J. Pharm. Pharmacol. 1996, 48: 601–606
Received June 14, 1995
Accepted December 5, 1995

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Pharmacodynamics of ZM 241385, a Potent A_{2a} Adenosine Receptor Antagonist, after Enteric Administration in Rat, Cat and Dog

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Abstract

4-(2-[7-Amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM 241385) is currently the most selective for the A_{2a} adenosine receptor antagonist. This paper describes the in-vivo activity of ZM 241385 after administration by both oral and intraduodenal routes.

In conscious spontaneously hypertensive rats, ZM 241385 (1–10 mg kg^{-1}) selectively attenuated the mean arterial blood pressure response produced by exogenous adenosine (1 mg kg^{-1} min^{-1}, i.v.) by up to 45% after oral administration. Activity of ZM 241385 was maintained for at least 6 h after 3 and 10 mg kg^{-1} (p.o.). In conscious normotensive cats, ZM 241385 attenuated the blood pressure responses to adenosine (0·6–1·0 mg kg^{-1}, i.v.) by 94% after 10 mg kg^{-1} (p.o.) and by up to 74% after 0·3 mg kg^{-1} (i.v.). Duration of action of ZM 241385 up to 12 h (36% inhibition) was observed after 3 mg kg^{-1} (p.o.). In anaesthetized dogs and cats, ZM 241385, after intraduodenal administration (1–10 mg kg^{-1}), produced a rapid (dose ratio 100-fold 15 min after administration of 10 mg kg^{-1} *in the cat*) and prolonged (dose ratio of 14 at 6 h after administration of 10 mg kg^{-1}) attenuation of the vasodilatation responses to adenosine receptor stimulation. When administered by this route ZM 241385 was six times more potent than theophylline in the cat and at least twice as potent as theophylline in the dog.

In conclusion, ZM 241385 is a potent, selective A_{2a} adenosine receptor antagonist which is orally active, with a good duration of action by the enteric route in cat, rat and dog. It could therefore be used to evaluate the role of adenosine A_{2a} receptors in the action of adenosine in-vivo.

Two functional adenosine receptor subtypes (A_1 and A_2) were first proposed by Londos & Wolff (1977) on the basis of the ability of adenosine either to inhibit or to activate adenylate cyclase; the proposal was confirmed by subsequent studies (Van Calker et al 1979; Bruns et al 1980). More recently, additional adenosine receptor sub-types (A_{2a}, A_{2b} and A_3) have also been characterized (Abbracchio et al 1993; Collis & Hourani 1994).

The potential of a particular adenosine receptor subtype to be involved in the action of adenosine has largely been limited to the use of selective agonists. The involvement of a specific receptor subtype in a physiological or pathophysiological functional response can, however, be elucidated only by use of selective antagonists for the receptor in question. The compound which is currently the most selective for the A_{2a} adenosine receptor antagonist described is 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[[2,3-a][1,3,5]triazin-5-ylamino]ethyl) phenol (ZM 241385 (Poucher et al 1995)). It is highly potent in A_{2a} adenosine receptor systems in-vitro (pA_2 of 9·02 in the guinea-pig isolated heart) and has 1000-fold A_{2a}:A_1 selectivity, 91-fold A_{2a}:A_{2b} selectivity and 5×10^5-fold A_{2a}:A_3 selectivity. ZM 241385 is also selective for the A_{2a} vascular adenosine receptor in both rat and dog in-vivo (Keddie et al 1995). An apparent loss of activity was, however, observed over 2 h after intravenous administration in the anaesthetized dog. This could limit the potential utility of the compound after intravenous administration for elucidation of the role of A_{2a} adenosine receptors in-vivo in protocols lasting more than 90 min. When using ZM 241385, therefore, care must be taken either to ensure that sufficient blockade of adenosine receptors is confirmed after a single administration of ZM 241385 by the intravenous route, or to develop dosing regimes to maintain the degree of adenosine receptor antagonism at the required level for the whole duration of the experimental protocol.

This paper describes the in-vivo cardiovascular activity and duration of action of ZM 241385 in three species, the rat, the cat and the dog by the oral, intravenous and intraduodenal routes.

