Pharmacological effects of the non-competitive NMDA antagonist CNS 1102 in normal volunteers

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- 1 Non-competitive antagonists at the glutamatergic *N*-methyl D-aspartate receptor significantly reduce the volume of ischaemic cerebral infarction in animals and are potential agents for the treatment of acute stroke in humans.
- 2 CNS 1102, a novel non-competitive NMDA antagonist, was administered as a 15 min intravenous infusion to healthy male volunteers in a double-blind, placebo-controlled, dose-ranging study. This was the first administration to man.
- 3 Clinically significant sedation, increased mean arterial pressure and pulse rate were seen at doses of 30 $\mu\text{g kg}^{-1}$ and above. Symptoms of sedation and central nervous excitation became unacceptable for conscious individuals at doses of 45 $\mu\text{g kg}^{-1}$ and above.
- 4 Rapid onset of central nervous system effects after administration is in keeping with rapid distribution of CNS 1102 to brain. Steady state volume of distribution was large (444 l) and terminal elimination half-life from plasma was approximately 4 h.
- 5 Pharmacokinetic properties are favourable for a potential neuroprotective therapy. The maximum tolerated dose for conscious individuals was 30 $\mu\text{g kg}^{-1}$ given intravenously over 15 min. Further assessment of CNS 1102 should seek methods of drug administration which maximise administered dose with minimal side effects.

Keywords *N*-methyl D-aspartate (NMDA) non-competitive antagonists CNS 1102 neuroprotection

Introduction

Acute ischaemic injury to the central nervous system (CNS) results in the release of excitatory amino acid (EAA) neurotransmitters from depolarised ischaemic neurones [1]. The most widely studied EAA is glutamate, for which four major classes of receptor exist (AMPA, kainate, metabotropic and *N*-methyl D-aspartate, or NMDA). Members of the NMDA class gate the entry of calcium and sodium ions via an integral ion channel [2]. In ischaemia, excess glutamate release in combination with post-synaptic membrane depolarisation permits unregulated entry of calcium and sodium [3]. The associated rise in intracellular free calcium is thought to initiate a cascade of events involving activation of multiple intracellular enzyme systems, ultimately leading to the loss of membrane integrity and neuronal death [4].

Neuroprotective therapy for cerebral ischaemia

aims to interrupt the cascade of events which lead to cell death. CNS 1102 (*N*-(1-naphthyl)-*N'*-(3-ethyl phenyl)-*N'*-methyl guanidine hydrochloride, or aptaguanal hydrochloride) is a novel non-competitive NMDA receptor antagonist which binds to a site within the ion channel, in a similar way to the compound dizocilpine (MK-801) [5, 6]. CNS 1102 reduced cerebral infarct volume measured histologically and by diffusion-weighted magnetic resonance imaging after permanent middle cerebral artery (MCA) occlusion in rats [6]. In this study, cortical infarct volume was reduced by 66%, comparable with the reductions of approximately 50% seen with dizocilpine [7]. Similar effects have been seen with CNS 1102 in other MCA permanent occlusion models, and in rat models of MCA occlusion and reperfusion (Cambridge NeuroScience Inc., unpublished observations). Efficacy has been

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demonstrated both for administration prior to ischaemia and post-ischaemic administration by a period of up to 60 min [8]. These results are similar to those obtained with other non-competitive or competitive NMDA receptor antagonists [7]. On the basis of the animal studies, CNS 1102 was felt to be a promising potential agent for the treatment of acute ischaemic stroke or neurotrauma in man.

We describe here the first administration of the compound to human volunteers in a dose-ranging, placebo-controlled, phase I study.

Methods

This was a single administration, placebo-controlled, dose-ranging study in healthy volunteers. Volunteers were recruited through the register of the Clinical Pharmacology Research Unit of the Department of Medicine and Therapeutics. All subjects gave written informed consent and the protocol was approved by the West Ethics Committee of Greater Glasgow Health Board.

