The protective action of chlormethiazole against ischaemia-induced neurodegeneration in gerbils when infused at doses having little sedative or anticonvulsant activity

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1 The effect of chlormethiazole administration on delayed neuronal death in gerbil hippocampus following transient global ischaemia has been examined. Chlormethiazole was administered either intraperitoneally or by intravenous infusion with either the dose or the time of infusion varied.

2 Chlormethiazole (600 $\mu\text{mol kg}^{-1}$, i.p.) given 60 min after ischaemia produced substantial (>60%) neuroprotection when damage was assessed 5, 14 or 21 days later, indicating the drug does not merely delay cell death.

3 Infusion protocols were developed which would result in sustained and defined plasma concentrations. Chlormethiazole (930 $\mu\text{mol kg}^{-1}$) was then infused intravenously for 30 min, 76.5 min or 110 min in ways resulting in sustained plasma concentrations of 200, 100 and 50 nmol ml^{-1} respectively. When treatment was initiated 30 min after the ischaemic episode all protocols provided effective neuroprotection. There was a dose-dependent decline in protection when plasma chlormethiazole concentrations of 50, 30 and 10 nmol ml^{-1} were sustained for 110 min with no protection observed at 10 nmol ml^{-1} .

4 In contrast, when a plasma concentration of 10 nmol ml^{-1} was sustained by infusion for 24 h, almost total neuroprotection against the ischaemic damage was achieved. This plasma concentration produced no sedative or anticonvulsant activity.

5 These data suggest that neuroprotection depends on both dose and duration of chlormethiazole administration and that excellent neuroprotection is possible in the absence of the sedative and anticonvulsant effects of the drug.

Keywords: Neuroprotection; neurodegeneration; ischaemia; chlormethiazole; sedation; hippocampus; anticonvulsant activity

Introduction

Chlormethiazole (INN: clomethiazole) has previously been shown to prevent the delayed neuronal degeneration that occurs in the gerbil hippocampus 4 days following transient forebrain ischaemia (Cross *et al.*, 1991). This protection was first demonstrated histologically (Cross *et al.*, 1991) and subsequently also biochemically and behaviourally (Baldwin *et al.*, 1993).

Neuroprotection was observed when a single dose of chlormethiazole (622 $\mu\text{mol kg}^{-1}$ = 100 mg kg^{-1}) was injected intra-peritoneally (i.p.) between 1 and 3 h after the ischaemic insult. A lower dose (300 $\mu\text{mol kg}^{-1}$) given 1 h post-ischaemia also provided some degree of neuroprotection (Cross *et al.*, 1991).

Since other pharmacological studies in this laboratory on the effects of chlormethiazole in rodents suggested that the drug had a short half-life (unpublished observations) it seemed possible that protection might be possible at lower doses if it were administered continuously over a period of time. Because intravenous administration would be the preferred route if the compound were to be given clinically to treat patients with acute ischaemic stroke, a study has now been undertaken on the dose-response characteristics of intravenously administered chlormethiazole. In addition, since chlormethiazole has sedative, hypnotic and anticonvulsant properties (see for example Evans *et al.*, 1986; Green & Murray, 1989), measures of sedative and anticonvulsant activity have also been made to examine whether these properties are present at the same plasma concentrations as

those required for neuroprotective activity. Results indicate that neuroprotection is possible when chlormethiazole is given by intravenous infusion and at plasma levels that are well tolerated in human subjects. Some of these results have been communicated in preliminary form to the British Pharmacological Society (Baldwin *et al.*, 1991).

Methods

Animals

Male Mongolian gerbils (Bantin and Kingman, Hull, U.K.) weighing 60–80 g were used. They were housed singly in a room with controlled environment and lighting (lights on: 0.7 h 00 min–19 h 00 min) and with free access to food and tap water.

Induction of ischaemia

Transient forebrain ischaemia was induced by bilateral carotid artery occlusion described in detail elsewhere (Cross *et al.*, 1991; Baldwin *et al.*, 1993). Briefly, gerbils were anaesthetized with Saffan (alphaxalone, 166 $\mu\text{mol kg}^{-1}$ plus alphadalone 47 $\mu\text{mol kg}^{-1}$, i.p.) and placed on a heating mat and under heating lamps to maintain body temperature (monitored by rectal probe) at $37.5 \pm 1.0^\circ\text{C}$ for at least 2 h post ischaemia. Carotid arteries were exposed and occluded with aneurysm clips for 5 min, followed by a reperfusion period of 4 days (unless otherwise stated).