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Methods and Materials

Oral activity of ZM 241385

Conscious spontaneously hypertensive rat. Female Alderley Park spontaneously hypertensive rats (240–260 g) were anaesthetized using a mixture of alphaxolone (9 mg mL^{-1}) and alphadolone acetate (3 mg mL^{-1}) (0·8–1·0 mL kg^{-1}, Saffan, Pitman Moore (UK), Uxbridge, UK) via the tail vein. The animals were prepared surgically for measurement of blood pressure and pulse rate responses to adenosine as described previously (Keddie et al 1995). Once the animal had become acclimatized to the restraining tubes, and resting blood pressure and pulse rate readings had been taken, the adenosine infusion (1 mg kg^{-1} min^{-1}) was started; it was maintained until stable bradycardic and depressor responses were achieved (usually 1–2 min). ZM 241385 or the non-selective adenosine antagonist 8-phenyltheophylline (8-PT (Collis et al 1985)) were then dosed orally in polyethylene glycol 400 (PEG 400, 2 mL kg^{-1}) and the adenosine infusion was repeated 1, 4 or 6 h after

administration. Inhibition of the adenosine depressor and bradycardic responses was then calculated.

Conscious normotensive cats. Mature male cats (2·5–4 kg) were anaesthetized using alphaxalone (9 mg mL^{-1}) and alphadolone acetate (3 mg mL^{-1}) (0·38–0·6 mL kg^{-1}, Saffan, i.v.); halothane (1·5–3·0%, Fluothane, Zeneca Pharmaceuticals, UK) was used to maintain anaesthesia. A right common carotid artery catheter (polythene/silicone rubber tubing) was advanced so that the tip lay in the aortic arch. Similarly the jugular vein was catheterized using a soft silicone rubber catheter with a guide wire placed in the catheter lumen to aid insertion of the catheter tip to a point corresponding to the 5th intercostal space. The positioning of both catheters was confirmed by fluoroscopy. Catheters were then exteriorized on the dorsal region of the neck and antibiotic and analgesic therapy administered (amoxycillin trihydrate, 15 mg kg^{-1} s.c., Clamoxil, Beecham Animal Health, Brentford, UK; buprenorphine hydrochloride, 6·5 $\mu g\ kg^{-1}$, Temgesic, Reckitt & Colman, Hull, UK). A minimum post-surgical recovery time of one week was allowed before cats were used for subsequent experiments.

For assessment of the oral activity of the adenosine antagonist, the unrestrained cats were placed individually in open-fronted Perspex boxes. The carotid artery catheter was connected to a pressure transducer (Bell and Howell L221, Basingstoke, UK) via a 70-cm length of polyvinyl chloride tubing (800/100/240, 0·76 mm i.d.) for the recording of blood pressure and pulse rate on a chart recorder (Lectromed MT2, St Peter, Jersey). A similar length and size of tubing was connected to the venous catheter, as this enabled intravenous administration without handling the animals. After a short period of stabilization a siting dose of 0·6 mg kg^{-1} adenosine was administered to assess the sensitivity of the cat to adenosine. On the basis of this result either 0·6 or 1·0 mg kg^{-1} adenosine was administered to the cat and three reproducible responses were obtained to give a minimum fall in diastolic blood pressure of 20 mmHg. Cats were then dosed orally, either with ZM 241385 (in polyethylene glycol 400 (PEG 400) in a hard gelatin capsule) or with the non-selective adenosine antagonist theophylline (Daly et al 1985) and adenosine challenges were repeated for up to 24 h. Subsequent adenosine-mediated reductions in diastolic blood pressure were calculated as percentage inhibition of the pre-antagonist response. Animals were also dosed intravenously with ZM 241385 (in PEG 400, 0·1 mL kg^{-1}).

Potency and duration of action of ZM 241385 following intraduodenal dosing

Anaesthetized dog. Female beagle dogs (12–18 kg) were anaesthetized with sodium pentobarbitone (45–50 mg kg^{-1}, i.v., Sagatal, Rhône Merieux, Harlow, UK). The trachea was intubated and the dogs were artificially ventilated (24 cycles min^{-1}, tidal volume 13–15 mL kg^{-1}) with room air. The animals were prepared and maintained for the assessment of the vasodilatation action of adenosine in the neurally and vascularly isolated right hind limb as described previously (Keddie et al 1995). The duodenum was also exposed for administration of adenosine antagonist (in PEG 400), and nitrobenzylthioinosine (0·5 mg kg^{-1}, i.v.) was administered to inhibit adenosine uptake. Adenosine was administered intra-arterially to the perfused hind limb, producing falls in hind limb perfusion pressure with negligible systemic effects. The dogs were sensitized to adenosine during the equilibration time after administration of nitrobenzylthioinosine by constructing an adenosine dose-response curve (0·001–0·3 mg kg^{-1}). During this time arterial blood gases and pH were measured (Corning 288 Blood Gas System, Medfield, MA, USA) and adjusted if necessary to within normal ranges by adjustment of tidal volume or by intravenous administration of 8.4% w/v sodium bicarbonate. The duration of the effect of adenosine antagonist was monitored for up to 6 h after intraduodenal administration. Rightward shifts in the adenosine dose-response curves were expressed as mean dose ratios for each dose of adenosine antagonist given.

