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Rat pharmacokinetics and pharmacodynamics of a sustained release formulation of the $GABA_A$ α5-selective compound L-655,708

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Non-standard abbreviations: HV-HPMC, high viscosity hydroxypropyl methylcellulose; LV-HPMC, low viscosity hydroxypropyl methylcellulose;

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Abstract

The pharmacokinetic and pharmacodynamic (i.e., receptor occupancy) properties of L-655,708, a compound with selectivity for α5- over α1-, α2- and α3 containing $GABA_A$ receptors, were examined in rats with the aim of developing a formulation that would give sustained (up to 6 hr) and selective occupancy of α5-containing $GABA_A$ receptors suitable for behavioural studies. Standard rat pharmacokinetic analyses showed that L-655,708 has a relatively short half-life with kinetics in the brain mirroring those in the plasma. In vivo binding experiments showed that plasma concentrations of around 100 ng/mL gave relatively selective in vivo occupancy of rat brain α5 versus α1, α2 and α3-containing $GABA_A$ receptors. This plasma concentration was therefore chosen as a target to achieve relatively selective occupancy of α5-containing receptors using subcutaneous implantations of L-655,708 (0.4, 1.5 or 2.0 mg) formulated into tablets of various size (20 or 60 mg) containing different amounts of L-655,708 and combinations of low and high viscosity hydroxypropyl methylcellulose (LV- and HV-HPMC). The optimum formulation, 1.5 mg L-655,708 compressed into a 60 mg tablet with 100% HV-HPMC, resulted in relatively constant plasma concentrations being maintained for at least 6 hr with very little difference between C_{max} concentrations (125-150 ng/mL) and plateau concentrations (100-125 ng/mL). In vivo binding experiments confirmed the selective occupancy of rat brain α5- over α1-, α2- and α3-containing $GABA_A$ receptors.

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γ-Amino butyric acid (GABA) is the major inhibitory neurotransmitter within the brain and its actions are mediated primarily by the $GABA_A$ receptor chloride channels and the G-protein coupled metabotropic $GABA_B$ receptors. $GABA_A$ receptors have attracted particular attention since in addition to their binding site for GABA they have recognition sites for clinically relevant drugs such as benzodiazepines, barbiturates, neurosteroids, ethanol and certain anaesthetics (Sieghart, 1995).

$GABA_A$ receptors are pentameters made up of subunits assembled from the 16 members (α1-6, β1-3, γ1-3, δ, ε, π and θ) of this gene family (Barnard et al., 1998; Simon et al., 2004). The majority (c. 75%) of $GABA_A$ receptors in the brain contain a benzodiazepine binding site and such receptors contain β and γ2 subunits along with either an α1, α2, α3 or α5 subunit (McKernan and Whiting, 1996). The benzodiazepine binding site occurs at the interface of the α and γ2 subunits with the α subunit being the major contributor to differences in the benzodiazepine pharmacology between the various $GABA_A$ receptor subtypes (McKernan and Whiting, 1996).

Classical benzodiazepines, typified by diazepam, have a number of pharmacological actions including anxiolytic, anticonvulsant, sedative and cognition impairing activities (Lüddens et al., 1995). Such compounds have equivalent affinity for the benzodiazepine binding site of $GABA_A$ receptors containing either an α1, α2, α3 and α5 subunit and the heterogeneity in behavioural effects is probably a consequence of modulation of these different receptors subtypes (Lüddens et al., 1995). As a corollary, particular behavioural properties may be associated with modulation of specific $GABA_A$ receptor subtypes. In this regard, the use of α subunit deleted or point-mutated mice have begun to delineate which $GABA_A$ subtypes are associated with which

pharmacological property of non-selective benzodiazepines such as diazepam (Rudolph et al., 1999; McKernan et al., 2000; Löw et al., 2002; Rudolph and Möhler, 2004).

A complimentary approach has been to use subtype-selective compounds to characterise the behaviour consequences of affecting only certain $GABA_A$ subtypes (McKernan et al., 2000; Atack et al., 2005b). In this regard, a number of compounds with higher affinity for the α5 compared to α1-, α2- or α3-containing $GABA_A$ receptors have been described. These include L-655,708 (Quirk et al., 1996), RY 80 (Skolnick et al., 1997) RY 023 (June et al., 2001) and RY 024 (Bailey et al., 2002). Clearly, such compounds should help further delineate the functions of the α5 subtype. However, since these compounds bind to the other subtypes, albeit with lower affinity, it is often not possible to unequivocally state that the observed in vivo effects are actually mediated via the α5 subtype or the much more abundant α1, α2 and/or α3 subtypes (Bailey et al., 2002). Thus, it is important to characterise the effects of such compounds at doses which selectively occupy the α5 subtype. For example, if plasma concentrations are too high, then there will be appreciable occupancy of α1-, α2- and α3- as well as α5-containing $GABA_A$ receptors, which would confound the interpretation of behavioural data. On the other hand, if the plasma concentrations are too low, although occupancy of α1-, α2- and α3-containing $GABA_A$ receptors would be minimal, occupancy of α5–containing $GABA_A$ receptors would also be relatively low and might not be sufficient to elicit a behavioural response.

In the present study, we examined the pharmacokinetics and $GABA_A$ receptor occupancy of L-655,708, a compound which because of its α5 binding selectivity and inverse agonist profile (Street et al., 2004) offers the potential to be an α5-selective cognition enhancer (Maubach, 2003). However, L-655,708 was found to have poor pharmacokinetic properties following bolus injections and therefore we developed a slow release formulation that would give relatively

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constant plasma concentrations, and therefore preferential occupancy of α5- versus α1-, α2- and α3-containing $GABA_A$ receptors, over a time period appropriate for subsequent behavioural evaluation.

