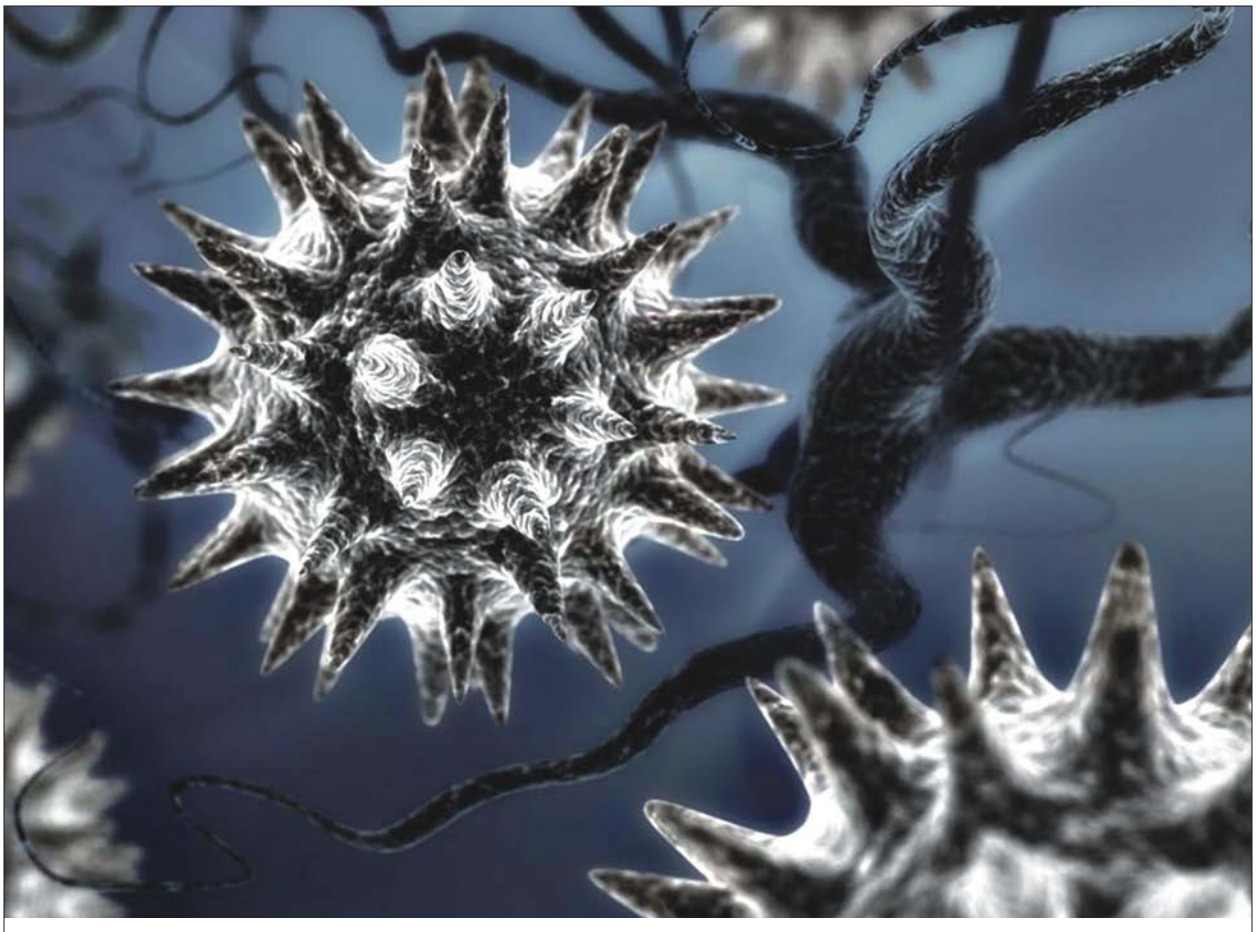

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Oseltamivir is Devoid of Specific Behavioral and Other Central Nervous System Effects in Juvenile Rats at Supratherapeutic Oral Doses

[1]C. Freichel, [2]E. Prinssen, [1]G. Hoffmann, [1]L. Gand, [3]M. Beck,
[1]T. Weiser and [1]A. Breidenbach
[1]Non-Clinical Safety,
[2]Discovery Neuroscience, Pharmaceuticals Division,
F. Hoffmann-La Roche Ltd., Basel, Switzerland
[3]WIL Research, Ashland, OH, USA

Abstract: In order to support the potential use of the antiviral oseltamivir in children aged <1 year, a non-clinical study was undertaken to identify any potential behaviourial and other effects of supratherapeutic doses of oseltamivir in juvenile rats in relation to plasma and brain drug exposure levels. Separate toxicology and toxicokinetic cohorts of 7-day old rats were divided into paired treatment groups such that individual dose groups from both cohorts received either vehicle or single oral doses of oseltamivir at 300, 500, 600, 700, 850 and 1000 mg kg^{-1}. All rats were observed twice daily for mortality and moribundity. Approximately 2 h after dosing, modified Functional Observational Battery (FOB) data were recorded for all dose groups in the toxicology cohort and clinical observations were performed in the toxicokinetic cohort for changes in posture, convulsions and tremors. Blood and brain samples were taken from the second cohort for toxicokinetic analysis. Post-necropsy assessments included microscopic examination of brain tissue. Young adult rats (aged approximately 6 weeks) were also included in the study (1000 mg kg^{-1} single oral dose only) for comparison of oseltamivir toxicokinetics. No effects on FOB parameters were recorded at 300 mg kg^{-1}. At 500 and 600 mg kg^{-1}, changes noted in the FOB were inconclusive because of inconsistent responses in the control group. Following doses of ≥600 mg kg^{-1}, drug-related changes indicative of non-specific systemic toxicity were noted. Therefore, in-life assessments did not identify any specific CNS behavior that would predict toxicity in these very young animals. Necropsy revealed no histological changes at any dose level evaluated. Compared to levels following therapeutic doses, very high oseltamivir and oseltamivir carboxylate plasma concentrations of up to approximately 42,000 and 9000 ng mL^{-1}, respectively, were already observed at the lowest dose of 300 mg kg^{-1} in juvenile rats. For all dose levels, the ratios of the oseltamivir and oseltamivir carboxylate concentrations in brain to those in plasma were low (≤0.3). The no observed effect level for single-dose oseltamivir was 300 mg kg^{-1}, which, because of the very high oseltamivir and oseltamivir carboxylate plasma levels, suggests a wide safety margin in children younger than 1 year old.