Anaesthetized cat. Male cats (2–3 kg) were anaesthetized with sodium pentobarbitone (45 mg kg^{-1}, i.p.). The right jugular vein (infusion of anaesthetic, sodium pentobarbitone, approx. 6 mg kg^{-1} h^{-1}), left jugular vein (administration of drugs) and right common carotid artery (monitoring blood pressure and pulse rate) were catheterized. The trachea was cannulated and the animals allowed to breathe spontaneously. A medial abdominal incision immediately below the sternum was made to reveal the duodenum for administration of adenosine antagonist. Blood gas status and pH were monitored and maintained within physiological limits throughout the experiment. Body temperature was maintained at $37·5 \pm 0·5$°C by means of a thermostatically controlled heating blanket. A control dose-response curve to 2-chloroadenosine (2-CADO, 0·3–30 $\mu g\ kg^{-1}$, i.v.) was constructed against the reduction in diastolic blood pressure. This was followed by further 2-CADO dose-response curves up to 6 h after adenosine antagonist administration (ZM 241385 or theophylline). Rightward shifts in the 2-CADO dose- response curves were expressed as mean dose ratios for each dose of adenosine antagonist given.

Pharmacokinetic studies

Conscious cats were prepared with catheters placed in the external jugular vein and common carotid artery as described above. ZM 241385 was administered either orally (1, 3 or 10 mg kg^{-1}) or via the external jugular vein (10 mg kg^{-1}) and blood samples (1 mL) were taken into heparinized syringes and the plasma removed by centrifugation. The samples were taken between 15 min and 24 h after oral administration or between 5 min and 24 h after intravenous administration. After C_{18} solid phase extraction the plasma samples were analysed by HPLC using either a Zorbax or Bakerbond C_8 column and a mobile phase comprising methanol – 0·1% phosphoric acid (9:11; adjusted to pH 7·0 with hexylamine) with UV detection at 255 nm. A standard curve for analysis of ZM 241385 was prepared with cat plasma containing ZM 241385 over the range 0·02–5·0 $\mu g\ mL^{-1}$, extraction and analysis being performed in a manner identical to that used for the samples.

Drugs and compounds used

ZM 241385 was prepared in the chemistry department by Dr Geraint Jones and Mr Peter Caulkett. It was administered intraduodenally to cats and dogs as a solution in 100% PEG 400. Conscious cats received ZM 241385 and theophylline as a solution or suspension in PEG 400 for oral dosing within a hard

gelatin capsule (Davcaps size 4). Conscious spontaneously hypertensive rats received ZM 241385 and standard antagonists as suspension or solution in PEG 400 given by oral gavage.

Adenosine, 2-chloroadenosine, theophylline, 8-phenyltheophylline and nitrobenzylthioinosine were obtained from Sigma Chemicals (Poole, UK). Adenosine for the conscious cat studies was dissolved in fresh sterile saline and filtered using Acro Discs (0·45 μm) before intravenous administration. Standard antagonists theophylline and 8-phenyltheophylline were dissolved in a 1:1 solution of PEG 400 in 50% 0·1 m NaOH.

Statistical analysis

All values are quoted as the mean ± s.e.m. Statistical analysis was undertaken using the Student's *t*-test for paired or unpaired data.

Results

Oral activity of 241385

Conscious spontaneously hypertensive rat. Control mean arterial blood pressure for the spontaneously hypertensive rats used in this study was 170 ± 1 mmHg (175 ± 3 mmHg for ZM 241385 treatment group) with a mean depressor response (fall in mean arterial blood pressure) on adenosine infusion of 65 ± 2 mmHg (69 ± 3 mmHg for ZM 241385 treatment group). Similarly, mean control pulse rate was 468 ± 4 beats min^{-1}, with a mean bradycardic response to adenosine of 89 ± 5 beats min^{-1} (84 ± 8 beats min^{-1} for ZM 241385 treatment group).

8-Phenyltheophylline (10 mg kg^{-1}, p.o.) attenuated both the adenosine mediated depressor and bradycardic responses 1 h after administration by 44 ± 5 and 95 ± 12%, respectively (Fig. 1). Inhibition of depressor response by 8-phenyltheophylline was maintained for at least 6 h, and inhibition of the bradycardic effect for 4 h (Fig. 1).

ZM 241385 (3 and 10 mg kg^{-1}, p.o.) inhibited adenosine's depressor response 1, 4 and 6 h after dosing. Lower doses of ZM 241385 (1 mg kg^{-1}, p.o.) attenuated the response at 4 h only (Fig. 1). ZM 241385 did not attenuate the bradycardic responses to adenosine at any of the doses studied (Fig. 1b). Indeed, there was a dose-dependent potentiation of the bradycardic response to adenosine after administration of ZM 241385 with the bradycardic response increased by as much as 2·5-fold after the highest dose of ZM 241385.