Twenty-eight healthy male volunteers aged 18–35 years were recruited and screened by history, physical examination and blood tests. All subjects had full biochemical profile, full blood count and serum ferritin results checked. Hospital laboratory normal reference ranges were used, and any abnormalities found at baseline had to be judged to be clinically insignificant. Twenty-seven subsequently underwent the full study protocol. All were non-smokers and within 15% of their ideal weight based on Metropolitan life tables. Volunteers were not considered for study if they used illicit drugs or took regular prescribed medication. Alcohol was prohibited for 48 h prior to study and breathalyser testing was performed on the day of study. Subjects remained under observation in the study unit for 24 h after drug administration, and were reviewed by physical examination, history and repeat blood and urine testing at 1 and 6–8 weeks after administration.

The volunteers were divided into dose groups of four individuals each. Within each group, one subject received placebo and three received active drug. Investigators and subjects were blinded to treatment. The dose levels were selected on the basis of efficacy in animal studies, and commenced at a level that was presumed to be free of adverse effects in man. Subsequent dose groups received increments of dose provided that clinically significant adverse events had not occurred at the preceding dose.

CNS 1102 was provided as a solution. The drug was prepared by the hospital pharmacist on the study day by dilution of CNS 1102 in normal saline to a volume of 40–50 ml. Drug or an equal volume of placebo (normal saline alone) was administered at constant rate by Braun Perfusor Secura E syringe drivers (B. Braun, Melsungen, Germany) over 15 or 30 min into a forearm vein. Doses were calculated in relation to the subject's body weight. Dose groups commenced at 3 mg kg⁻¹, and progressed to 10, 30 and 100 mg kg⁻¹, all administered over 15 min. At the 100 mg kg⁻¹ dose, unacceptable symptoms and signs

were encountered. Since only mild effects had been seen at 30 mg kg⁻¹, two further dose groups were assigned to intermediate doses of 60 and 45 mg kg⁻¹. One subject did not proceed to drug administration after the finding of haematuria on urine dipstick testing on the morning of study. He was not replaced, and the 60 mg kg⁻¹ dose group thus consisted of only two active and one placebo treated subjects. A further dose group received 45 mg kg⁻¹ over 30 min.

During the study, pulse rate and blood pressure were determined using Dinamap (Critikon Inc., Tampa, Florida) automated oscillometric blood pressure and pulse rate recorders at predetermined intervals. A single observation was recorded at each time point. Continuous electrocardiographic (ECG) monitoring was undertaken throughout the infusion period. Subjects were asked frequently how they felt and all spontaneous complaints were reported as possible adverse effects. Additional clinical signs were recorded by the observers.

Sedation was self-assessed using a visual analogue scale: a 10 cm unmarked line was presented with 'awake' and 'asleep' marked at opposite ends, the direction of the line being randomly reversed. A number of simple psychomotor tests were administered in an attempt to screen for effects on CNS function. Specific abnormalities were not being sought, and accordingly a battery of simple bedside tests for various aspects of CNS function was devised. Trail-making involved the sequential connection of a random scattering of numbered dots, with the time taken for completion of the sequence being recorded. Digit span was tested by asking subjects to repeat random sequences of numbers: the number of digits recalled forwards and backwards was recorded. Finger-tapping frequency was assessed by recording the number of strikes on a pocket calculator keypad in 60 s. Gait was assessed by asking subjects to walk for 3–4 m.

Plasma drug concentrations were determined by high-performance liquid chromatography with ultraviolet detection, validated in the range 1.25 ng ml⁻¹–100 ng ml⁻¹ using 1.0 ml plasma samples analysed in duplicate. Error for the mean of the standard calibration at the lower limit of quantification was 0.0%, with coefficient of variation of 8.4%. Corresponding values for the highest mean of the standard calibration were -0.1% and 1.48% respectively.

Intra-assay coefficients of variation for low quality controls ranged from 2.20–2.68%, and for medium and high quality controls 0.69–1.28% and 0.92–1.07% respectively. Inter-assay coefficients of variation were 3.87, 1.65 and 2.03% respectively.

Standard calibration was linear in the range 1.25 ng ml⁻¹ to 100 ng ml⁻¹. Correlation coefficients and coefficients of determination were greater than 0.999 in all instances.

Blood was withdrawn for plasma concentration assay at 0, 5, 10, and 15 min during infusion, and at 10, 20, 40, and 60 min, and at 2, 3, 4, 6, 8, 12 and 24 h after the end of the infusion.