Key words: Oseltamivir, toxicology, toxicokinetics, central nervous system, behavioral effects

Corresponding Author: Christian Freichel, DVM, PhD, DABT, F. Hoffmann-La Roche Ltd., Pharmaceuticals Division, Non-Clinical Safety, Grenzacherstrasse 124, CH-4070 Basel, Switzerland

INTRODUCTION

The neuraminidase inhibitor, oseltamivir (Tamiflu®), is the antiviral agent most widely used for the treatment and prevention of influenza A and B infections. Although, it is currently approved for these indications in adults and children aged 1 year and older, there is growing interest in demonstrating that the drug is safe and well tolerated in infants less than 1 year old. In this age group, influenza is associated with higher rates of hospitalization and even mortality than in older children (Bhat *et al*., 2005; Neuzil *et al*., 2000; Izurieta *et al*., 2000) and young infants will be widely exposed to virus during any future influenza pandemic. A clinical study (number NCT 00391768; sponsored by the US National Institute of Allergy and Infectious Diseases (NIAID)) is now in progress to define the pharmacokinetics, safety and efficacy of oseltamivir and oseltamivir carboxylate in children aged less than 2 years with confirmed influenza.

Although, single dose toxicology data in rats of various ages were generated during the development of oseltamivir, a dedicated safety study in juvenile rats to assess potential behavioral and other Central Nervous System (CNS) effects was considered necessary to support the NIAID study. Therefore, prior to the start of the clinical study, Roche, the manufacturer of oseltamivir, performed this single-dose study in juvenile rats dedicated to identify biomarkers of potential behavioral and other CNS effects. Oseltamivir, the ingredient in pharmaceutical preparations of Tamiflu, is an ethyl ester prodrug that is delivered orally as a phosphate salt; it is hydrolyzed in the body by high-capacity human carboxylesterases (HCEs) to the active metabolite oseltamivir carboxylate (He *et al*., 1999; Doucette and Aoki, 2001).

The dose levels selected for use in 7 day old rats in this study were based on the results of a previous single-dose toxicology study in rats of various ages (7, 14, 24 and 42 days) that were given supratherapeutic oseltamivir doses of 381, 533 and 761 mg kg^{-1} free base (Roche, data on file). In that study, the no observed effect level in 7 day old rats was 381 mg kg^{-1}, while oseltamivir at doses of 533 and 761 mg kg^{-1} was not tolerated in 7 day old rats, but had no effects in older rats (up to 42 days old). Thus, a lowest dose of 300 mg kg^{-1} was selected for the present study, slightly below the no observed effect level from the previous study, but still supratherapeutic by a factor of about 50 and included five higher doses up to 1000 mg kg^{-1}. Rats aged approximately 6 weeks were also included in the study (1000 mg kg^{-1} single oral dose only) to enable comparison of oseltamivir toxicokinetics with juveniles.

The objectives of this study were: (1) to identify any specific behavioral markers that might occur at doses lower than those inducing overt toxicity, (2) to examine how the toxicokinetic profile of the drug in plasma and brain relates to the observed effects and (3) to indicate what the likely safety margin might be for oseltamivir when used for the treatment of infants.

MATERIALS AND METHODS

This study was conducted between 17 April and 13 December 2007. All parts of the study were carried out at WIL Research Laboratories, Ashland, OH, USA except for the bioanalytical and toxicokinetic evaluations. All parts of the study were conducted according to Good Laboratory Practice (GLP) except the quantification of brain tissue concentrations, which was performed under non-GLP conditions using a validated method.

Animals

The study was conducted in two groups of 7 day old rats of both sexes: a toxicology cohort and a toxicokinetics cohort. In the toxicokinetics cohort, a group of young adult rats (approximately 6 weeks old) of both sexes was also included.

The juvenile rats used in the study were the offspring of pregnant female Crl:CD (Sprague-Dawley) rats that were received at the test facility when they were 11-15 days into gestation (Charles River Labs, Raleigh, NC, USA) and allowed to deliver and rear the pups naturally. Crl:CD (Sprague-Dawley) rats (27 of each sex) aged approximately 6 weeks were ordered (Charles River, Raleigh, NC, USA) for toxicokinetic evaluation only. Animals were maintained in accordance with the guide for the care and use of laboratory animals (National Research Council, 1996). The animal facilities at WIL research laboratories, LLC are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

Selection Criteria and Intervention

After physical examination, juveniles weighing 12-20 g were randomly assigned to a dose group in either the toxicology or toxicokinetics cohort, using a computerized randomization procedure; adults weighing 130-300 g (males) and 115-250 g (females) were assigned to a single dose group.

In the toxicology cohort, 20 juveniles each (10 of each sex) were assigned to receive oseltamivir at one of six dose levels (300, 500, 600, 700, 850 and 1000 mg kg^{-1}) or vehicle alone (0 mg kg^{-1}). In the toxicokinetic cohort, 96 juveniles each (48 of each sex) were assigned to the same dose levels and 48 adult rats (24 of each sex) received oseltamivir at a dose of 1000 mg kg^{-1} b.wt. All test doses are expressed as oseltamivir free base. In the toxicology cohort, no more than 1 juvenile of each sex from each litter was assigned to any dose group, while in the toxicokinetic cohort, all juveniles from each litter were assigned to the same dose group.