Conscious normotensive cat. The capacity of ZM 241385 to antagonize the vascular effects of adenosine was assessed for up to 24 h after oral administration (0·1–10·0 mg kg^{-1}) and for up to 6 h after intravenous administration (0·1–1 mg kg^{-1}).

After oral administration the arterial blood pressure of the cats studied was 127 ± 3/83 ± 3 mmHg (systolic/diastolic, n = 50); the corresponding reduction in diastolic blood pressure after administration of adenosine was 32·6 ± 1·7 mmHg. ZM 241385 caused dose-dependent inhibition of the depressor response induced by adenosine. A high level of oral efficacy was observed, maximum inhibition of 94 ± 11% being observed 2 h after administration of 10 mg kg^{-1} ($P < 0·05$, n = 7, Fig. 2). Compound activity was observed for 12 h after administration of the highest dose given. After both 1 and 3 mg kg^{-1}, ZM 241385 activity was maintained for up to 9 h (42 ± 12%, n = 7) and 12 h (36 ± 9%, n = 15), respectively. In comparison, theophylline (3 mg kg^{-1}) resulted in significant attenuation of the depressor response after 2 h only (48 ± 9%, $P < 0·05$, n = 5, Fig. 2). In the group used for intravenous administration of ZM 241385, blood pressure was 110 ± 3/81 ± 2 mmHg (n = 18).

Administration of adenosine resulted in a decrease in

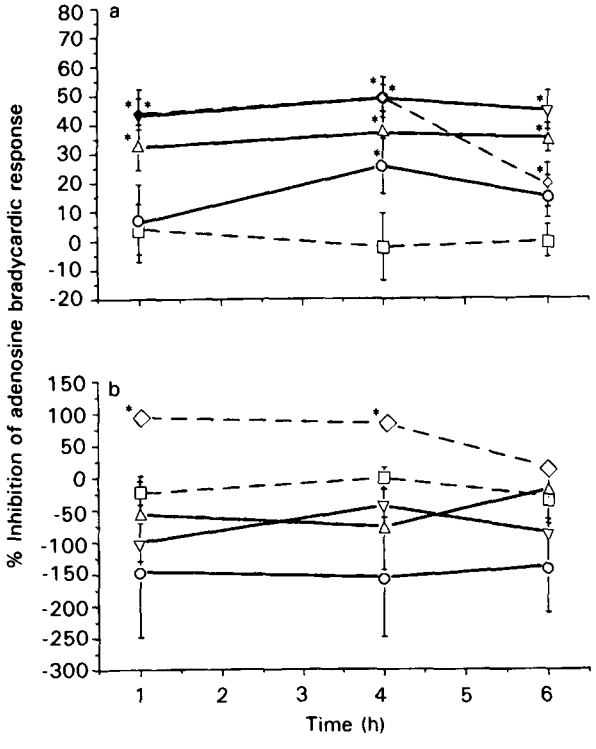

FIG. 1. The effect of orally administered adenosine receptor antagonists 8-phenyltheophylline and ZM 241385 upon adenosine-mediated blood pressure (a) and bradycardic (b) responses in conscious spontaneously hypertensive rats. □, Vehicle control (n = 8); ○, ZM 241385 1 mg kg^{-1} (n = 6); △, ZM 241385 3 mg kg^{-1} (n = 7–13); ▽, ZM 241385 10 mg kg^{-1} (n = 11–14); ◇, 8-phenyltheophylline 10 mg kg^{-1} (n = 23). Each point is the mean ± s.e.m. *P <0·05, significantly different compared with the vehicle control response (using Student's unpaired *t*-test, 1-tailed for blood pressure, 2-tailed for bradycardia).

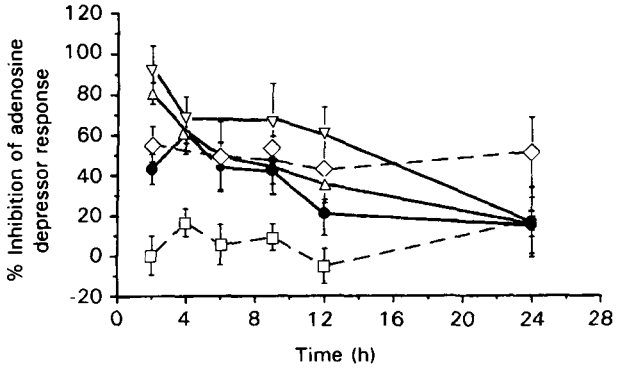

FIG. 2. The effect of orally administered adenosine receptor antagonists theophylline and ZM 241385 upon adenosine-mediated hypotension in conscious normotensive cats. □, Vehicle control (n = 17); ●, ZM 241385 1 mg kg^{-1} (n = 6); △, ZM 241385 3 mg kg^{-1} (n = 15); ▽, ZM 241385 10 mg kg^{-1} (n = 7); ◇, theophylline 10 mg kg^{-1} (n = 5). Each point is the mean ± s.e. of the mean. With the exception of the point 24 h after administration of ZM 241385 all points are significantly different from the vehicle control response (P <0·05 using Student's unpaired, 1-tailed *t*-test).

