5IT was out of the range of autoregulation. Hypotension is a recognized detrimental factor for cerebral ischemia especially when occurring in the first 2 h after middle cerebral artery occlusion, the period critical in determining the final outcome of ischemic insult (Zhu and Auer, 1995). However, the greatest neuroprotection was achieved at 1.85 mg/kg 5′d-5IT which was associated with the lowest blood pressure in the present study, suggesting that the beneficial effect of 5′d-5IT via other favorable mechanisms outweighs this disadvantage.

Though DMSO was reported to be a free radical scavenger (Ashwood-Smith, 1967; Panganamala et al., 1967; Del Maestro et al., 1980) and may be beneficial in cerebral ischemia (Gisvold and Steen, 1985), no beneficial effect was found in our present study at the dose of 5 ml/kg 10% DMSO in normal saline compared to our previous data with saline treatment (data not shown). Thus, DMSO can be used as a solvent at the present dose for water-insoluble agents to be evaluated for neuroprotection.

A note of caution in the interpretation of our data is that we evaluated cerebral tissue at 2 days after onset of ischemia. Therefore, we cannot exclude the possibility that intervention with 5′d-5IT delays the maturation of the lesion and provides only temporary (2 day) improvement in the outcome after stroke, similar to the transient beneficial effect of the NMDA antagonist MK-801 on focal cerebral ischemia (Persson et al., 1992; Dezsi et al., 1994).

Although its efficacy remains to be investigated in reducing ischemic cell damage when given after onset of ischemia, 5′d-5IT or other adenosine kinase inhibitors may have clinical potential as an ischemic prophylaxis in situations with a high risk of cerebral ischemia such as coronary artery bypass surgery (Brillman, 1993; Harrison, 1995) and carotid endarterectomy (Ammar and Hanosh, 1991; Gelabert and Moore, 1991).

In summary, we have demonstrated that pretreatment with the adenosine kinase inhibitor 5′d-5IT significantly reduces ischemic infarct damage 48 h after transient (2 h) focal cerebral ischemia which is accompanied by improved neurological and physiological functions. These findings provide further support for a role of adenosine in the salvage of ischemic tissue after stroke consistent with previous studies (Block and Pulsinelli, 1987; Bielenberg, 1989; Miller and Hsu, 1992; Rudolphi et al., 1992). They further suggest that manipulation of adenosine catabolism by inhibiting adenosine kinase may be an effective therapeutic approach to the clinical treatment of stroke. Additional studies are in progress to determine whether this agent remains effective when initiation of the treatment is delayed and brain temperature is kept within physiological range.

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