Pharmacokinetics of 619C89, a novel neuronal sodium channel inhibitor, in acute stroke patients after loading and discrete maintenance infusions

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- 1 This was a multi-centre, placebo controlled, randomized, dose-escalating design study in which five dosing regimens of 619C89/placebo were evaluated in 48 stroke patients. Loading infusions of 0.5, 1, 1.5, 2 and 2.5 mg kg⁻¹ over 1 h were followed by respective maintenance infusions of 0.25, 0.5, 0.75, 1 and 1.25 mg kg⁻¹ over 30 min at 8 hourly intervals for 3 days.
- 2 Plasma concentrations of 619C89 and its *N*-oxide, 341C90, and *N*-demethylated, 78C90, metabolites were assayed using an LC–MS–MS method. Plasma concentration-time profiles after the final maintenance infusion were subjected to conventional noncompartmental pharmacokinetic analysis.
- 3 For 619C89, geometric CL means ranged between 0.71 and 0.99 l h⁻¹ kg⁻¹ for maintenance infusions up to 1.25 mg kg⁻¹ over 30 min, with an overall mean of 0.85 l h⁻¹ kg⁻¹ (95% CI: 0.70–1.04 l h⁻¹ kg⁻¹). Geometric *V*_{ss} means ranged between 13.2 and 27.9 l kg⁻¹ for the same doses, with an overall mean of 22.5 l kg⁻¹ (95% CI: 16.4–30.9 l kg⁻¹). The ANOVA results revealed that neither CL, *V*_{ss} nor *t*_{1/2} were significantly different across the five dosing regimens (*P* values: 0.82, 0.54 and 0.61, respectively).
- 4 Average AUC for 341C90 was 270% and that for 78C90 was 62% of the AUC for 619C89. The AUC_m/AUC_p-ratios were similar at all dose levels for each metabolite. Values of *t*_{1/2} for 341C90 were similar to those of 619C89 whereas *t*_{1/2} for 78C90 was about three-fold longer than that of parent drug.
- 5 In conclusion, the pharmacokinetics of 619C89 are independent of dose in acute stroke patients. The pharmacokinetics of 341C90 are probably formation rate-limited and those of 78C90 are elimination rate-limited and are also dose-independent.

Keywords 619C89 341C90 78C90 stroke pharmacokinetics

Introduction

Stroke is the third leading cause of death in the western world after ischaemic heart disease and cancer [1] and is also a major cause of disability with at least one third of survivors incapable of an independent existence. Ischaemic stroke due to thromboembolism, or intracerebral haemorrhage (ICH) is associated with glutamate release and cerebral oedema. Therefore, therapies aimed at inhibiting one or both of these factors may prove efficacious. There is currently no effective treatment which, when administered acutely, will reliably influence

neuronal or functional outcome after ischaemic stroke or ICH.

Following cerebral ischaemia, neuronal damage occurs central to the initiating event and also as a radiating wave of evolving cell loss- the 'ischaemic penumbra' [2], which represents an area of brain tissue for potential salvage and thus is a target for therapeutic intervention. Release of glutamate and aspartate has been shown to occur in experimental animal models of cerebral ischaemia [3, 4] and subdural haematoma [5]. Such substances are neurotoxic in excess [6] and prevention of their release or limitation of their

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effects provides a potential strategy to limit ischaemic damage. In animal models, 619C89 (4-amino-2-(4-methylpiperazin-1-yl)-5-(2,3,5 trichlorophenyl) pyrimidine) inhibits burst firing and transmitter release in depolarized/anoxic neurones by an interaction with voltage-gated sodium channels [7]. This activity results in inhibition of glutamate release from pre-synaptic nerve terminals and represents a possible therapeutic intervention to limit neuronal death. Experiments in the rodent model of permanent middle cerebral artery occlusion have shown a reduction in infarct size and accordingly, if the compound produces similar activity in man, 619C89 might be expected to reduce the frequency of death and disability following stroke.