Model independent methods were used to estimate the maximum observed plasma concentration of CNS

1102 ($C_{\max}$) and terminal half-life ($t_{1/2}$). The area under the plasma concentration-time curve from dosing until 24 h was calculated using the trapezoidal method. Clearance (CL) was calculated from AUC and dose ($CL = \text{Dose}/\text{AUC}$). Volume of distribution at steady state (V_{ss}) and volume of the central compartment (V_1) were estimated by model dependent methods.

Pharmacokinetics were modelled using a two-compartment open model with zero order input and first order elimination from the central (plasma) compartment. The biomedical statistics package BMD-PAR was used to model the data, using weighted least squares non-linear regression analysis.

In view of the small numbers of subjects at each dose level, inferential statistical analysis was not appropriate.

Results

Tolerability

No life-threatening adverse events occurred during the study period. Low doses (up to and including $30 \mu\text{g kg}^{-1}$) were well tolerated with only minor

symptoms reported—headache, light-headedness, peripheral and perioral paraesthesiae and mild sedation. All of these events were also reported by subjects in the placebo group. Significant symptoms and signs were seen at doses of $45 \mu\text{g kg}^{-1}$ and greater.

Clinical events fell into four broad categories: cardiovascular, central nervous system (CNS) depression and excitation, and general effects. Details of events experienced are given in Table 1. These occurred in a dose-dependent manner, and at $100 \mu\text{g kg}^{-1}$, adverse events of CNS depression were such that dose escalation was abandoned and subsequent groups received lower doses. 'Catatonia' encountered at the highest doses was felt to be the most accurate descriptive term for the condition experienced by subjects whereby they were unable to speak or move, but eye opening and pain responses were preserved. During this time, subjects' limbs could be placed in a position in which they were maintained. This state lasted for a maximum period of 3 h from the end of the infusion, and was seen in four subjects.

Mild sedative effects including disorientation, disinhibition and tiredness were noted in lower dose groups. Signs of CNS stimulation—nystagmus,

Table 1 Adverse events seen with CNS 1102 infusion. Numbers experiencing event at each dose level shown

	Placebo	Dose group ($\mu\text{g kg}^{-1}$)						
		3	10	30	45	45	60	100
Infusion duration (min)	—	15	15	15	15	30	15	15
Number of subjects	7	3	3	3	3	3	2	3
<i>Events</i>								
<i>General</i>								
Headache	1	1	1			2	1	1
Weakness			1					
Light headedness				2	2	3	2	3
Sweating				1	1		1	
Nausea					2	2		2
Vomiting					1		1	2
Dry mouth						2	2	1
Paraesthesiae			1	2	2	2	2	3
<i>Cardiovascular</i>								
Cold extremities	1				1	1	2	3
Flushing				1	1	1	1	1
<i>CNS depression</i>								
Sedation	2		1	2	1		1	3
Disinhibition					1	1		
Dulled senses					1	1		
Blurred vision					1	3	2	1
Disorientation							1	2
Dysarthria				1			1	1
Catatonia							2	2
Laboured respiration							1	1
<i>CNS excitation</i>								
Nystagmus						3	2	3
Diplopia					1	2	1	1
Heightened senses					1	1		
Photophobia						1	1	
Anxiety						1		
Paranoia							1	
Choreiform movements								1

diplopia and choreiform movements were also seen from doses of $45 \mu\text{g kg}^{-1}$. Subjects who had been heavily sedated or catatonic after administration of CNS 1102 had no subsequent recollection of the period during which they had been drowsy or catatonic.

Administration of drug over 30 min instead of 15 min did not alter the incidence of adverse events.

Laboratory indices

No significant abnormalities were seen in biochemical or haematological indices over the study period. One subject had persistently elevated bilirubin compatible with Gilbert's syndrome, and another persistently elevated alkaline phosphatase, thought to reflect continued bone growth (the subject was aged 18 years). Urinalysis was normal except for the presence of ketones 24 h after the study day in subjects who experienced nausea and vomiting and thus fasted for a prolonged period.