A single oral dose was given by gastric incubation on study day 0, when juveniles were 7 days old and adults approximately 6 weeks old. Solutions of oseltamivir for oral dosing were dissolved in vehicle (deionized water adjusted to pH 4 with 0.1M hydrochloric acid) to produce concentrations of 30 to 100 mg mL^{-1}. The control groups received vehicle only. For all dose groups (juveniles and adults), the volume administered was 10 mL kg^{-1}.

Outcomes Evaluated and Assessment Schedule

Juvenile rats were kept in the same cage as mothers throughout the study. Adult animals were housed individually. On study days 0 and 1 (the 24 h period after dosing), all rats were observed twice daily (morning and afternoon) for mortality and moribundity. All juveniles were weighed on study day 0 and surviving animals weighed on study day 1.

In the toxicology cohort, behavior was assessed using a Functional Observational Battery (FOB) based on previously developed protocols (Gad, 1982; Haggerty, 1989; Irwin, 1968; Moser *et al*., 1988, 1991; O'Donoghue, 1989). Testing was performed by personal without knowledge of the animal's group assignment. The FOB was performed in a sound-attenuated room equipped with a white-noise generator set to operate at 70±10 dB. Parameters in the complete FOB utilized at WIL Research that were inappropriate for the age group to monitor (e.g., mobility, rearing, gait, approach response and startle response) were excluded. FOB assessments consisted of behaviourial observations in the home cage, observations made during handling and for 2 min in an open field setting and sensory function responses. In the toxicokinetic cohort, clinical observations were made outside the home cage only, with special emphasis on changes in body posture, tremors and convulsion.

In both cohorts, the observations described took place approximately 2 h after dosing. Therefore, due to the sampling time points being scheduled prior to the observations, observations could only be conducted in 24 out of the 48 animals initially dosed per sex/group.

In the toxicokinetic cohort, sampling time points for juveniles and adults were 0.25, 0.5, 1, 1.5, 2, 4 and 8 h after dosing on study day 0 and 24 h after dosing on study day 1. At each time point, blood from 2 or 3 juveniles of each sex was pooled for analysis (separately for each sex); brains were pooled in the same way. Pooling of samples from non-siblings was permitted. Three adults per sex were sampled at each time point, but no pooling of blood or brain samples from these animals took place.

On study day 1 (24 h after dosing), all surviving juveniles in the toxicology cohort were subjected to necropsy evaluations. All animals that did not survive to the scheduled necropsy in either cohort also underwent necropsy. Macroscopic examination was performed on all juvenile animals and the brains of selected juveniles in the toxicology cohort (those in the 0, 850 and 1000 mg kg^{-1} dose groups only) were examined microscopically. No necropsy was performed on adult rats.

Plasma and brain samples were analyzed with selective LC-MS/MS methods with lower limits of quantification of 1 ng mL^{-1} and 37.5 ng g^{-1} for oseltamivir and 10 ng mL^{-1} and 37.5 ng g^{-1} for oseltamivir carboxylate, respectively. Toxicokinetic parameters were evaluated with non-compartmental methods using composite profiles.

Statistical Analyses

Body weights, body weight gains and continuous FOB data for toxicology phase juvenile animals were subjected to a parametric one-way Analysis of Variance (ANOVA) to determine intergroup differences. Following statistically significant intergroup variance ($p<0.05$). Dunnett's test was used to compare the test item-treated groups to the control group. FOB parameters that yielded scalar or descriptive data were analyzed using Fisher's Exact test. Analyses were conducted using two-tailed tests for minimum significance levels of 5%, comparing each test item-treated group to the control group by sex. Statistical analyses were not conducted if the number of animals was 2 or less.

RESULTS AND DISCUSSION

On the day of dosing, weights of juvenile rats in the toxicology cohort ranged from 12.7-21.8 g (males) and from 12.7-19.9 g (females). In the toxicokinetics cohort, juvenile weights ranged from 6.1-21.7 g (males) and from 10.2-20.3 g (females). Due to inherent variations in the offspring growth, some study animals were outside the expected body weight range on the day of dosing (including one 700 mg kg^{-1} group male assigned to the toxicokinetic phase weighing 6.1 g on the day of dose administration only).

In-Life Observations: 300 mg kgG¹ Dose

Study results are presented below in three sections: in-life observations (behavior, lethality and body weight) grouped by dose level, necropsy findings and toxicokinetics results.

In the FOB assessment (n = 20 per dose group), no effects on behavior were recorded in the home cage, handling and open field setting. Results in sensory function tests did not differ significantly from time matched controls (Table 1), except for surface righting response, which was present in all female animals at 300 mg kg^{-1}. No effects were noted during clinical observations in animals in the toxicokinetic cohort at 300 mg kg^{-1} (Table 2).