Single dose infusions of 619C89 up to 1 mg kg^{-1} over 30 min and loading and maintenance infusions up to 0.9 mg kg^{-1} over 1 h and $0.3 \text{ mg h}^{-1} \text{ kg}^{-1}$ over 5 h, respectively, are well tolerated in healthy young and elderly volunteers (data to be published). The general findings from the studies indicate dose-independent pharmacokinetics of 619C89 in young and elderly volunteers showing no age-related differences in the pharmacokinetics. Also, 619C89 is metabolized throughout *N*-oxidation to an inactive metabolite, 341C90, and *N*-demethylation to a neuroprotective metabolite, 78C90 (Figure 1). On average, the elimination half-lives for 619C89 and 341C90 were similar at approximately 18 h whereas that of 78C90 is much longer at approximately 70 h. Clearance and steady-state distribution volume of 619C89 are approximately $0.95 \text{ l h}^{-1} \text{ kg}^{-1}$ and 18 l kg^{-1} , respectively. These findings suggest that 619C89 is a moderate-to-highly extracted drug with a large volume of distribution over the dose-range examined. The formation and elimination of the metabolites are also dose-independent. Only traces of 619C89 were excreted in urine whereas approximately 1% and 15% of the dose were recovered as 78C90 and 341C90 respectively.

We now report the pharmacokinetics of 619C89 and its 341C90 and 78C90 metabolites in the first study of 619C89 in patients with acute stroke. Plasma concentration-time profiles after the final maintenance infusion were subjected to conventional noncompartmental pharmacokinetic analysis.

Figure 1 Proposed metabolic pathway of 619C89 in humans.

Methods

Study protocol

This was a multi-centre, randomized, double-blind (with respect to active drug and placebo but not with respect to regimen), placebo controlled, dose-escalating design study to assess the safety, efficacy and pharmacokinetics of 619C89 in stroke patients. Males or females over 21 years of age presenting with symptoms of acute stroke were eligible for inclusion in the study and treatment was initiated within 12 h of the onset of symptoms. Stroke was defined as symptoms of rapid onset reflecting a focal disturbance of cerebral function, excluding isolated impairment of higher function, and of presumed vascular origin, and classified acutely by the National Institute of Health (NIH) stroke scale. Patients were randomized to receive either 619C89 or matching placebo for 3 days and followed up for disability outcome over 3 months. Loading infusion doses of 0.5, 1, 1.5, 2 and 2.5 mg kg^{-1} over 1 h were followed by respective maintenance infusion doses of 0.25, 0.5, 0.75, 1 and 1.25 mg kg^{-1} over 30 min at 8 hourly intervals for 3 days. The five dosing regimes were administered sequentially to separate groups of patients. Successive groups did not receive medication until the safety of the preceding regimen had been established. Prior to administration, the appropriate volume of concentrate was made up with 5% glucose to give a total volume of 250 ml for both the loading infusion and each maintenance infusion. 619C89 was supplied in sterile ampoules containing 5 ml at 10 mg ml^{-1} by Wellcome (Dartford, UK). Sterile ampoules of placebo were identical in appearance to active and contained 5% glucose solution. The actual body weight of each patient was classified within a 5 kg weight group (i.e., 45, 50, 55, 60 kg etc.) and then infused accordingly. For a patient classified as a 70 kg, the infusate volumes for loading infusions of 0.5, 1, 1.5, 2 and 2.5 mg kg^{-1} were 3.5, 7, 10.5, 14, and 17.5 ml over 1 h. The corresponding infusate volumes for maintenance infusions of 0.25, 0.5, 0.75, 1 and 1.25 mg kg^{-1} were 1.8, 3.5, 5.3, 7, and 8.8 ml over 30 min.

Study population

Prior to study initiation the study protocol was approved by hospital's Local Research Ethics Committee. Patients were excluded if of child-bearing potential, in coma or with life threatening illness, non-stroke neurological pathology, renal or hepatic dysfunction, or pre-existing functional impairment resulting in dependence. Anticoagulation was not permitted within the first 7 days. All patients were assessed by electrocardiograph, biochemistry, complete blood count and coagulation analysis pre-treatment, and at days 7, 14, and 28 post-stroke.

Each of the five dosing regimens was assessed in cohorts of eight patients, six receiving active drug and two placebo randomized in blocks of four. The fourth dosing regimen ($2 \text{ mg h}^{-1} \text{ kg}^{-1}$ loading + 1 mg kg^{-1}

over 30 min maintenance) was assessed in a second cohort which actually comprised nine subjects due to a randomization error.