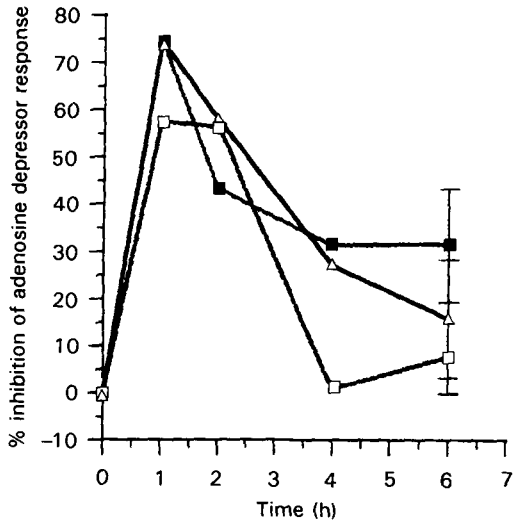

FIG. 3. The effect of ZM 241385 (□, 0·1; ■, 0·3; △, 1·0 mg kg^{-1}, i.v.) upon adenosine-mediated hypotension in conscious normotensive cats. Each point is the mean ± s.e. given only for the 6 h value. In the vehicle-treated group, percentage inhibition was − 11·1 ± 14·9%, 20·7 ± 4·7%, 5·4 ± 10·3% and 25·5 ± 4·8% 1, 2, 4 and 6 h after administration of antagonist.

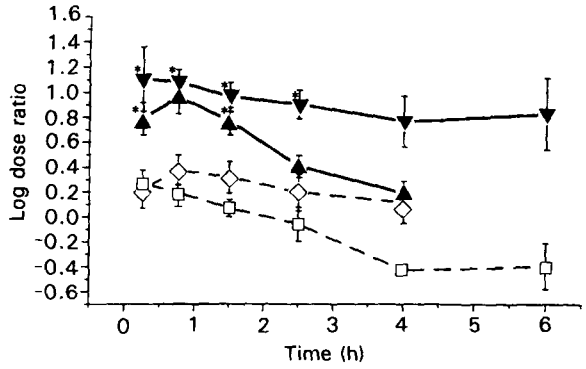

FIG. 4. The effect of adenosine receptor antagonists theophylline and ZM 241385, administered intraduodenally, upon the adenosine-mediated reduction of hind limb perfusion pressure in anaesthetized dogs. □, Vehicle control (n = 4); ▲, ZM 241385 3 mg kg^{-1} (n = 4); ▼, ZM 241385 10 mg kg^{-1} (n = 4); ◇, theophylline 6 mg kg^{-1} (n = 4). Each point is the mean ± s.e.m. *$P < 0.05$, significantly different from result for theophylline (using Student's unpaired, 2-tailed t-test).

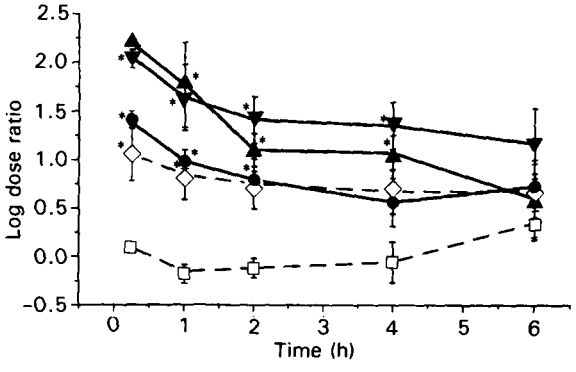

FIG. 5. The effect of adenosine receptor antagonists theophylline and ZM 241385, administered intraduodenally, upon the adenosine-mediated reduction of diastolic blood pressure in anaesthetized cats. □, Vehicle control (n = 3); ●, ZM 241385 1 mg kg^{-1} (n = 3); ▲, ZM 241385 3 mg kg^{-1} (n = 3); ▼, ZM 241385 10 mg kg^{-1} (n = 3); ◇, theophylline 6 mg kg^{-1} (n = 3). Each point is the mean ± s.e.m. *$P < 0.05$, significantly different from vehicle control (using Student's unpaired, 1-tailed t-test).