Vital signs

A dose-dependent rise in systolic, diastolic and mean arterial blood pressure, and in pulse rate was seen at doses of $30 \mu\text{g kg}^{-1}$ and greater (Figures 1 and 2). The rise in blood pressure began shortly after the end of the infusion, reached a peak at 40–60 min after the end of the infusion, and had returned to baseline values by 4 h. Elevation in pulse rate began coincidentally but persisted for up to 12 h. The maximum mean rise in blood pressure, in the $100 \mu\text{g kg}^{-1}$ group, was 30/20 mm Hg over baseline.

Table 2 Pharmacokinetics of CNS 1102 by dose group

	Dose group ($\mu\text{g kg}^{-1}$)				
	30	45	45	60	100
Infusion duration (min)	15	15	30	15	15
Number of subjects	3	3	3	2	3
Mean weight (kg)	73	77	75	99	78
Mean height (cm)	177	178	171	178	180
C_{max} (ng ml $^{-1}$)	16.1	18.5	16.8	41.4	40.4
AUC (ng ml $^{-1}$ h)	36.1	38.6	39.1	77.9	108.1
Clearance (l h $^{-1}$)	60.5	93.7	89.7	77.4	72.6
V_{ss} (l)	346	530	370	448	528
$t_{1/2}$ (h)	4.3	3.8	3.0	6.1	4.5

Data are not included for 3 and $10 \mu\text{g kg}^{-1}$ groups.

Psychomotor tests

The tests used in the study were insensitive in detecting symptomatic CNS depression. Sedation showed a dose-dependent increase by visual analogue scale. All tests tended to show an impaired response to testing at higher doses of CNS 1102, but the inability of subjects in the higher dose groups to complete the tests precluded useful assessment.

Pharmacokinetics

The pharmacokinetics are summarised in Table 2. Plasma concentrations of CNS 1102 barely exceeded the limit of detection in subjects receiving $3 \mu\text{g kg}^{-1}$,

Figure 1 Change from baseline mean arterial pressure after CNS 1102 infusion (■ $30 \mu\text{g kg}^{-1}$; ▲ $45 \mu\text{g kg}^{-1}$; ● $60 \mu\text{g kg}^{-1}$; ▼ $100 \mu\text{g kg}^{-1}$; ○ placebo). Doses below $30 \mu\text{g kg}^{-1}$ are omitted for clarity.

Figure 2 Change from baseline pulse rate following CNS 1102 infusion (■ 30 $\mu\text{g kg}^{-1}$; ▲ 45 $\mu\text{g kg}^{-1}$; ● 60 $\mu\text{g kg}^{-1}$; ▼ 100 $\mu\text{g kg}^{-1}$; ○ placebo). Doses below 30 $\mu\text{g kg}^{-1}$ are omitted for clarity and curves have been smoothed by plotting the mean of three readings.

and were too low to assess pharmacokinetics reliably at 10 $\mu\text{g kg}^{-1}$.

C_{max} and AUC rose linearly with dose administered. Clearance showed no dependency on body weight ($r^2 = 0.01\%$). Clearance was $79 \pm 16 \text{ l h}^{-1}$ (mean $\pm$ s.d., $n = 14$). No relation was demonstrated between body weight and V_{ss} ($r^2 = 0.4\%$) or between body weight and V_1 ($r^2 = 0.9\%$). Volume of the central compartment was estimated to be $87 \pm 61 \text{ l}$ and V_{ss} was $444 \pm 119 \text{ l}$ (mean $\pm$ s.d., $n = 14$).

Discussion

The NMDA receptor appears to function both as a simple receptor-operated ion channel and additionally as an important mediator of neuronal plasticity with functions relating to memory [9]. CNS 1102 exhibits effects similar to the general anaesthetic agent ketamine, which also blocks the NMDA ion channel site [10]. As for other agents of this type, the neurological response progressed through depressant features (sedation, light-headedness, disorientation, disinhibition) from doses of 30 $\mu\text{g kg}^{-1}$ upwards, to excitatory features (nystagmus, diplopia, photophobia, paranoia, chorea) evident from 45 $\mu\text{g kg}^{-1}$. CNS depression progressed to effective catatonia at the highest doses. Given the role of NMDA receptors in long-term potentiation and memory, it is of interest that subjects at the highest doses (60 and 100 $\mu\text{g kg}^{-1}$) had no recollection of events during drug administration and for the duration of symptoms of major sedation or catatonia.