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Measurement of hippocampal damage

Gerbils were anaesthetized, perfused with formaldehyde, the brains removed and brain sections prepared and stained with Cresyl Violet as described by Cross *et al.* (1991) and Baldwin *et al.* (1993). The extent of neurodegeneration was measured by a method similar to that described by Gill *et al.* (1987) and detailed by Cross *et al.* (1991). The length of degenerated CA1/CA2 region was expressed as a percentage of the entire CA1/CA2 region. All assessments of histological sections were made by an observer unaware of drug treatment of the animals.

Measurement of chlormethiazole in plasma and brain

Series of gerbils were infused with chlormethiazole as described below. At various times following the start of infusion the animals were either killed and trunk blood collected or, in the case of the 24 h infusion, blood samples collected from the uncatheterized jugular vein following brief anaesthesia with halothane in a N₂O/O₂ mixture.

Plasma was obtained and chlormethiazole extracted by solid phase extraction using a C8 sorbent and vacuum manifold. The eluate was used for analysis of chlormethiazole by high performance liquid chromatography as described by Kim & Khanna (1983).

For analysis of the chlormethiazole concentration in brain tissue, the brain was homogenized in H₂O with an Ultra Turrax homogenizer. The homogenate was centrifuged at 13,000 r.p.m. for 15 min and the supernatant treated in the same way as plasma for chlormethiazole analysis.

Measurement of the anticonvulsant action of chlormethiazole

The anticonvulsant activity was examined in groups of gerbils 90 min after the start of continuous infusion of chlormethiazole to conscious animals at doses designed to achieve plasma levels of chlormethiazole of 10 nmol ml⁻¹, 30 nmol ml⁻¹ or 50 nmol ml⁻¹. The gerbils were injected with N-methyl-DL-aspartate (NMDLA) at a dose of 6.8 nmol kg⁻¹, i.p. and the latency to seizure recorded compared to saline-injected control animals.

Measurement of sedative activity following chlormethiazole administration

Locomotor activity of individual gerbils was measured in perspex boxes (40 × 40 × 35 cm) mounted with infrared photocell beams and linked to a computer (PMS Instruments, Maidenhead). The number of beam breaks made by the animal in a test period was used as a measure of locomotor activity.

Activity was measured for a 5 min period both 45 min and 90 min following the start of the chlormethiazole infusions (to achieve a plasma concentration of 10, 30, 50 or 100 nmol ml⁻¹). Activity values were summed to give an index of inhibition of locomotor activity compared to saline-infused control animals.

Catheterization of jugular vein for infusion studies in freely moving animals

Gerbils were anaesthetized with Saffan and the jugular vein exposed. The scalp was incised and skull exposed. Plastic tubing (i.d. = 0.28 mm; o.d. = 0.64 mm) was passed under the skin to emerge close to the scalp; the other end emerged at the jugular and was used for catheterizing the vessel. The tubing emerging from the scalp was held to the skull with dental cement, 10 cm being left for subsequent manipulation, the free end being sealed with hot plastic. The animals were placed in perspex boxes with free access to food and water.

On alternate days, 200 µl heparinised saline (100 u ml⁻¹)

was flushed through the tubing. After 7 days recovery the animals were used for further investigation.

Protocol for 24 h infusion study

Gerbils were divided into 4 treatment groups following induction of ischaemia (as detailed above). One group received a single i.p. injection of chlormethiazole (600 µmol kg⁻¹) 60 min post-ischaemia while a second group received saline i.p. at the same time. A third group received an intravenous infusion of chlormethiazole (62.1 µmol kg⁻¹ in saline) at the following rates; 6.3 ml kg⁻¹ h⁻¹ for the first 10 min; 2.32 ml mg⁻¹ h⁻¹ for 10 min–120 min and 1.74 ml kg⁻¹ h⁻¹ for 120 min until 24 h. The infusion was started 30 min post-ischaemia and the total chlormethiazole dose over this time was 2702 µmol kg⁻¹. The fourth group of gerbils received saline infused at the same rate.