Blood sampling for drug analysis

Venous blood (5 ml) was scheduled to be taken from each patient before and at the end of the loading infusion, before and at the end of the daytime maintenance infusions on day 2, and pre-dose and at 0.5, 1, 2, 4, 6, 8, 12, 16, 24, 36, 48, and 72 h after the start of the final maintenance infusion on day 3. Sampling after the final dose was undertaken only when convenient which depended on the time of the start of the treatment. Samples were centrifuged and plasma was harvested and stored frozen at -20°C until analysis.

Analytical methodology

Plasma concentrations of 619C89 and its 341C90 and 78C90 metabolites were determined by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS-MS) with Atmospheric Pressure Chemical Ionisation (APCI) [8]. The analytes and the internal standard (IS) were extracted from plasma and concentrated by solid phase extraction and then eluted with a solution of 25% 10 mM ammonium formate, adjusted to pH 3.5 with formic acid, and 75% acetonitrile followed by an acetonitrile wash. An aliquot of the elute was analysed by LC-MS-MS with positive ion APCI, using multiple reaction monitoring. A Hewlett-Packard Model HP1090A liquid chromatograph equipped with a variable-volume injector, auto sampler and ternary DR5 solvent delivery system was used in conjunction with a PE-SCIEX API-III triple quadrupole mass spectrometer. The latter was equipped with an API source and a heated nebulizer LC-MS interface and Macintosh computers were used for instrument control, data acquisition and data processing.

For the determination of 619C89, 341C90 and 78C90 in plasma, separations were achieved on a 15 mm SCX in-line filter (Hichrom Ltd, Theale, Reading, Berks., UK). A mobile phase consisting of 80% h.p.l.c. grade acetonitrile and 20% 10 mM ammonium formate, adjusted to pH 3.5 with formic acid, was used at a flow rate of 1.0 ml min^{-1} . The in-line filter was operated at a temperature of 40°C resulting in a typical analysis time of 2 min achieving the required separation of the *N*-oxide 341C90 from the other analytes. The API instrument was operated in the positive ion APCI mode with a corona discharge current of $3\text{ }\mu\text{A}$. Medical grade air at an opening pressure of 60 p.s.i. was used as the nebulizing gas with an overall flow rate of approximately 0.6 l min^{-1} . High purity nitrogen (99.999%) was used as the curtain gas at a flow rate of 1.0 l min^{-1} . The heated nebulizer interface was maintained at 450°C . MRM was used for the determination of 619C89, 341C90 and 78C90, monitoring the dissociation of the protonated molecules (m/z 372, 388 and 358 amu, respectively) and the IS, 481C90 (m/z 338 amu) to

produce ions at m/z 315, 301, 315 and 288 amu respectively, with a dwell time of 150 ms/transition. A collision energy of approximately -40 eV was used, with argon as the target gas.

Pharmacokinetic analysis

The pharmacokinetic parameters of each analyte were estimated from plasma profiles after the last maintenance infusion on day 3 by conventional methods [9]. The parameters included pre-dose and 8 h trough plasma concentrations ($C_{\min,0h}$ and $C_{\min,8h}$, respectively), maximum plasma concentration ($C_{\max}$), time to reach $C_{\max}$ ($t_{\max}$), area under the plasma concentration-time curve within an 8 h dosing interval (AUC), and elimination half-life ($t_{1/2}$). Additional parameters were derived for 619C89 which included mean residence time (MRT), clearance (CL), apparent volume of distribution (V_{β}), and steady-state volume of distribution (V_{ss}). All parameters were then log transformed.

Statistical analysis

For 619C89, overall geometric means and 95% confidence intervals (CI) at each dose level were calculated for the ratio $C_{\min,8h}/C_{\min,0h}$, CL, V_{ss} and $t_{1/2}$. For the metabolites 341C90 and 78C90, overall geometric means and 95% CI were calculated for the ratio $C_{\min,8h}/C_{\min,0h}$, and for the ratios of $\text{AUC}_m/\text{AUC}_p$ and $t_{1/2}$ of each metabolite to that of 619C89. Differences between these pharmacokinetic parameters at the five dose levels were investigated using analysis of variance (ANOVA), taking into account variation due to the random effect of subject and the fixed effect of scheduled dose.