Methods

Bolus dosing pharmacokinetic properties. Following overnight food removal, six male Sprague-Dawley rats (approximate weight 280 g) were anaesthetised with Isoflurane and their tail arteries were cannulated. Animals were pretreated with heparin and allowed to recover for at least 30 minutes prior to dosing with L-655,708 (Figure 1). The compound was administered i.v. as a solution (3 mg/mL) in water:1-methyl-2-pyrrolidinone:propylene glycol (56:22:22 v/v), and orally as a suspension (0.8 mg/mL) in aqueous methylcellulose (0.5% w/v HV-HPMC; Colorcon, Dartford, UK). Blood samples (approximately 600 μL) were taken from each rat via the tail artery cannula at 0.017, 0.167, 0.5, 1, 2, 3 and 4 hr following the i.v. dose and at 0.083, 0.25, 0.5, 1, 2, 3 and 4 hr following oral dosing. After each sample an equivalent volume of heparinised saline was injected into the rat via the cannula. Plasma samples were frozen (-20°C) before being analysed by HPLC with UV detection as described below. Standard methods were used to estimate plasma pharmacokinetic parameters.

Receptor occupancy. *Occupancy after bolus i.p. dosing of L-655,708.* L-655,708 was dosed to male Sprague-Dawley rats (0.3-3 mg/kg i.p., 0.5% HV-HPMC). Fifteen min. later (a time point chosen based on the time course of occupancy in the mouse; Atack et al., 2005a), animals were dosed i.v. with either [^{3}H]L-655,708 (synthesised in house: Quirk et al., 1996; also commercially available from American Radiolabeled Chemicals, St. Louis, MO) or [^{3}H]Ro 15-1788 (Perkin-Elmer Life Sciences, Boston, MA) to measure *in vivo* binding of either α5- or the

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combined α1-, α2- plus α3-containing $GABA_A$ receptor populations, respectively as described in more detail elsewhere (Atack et al., 1999, 2005a). Strictly speaking, [^{3}H]Ro 15-1788 also labels α5-containing receptors but since these constitute a minority of brain $GABA_A$ receptors (McKernan and Whiting, 1996), the binding of [^{3}H]Ro 15-1788 in vivo is effectively measured binding to the combined population of α1- α2- and α3-containing receptors with a negligible contribution from α5-containing receptors. Briefly, rats were killed by decapitation either 1 min after [^{3}H]L-655,708 or 3 min after [^{3}H]Ro 15-1788 injections. Trunk blood was collected and brains were then removed and homogenised in 10 volumes of phosphate buffer. Aliquots of homogenate (300 μL) were filtered and washed (10 mL phosphate buffer) over Whatman GF/B glass fibre filters which were then placed in scintillation vials. Scintillation fluid was added and filters were counted (membrane-bound radioactivity). For both [^{3}H]L-655,708 and [^{3}H]Ro 15-1788, non-specific binding was defined in rats dosed with bretazenil, which at a dose of 5 mg/kg i.p. (in PEG 300 vehicle) occupies essentially all benzodiazepine binding sites. Blood samples were centrifuged and plasma removed and analysed for L-655,708 concentrations as described below.

Sustained release formulation. Initially, L-655,708 was administered subcutaneously in either an aqueous (0.5% HV-HPMC) or oil-based (sesame oil) 2.5 mg/mL suspension. Since neither of the subcutaneous formulations proved to give adequate, prolonged exposure, we next focussed on developing a sustained release formulation, the most common method of which is to formulate compound into a tablet containing a hydrophobic polymer matrix, such as HPMC (Lordi, 1987). These tablets are usually administered orally, but given the short GI transit time in rats (approximately 90 min in rat; Davies and Morris, 1993) it was decided to administer the tablets subcutaneously. Hence, L-655,708 was formulated as a solid in tablets (20 or 60 mg)

containing various compositions or either low viscosity HPMC (LV-HPMC; Pharmacoat 606, Shin-Etsu, Japan) and/or HV-HPMC (Colorcon). The rationale for tablet formulation was that when placed in an aqueous environment, water diffuses into the solid tablet and the HPMC forms a gelatinous layer on the tablet surface followed by a progressive swelling and gelation of the tablet matrix. The rate of release of L-655,708 from the tablet is related to its rate of diffusion through the hydrated HPMC and the erosion of the hydrated polymer. Tablets were made by grinding the appropriate proportions of L-655,708 and HPMC in a pestle and mortar and then compressing the mixture with a hand press.

Sample Preparation and Analysis. *Plasma samples*. Aliquots of plasma were applied to C_2 solid phase extraction cartridges preconditioned with 2 mL methanol and 2 mL water. Cartridges were washed with water and samples eluted with 1 mL acetonitrile:0.1% acetic acid (90:10 v/v) + 0.4% triethylamine. Eluates were evaporated to dryness (60^{o}C, N_2), and then dissolved in 300 µL of 25 mM triethylamine phosphate pH 3.2/acetonitrile (75:25 v/v). For calibration, 1 mL aliquots of control rat plasma were spiked to give standards within the range of 5-5000 ng/mL of L-655,708. These were extracted as for the samples above. Calibration curves obtained were linear (r = 0.99 or greater), with a limit of detection in the region of 5 ng/mL.

Brain samples. Two mL of acetonitrile was added to half of the brain tissue and then homogenised with a sonic probe, shaken and centrifuged for 10 min. The supernatant was removed and evaporated to dryness (60^{o}C, N_2) and the residue was dissolved in 300 µL of 25 mM triethylamine phosphate pH 3.2/acetonitrile (75:25 v/v) and filtered through a 0.2 µm LC filter. For calibration purposes, control brain tissue was spiked to give standards equivalent to 5-500 ng/sample of L-655,708. These were extracted using the same methods as for the samples. The calibration curves were linear (r>0.99) with a limit of detection of 5 ng/sample.

HPLC analysis. HPLC analysis was performed using a Gilson binary gradient system with 231/401 autosampler and UV detection. Samples were run on a Hichrom RPB 100 x 4.6 mm column at 45^{o}C using a mobile phase of 25 mM triethylamine phosphate (pH 3.2)/acetonitrile (75/25 v/v) with a flow rate of 1.5 mL/min. The injection volume was 100 µL and detection was performed at a wavelength of 250 nm using a ABI 759A UV detector. Signals were processed using a VG Multichrom system assuming a retention time for L-655,708 defined by the use of calibration standards (generally in the region of 5.0-6.5 min).