diastolic blood pressure of 35·4 ± 1·9 mmHg. PEG 400 vehicle given by the intravenous route transiently reduced the arterial blood pressure. After 1 h, however, diastolic blood pressure had returned to pre-dose levels (83 ± 3 vs 81 ± 5 mmHg, n = 6) and the depressor response to adenosine was unaffected (89 ± 15% of pre-PEG 400). The adenosine response was inhibited by 57·5 ± 15·3% (n = 6) and 74·5 ± 15·8% (n = 6) 1 h after intravenous administration of ZM 241385 at 0·1 and 0·3 mg kg^{-1}, respectively. Following this route of administration it was, however, apparent that the compound was cleared and/or metabolized rapidly because neither of the doses given maintained a level of inhibition greater than 50% 4 h after dosing (Fig. 3).

Potency and duration by intraduodenal route

Anaesthetized dog. The dose ratio from the adenosine dose-response curves upon hind limb perfusion pressure before and after intraduodenal administration of ZM 241385 were calculated after periods from 15 min to 6 h. The mean dose ratios after each time period are shown in Fig. 4 for ZM 241385 (3–10 mg kg^{-1}), theophylline (6 mg kg^{-1}) and their vehicle (PEG 400). The maximum adenosine antagonist effect of ZM 241385 was observed between 15 and 45 min. ZM 241385 maintained good activity for 6 h (mean dose response = 6·7, n = 4) after the 10 mg kg^{-1} dose. Theophylline by comparison produced a weak adenosine antagonism dose response < 3·0 at 45 and 90 min with no activity 4 h after administration. ZM 241385 3 mg kg^{-1} resulted in similar antagonism to 10 mg kg^{-1} at 15 and 45 min, but this activity had rapidly diminished 4 h after administration (Fig. 4).

Anaesthetized cat. The dose ratio from the 2CADO dose-response curves before and after intraduodenal administration of ZM 241385 were calculated after periods from 15 min to 6 h. The mean dose ratios after each time period are shown in Fig. 5 for ZM 241385 (1–10 mg kg^{-1}), theophylline (6 mg kg^{-1}) and their vehicle (PEG 400). The maximum effect observed was a 100-fold shift 15 min after administration of 3 and 10 mg kg^{-1} ZM 241385, indicating rapid absorption of the compound by this route. ZM 241385 10 mg kg^{-1} resulted in significant activity 6 h after administration (mean dose response = 14). Theophylline (6 mg kg^{-1}) was also rapidly absorbed, resulting in a mean dose response of 11 after 15 min; this, however, was the peak effect observed and the adenosine antagonism had diminished to a mean dose response of 4·5 after 6 h. This adenosine antagonism profile of theophylline (6 mg kg^{-1}) paralleled that observed for 1 mg kg^{-1} ZM 241385.

Pharmacokinetic studies

After oral administration of ZM 241385 in PEG 400/NaOH the compound appeared to be absorbed rapidly, with peak plasma concentrations appearing within 60 min of administration to all cats (Table 1). After both oral and intravenous administration, however, ZM 241385 was also rapidly cleared from the plasma. Although there was no detectable ZM 241385 present in the plasma 4 h after oral administration at both 3 and 1 mg kg^{-1}

this did not correlate with a lack of biological activity (Fig. 2). The limit of detection of the assay was 10 ng mL^{-1}, whereas the pA_2 value for ZM 241385 at the A_{2a} adenosine receptor is 9·02 or 0·34 ng mL^{-1}, i.e. 70-fold lower than the limit of detection. From the area under the curve for the 10 mg kg^{-1} dose level, oral bioavailability was found to be 18·2%.

Discussion

The results of this study demonstrate that the non-xanthine, A_{2a}-selective antagonist ZM 241385 inhibits the vascular effects of adenosine receptor stimulation in in-vivo preparations. Prolonged antagonism could not, however, be demonstrated after intravenous administration. This observation was supported by the rapid clearance observed in the conscious cats. Good duration of activity was, however, demonstrated after oral administration to conscious rats and cats, and by the intraduodenal route in anaesthetized cats and dogs. This may be because of prolonged absorption of the compound.