Table 1: Behavioral changes in juvenile rats at each dose level in the toxicology cohort (Functional Observational Battery (FOB))

		Dose (mg kg^{-1})						
Behavioral changes	Sex	0	300	500	600	700	850	1000
Observations (Cage, handling, open field)								
Low respiratory rate	Male	0/10	0/10	0/10	0/10	0/10	2/10	1/7
	Female	0/10	0/10	0/10	0/9	0/9	1/9	1/4
Gasping	Male	0/10	0/10	0/10	0/10	0/10	1/10	0/7
	Female	0/10	0/10	0/10	0/9	0/9	1/9	0/4
Pallor of mucous membrane	Male	0/10	0/10	0/10	0/10	0/10	2/10	0/7
	Female	0/10	0/10	0/10	0/9	0/9	2/9	0/4
Pallor of skin	Male	0/10	0/10	0/10	0/10	0/10	2/10	0/7
	Female	0/10	0/10	0/10	0/9	0/9	1/9	0/4
Impaired or low arousal	Male	0/10	0/10	0/10	0/10	2/10	3/10	4/7*
	Female	0/10	0/10	0/10	1/9	3/9	3/9	1/4
Whole body tremor	Male	0/10	0/10	0/10	0/10	1/10	0/10	0/7
	Female	0/10	0/10	0/10	0/9	1/9	0/9	0/4
Sensory function tests								
Absent surface righting response	Male	2/10	1/10	3/10	3/10	6/10	4/10	5/7
	Female	5/10	0/10*	2/10	3/9	4/9	4/9	3/4
Absent cliff aversion response	Male	4/10	6/10	6/10	6/10	10/10*	9/10	7/7*
	Female	5/10	4/10	7/10	7/9	9/10*	6/10	4/4
Absent olfactory orientation	Male	3/10	6/10	8/10	8/10	9/10*	8/10	7/7*
	Female	4/10	5/10	8/10	8/9	9/9*	9/9*	4/4
Absent forelimb extension	Male	1/10	0/10	1/10	1/10	3/10	4/10	3/7
	Female	0/10	1/10	0/10	4/9*	2/9	1/9	1/4
Absent hind limb extension	Male	8/10	8/10	10/10	10/10	8/10	10/10	7/7
	Female	5/10	9/10	10/10	9/9	6/9	9/9	4/4
Absent negative geotaxis response	Male	7/10	8/10	7/10	7/10	8/10	9/10	7/7
	Female	8/10	8/10	10/10	9/9	8/9	9/9	4/4

Data displayed as number of animals not showing the expected response/total number of animals evaluated in group; *p<0.05 for difference compared to control group

Table 2: Results of clinical observations in juvenile rats in the toxicokinetic cohort

		Dose (mg kg^{-1})						
Clinical observation	Sex	0	300	500	600	700	850	1000
Hypoactivity	Male	0/24	0/23	0/24	0/24	1/22	3/23	3/16
	Female	0/24	0/24	0/24	0/22	1/22	1/20	1/18
Clonic convulsions	Male	0/24	0/23	0/24	0/24	0/22	0/23	0/16
	Female	0/24	0/24	0/24	0/22	0/22	1/20	0/18
Body coolness	Male	0/24	0/23	0/24	1/24	0/22	1/23	0/16
	Female	0/24	0/24	0/24	0/22	1/22	0/20	0/18
Body pallor	Male	0/24	0/23	0/24	0/24	1/22	3/23	2/16
	Female	0/24	0/24	0/24	0/22	1/22	0/20	0/18
Labored breathing	Male	0/24	0/23	0/24	0/24	1/22	1/23	0/16
	Female	0/24	0/24	0/24	0/22	0/22	0/20	0/18
Gasping	Male	0/24	0/23	0/24	0/24	0/22	3/23	2/16
	Female	0/24	0/24	0/24	0/22	1/22	0/20	0/18

Data displayed as number of animals showing the respective finding/total number of animals evaluated in group (N.B. 48 animals were initially dosed per dose level, and 16-24 animals at each dose were for sampling during the 24 h of the study)

No treatment related mortality occurred at 300 mg kg^{-1}. The mean weight gains over 24 h (males, 2.1g; females, 2.2 g) were not statistically significantly different from those in controls (2.5 and 2.2 g, respectively).

In-Life Observations: 500 and 600 mg kgG^{1} Doses

FOB results were recorded for 20 animals at the 500 mg kg^{-1} dose and 19 animals at the 600 mg kg^{-1} dose. In the home cage, handling and open field assessment, no effects on

behavior were noted at either dose except for one animal with low arousal in the 600 mg kg^{-1} group. In the FOB sensory function tests, impairment or absence of some responses was recorded in animals at 500 and 600 mg kg^{-1}, but as with the 300 mg kg^{-1} animals, the incidence of impaired responses was not statistically significantly different to that in controls, except for absent forelimb extension in females on 600 mg kg^{-1} (4/9 vs 0/10; $p<0.05$) (Table 1). No behavioral effects were observed in animals in the toxicokinetic cohort. One juvenile male presented with cool body in the 600 mg kg^{-1} group (Table 2).

In the toxicology cohort, three rats that received 600 mg kg^{-1} (including the animal with low arousal) did not survive to the scheduled necropsy. In the toxicokinetic cohort, 3 and 5 rats in the 500 and 600 mg kg^{-1} groups, respectively, did not survive to the scheduled necropsy.

In the 500 mg kg^{-1} group, mean weight gains in both sexes (male, 1.8 g; female, 2.1 g) did not differ statistically significantly from those calculated in controls. At 600 mg kg^{-1}, weight gains were statistically significantly lower than in controls (1.4 g for males and females; $p<0.05$).

In-Life Observations: 100, 850 and 1000 mg kgG¹ Doses

In the toxicology cohort, FOB results were recorded for 19 animals per dose group at doses of 700 and 850 mg kg^{-1}, but only for 11 animals at 1000 mg kg^{-1} because of the low tolerability at that dose level. In the home cage, handling and open field assessments, the most frequent observation in the 3 high-dose groups was low arousal, seen in 5/19, 6/19 and 5/11 animals at doses of 700, 850 and 1000 mg kg^{-1}, respectively; 13 of the 16 affected animals did not survive to the scheduled necropsy (Table 1). Comparison to the control group (0/20 with reduced arousal) showed a statistically significant difference only in the 1000 mg kg^{-1} group ($p<0.05$). Most of the other changes observed occurred in the 850 mg kg^{-1} group (Table 1) and all affected animals did not survive to the scheduled necropsy. One of two animals in the 700 mg kg^{-1} group that displayed tremors did not survive to the scheduled necropsy.