Protocol for infusion of chlormethiazole up to 120 min duration

Thirty min after the carotid artery occlusion chlormethiazole or saline was infused for various periods of time by exposing the left jugular vein and inserting a 27 gauge needle, the needle being attached by plastic tubing to a Hamilton syringe in an infusion pump. The needle was held in the jugular vein with adhesive tape. At the end of the infusion gerbils were sutured and left to recover for various times before killing and removal of brains for histological evaluation or killing and removal of trunk blood for measurement of plasma chlormethiazole levels.

The infusion protocols used to achieve the desired plasma levels are detailed in Table 1. The pharmacokinetic assumptions and calculations used to construct these infusion protocols were based on the method of Wagner (1974).

Drugs

Dichlormethiazole edisylate ('chlormethiazole') was obtained from Astra Arcus, Södertälje, Sweden and Saffan (alphaxalone plus alphadalone) from Veterinary Drug Company, U.K. Doses of chlormethiazole refer to the free base.

Statistics

Data were evaluated by Student's *t* test or one way ANOVA as appropriate. In the latter case, individual group differences were determined by adjusted *t* tests with significance levels corrected by the Bonferroni method.

Results

Effect of a single injection of chlormethiazole (600 µmol kg⁻¹, i.p.) on hippocampal neurodegeneration

Initial experiments were undertaken to confirm the neuroprotective effect of a single i.p. dose of chlormethiazole of 600 µmol kg⁻¹. Gerbils were subjected to a 5 min bilateral carotid artery occlusion and chlormethiazole injected 60 min later.

A marked reduction (control 76 ± 8%, treated 30 ± 7%) in hippocampal damage was observed 5 days later (Figure 1). Similar protection was observed in other groups of animals when damage was assessed 14 (31 ± 9% damage) or 21 days (28 ± 12% damage) after the ischaemic insult (Figure 1).

Plasma chlormethiazole concentrations following intraperitoneal and intravenous administration

Following an intraperitoneal injection of chlormethiazole (600 µmol kg⁻¹) plasma chlormethiazole concentrations peaked within 2 min with no drug being detectable by 240 min

Table 1 Infusion protocols to achieve various plasma chlormethiazole (CMZ) concentrations in gerbils

Total dose of CMZ administered ($\mu\text{mol kg}^{-1}$)	Duration of infusion (min)	Plasma concentration required (nmol ml^{-1})	Infusion protocol details
930	30	200	CMZ ($62.1 \mu\text{mol ml}^{-1}$) infused at $0.86 \text{ ml kg}^{-1} \text{ min}$ for 10 min and $0.323 \text{ ml kg}^{-1} \text{ min}$ for 20 min
930	76.5	100	CMZ ($62.1 \mu\text{mol ml}^{-1}$) infused at $0.43 \text{ ml kg}^{-1} \text{ min}$ for 10 min and $0.16 \text{ ml kg}^{-1} \text{ min}$ for 66.5 min
930	110	50	CMZ ($45.7 \mu\text{mol ml}^{-1}$) infused at $0.427 \text{ ml kg}^{-1} \text{ min}$ for 10 min and $0.161 \text{ ml kg}^{-1} \text{ min}$ for 100 min
630	110	30	CMZ ($31.1 \mu\text{mol ml}^{-1}$) infused at $0.427 \text{ ml kg}^{-1} \text{ min}$ for 10 min and $0.161 \text{ ml kg}^{-1} \text{ min}$ for 100 min
330	110	10	CMZ ($15.2 \mu\text{mol ml}^{-1}$) infused at $0.427 \text{ ml kg}^{-1} \text{ min}$ for 10 min and $0.161 \text{ ml kg}^{-1} \text{ min}$ for 100 min

Figure 1 The effect of chlormethiazole ($600 \mu\text{mol kg}^{-1}$, i.p.) injected 60 min following 5 min transient ischaemia on hippocampal CA1/CA2 degeneration, measured 5, 14 or 21 days later. Columns represent mean % damage with s.e.mean; $n = 7-9$. ANOVA $F = 7.34$, d.f. 3,28, $P < 0.001$. All treatment groups are significantly different from control ($*P < 0.05$) but not different from each other.

(Figure 2a). The peak plasma concentration was 200 nmol ml^{-1} and $t_1 = 25 \text{ min}$.

To generate kinetic data in order to be able to calculate infusion protocols, a study was also performed on the rate of disappearance of a dose of chlormethiazole ($370 \mu\text{mol kg}^{-1}$) infused intravenously over 5 min (Figure 2b). These data were fitted to a two compartment model which generated the following parameters; t_1 : 15 min; Clearance: $102.6 \text{ ml min}^{-1} \text{ kg}^{-1}$. Volume of distribution (Vd): 2.21 kg^{-1} ; area under curve (AUC): $60.1 \mu\text{mol h l}^{-1}$.