Oral activity was demonstrated for 3 and 10 mg kg^{-1} ZM 241385 in spontaneously hypertensive rats with at least 6 h inhibition of the adenosine depressor responses and no antagonism of adenosine's bradycardic action. There was however a trend for the bradycardic effect of adenosine to be potentiated by administration of ZM 241385. Since the conscious rats were fully reflexic in the presence of ZM 241385, the enhanced bradycardic effects of adenosine may be accounted for by the reduction or loss of both the vagal withdrawal and increased sympathetic efferent nerve responses to the heart during administration of adenosine. In the presence of a vascular selective adenosine receptor antagonist, therefore, the direct inhibitory action of adenosine on the sino-atrial node is not reduced by the normal reflex response to hypotension. The potency of ZM 241385 would appear to be slightly greater in the conscious cat paradigm than in the spontaneously hypertensive rat, with 6 h duration of antagonism of adenosine's depressor action at 1 and 3 mg kg^{-1}, orally. When administered intravenously to conscious cats, activity of ZM 241385 had diminished at all doses (0·1–0·3 mg kg^{-1}) after 4 h, and was less than the activity seen for the equivalent oral dose, indicating rapid clearance from the biophase. Thus for an equivalent dose of ZM 241385 the duration of antagonism in the cat is greater after oral administration.

Because of their intact autonomic reflexes, however, the conscious rat and cat cannot be used to determine the absolute potency of ZM 241385 or, indeed, other adenosine receptor antagonists. Only the potency of a compound relative to an internal standard (such as theophylline or 8-phenyltheophylline) can be determined using a fixed dose of agonist (adenosine). On the basis of full dose-response curves for adenosine receptor agonists in anaesthetized cats (see Fig. 1 in Poucher (1996)), a 50% inhibition of ED50 dose is seen when the rightward dose shift is 2·6-fold, whereas 100% inhibition of the response could be observed after administration of a dose of antagonist capable of producing either a 7·6-fold or a 76-fold rightward shift. In addition, the use of a single dose of agonist does not enable estimation of the slope, and hence sensitivity to agonist, of a dose-response curve. It is, consequently, not possible to estimate the dose shift from the percentage inhibition of response to single dose of agonist. To determine the absolute potency of ZM 241385 by the enteric route, therefore, the compound was dosed intraduodenally to anaesthetized cats and dogs in which full adenosine receptor agonist responses were determined before, and for up to 6 h after, administration of antagonist. Extrapolating such information from the anaesthetized animal to conscious animal assumes that the anaesthetic agent does not modify either gut motility or splanchnic blood flow. Pilot studies measuring plasma concentrations of other structurally related adenosine antagonists after either oral administration to conscious dogs or intraduodenal dosing to anaesthetized dogs revealed, however, a decrease in exposure of the animals to the compound given orally compared with those to which it was administered by the intraduodenal route (McKillop, unpublished observation). This difference is possibly because of better absorption from a liquid formulation. Apparent rapid absorption was demonstrated after intraduodenal administration of ZM 241385 to anaesthetized dogs and cats; peak activity was seen 15 min after administration, and potency and duration of action were good in comparison with theophylline. The peak effect observed in the cats after administration of ZM 241385 was higher than that observed in the dogs; the greater potency and duration in the cat were particularly apparent for a dose of 3 mg kg^{-1}, which could reflect more complete initial absorption of the compound in the cat or greater sensitivity of the cat to antagonism by ZM 241385. The dose of ZM 241385 required for a twofold rightward shift in the anaesthetized cat, however, gives a value of 0·04 ± 0·02 mg kg^{-1} (50 min after administration of

Table 1. Plasma concentration of ZM 241385 ($\mu g\ mL^{-1}$) after administration to conscious cats by oral and intravenous routes.

Time post dose (h)	Dose of ZM 241385 10 mg kg^{-1} (i.v.) (n = 3)	10 mg kg^{-1} (p.o.) (n = 3)	3 mg kg^{-1} (p.o.) (n = 1)	1 mg kg^{-1} (p.o.) (n = 1)
0	ND	ND	ND	ND
0.08	4·24 ± 0·27	–	–	–
0.25	1·61 ± 0·19	0·15 ± 0·05	0·188	0·029
0·5	0·89 ± 0·05	0·17 ± 0·02	0·081	0·025
0·75	0·50 ± 0·06	–	–	–
1·0	0·33 ± 0·03	0·10 ± 0·03	0·024	0·011
1·5	0·12 ± 0·02	–	–	–
2	–	0·03 ± 0·01	0·012	0·007
4	0·02 ± 0·01	0·01 ± 0·01	0·008	ND
6	0·02 ± 0·01	0·01 ± 0·01	ND	ND
12	ND	ND	ND	ND
24	ND	ND	ND	ND

Values are means ± s.e. ND, not detected; –, not measured.