In the FOB sensory function tests, impairment or absence of some responses was recorded in many animals at doses of $\geq$700 mg kg^{-1}, including absent surface righting, cliff aversion or olfactory orientation responses (Table 1). However, impaired or absent sensory function responses were also observed in several control animals. For two tests (cliff aversion response and olfactory orientation), incidence of impaired responses was significantly different to controls ($p<0.05$) in at least two dose groups. Many animals with impaired sensory responses in the FOB did not tolerate treatment. In total, 7/20, 11/20 and 19/20 animals in the toxicology cohort did not survive to the scheduled necropsy at the 700, 850 and 1000 mg kg^{-1} doses, respectively.

Clinical observations such as body pallor and coolness, respiratory difficulties, hypoactivity and convulsions were recorded in 16 juvenile rats in the toxicokinetic cohort at doses of $\geq$700 mg kg^{-1} (Table 2), 14 of which did not survive to the scheduled necropsy. In total, 12, 33 and 40 animals did not tolerate test item application at the 700, 850 and 1000 mg kg^{-1} doses, respectively. In contrast, mortality did not occur in adult rats in the toxicokinetic cohort after single oral doses of 1000 mg kg^{-1}.

Mean weight gains in the 24 h after dosing were statistically significantly lower than controls in females at 700 mg kg^{-1} (1.1 g; $p<0.01$) and in both sexes at 850 mg kg^{-1} (males, 1.2 g; females, 0.6 g; $p<0.01$). In the 1000 mg kg^{-1} dose group, weight gain could only be calculated for one animal (0.3 g, male).

Necropsy Findings

No treatment-related changes were noted on macroscopic examination in juvenile rats from the toxicology cohort examined at the scheduled necropsy 24 h after dosing and in juvenile animals from the toxicology and toxicokinetics cohort that did not survive to the scheduled necropsy. In 10 animals from the toxicology cohort that had received doses of 850 or 1000 mg kg^{-1}, microscopic examination of brain tissue revealed no histopathological changes.

Toxicokinetics

In all juvenile rats administered any of the test doses, oseltamivir and oseltamivir carboxylate were measurable in plasma samples and oseltamivir was measurable in all brain samples at all sampling time points. However, oseltamivir carboxylate was quantifiable in brain samples from 1 h post dose onwards. Overall, concentrations were comparable for males and females. As described above, the reduced number of animals in the 850 and 1000 mg kg^{-1} groups available during the 24 h after dosing limited the number of plasma and brain samples for testing. In the 850 mg kg^{-1} group, parameter values at time points from 4 h onwards are based on means of 1-3 animals (instead of 4, as for other time points) and in the 1000 mg kg^{-1} group, due to the low number of animals, samples were insufficient to allow valid calculation of maximum plasma concentration (C_{max}) or area under the concentration-time curve in the 24 h after dosing (AUC_{0-24}) values.

For oseltamivir, plasma C_{max} and AUC_{0-24} values rose with increasing oseltamivir dose up to 850 mg kg^{-1} in a roughly dose-proportional manner. In brain homogenates, a similar dose-dependent rise in C_{max} and AUC_{0-24} values was seen, up to a dose of 700 mg kg^{-1}. These results are shown graphically in Fig. 1. Concentrations of oseltamivir carboxylate were lower in plasma and brain than those of oseltamivir over the whole sampling period. Mean plasma C_{max} and AUC_{0-24} for the carboxylate were roughly dose-proportional up to 600 mg kg^{-1}, with lower than dose-proportional values in the 700 and 850 mg kg^{-1} groups. A similar pattern of results was also seen in brain homogenates, with dose-proportionality only at the lower doses (Fig. 2). Plasma and brain data for oseltamivir and oseltamivir carboxylate are shown in Table 3.

In 7 day old rats in the 300 mg kg^{-1} dose group, the ratio of the oseltamivir concentration (C_{max}) in brain to that in plasma was 0.25 (at higher doses, ratio ranged from 0.19-0.28). In adult rats that received 1000 mg kg^{-1}, however, the brain-to-plasma ratio was smaller, at 0.11 (Table 3). Calculating the same ratio using AUC_{0-24} values also showed a higher ratio in juveniles than adults (0.31, 0.22). The brain-to-plasma ratios for oseltamivir carboxylate were also higher in juveniles, the contrast to adult rats being even greater than for oseltamivir: 0.057 (juveniles) and 0.013 (adults) based on C_{max} and 0.055 and 0.014, respectively, based on AUC_{0-24} (Table 3). Expressing the difference in these ratios as a (juveniles-to-adults ratio) gave values of 2.3 for oseltamivir and 4.4 for oseltamivir carboxylate, based on C_{max} and 1.4 and 3.9, respectively, based on AUC_{0-24} values.

When comparing the relationship between mortality and plasma exposure across the range of doses tested in the 7 day old rat study, there was an apparent linear relationship up to the 850 mg kg^{-1} dose for oseltamivir levels but not for oseltamivir carboxylate (Fig. 3).