Results and Discussion

Bolus dose pharmacokinetic parameters. The plasma concentrations of L-655,708 following bolus dosing L-655,708 via either the i.v. or oral route are illustrated in figure 2. The pharmacokinetic parameters calculated from the mean data are summarised in Table 1, which shows that despite the relatively low plasma clearance rate (19 mL/min/kg), L-655,708 had a very short half life (0.5 h), presumably as a consequence of cleavage of the ester by blood esterases and the fact that it has a relatively low steady state volume of distribution of 0.7 L/kg, which, for example, compares to a volume of distribution of 19.3 L/kg for diazepam in rats (Friedman et al., 1986). Following oral dosing C_{max} was achieved rapidly (t=0.25 hr) after which plasma concentrations fell rapidly. These data suggest that oral absorption is rapid but is not sustained. Since the animals used for this study were cannulated, brain samples were only available at completion of the study and therefore in this experiment it was not possible to assess the pharmacokinetics of L-655,708 in rat brain.

Receptor occupancy as a function of L-655,708 dose. When dosed i.p. as a bolus in a HV-HPMC suspension, L-655,708 gave greater occupancy of α5- versus α1-, α2- and α3-containing $GABA_A$ receptors Figure 3A). For example, at a dose of 1 mg/kg L-655,708 gave 64% occupancy of α5 receptors versus 18% occupancy at α1, α2 and α3 receptors whereas at 3 mg/kg occupancy at α5 receptors was 75% and at α1, α2 and α3 receptors it was 33%.

Trunk blood samples were collected from each animal and the plasma concentrations of L-655,708 determined and the relationship between plasma drug concentrations and occupancy is shown in Figure 3B. The α5 selectivity of L-655,708 observed in vitro was also reflected by in rat brain in vivo in that the occupancy at α5-containing receptors was greater than at those

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containing α1, α2 and α3 subunits. Hence, the plasma EC_{50} for α5 occupancy, 35 ng/mL, was much lower than that for α1, α2 and α3 receptors (346 ng/mL). These data are consistent with observations in mice which showed that the ID_{50} for [^{3}H]L-655708 binding was much lower than that for [^{3}H]Ro 15-1788 binding to α1 or α2/α3 receptors (respective ID_{50} values = 0.2, 1.1 and 4 mg/kg; Atack et al., 2005a). Based on these data, it was estimated that a plasma drug concentration of 100 ng/mL should result in an inhibition of [^{3}H]L-655,708 and[^{3}H]Ro 15-1788 binding by around 75% and 20%, respectively, thereby producing relatively selective occupancy of α5-containing receptors.

Compounds which bind with higher affinity to α5- compared to α1-, α2- and α3-containing $GABA_A$ receptors, such as L-655,708 (Quirk et al., 1996), RY 80 (Skolnick et al., 1997) RY 023 (June et al., 2001) and RY 024 (Bailey et al., 2002) are valuable tools for characterising the roles of the $GABA_A$ α5 subtype. However, in order to ascribe particular functions of an α5-selective compound to the α5-containing $GABA_A$ receptors requires careful selection of the dose. Thus, although at relatively high doses there will be substantial occupancy of α5-containing receptors, there will also be significant occupancy of the lower affinity α1, α2 and α3-containing $GABA_A$ receptors. Moreover, although compared to the α5 subtype the occupancy of α1, α2 and α3 receptors will be less, the much greater abundance of these latter subtypes in the brain as a whole (McKernan and Whiting, 1996) will complicate the interpretation of behavioural responses mediated by the α5 subtype. On the other hand, if a lower dose is used such that there is only modest occupancy of α5-containing $GABA_A$ receptors, this lower level of occupancy of the α5 subtype may be insufficient to elicit a behavioural response. Consequently, in order to ascribe particular L-655,708-mediated effects to the α5 subtype, it is necessary to select a dose of

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compound which gives preferential, sustained and appreciable occupancy at α5- compared to α1-, α2- and α3-containing $GABA_A$ receptors.

Brain and plasma concentrations of L-655,708 following s.c. dosing. Although the plasma kinetics of L-655,708 after oral dosing are relatively rapid, it is possible that plasma concentrations may be more sustained if compound is dosed subcutaneously as a suspension. Furthermore, L-655,708 may become sequestered within the brain such that brain concentrations do not reflect plasma kinetics (Jack et al., 1983). Therefore the kinetics of brain and plasma L-655,708 were measured in rats dosed s.c. in either an aqueous (0.5% HV-HPMC) or oil-based (sesame oil) vehicle. Rats were killed at various times after dosing and the brain and plasma profiles of these experiments are presented in Figure 4. The plasma profile of L-655,708 is comparable whether compound is dosed (5 mg/kg s.c.) as an aqueous (0.5% HV-HPMC) or oil-based (sesame oil) suspension (Figs. 4A and B). Moreover, the kinetics in either vehicle are rapid, with a C_{max} at around 0.5 hr, after which compound is rapidly cleared. Thus, s.c. dosing of L-655,708 in a lipophilic rather than aqueous suspension does not produce a prolonged absorption phase. Nevertheless, both these formulations do give a degree of sustained absorption since at lower doses, 1 and 0.6 mg/kg s.c. in 0.5% HV-HPMC, the kinetics of plasma L-655,708 were even more rapid, with C_{max} (around 325 and 250 ng/mL at 1 and 0.6 mg/kg, respectively) at 0.25 hr and thereafter a rapid clearance of compound such that by 2 hr post-dosing, plasma concentrations were in the region of 25-20 ng/mL. Thus, compared to administration via the oral route, bolus dosing in an aqueous suspension via a subcutaneous route did not appreciably prolong or flatten the pharmacokinetic profile of L-655,708.

For all subcutaneous bolus doses the time course of brain concentrations of L-655,708 mirrored the corresponding plasma concentrations. Thus, there was no tendency for L-655,708 to

be retained in the brain as compound is cleared from the plasma. The comparable kinetics of L-655,708 in brain and plasma, is similar to that seen in rodents for other benzodiazepines such as lorazepam, clonazepam and diazepam and its metabolites desmethyldiazepam and oxazepam (Friedman et al., 1986; Miller et al., 1987; Barnhill et al., 1990; Greenblatt and Sethy, 1990), Similarly, in cats the kinetics of a variety of benzodiazepines (including diazepam, desmethyldiazepam, midazolam, lorazepam, alprazolam, triazolam, flunitrazepam and clobazam) were similar in plasma and CSF (Arendt et al., 1983). These data suggest that, like other benzodiazepines, L-655,708 does not become sequestered in the brain.