antagonist (Poucher 1996)) compared with $0{\cdot}04 \pm 0{\cdot}02$ mg kg^{-1} (15 min after administration of antagonist) and $0{\cdot}14 \pm 0{\cdot}06$ mg kg^{-1} (45 min after administration of antagonist) in the anaesthetized dog (unpublished observation). The degree of reduction in the apparent dose of ZM 241385 required for a twofold rightward shift between 15 min and 45 min in the dog parallels the $3{\cdot}2 \pm 0{\cdot}18$-fold ($n = 3$) reduction in plasma concentration of ZM 241385 found in cats after intravenous administration at 10 mg kg^{-1} over this time period. The rate of appearance of ZM 241385 in the plasma after intraduodenal administration was not, however, determined after these doses. Significant potency of intraduodenal ZM 241385 (10 mg kg^{-1}) in the dog was, nevertheless, demonstrated, and the duration of action exceeded 6 h. Theophylline, by comparison, was significantly less potent than ZM 241385 in both species. It was demonstrated that the duration of theophylline in the dog was limited to 90 min; in the cat theophylline 6 mg kg^{-1} was far less potent than ZM 241385, and resulted in a profile of activity equivalent to that from ZM 241385 at 1 mg kg^{-1}, intraduodenally. Although it was not the aim of this experiment to address the selectivity of the compound, it would not be expected that the cardiac adenosine receptors would be affected by the doses used. This conclusion is based upon the 140-fold vascular:cardiac selectivity of the compound observed in the anaesthetized dog after intravenous administration (Keddie et al 1995) and the 1000 fold A_{2a}:A_1 selectivity observed using in-vitro preparations (Poucher et al 1995).

In conclusion, the results of this study indicate that the non-xanthine, adenosine receptor antagonist ZM 241385 is an orally active adenosine antagonist with good duration of action after administration to conscious cats and rats. In addition, when used in preparations suitable for anaesthetized animals, a single dose of ZM 241385 is rapidly absorbed following intraduodenal administration and this is followed by a long duration of activity. This latter route of administration of the compound may be suitable for investigation of the role of A_{2a} adenosine receptors in long-term anaesthetized preparations. If ZM 241385 is administered intravenously, it is essential that antagonism of the adenosine receptors by the compound is corroborated using a suitable adenosine receptor agonist challenge during the protocol.

Acknowledgements

We thank Helen Musgrove and Cheryl Harding for their technical assistance in undertaking the conscious rat studies, and Rachel Pleeth and Carol Jones for their assistance in the conscious cat studies. We thank Gary Boyle for the development of the HPLC assay for ZM 241385.

References

Abbracchio, M. P., Cattabeni, F., Fredholm, B. B., Williams, M. (1993) Purinoceptor nomenclature: a status report. Drug Dev. Res. 28: 207–213

Bruns, R. F., Daly, J. W., Snyder, S. H. (1980) Adenosine receptors in brain membranes: binding of N^6-cyclohexyl[^{3}H]adenosine and 1,3-diethyl-8-[^{3}H] phenylxanthine. Proc. Natl. Acad. Sci. USA 77: 5547–5551

Collis, M. G., Hourani, S. M. O. (1994) Adenosine receptor subtypes. Trends Pharmacol. Sci. 14: 360–366

Collis, M. G., Palmer, D. B., Saville, V. L. (1985) Comparison of the potency of 8-phenyltheophylline as an antagonist at A_1 and A_2 adenosine receptors in atria and aorta from the guinea-pig. J. Pharm. Pharmacol. 37: 278–280

Daly, J. D., Padgett, W., Shamim, M. T., Butts-Lamb, P., Waters, J. (1985) 1,3-Dialkyl-8(*p*-sulphophenyl)xanthines: potent water soluble antagonists for A_1 and A_2 adenosine receptors. J. Med. Chem. 28: 487–492

Keddie, J. R., Poucher, S. M., Shaw, G. R., Collis, M. G. (1995) The in-vivo cardiovascular pharmacology of ZM 241385, a novel, non-xanthine, adenosine antagonist. Br. J. Pharmacol. 115: 52P

Londos, C., Wolff, J. (1977) Two distinct adenosine-sensitive sites on adenylate cyclase. Proc. Natl. Acad. Sci. USA 74: 5482–5486

Poucher, S. M. (1996) The role of the A_{2a} adenosine receptor subtype in functional hyperaemia in the hind limb of anaesthetized cats. J. Physiol. In press

Poucher, S. M., Keddie, J. R., Singh, P., Stoggall, S. M., Caulkett, P. W. R., Jones, G., Collis, M. G. (1995) The in-vitro pharmacology of ZM 241385, a potent, non-xanthine, A_{2a} selective adenosine antagonist. Br. J. Pharmacol. 115: 1096–1102

Van Calker, D., Muller, M., Hamprecht, B. (1979) Adenosine regulates, via two different types of receptor, the accumulation of cyclic cAMP in cultured brain cells. J. Neurochem. 33: 995–1005