The objective of this single-dose study of oral oseltamivir at supratherapeutic doses in juvenile rats was to investigate potential effects on behavior in relation to plasma and brain exposure levels and to compare the toxicokinetic profile in juveniles to that in adults. Application of oseltamivir did not induce any effects in 7 day old rats at 300 mg kg^{-1} or adult rats at 1000 mg kg^{-1}. Furthermore, no unusual changes in necroscopy, including microscopic

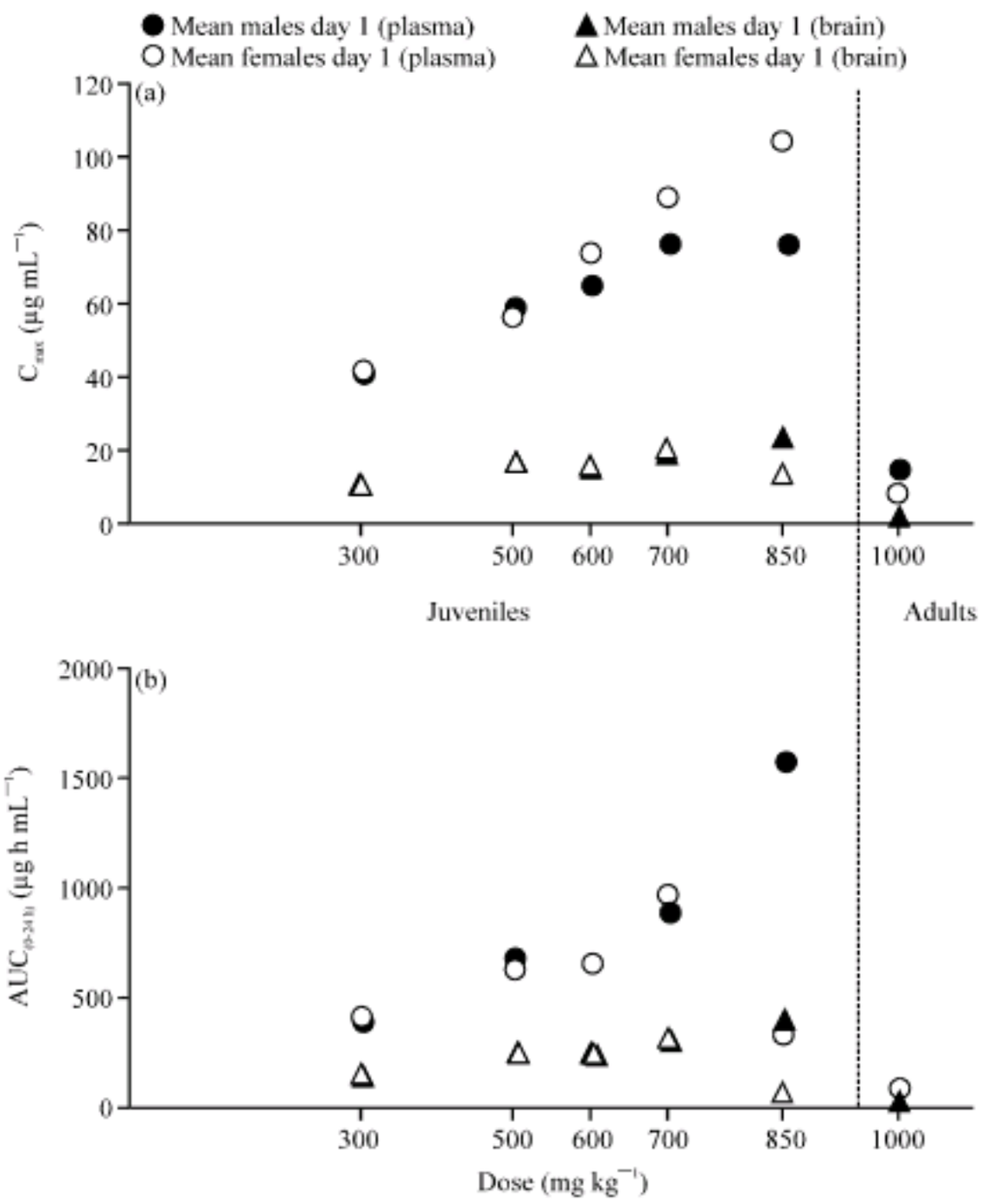

Fig. 1: Maximum Concentration (C_{max}) (a) and area under the concentration-time curve (b) in the 24 h after dosing (AUC_{0-24}) for oseltamivir in plasma and brain after single doses of oseltamivir at doses from 300 to 850 mg kg^{-1} in juvenile rats and 1000 mg kg^{-1} in adults

examination of brain samples, were found in any animal examined, even at the highest dose. These results are in line with those of a previous single-dose study in animals of the same age (Roche, data on file).

As the present study was designed to assess the occurrence of potential specific CNS-related behavioral effects, the in-life assessments did not identify any such effects at non-toxic dose levels that would predict toxicity in very young animals at higher dose levels. At the three highest doses (700-1000 mg kg^{-1}), changes in behavior were seen in the FOB that signified general moribundity, such as low arousal, respiratory difficulty and pale skin and/or mucous membranes, as opposed to a CNS-specific effect. At least one of these changes was seen in 18 of 49 animals in these dose groups, 14 of which did not survive to the scheduled necropsy (13 of those with low arousal). A very similar set of effects was observed at these doses in the toxicokinetic cohort, with 14 of 16 affected animals not surviving to the scheduled necropsy. Therefore, these observations might reflect systemic toxicity and are less clearly indicative of neurotoxicity according to established definitions, e.g., the occurrence of convulsions at dose levels at which animals are otherwise severely