Neuroprotection following an intravenous dose of chlormethiazole of $930 \mu\text{mol kg}^{-1}$, infused for varying periods of time

The peak plasma concentration of chlormethiazole following an i.p. injection was approximately 200 nmol ml^{-1} (Figure 2a). An experiment was therefore designed to produce this concentration in plasma for a 30 min period. Subsequent experiments investigated the effects of administering the same total dose but infused at concentrations which resulted in a sustained plasma concentration of 100 nmol ml^{-1} (infusion period: 76.5 min) or 50 nmol ml^{-1} (infusion period: 110 min). The protocols used (see Methods) resulted in the required plasma levels being achieved, although some accumulation occurred at the end of each infusion period (Figure 3). All 3 infusion protocols resulted in a similar degree of neuroprotection (Table 2).

Figure 2 Plasma chlormethiazole concentration in the 120 min following injection of (a) chlormethiazole, $600 \mu\text{mol kg}^{-1}$, i.p. or (b) chlormethiazole $370 \mu\text{mol kg}^{-1}$ infused intravenously over 5 min. Results shown as mean $\pm$ s.e.mean ($n = 4-5$). Kinetic parameters calculated from these data are given in the text.

Neuroprotection following a 2 h infusion period of chlormethiazole designed to produce different plasma levels

In the next experiments chlormethiazole was infused at doses which produced sustained plasma levels of drug of 30 and

10 nmol ml⁻¹ over a 120 min period (Figure 3). Compared with a 50 nmol ml⁻¹ plasma concentration, less neuroprotection was achieved by a 30 nmol ml⁻¹ plasma level and no protection when the plasma concentration of chlormethiazole was kept at 10 nmol ml⁻¹ (Table 3).

Measurement of sedative and anticonvulsant activity following chlormethiazole infusion for 90 min at varying plasma drug levels

By use of indwelling catheters in the jugular vein, studies could be performed on conscious freely moving gerbils. Infusions were performed to achieve sustained plasma chlormethiazole levels of 50, 30 and 10 nmol ml⁻¹. In some animals locomotor activity was measured both 45 min and 90 min after the start of the infusion, while others were injected with NMDLA (6.8 nmol kg⁻¹) immediately after the 90 min infusion in order to determine the anticonvulsant effect of the chlormethiazole.

The modest increase in mean time to convulsion seen in gerbils with plasma chlormethiazole concentrations of 10 and

30 nmol ml⁻¹ was not statistically significant (Table 4). There was a significant increase in threshold in the group with plasma concentration of 50 nmol ml⁻¹, no seizures being observed in the 15 min period of observation (Table 4).

With regard to locomotor activity, animals with a plasma chlormethiazole level of 10 nmol ml⁻¹ showed normal locomotor activity (Figure 4). However, gerbils with plasma levels above 30 nmol ml⁻¹ had reduced locomotor activity and were clearly sedated (Figure 4).

Effect of a sustained plasma concentration of chlormethiazole of 10 nmol ml⁻¹ for 24 h

We next investigated whether a sustained low plasma concentration of chlormethiazole of 10 nmol ml⁻¹ would be protective if the infusion were continued for 24 h rather than 2 h. Details for the infusion protocol are given in the Methods, but briefly the infusion was initiated 30 min after the removal of the carotid artery occlusion clips. The design of the experiment allowed chlormethiazole to be infused into awake freely moving animals. A 'positive' control study was also undertaken with a group of gerbils receiving either an i.p. injection of saline ($n = 10$) or chlormethiazole (600 μ mol kg⁻¹, $n = 10$) 60 min after the 5 min bilateral carotid occlusion.

The sustained chlormethiazole infusion resulted in plasma chlormethiazole concentrations just below 10 nmol ml⁻¹ for the duration of the infusion (Figure 5a). Rectal temperature

Figure 3 Plasma chlormethiazole concentrations achieved by infusion of chlormethiazole at various doses and times detailed in Table 1. Values show a mean for groups of animals ($n = 4-5$).

Figure 4 The degree of sedation in gerbils at defined plasma chlormethiazole concentrations. Activity is shown as the sum of a 5 min measure both 45 min and 90 min after the infusion as a % inhibition compared to saline-injected controls.