However, brain:plasma ratio for L-655,708, which was around 0.4, is in marked contrast to other benzodiazepines, such as diazepam, desmethyldiazepam, clonazepam, lorazepam, midazolam, triazolam and oxazepam which have brain:plasma ratios in the region of 2-5 (Friedman et al., 1986; Arendt et al., 1987; Barnhill et al., 1990; Greenblatt and Sethy, 1990). Moreover, although flunitrazepam and alprazolam differ from this group of benzodiazepines by virtue of their lower brain:plasma ratios (0.9-1.1; Arendt et al., 1987), they nevertheless have brain:plasma ratios considerably greater than the value of 0.4 for L-655,708. The physicochemical properties which render L-655,708 less brain penetrant than other benzodiazepines (e.g. LogD for L-655,708 of 1.6 versus 2.8 for diazepam; Worboys et al, 1997) may also contribute to the relatively low volume of distribution (0.7 L/kg) of this compound, although lipophilicity *per se* does not seem to be the major determinant of benzodiazepine brain:plasma ratios (Arendt et al., 1987).

Obviously, the plasma pharmacokinetics of L-655,708 are unsuitable to provide sustained and selective occupancy of α5-containing $GABA_A$ receptors (i.e. relatively constant plasma concentrations of around 100 ng/mL). Hence, although plasma concentrations of L-655,708 in

the region of 100 ng/mL can be obtained 4 hr. after dosing with 5 mg/kg (c.80 ng/mL), the high peaks concentrations required to achieve such sustained concentrations (around 600 ng/mL) would produce appreciable occupancy of α1, α2 and α3-containing $GABA_A$ receptors as well as those containing an α5 subunit, thereby confounding the interpretation of behavioural results.

Pharmacokinetics of hydroxypropylmethylcellulose (HPMC) tablets. The plasma versus time profiles for 0.4 mg L-655,708 administered via subcutaneous implantation in a HPMC tablet are shown in Figure 5. The equivalent dose of compound (0.4 mg) gave less intra-individual variability when dosed in a 60 mg rather than 20 mg tablet. However, there remained considerable inter-individual variability, especially at the earlier time points and plasma concentrations in each animal fell progressively from the time of C_{max} (t=0.5-1 hr), suggesting that a tablet containing 0.4 mg of compound was insufficient to produce a continual release from the tablet. In addition, the relatively early Cmax suggests that compound leaves the tablet relatively rapidly such that plasma concentrations at 6 hr were much lower than peak concentrations obtained around 1 hr. Therefore, if dissolution of the tablet is slower, such as by the inclusion of a high viscosity form of HPMC, then the plasma profile of L-655,708 may be more prolonged. Furthermore, the plasma profile for the 60 mg tablet was flatter than the 20 mg tablet presumably due to the greater HPMC:L-655,708 ratio of the former resulting in slower drug release and accordingly a 60 mg tablet size was used for subsequent formulations. Accordingly, in the next series of experiments, 60 mg tablets were made containing 2 mg L-655,708 and varying ratios of LV- and HV-HPMC.

Pharmacokinetics of HPMC tablets containing high viscosity HPMC. Increasing the proportion of HV-HPMC resulted not only in a flattening of individual rat plasma L-655,708 versus time profiles, but also greatly reduced inter-individual variability. The effect of the HV-HPMC on tablet dissolution is reflected in the lower mean peak plasma concentrations observed in tablets containing 100% compared to 5% HV-HPMC (peak mean plasma concentrations = ~150 and ~450 ng/mL, respectively).

Two mg of L-655,708 was administered to rats in subcutaneous tablets with varying ratios of LV-HPMC and HV-HPMC and the corresponding plasma versus time curves for individual animals are shown in Figure 6. The tablet containing the lowest proportion of HV-HPMC (5%) gave marked inter-individual variability (Fig. 6A), especially at later time points (4-6 hr). Moreover, there was a distinct peak in plasma concentrations around 1-2 hr. However, increasing the proportion of HV-HPMC in the tablet to 20, 40, 60, 80 or 100% reduced both of these features, such that each of these formulations gave relatively reproducible plasma kinetic profiles between animals and there was not a pronounced peak of L-655,708 concentrations at early time points. The sustained plasma concentrations achieved ranged from 250-300 ng/mL to 100-150 ng/mL for the 20% and 100% HV-HPMC tablets, respectively (Figs. 6B and F), indicating that increasing the proportion of HV HPMC results in reduced absolute plasma concentrations. With the 80% HV-HPMC tablet (Fig 6E), there was some inter-individual variability with one out of four of the rats having plasma concentrations of around half those of the other three. However, using the 100% HV-HPMC tablet, all four rats had very similar plasma L-655,708 pharmacokinetic profiles (Fig 6F).

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Optimum tablet formulation. As a final iteration, 1.5 mg of L-655,708 were compressed into a 60 mg tablet containing 100% HV-HPMC as excipient. The plasma L-655,708 versus time profiles for four rats implanted with this type of tablet are shown in Figure 7. As with the HV-HPMC tablet containing 2 mg L-655,708 (Fig. 6F), 1.5 mg L-655,708 in a 60 mg HV-HPMC tablet gave very similar plasma pharmacokinetics in the different animals with little variation in the absolute plasma concentrations (Fig. 7) and minimal difference between peak concentrations at 1 hr and concentrations at 6 hr (~150 and ~100 ng/mL, respectively). Thus, mean plasma concentrations were around 125 ng/mL at 1-2 hr and thereafter achieved relatively constant concentrations of around 100 ng/mL for the duration of plasma sampling (6 hr),

In a separate group of rats implanted with these tablets, receptor occupancy was measured. As shown in Figure 8, 1.5 mg L-655,708 in a 60 mg HV-HPMC tablet gave relatively selective occupancy at α5 versus α1, α2 and α3 subunit-containing $GABA_A$ receptors. Thus, occupancy of α5 containing receptors measured using in vivo [^{3}H]L-655,708 binding was 83% whereas occupancy at α1, α2 and α3 receptors was 18%.