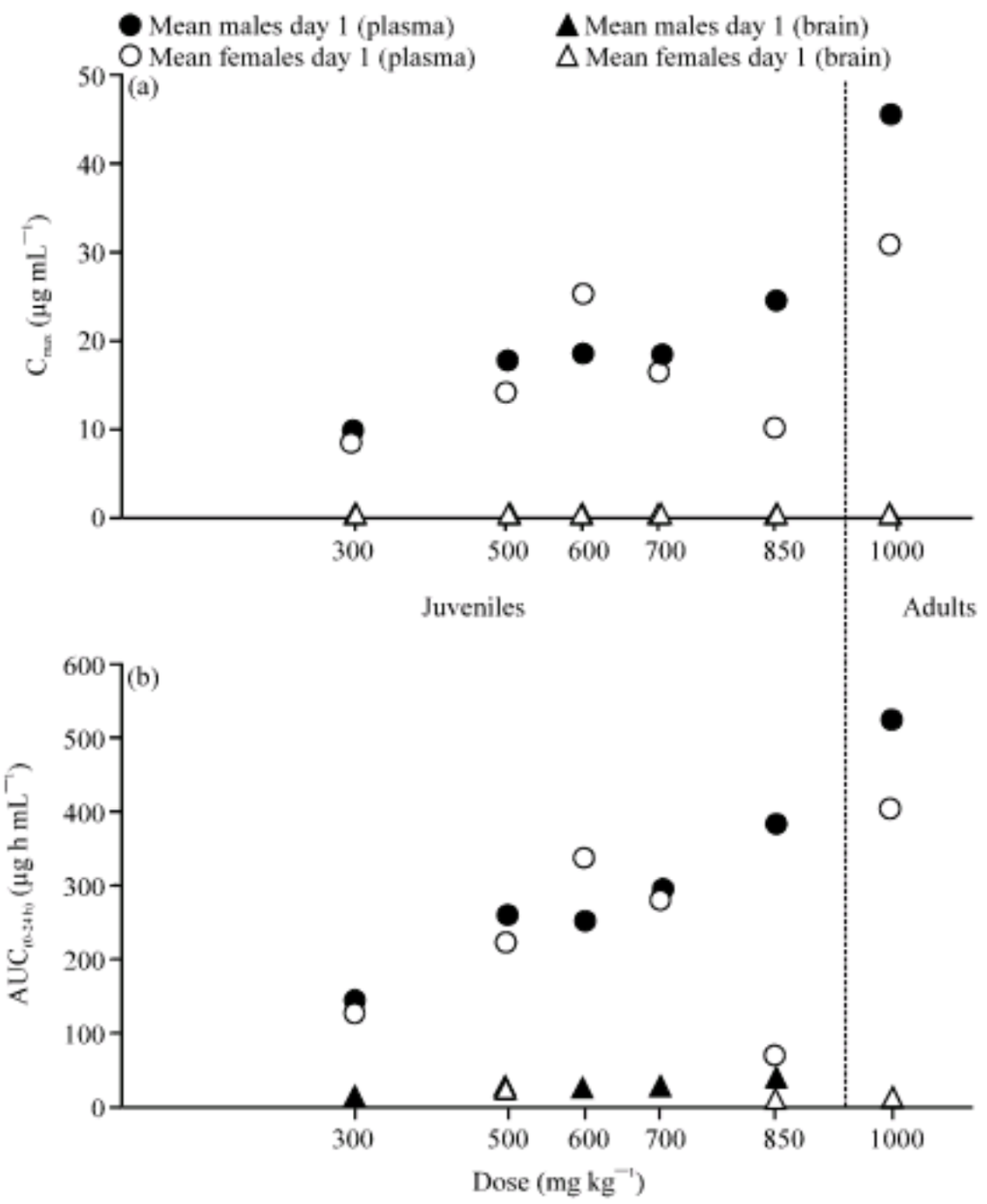

Fig. 2: Maximum Concentration (C_{max}) (a) and area under the concentration-time curve (b) in the 24 h after dosing (AUC_{0-24}) for oseltamivir carboxylate in plasma and brain after single doses of oseltamivir at doses from 300 to 850 mg kg^{-1} in juvenile rats and 1000 mg kg^{-1} in adults

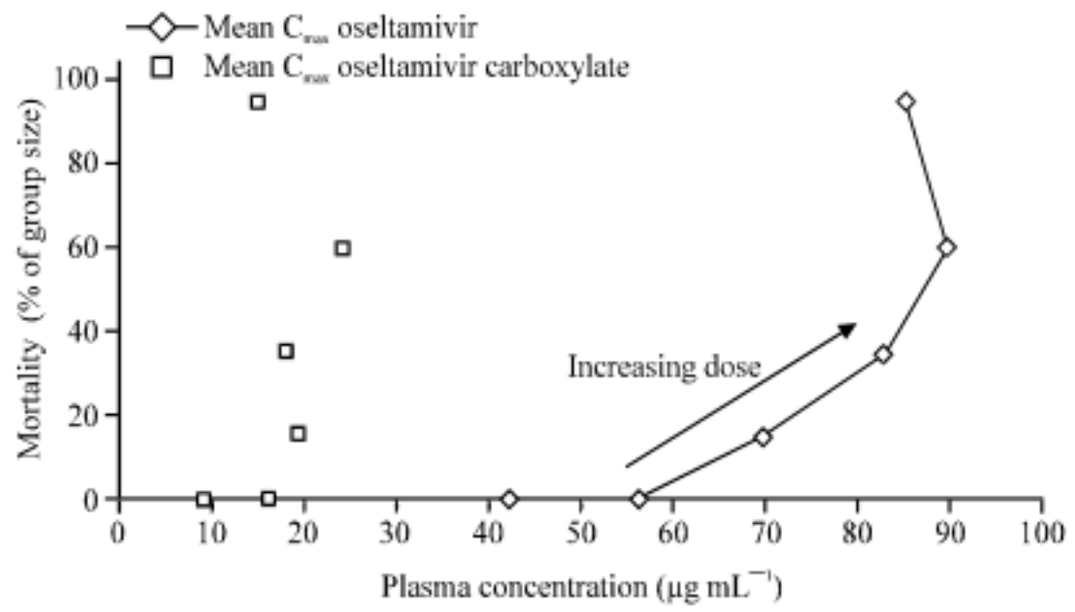

Fig. 3: Exposure-non survivor dependence: animals of the toxicology cohort compared to plasma concentrations from animals of the toxicokinetic cohort for doses 300 to 850 mg kg^{-1}

Table 3: Concentrations of oseltamivir and oseltamivir carboxylate in plasma and brain in juvenile and adult rats. In each dose group, data are means of 4 (2 male and 2 female) juvenile rats, except for 850 mg kg^{-1} (4 rats up to 2 h time point; 1-3 rats thereafter) and 6 (3 male and 3 female) adult rats