Results

Patient population

A total of 48 stroke patients were enrolled into the study at three centres. All patients were receiving concomitant therapy with at least one drug, mostly aspirin, β -adrenoceptor blockers and benzodiazepine sedatives. Thirty-six patients received 619C89 and 12 patients received a matching placebo. Of the 36 patients receiving 619C89, full plasma concentration-time profiles were available from 23 Caucasian patients only (22 males and 1 female). The mean $\pm$ s.d. age was 70.4 ± 10.4 years (range: 50.3–87.9 years) and body weight was $73.6 \pm 14.2\text{ kg}$ (range: 48.5–110 kg). The remaining 13 patients who received 619C89 had either sparse samples ($n=2$) or no samples were collected ($n=11$).

Assay validation

The lower limit of quantification was 0.5 ng ml^{-1} for 619C89 and 78C90 and 1.0 ng ml^{-1} for 341C90 with a

0.5 ml sample aliquot. A least squares linear regression weighted $1/X$ was used for the calibration for all three analytes. Assay results for plasma quality control samples showed intra- and inter-assay coefficients of variation within 16%, 19% and 13% for 619C89, 341C90 and 78C90, respectively. 619C89 and metabolites were stable in frozen human plasma quality control samples as well as in their extracts reconstituted and standing at room temperature.

Pharmacokinetics

Representative mean plasma concentration-time profiles for 619C89 and the metabolites 341C90 and 78C90 after the final maintenance infusion (MI) are presented in Figure 2. After the infusion cessation the decline in 619C89 plasma concentrations was bi-exponential characterising the rapid distribution and moderate elimination of 619C89. Plasma concentrations of the metabolites 341C90 and 78C90 peaked at approximately the same time 0.5–4 h later than parent drug. However, 341C90 plasma levels then declined parallel to those of 619C89 whereas 78C90 plasma concentrations remained fairly flat throughout the 72 h sampling period, declining late in the sampling period for only few patients.

Median [range] pharmacokinetic parameters for 619C89 are presented in Table 1. The geometric mean $C_{\min,8h}/C_{\min,0h}$ ratios ranged between 0.78 and 1.23 and none of the ratios was significantly different from unity. The ANOVA results showed the ratios to be dose-independent across all doses (P value: 0.25) with an overall mean of 0.95 (95% CI: 0.83–1.08). Geometric CL means ranged between 0.71 and 0.99 $l\ h^{-1}\ kg^{-1}$ for doses up to 1.25 $mg\ kg^{-1}$ over 30 min, with an overall mean of 0.85 $l\ h^{-1}\ kg^{-1}$ (95% CI: 0.70–1.04 $l\ h^{-1}\ kg^{-1}$). Geometric V_{ss} means ranged between 13.2 and 27.9 $l\ kg^{-1}$ for the same doses, with an overall mean of 22.5 $l\ kg^{-1}$ (95% CI: 16.4–30.9 $l\ kg^{-1}$). Geometric $t_{1/2}$

means ranged between 20.4 and 28.3 h with an overall mean of 22.9 h (95% CI: 19.3–26.5 h). The ANOVA results revealed that neither CL, V_{ss} nor $t_{1/2}$ were significantly different across the five dosing regimens (P values: 0.82, 0.54 and 0.61, respectively).

Median [range] pharmacokinetic parameters for 341C890 and 78C90 are presented in Table 2. Median $t_{\max}$ values for 341C90 ranged between 1.3 and 4.1 h. The geometric mean $C_{\min,8h}/C_{\min,0h}$ ratios ranged between 0.79 and 1.22 and none of the ratios was significantly different from unity. The ANOVA results showed the ratio not to be significantly different across the doses (P value: 0.21) with an overall mean of 0.93 (95% CI: 0.83–1.04). The geometric mean AUC_m/AUC_p -ratios ranged between 1.71 and 3.48 with an overall mean ratio of 2.66 (95% CI: 2.20–3.21) and the ratios were similar across all five dose (P value: 0.16). Geometric $t_{1/2}$ -ratios of 341C90/619C89 ranged between 0.89 and 0.96 and were not statistically significant across doses; the overall mean was 0.92 (95% CI: 0.88–0.96).