Functions of α5 subtype selective compounds. The in vivo properties of a number of compounds which have higher affinity for the α5 compared to α1, α2 and α3 $GABA_A$ subtypes have been described (Liu et al., 1996; June et al., 2001; Bailey et al., 2002; Navarro et al., 2002). For example, such compounds have been described as being convulsant and producing anxiogenic and fear-like behaviours (Liu et al., 1996; Bailey et al., 2002, Navarro et al., 2002). However, none of these studies have measured levels of receptor occupancy and so it remains unknown to what extent these effects might be mediated via the α5 subtype (Bailey et al., 2002). Clearly, this issue is of considerable importance in defining the suitability or not of this $GABA_A$

receptor subtype as a potential target for novel therapeutics (Maubach, 2003). In the present studies, we have achieved a formulation of L-655,708 that gives sustained and constant plasma concentrations which gives high and relatively selective occupancy of the α5 subtype (83% occupancy) with minimal occupancy at the α1, α2 and α3 subtypes (18%). In further studies, this formulation be used to characterise the pharmacological properties of L-655,708 and thereby help define the behavioural consequences of selective modulation of the $GABA_A$ receptor α5 subtype.

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Figure Legends

Figure 1. Structure of L-655,708

Figure 2. Concentrations of L-655,708 in plasma obtained via tail artery cannulation after bolus injection of A. 3 mg/kg i.v. (in water:1-methyl-2-pyrrolidinone:propylene glycol; 56:22:22 v/v; n=3) and B. 4 mg/kg p.o. (in 0.5% HV-HPMC; n=3). Values shown are mean ± SEM (n=3/group).

Figure 3. Rat brain benzodiazepine binding site occupancy as a function of L-655,708 dose and plasma concentration. A. Receptor occupancy of α5- and the combined population of α1-, α2- plus α3-containing $GABA_A$ receptors was measured using in vivo binding of [^{3}H]L-655,708 and [^{3}H]Ro 15-1788, respectively, 15 min after administration of various doses of L-655,708 given i.p. in a 0.5% HV-HPMC suspension. Values shown are mean ± SEM (n=7/group). B. For each animal, occupancy was plotted as a function of plasma concentration of L-655,708. Respective EC_{50} values for the inhibition of [^{3}H]L-655,708 and [^{3}H]Ro 15-1788 binding were 35 ng/mL and 346 ng/mL.

Figure 4. Plasma concentration versus time profiles of L-655,708 dosed at 5 mg/kg s.c. in either HV-HPMC or sesame oil (panels A and B, respectively) or dosed at 1 or 0.6 mg/kg s.c. in HV-HPMC (panels C and D, respectively). Insets show the corresponding brain:plasma ratios. Values shown are mean ± SEM (n=3/time point).

Figure 5. Concentration versus time profiles of 0.4 mg L-655,708 administered by subcutaneous implantation of L-655,708 contained within a low viscosity hydroxypropylmethylcellulose (LV-HPMC) tablet. A. 0.4 mg L-655,708 in a 20 mg LV-HPMC tablet. B. 0.4 mg L-655,708 in a 60 mg LV-HPMC tablet. Values shown are plasma concentrations of each of 3 individual, cannulated rats and the inset shows the mean ± SEM of plasma concentrations for each tablet formulation (n=3).

Figure 6. Concentration versus time profiles of 2 mg L-655,708 administered by subcutaneous implantation of 60 mg tablets containing A. 5%; B. 20%; C. 40%; D. 60%; E. 80%; and F. 100% HV-HPMC. Values shown are plasma concentrations of each of 4 individual, cannulated rats and the inset shows the mean ± SEM of plasma concentrations for each tablet formulation (n=4).

Figure 7. Plasma concentration versus time profiles of L-655,708 administered by subcutaneous implantation in a 60 mg tablet containing 1.5 mg L-655,708 and 100% HV-HPMC. Values shown are plasma concentrations of each of 4 individual, cannulated rats and the inset shows the mean ± SEM of plasma concentrations (n=4).

Figure 8. Four hr after subcutaneous implantation of rats with 60 mg tablets containing 1.5 mg L-655,708 and 100% HV-HPMC, occupancy of α5- and the combined population of α1-, α2- and α3-containing $GABA_A$ receptors was measured using in vivo binding of [^{3}H]L-655,708 and [^{3}H]Ro 15-1788, respectively. Values shown are mean ± SEM (n=4-5).

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Table 1: Plasma pharmacokinetic parameters of L-655,708 after dosing with 3 mg/kg i.v. and 4 mg/kg p.o. in rat

Parameter	Value
Plasma clearance	19 mL/min/kg
Plasma half life (0.5-4h)	0.5 h
Steady state volume of distribution	0.7 L/kg
Oral bioavailability	45 %
Oral C_{max}	733 ng/mL
Oral T_{max}	0.25 h