In animals, experimental interactions of agents active at NMDA receptors with the regulation of the cardiovascular system are complex and dependent on the site of administration and the state of anaesthesia. Non-competitive NMDA receptor antagonists prevent glutamate-stimulated blood pressure rises in the rat if administered intrathecally [11], probably by blockade of spinal NMDA receptors which mediate sympathetic vasoconstrictor outflow from the medulla. However, direct injection of NMDA antagonists into the dorsal ventrolateral medulla is associated with elevation of blood pressure [12].

The cardiovascular effects observed with CNS 1102 in animal studies were variable and species dependent. Intravenously administered CNS 1102 caused hypotension in anaesthetised rats, but moderate rises in systolic blood pressure in pigs and baboons (Cambridge NeuroScience Inc., unpublished observations). In this study in man, the cardiovascular effects seen with CNS 1102 were compatible with a centrally-mediated pressor response. The magnitude of the blood pressure rise (up to 30/20 mm Hg over baseline) was clinically significant in normotensive individuals. Clinical doubt remains regarding the relevance of systemic blood pressure changes after stroke, particularly since changes in cerebrovascular resistance vessels may be important in determining collateral flow following cerebral ischaemia. It remains unproven whether elevated systemic blood pressure after acute stroke is desirable, but anecdotal evidence from positron emission tomographic studies in man suggests that low systemic blood pressure is associated with poor peri-infarct perfusion and that increasing blood

pressure may correct this with good clinical outcome [13]. Clarification of the effects of CNS 1102 on cerebral blood flow is desirable.

The pharmacokinetics of CNS 1102 are compatible with a highly lipid soluble compound, rapidly distributed throughout a large volume of distribution. Clearance of the unchanged drug was variable among the small numbers of subjects studied. The observed efficacy of non-competitive NMDA antagonists in animal studies has been significantly influenced by the pharmacokinetics of the agent used. While administration of lipophilic compounds such as dizocilpine may have equivalent effects on infarct volume when administered either prior to ischaemia or 2 h after [14], compounds which are less rapidly distributed to the brain are notably less effective when administered even 1 h after induction of ischaemia [15]. In the clinical setting, the rapid distribution to the central nervous system evident with CNS 1102 will be desirable in order to maximise tissue concentrations as rapidly as possible after ischaemic injury.

Adverse events were similar to those predicted from animal studies [15]. All NMDA antagonists produce similar behavioural changes in all animal species in which trials have been conducted, including sedation, impairment of learning, and ataxia [15]. These effects have been seen at doses similar to the neuroprotective dose. For future clinical development, it will be important to attain drug levels as close to the anticipated neuroprotective dose as

possible while minimising adverse effects. CNS depression and excitation manifested themselves in a manner which may be clinically undesirable in stroke patients at doses greater than $30 \mu\text{g kg}^{-1}$. Slowing the administration of the drug from 15 to 30 min did not significantly alter these effects. In subsequent studies it has been possible to administer substantially higher doses in head injured patients, whose management often requires paralysis and sedation.

CNS 1102 exhibits significant haemodynamic effects which may or may not be desirable after acute stroke. Dose-related symptoms and signs of nervous system excitation and depression were also seen. It is tolerated to doses which approach the dose anticipated to be neuroprotective from experimental models, and its potential as a therapeutic agent in focal and global cerebral ischaemia deserves further exploration. Methods of drug administration which maximise brain concentrations in the immediate post-ischaemic period while minimising adverse events require further investigation.

We wish to thank Cambridge NeuroScience Inc. for financing the study and supplying drugs; Dr Peter Meredith and Charles McNeill for assaying CNS 1102; Wendy Fallon for drug preparation and randomisation; Sister Jean Fenton and the staff of the Clinical Investigation and Research Unit for conducting the study; and Dr Martin Brodie for assistance with psychomotor tests.

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(Received 25 November 1993,
accepted 2 March 1994)