Median $t_{\max}$ values for 78C90 ranged between 0.6 and 4.1 h. The geometric mean $C_{\min,8h}/C_{\min,0h}$ ratios ranged between 0.80 and 1.32 and none of the ratios was significantly different from unity. The ANOVA results showed a similar ratio across the doses (P value: 0.11) with an overall mean of 1.00 (95% CI: 0.90–1.11). The geometric mean AUC_m/AUC_p -ratios ranged between 0.56 and 0.73 with an overall mean ratio of 0.62 (95% CI: 0.52–0.73) and were similar across all five dose cohorts (P value: 0.89). Individual $t_{1/2}$ of 78C90 were not obtainable for some patients for whom plasma concentrations were sustained even 72 h after the final dose and not declining.

Discussion

This placebo-controlled study is the first in which escalating doses of 619C89 were given to stroke patients. Each dosing regimen consisted of a loading intravenous infusion over 1 h followed by eight discrete maintenance infusions of 50% of the loading infusion infused over 30 min at 8 hourly intervals. The pharmacokinetics of 619C89 and its major inactive *N*-oxide metabolite, 341C90, and *N*-demethylated metabolite, 78C90, are hereby characterized for the first time in the stroke patient population.

The decay in plasma profiles of 619C89, following the final dose on day 3, was bi-exponential with a relatively short distribution phase. This was followed by an elimination phase with a $t_{1/2}$ of approximately 24 h. For four patients, across the 0.25–1 $mg\ h^{-1}\ kg^{-1}$ maintenance dosing regimens, plasma concentrations at the end of infusion ($C_{\max}$) were much higher than values occurring after preceding infusions, including the loading infusion and those of other patients within the same dosing regimen. Instructions were outlined in the protocol to collect one of the blood samples immediately before the end of infusion. It is likely that for these four patients the sample was collected soon after flushing the

Figure 2 Representative mean ($\pm$ s.d.) plasma concentration-time profiles for 619C89 (○), 341C90 (◆) and 78C90 (■) following the last maintenance infusion for the 2 $mg\ kg^{-1}\ h^{-1}$ loading and 1 $mg\ kg^{-1}$ over 30 min maintenance infusion dosing regimen ($n=7$).

Table 1 Median [range] noncompartmental pharmacokinetic parameters for 619C89 in stroke patients

Dose LI/MI (mg kg ⁻¹ h ⁻¹)	0.5/0.25 (n=4)	1/0.5 (n=5)	1.5/0.75 (n=4)	2.0/1.0 (n=7)	2.5/1.25 (n=3)
<i>Parameter</i>					
<i>C</i> _{max} (ng ml ⁻¹)	95 [50–327]	157 [76–368]	348 [77–1061]	266 [132–376]	269 [229–365]
AUC (ng ml ⁻¹ h)	321 [254–602]	545 [407–974]	892 [375–1552]	1363 [643–1790]	1370 [1161–1467]
<i>C</i> _{min,0h} (ng ml ⁻¹)	24 [19–26]	46 [34–62]	72 [47–143]	163 [65–203]	93 [90–114]
<i>C</i> _{min,8h} (ng ml ⁻¹)	24 [18–37]	37 [26–69]	62 [27–140]	135 [49–198]	130 [97–141]
<i>t</i> _{1/2} (h)	21.7 [14.1–26.3]	25.0 [15.2–34.7]	33.7 [14.1–42.0]	19.7 [‡] [16.8–34.9]	18.5 [14.1–34.8]
MRT (h)	23.7 [6.8–32.5]	29.8 [12.7–53.3]	31.7 [15.4–40.0]	25.9 [20.5–35.3]	25.4 [22.7–45.0]
CL (l h ⁻¹ kg ⁻¹)	0.78 [0.42–0.99]	0.92 [0.51–1.23]	1.18 [0.48–2.00]	0.73 [0.56–1.55]	0.91 [0.85–1.08]
<i>V</i> _β (l kg ⁻¹)	21.7 [14.1–31.1]	30.3 [11.3–53.0]	37.0 [28.6–69.0]	24.4 [19.3–37.8]	22.7 [22.0–45.8]
<i>V</i> _{ss} (l kg ⁻¹)	20.0 [2.8–26.7]	29.5 [6.5–48.9]	25.1 [14.6–66.8]	20.4 [17.4–41.2]	27.4 [19.3–41.0]

[‡]*n*=6. LI=Loading infusion of 619C89. MI=Maintenance infusion of 619C89.