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Figure 1

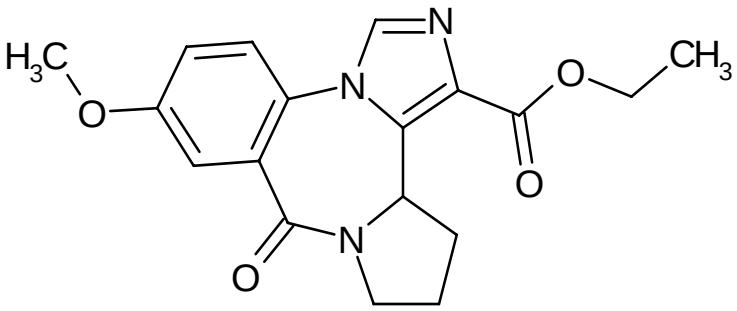

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Figure 2

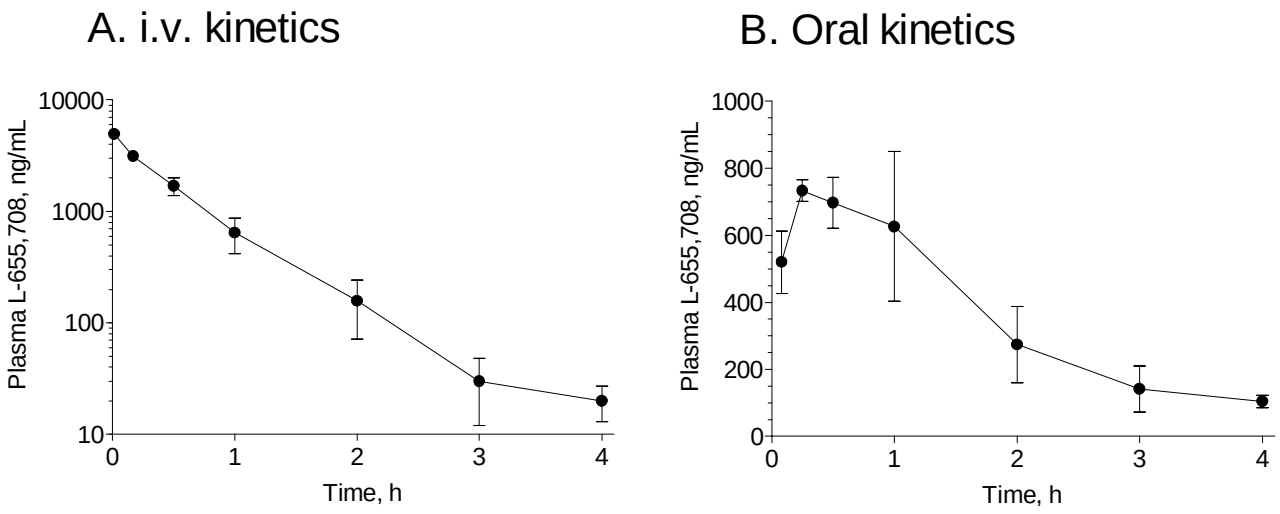

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Figure 3