Table 2 Effect of varying the duration of intravenous infusion of chlormethiazole (CMZ) on the neuroprotective activity of the drug when given at a fixed dose of 930 μ mol kg⁻¹ to achieve defined plasma concentrations

Duration of infusion (min)	Approx. plasma CMZ concn. during infusion (nmol ml ⁻¹)	% neuronal damage
30	0 (Saline)	82 $\pm$ 8 (6)
30	200	16 $\pm$ 5 (6)**
76.5	100	36 $\pm$ 9 (8)**
110	50	9 $\pm$ 4 (11)**

Hippocampal damage is the mean $\pm$ s.e.mean, number of observations in parentheses. ANOVA, $F = 22.6$, d.f., 3,27, $P < 0.01$. ** $P < 0.01$ vs saline.

Table 3 Effect of an infusion of chlormethiazole (CMZ) for 110 min at various doses to achieve defined plasma concentrations on the neuroprotective activity of the drug

Total dose of CMZ (μ mol kg ⁻¹)	Approx. plasma CMZ concn. during infusion (nmol ml ⁻¹)	% hippocampal neuronal damage
-	0 (Saline)	70 $\pm$ 5 (12)
930	50	9 $\pm$ 4 (11)**
630	30	44 $\pm$ 10 (9)*
330	10	68 $\pm$ 21 (6)

Hippocampal damage is the mean $\pm$ s.e.mean, number of observations in parentheses. ANOVA, $F = 11.1$, d.f., 3,34, $P < 0.01$. ** $P < 0.01$; * $P < 0.05$ vs saline.

Table 4 Effect of chlormethiazole (CMZ) on convulsant action of N-methyl-DL-aspartate (6.8 nmol kg⁻¹)

Plasma CMZ concentration (nmol ml ⁻¹)	Time (min) to tonic convulsion
0	7.25 $\pm$ 0.63 (6)
10	10.67 $\pm$ 1.23 (6)
30	11.50 $\pm$ 1.64 (6)
50	> 15* (6)

*Different from control group $P < 0.05$. No significant difference in threshold at either of the other two plasma concentrations. 'Cut off' time of 15 min used.

was also maintained at normal values during the first 6.5 h following the occlusion (Figure 5b) and was similar in control (average temperature $38.1 \pm 0.21^\circ\text{C}$) and chlormethiazole-treated (average temperature $38.1 \pm 0.17^\circ\text{C}$) groups throughout the study period.

Intravenous drug administration provided essentially total protection from the ischaemia-induced degenerative changes

in the hippocampus (Figure 5c). The protection observed was greater than that achieved by a single intraperitoneal dose of chlormethiazole of $600 \mu\text{mol kg}^{-1}$ (Figure 5c).

Brain chlormethiazole concentration at the end of the infusion period

The brain chlormethiazole concentration at the end of the infusion period (24 h) was $16.0 \pm 3.8 \text{ nmol g}^{-1}$ (mean $\pm$ s.e.mean, $n = 3$).

Discussion

In gerbils, chlormethiazole was observed to have a short plasma half life ($t_{1/2} = 15 \text{ min}$) and rapid clearance ($102.6 \text{ ml min}^{-1} \text{ kg}$) in comparison with human volunteers ($t_{1/2} = 6.6 \text{ h}$; clearance = $15.1 \text{ ml min}^{-1} \text{ kg}$; Pentikäinen *et al.*, 1980). Despite these possible pharmacokinetic limitations to therapeutic efficacy in the gerbil, a single intraperitoneal dose of the drug of $600 \mu\text{mol kg}^{-1}$ given 60 min after an ischaemic insult resulted in substantial neuroprotection to the hippocampus when measured 5 days after the insult, thereby confirming previous observations (Cross *et al.*, 1991; Baldwin *et al.*, 1993). Protection has now also been observed when hippocampal tissue was examined 14 and 21 days later, thereby showing that the drug is preventing cell death, not merely retarding it. When the drug was infused at a fixed dose, but over varying periods of time, similar neuroprotection was achieved, even when the mean plasma concentration was only 25% the peak concentration achieved after an i.p. injection (Table 2). This suggests that the neuroprotective effect of the drug is related not only to drug concentration but also duration of administration.