Table 2 Median [range] noncompartmental pharmacokinetic parameters for the metabolites of 619C89, 341C90 and 78C90, in stroke patients

Dose LI/MI (mg kg ⁻¹ h ⁻¹)	0.5/0.25 (n=4)	1/0.5 (n=5)	1.5/0.75 (n=4)	2.0/1.0 (n=7)	2.5/1.25 (n=3)
<i>Parameter</i>					
<i>341C90</i>					
<i>C</i> _{max} (ng ml ⁻¹)	98 [84–113]	262 [234–821]	370 [161–607]	594 [315–860]	584 [506–982]
AUC (ng ml ⁻¹ h)	624 [475–752]	1737 [1571–4014]	2406 [755–2976]	3844 [1727–6417]	3796 [3316–6663]
<i>C</i> _{min,0h} (ng ml ⁻¹)	72 [62–82]	207 [160–378]	300 [124–449]	423 [211–566]	358 [326–472]
<i>C</i> _{min,8h} (ng ml ⁻¹)	61 [46–86]	162 [147–266]	283 [75–345]	403 [152–689]	495 [311–651]
<i>t</i> _{1/2} (h)	19.9 [13.9–26.4]	24.9 [15.0–30.7]	28.5 [13.9–36.2]	18.6 [‡] [13.0–28.1]	16.3 [12.3–31.7]
<i>78C90</i>					
<i>C</i> _{max} (ng ml ⁻¹)	33 [21–35]	51 [35–65]	89 [57–145]	120 [87–155]	117 [113–200]
AUC (ng ml ⁻¹ h)	215 [137–238]	304 [251–449]	441 [290–980]	741 [558–1056]	840 [815–1325]
<i>C</i> _{min,0h} (ng ml ⁻¹)	24 [21–33]	36 [24–56]	80 [45–99]	89 [71–147]	93 [73–114]
<i>C</i> _{min,8h} (ng ml ⁻¹)	24 [20–35]	34 [19–54]	53 [35–122]	86 [73–141]	113 [110–144]
<i>t</i> _{1/2} (h)	61 (<i>n</i> =3) [38–96]	58 (<i>n</i> =3) [36–100]	74 (<i>n</i> =1)	45 (<i>n</i> =2) [40–49]	33 (<i>n</i> =2) [29–36]

[‡]*n*=6. LI=Loading infusion of 619C89. MI=Maintenance infusion of 619C89.

infusion cannula resulting in the high outlier *C*_{max} values. Other pharmacokinetic parameters were not affected by the outliers.

Based on the averaged elimination half-life of 619C89 of 24 h, achievement of steady-state would be predicted

to take 96–120 h after multiple dosing of 619C89. However, steady-state was generally reached after the final discrete infusion of 619C89 at 72 h, as indicated by the ratio of the pre-dose and end of dosing interval trough plasma concentrations (*C*_{min,0h} and *C*_{min,8h},

respectively) which was not significantly different from unity at all five dose levels. This apparent early achievement of steady-state resulted from the loading infusion of 619C89 and the fact that almost 50% of 619C89 AUC is attributed to the distribution phase. Indeed, simulations for similar dosing regimens, using pharmacokinetic parameters from healthy subjects, showed that steady-state is to be reached after the fourth maintenance infusion.