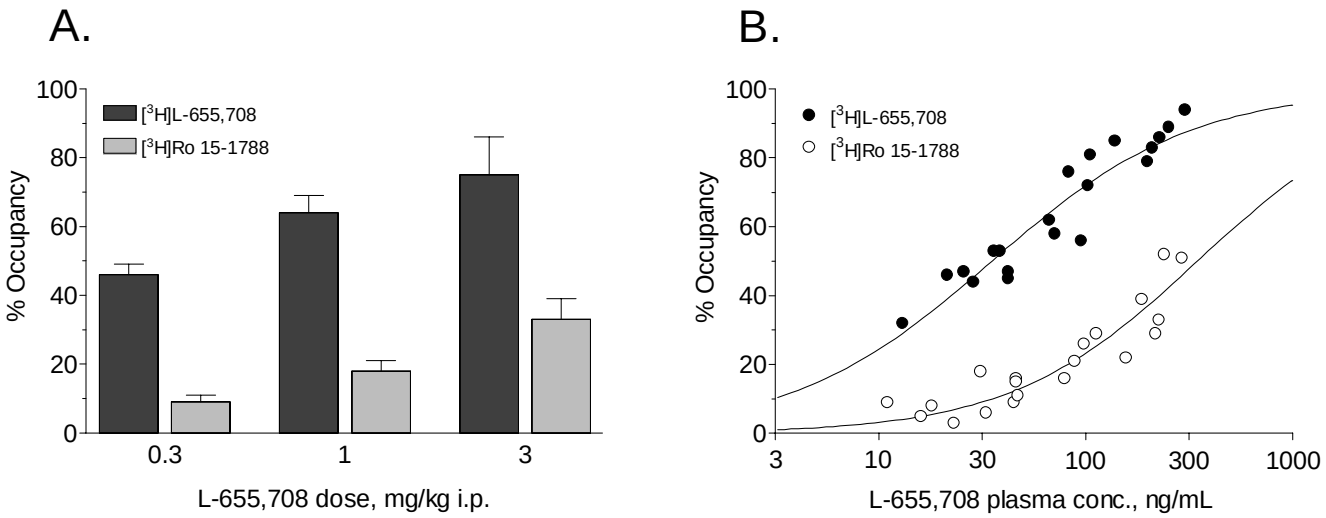

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Figure 4

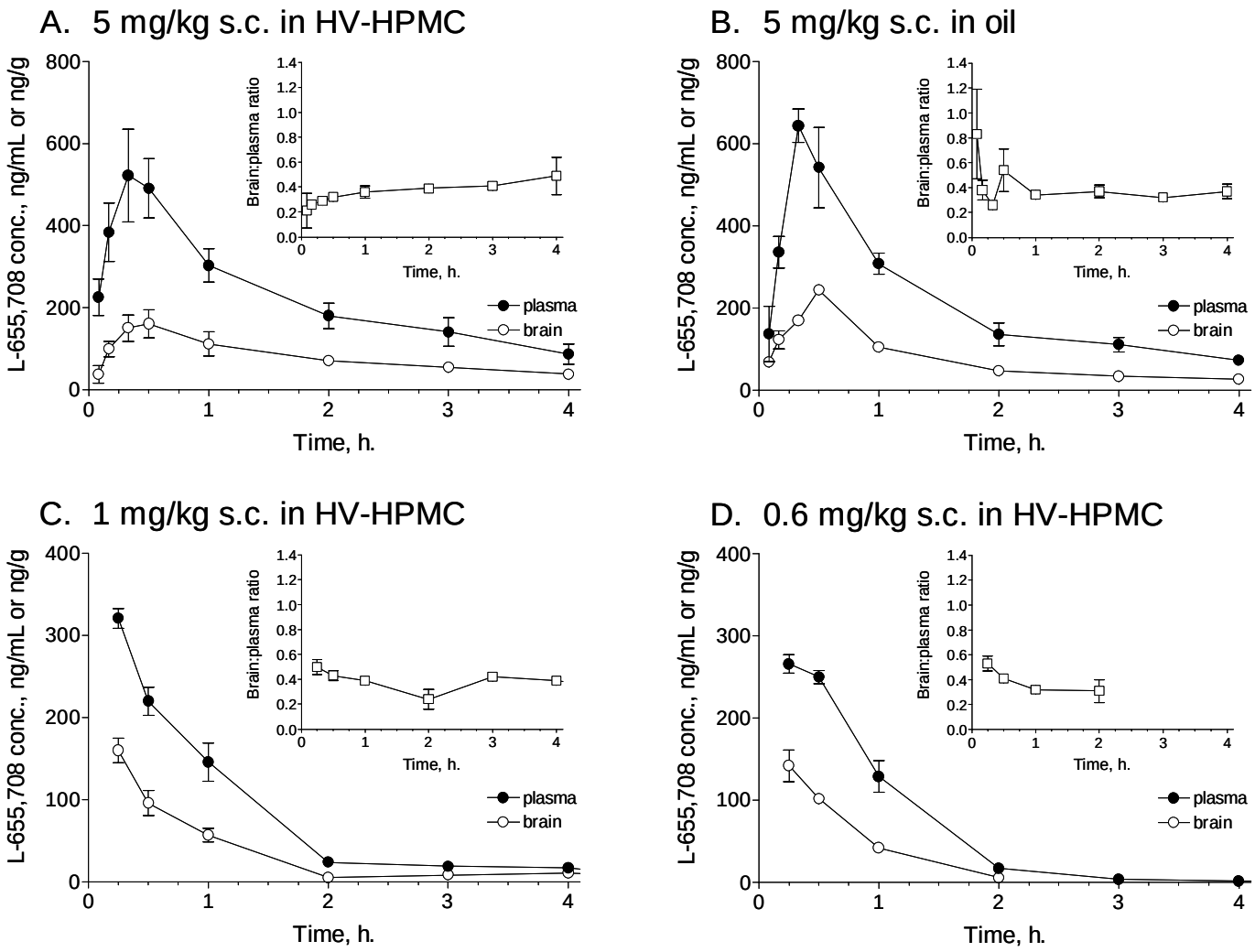

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Figure 5

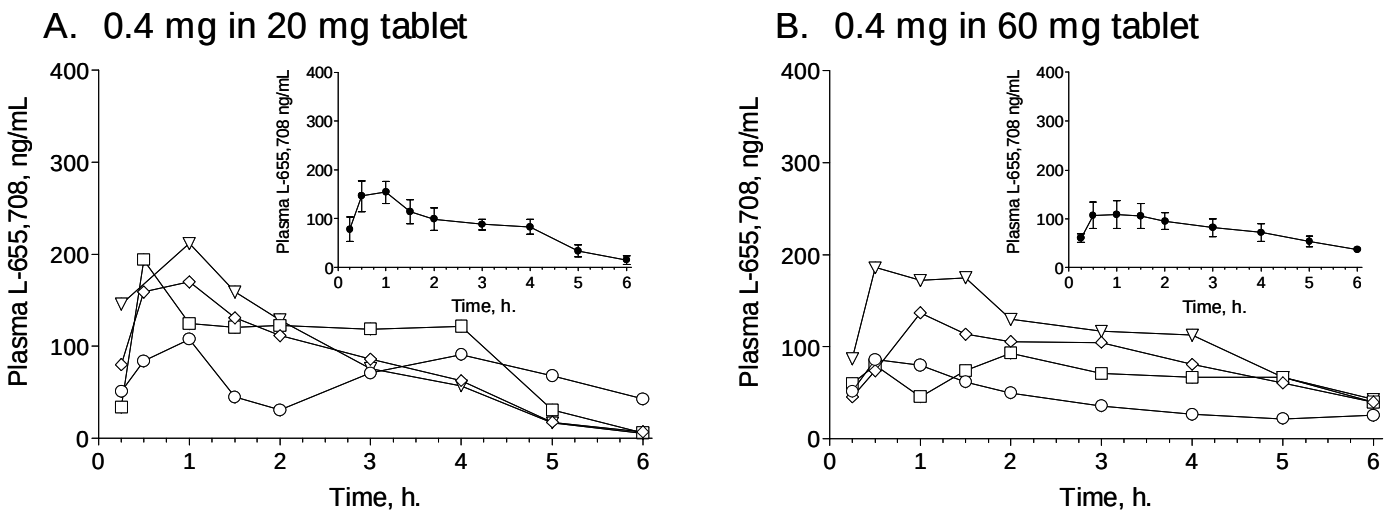