In the next experiments, when the time of administration was kept constant but the dose was lowered, protection was found to be dose-dependent. Only the lowest dose (plasma concentration 10 nmol ml^{-1} over 120 min) failed to provide some protection. Nevertheless, in support of the contention that both duration of drug administration and dose are important, maintaining the plasma concentration of chlormethiazole at 10 nmol ml^{-1} for 24 h resulted in almost total neuroprotection. The fact that the brain chlormethiazole concentration at the end of the infusion was only slightly higher than the plasma levels suggests that there was no significant accumulation of the drug in the brain during the period of administration which would account for this result.

Significant sedation was observed in gerbils with plasma chlormethiazole concentrations of 50 or 30 nmol ml^{-1} . A steady plasma concentration of 10 nmol ml^{-1} , in contrast, produced no inhibition of locomotor activity. Chlormethiazole can therefore be given by infusion and provide protection against ischaemic cell death at plasma concentrations at which there is no significant sedation. Our results also suggest that the neuroprotection is unrelated to the anticonvulsant activity of the drug. It is also important to note, given the evidence that hypothermia can be neuroprotective (Busto *et al.*, 1987), that there was no indication of any reduction in body temperature during the infusion.

We have shown previously that a single intraperitoneal administration of chlormethiazole is effective when given up to 3 h post-ischaemia (Cross *et al.*, 1991). The present experiments demonstrate that plasma chlormethiazole concentrations need to be maintained above 30 nmol ml^{-1} during this period to obtain a significant neuroprotective effect. Following transient forebrain ischaemia in the gerbil, hippocampal CA1 neurones do not show degenerative changes until 2 days post-ischaemia (Kirino, 1982). It would seem likely that the administration of chlormethiazole up to 3 h post-ischaemia interferes with the sequence of events leading to delayed neuronal death. The observation that prolonged infusion of chlormethiazole at low (10 nmol ml^{-1}) plasma concentrations is also neuroprotective suggests that in the

Figure 5 The effect of infusion of chlormethiazole (CMZ) for 24 h on (a) plasma chlormethiazole concentration, (b) rectal temperature and (c) neurodegeneration of hippocampal CA1/CA2 regions. Chlormethiazole was infused for 24 h as described in the Methods starting 30 min after a 5 min bilateral carotid occlusion. Results show (a) the plasma chlormethiazole concentration throughout the infusion period with mean $\pm$ s.e.mean values ($n = 4$), (b) the rectal temperature of control and experimental groups for the first 6 h following occlusion and (c) the % hippocampal neurodegeneration in the control (ischaemia + saline infusion, $n = 10$) and experimental (ischaemia + chlormethiazole, $n = 7$) groups. Also shown is a parallel 'positive control' study with the % neurodegeneration in a saline (i.p.) injected group ($n = 10$) and a chlormethiazole ($600 \mu\text{mol kg}^{-1}$, i.p.) injected group ($n = 10$). Results shown as mean $\pm$ s.e.mean. ANOVA $F = 18.0$, d.f. 3,33, $P < 0.001$. * $P < 0.001$ versus saline control (Bonferroni corrected t test).

gerbil the period during which intervention with chlormethiazole is effective may be longer than 3 h. In this regard it is interesting to note that the N-channel calcium antagonist, SNX-111, may also exert a neuroprotective effect when given several hours after transient forebrain ischaemia in the gerbil (Valentino *et al.*, 1993).

Chlormethiazole has been shown to be neuroprotective in several animal models of cerebral ischaemia, namely transient global ischaemia in the gerbil (Cross *et al.*, 1991; Baldwin *et al.*, 1993), transient focal ischaemia following middle cerebral artery occlusion (Sydserff *et al.*, 1995) and permanent focal ischaemia in the rat following a photochemically induced thromboembolism (Snape *et al.*, 1993; Baldwin *et al.*, 1994). These data have been reviewed elsewhere (Green & Cross, 1994) and support our contention that chlormethiazole may

be useful in the treatment of acute stroke (Cross *et al.*, 1991; Snape *et al.*, 1993). The current data strengthen this contention since the plasma drug levels necessary for neuroprotection in the gerbil are well below levels reported to be safely tolerated in clinical studies. For example, plasma chlormethiazole concentrations as high as 120 nmol ml⁻¹ for more than 24 h have been used safely (Scott *et al.*, 1980). It should therefore be possible to test the efficacy of chlormethiazole in acute ischaemic stroke using a treatment regimen known to be effective in experimental models.

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