The means of CL, V_{ss} and $t_{1/2}$ obtained from the noncompartmental analysis were not affected by dose-escalation indicating dose-independent pharmacokinetics of 619C89 over the studied dosing regimens. The linear pharmacokinetics of 619C89 will allow the prediction of plasma concentration-time profiles with various dosing regimens in future studies in stroke patients, within the doses and regimens studied. 619C89 steady-state clearance in stroke patients ($0.92 \text{ l h}^{-1} \text{ kg}^{-1}$) appears to be similar to the average CL values obtained in healthy young and elderly volunteers ($\approx 0.95 \text{ l h}^{-1} \text{ kg}^{-1}$) after single intravenous infusions of up to 1 mg kg^{-1} over 30 min [data to be published]. However, 619C89 distribution in stroke patients was higher than in healthy elderly and young volunteers (V_{ss} : 27 vs 18 l kg^{-1}). Nevertheless, the similarity in clearance between the two populations should result in similar average steady-state plasma concentrations of 619C89 in stroke patients and healthy elderly volunteers receiving the same dosing regimen. For example, with a continuous infusion of $0.375 \text{ mg kg}^{-1} \text{ h}^{-1}$ the steady-state plasma concentrations of 619C89 are predicted at 408 and 395 ng ml^{-1} in stroke patients and elderly volunteers, respectively.

The average CL value of $0.92 \text{ l h}^{-1} \text{ kg}^{-1}$ and V_{ss} value of 27 l kg^{-1} in stroke patients highlights two fundamental pharmacokinetic characteristics of 619C89. First, 619C89 is a highly cleared drug, mainly by undergoing extensive hepatic biotransformation. Previous studies in healthy volunteers showed that no intact 619C89 was excreted in urine (data to be published). Even though plasma protein binding for 619C89 is high ($\approx 96\%$) the plasma clearance of 619C89 is approximately 65% of hepatic plasma flow ($1.35 \text{ l h}^{-1} \text{ kg}^{-1}$) which suggests that 619C89 is a drug with a moderate-to-high extraction ratio in humans. Second, the steady-state volume of distribution of 619C89 is large, apparently related to a greater influx clearance of 619C89 into the tissue compartment than efflux clearance. The combination of high clearance and large volume of distribution resulted in the moderate 619C89 elimination half-life of approximately 24 h. The apparent volume of distribution (V_{β}) is also high ($\approx 31 \text{ l kg}^{-1}$) suggesting that 619C89 readily crosses membranes with high binding and/or partitioning into lipid tissues, such as brain and fat.

The $\text{AUC}_m/\text{AUC}_p$ -ratio for 341C90 was dose-independent at approximately 2.7 and similar to that seen in healthy elderly volunteers (≈ 2.8) receiving single dose infusions up to 1 mg kg^{-1} over 30 min (data on file). The formation, by *N*-oxidation of 619C89, and elimination of 341C90 obey linear kinetics across a wide range of doses. At each dose level the decline in 341C90 plasma concentrations was parallel to the decline in

619C89 levels such that the ratio between the terminal half-lives of 341C90 and 619C89 was not significantly different from unity. Such findings suggest that the pharmacokinetics of the *N*-oxide metabolite are formation rate-limited with its terminal half-life reflecting the elimination half-life of 619C89 and not its true elimination half-life which is shorter than that of parent drug. The formation rate-limited pharmacokinetics of 341C90 and its plasma concentrations being higher than those of parent drug also indicate that the clearance of the *N*-oxide metabolite is lower and the volume of distribution is smaller than those of 619C89. The $\text{AUC}_m/\text{AUC}_p$ -ratio between 78C90 and 619C89 was dose-independent at approximately 0.64, suggesting that both the formation and elimination of 78C90 were linear over the range of studied doses. Due to the long half-life of 78C90, as seen in healthy young volunteers at approximately 60–70 h, plasma concentrations of 78C90 hardly declined after the final dose of 619C89, and it was therefore difficult to accurately estimate the elimination half-life of 78C90 in most stroke patients with plasma sampling only up to 72 h after the final dose. However, data from this study and in healthy young volunteers showed that the decline in 78C90 plasma concentrations was not parallel to the decline in 619C89 levels, suggesting that the pharmacokinetics of the *N*-demethylated metabolite, 78C90, are elimination rate-limited with the approximately 60–70 h terminal half-life representing the true elimination half-life of 78C90.

In conclusion, in acute stroke patients the pharmacokinetics of 619C89 are independent of dose. The pharmacokinetics of 341C90 are probably formation rate-limited and those of 78C90 are elimination rate-limited and also are dose-independent.

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