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Figure 6

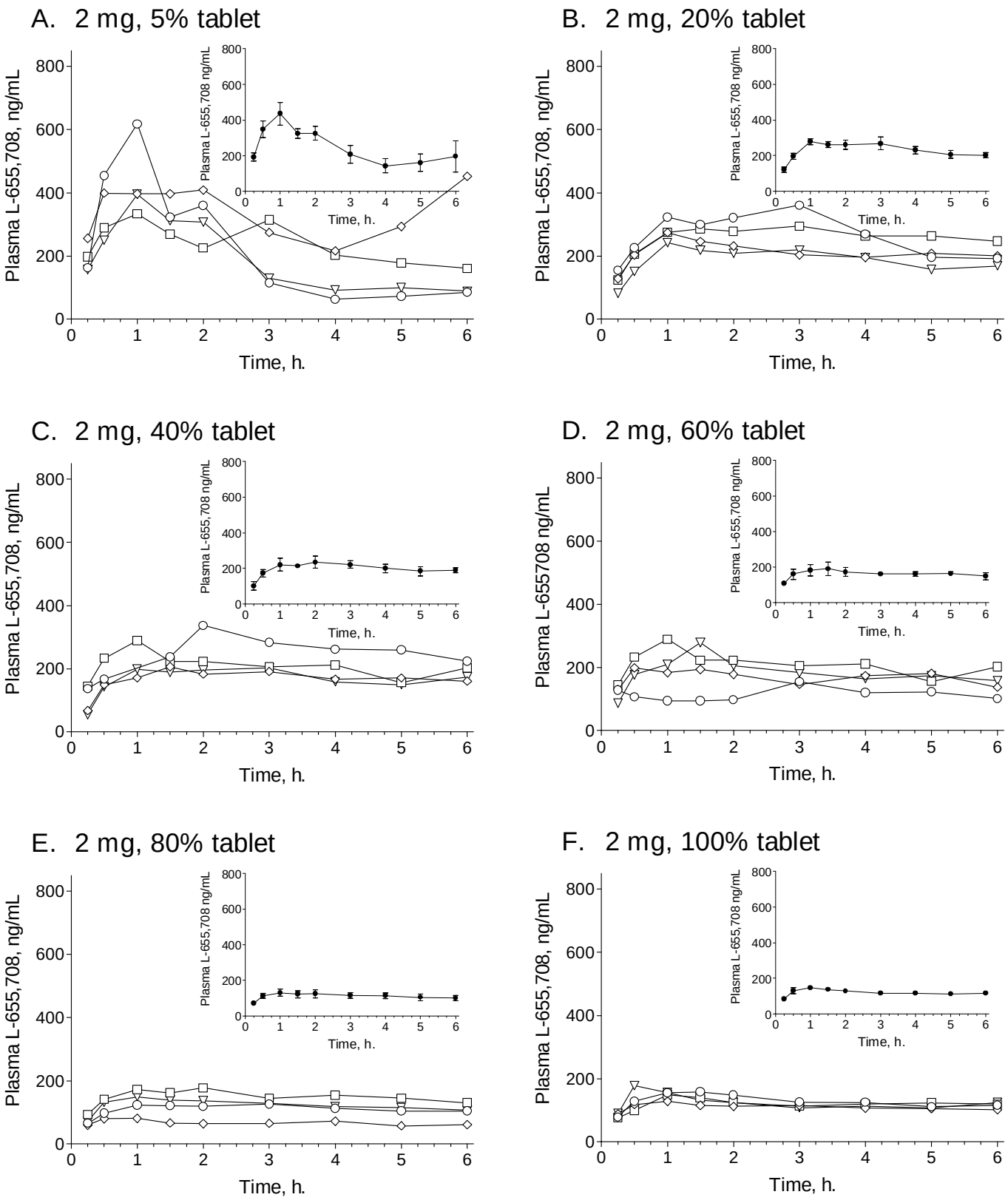

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Figure 7

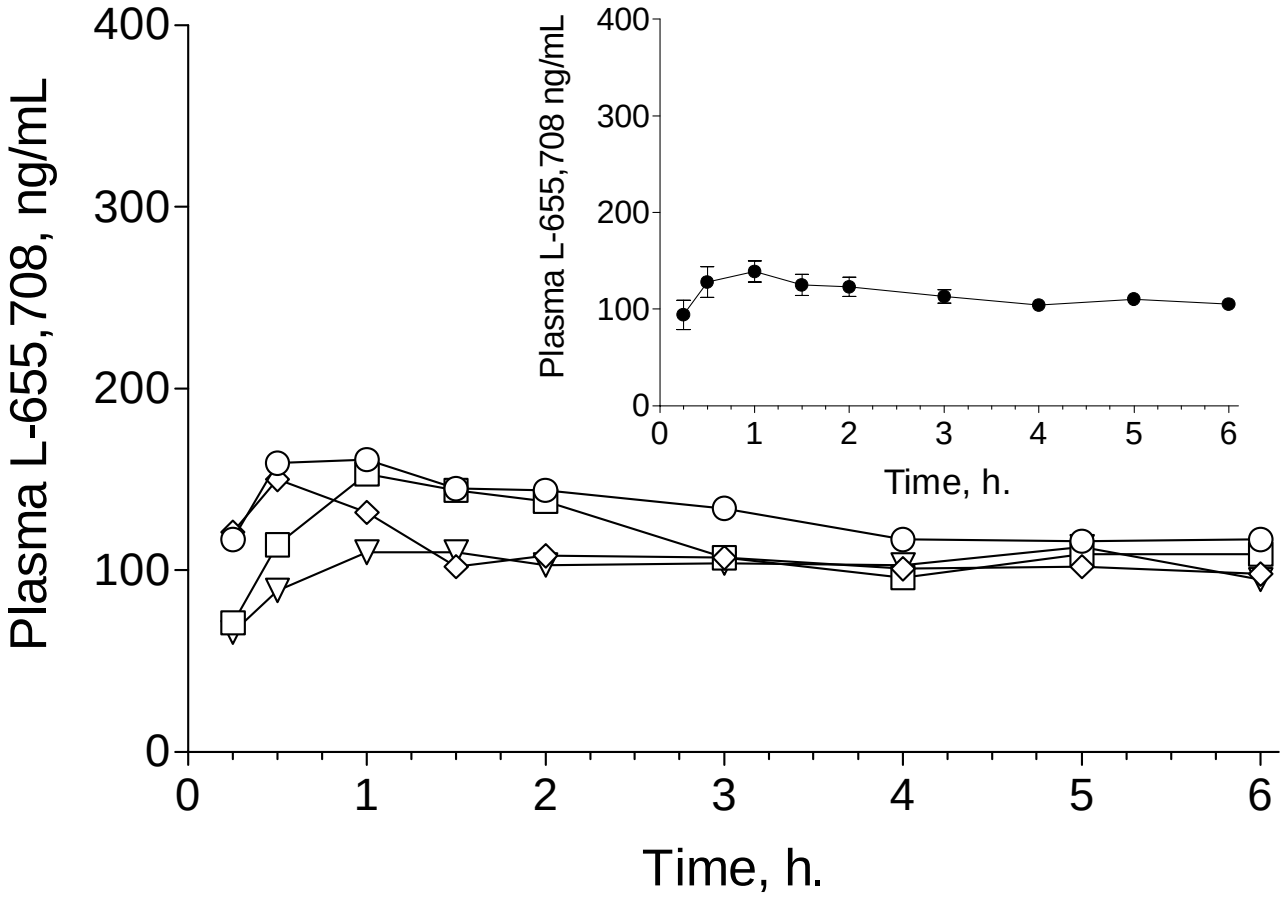

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Figure 8