	Brain		Plasma		Ratio, brain:plasma	
Dose (mg kg^{-1})	C_{max} (ng g^{-1})	AUC_{0-24} (ng h g^{-1})	C_{max} (ng mL^{-1})	AUC_{0-24} (ng h mL^{-1})	C_{max}	AUC_{0-24}
Oseltamivir						
Juveniles						
300	10,700	126,000	42,400	410,000	0.252	0.307
500	16,300	231,000	58,000	671,000	0.281	0.344
600	14,100	221,000	70,200	670,000	0.201	0.33
700	18,700	285,000	83,100	946,000	0.225	0.301
850	17,600	206,000	91,000	775,000	0.193	0.266
1000	NC*	NC*	NC*	NC*	-	-
Adults						
1000	1280	18,200	11,500	82,700	0.111	0.22
Oseltamivir carboxylate						
Juveniles						
300	530	7670	9380	139,000	0.057	0.055
500	883	12,300	16,100	242,000	0.055	0.051
600	874	14,100	22,100	299,000	0.04	0.047
700	819	13,700	17,800	291,000	0.046	0.047
850	820	12,400	17,300	228,000	0.047	0.054
1000	NC*	NC*	NC*	NC*	-	-
Adults						
1000	518	6650	38,400	467,000	0.013	0.014

*Insufficient samples were collected to allow calculation of valid C_{max} and AUC_{0-24} values in the 1000 mg kg^{-1} dose group. AUC_{0-24}: Area Under the Concentration-time curve for 24 h period after dosing; C_{max}: Maximum Concentration; NC: Not Calculated

compromised (US Environmental Protection Agency, 1998). This is further supported by the observation that, at these higher dose levels, weight gains were often lower than in controls. Also observed during the FOB at the three highest oseltamivir doses was a decrease in responses to several sensory reflex observations, e.g., cliff avoidance and olfactory orientation, compared to the control group, most of which were statistically significant. As the majority of animals with abnormal responses to these tests did not survive to the scheduled necropsy, moribundity is likely to have contributed to the impairment of responses to these sensory tests and the effects are therefore unlikely to have been functionally specific to the CNS.

At doses of 500 and 600 mg kg^{-1}, changes observed in the FOB consisted of absent sensory reflex responses only (except for one female at 600 mg kg^{-1} also showing low arousal), but the high rate of absent responses to some sensory function tests in the control group confounded further interpretation of these findings. For example, only 6/10 males and 5/10 females in the control group showed cliff aversion and only 3/10 male and 2/10 control females displayed negative geotaxis. This might indicate that 7 day old rats were not sufficiently mature to conduct these tests; indeed, a cliff aversion response is usually acquired between days 6 and 9 of life in untreated CRL:CD(SD) rats (Drago *et al*., 1999). It is generally accepted that in an FOB reflex or reaction test in which a significant number of normal test subjects do not respond to the test stimulus the results are considered to have limited value (US Environmental Protection Agency, 1996). In designing the present study, it was anticipated that some FOB tests would not be suitable for 7 day old rats, hence the decision to omit components such as mobility, gait and grip strength. Other tests, for which age dependent effects on proper conductance could not be as surely predicted, were retained in the expectation that data could still be of value, even if some confounding of results

occurred. However, due to the prominent occurrence of absent responses to various sensory function tests in the control group, a relationship between oseltamivir administration and the effects observed at 500 and 600 mg kg^{-1} cannot be concluded.

In 7-day old juvenile rats, in contrast to adult animals, oseltamivir plasma levels were higher than those of oseltamivir carboxylate, suggesting that the Capacity of the Involved Esterase HCE1 (Shi *et al*., 2006) to convert oseltamivir pro-drug to the active metabolite is not fully developed in rats of this age. In contrast in adult rats, oseltamivir carboxylate plasma levels were over 3 times those of oseltamivir, whereas in 7 day old rats, oseltamivir was dominant (plasma levels roughly four times those of oseltamivir carboxylate). The higher oseltamivir and oseltamivir carboxylate brain to plasma ratios in juvenile animals (greater than those in adults by a factor of 2.3 and 4.4, respectively) may be due to the non-maturity of the blood-brain barrier (Watson *et al*., 2006). Nevertheless, even in juvenile rats, the brain to plasma ratio concentrations were low (≤0.3) for oseltamivir and oseltamivir carboxylate in all cases. The latter is consistent with findings of a previous investigation in juvenile rats (Roche, data on file) and with a published study in juvenile mice (Ose *et al*., 2008). In 7-day old juvenile rats, the highest dose level of oseltamivir that was not associated with any adverse effects was 300 mg kg^{-1}. Comparing the plasma exposure achieved at this dose level with steady-state data currently available for the youngest humans in which kinetic data have been obtained (6-8 months of age, 3 mg kg^{-1} b.i.d.) shows the value in the rat to be approximately 280 and 20 times the human value for oseltamivir and oseltamivir carboxylate, respectively (Roche, data on file). As the cause of toxicity in young rats seems to be oseltamivir and not oseltamivir carboxylate levels, based on the relationship between mortality and plasma exposure across the range of doses tested in the 7 day old rat study, this suggests a wide safety margin in children younger than 1 year old.

In conclusion, this study demonstrated that the no observed effect level for a single oral dose of oseltamivir in juvenile rats was 300 mg kg^{-1}. Because of the very high associated oseltamivir and oseltamivir carboxylate plasma levels, this finding suggests that there is a wide safety margin for the use of the drug in young children at the doses being tested in the NIAID-sponsored study of oseltamivir in infants aged <2 years. That study will provide more direct evidence of the safety and tolerability of the drug in children less than 1 year old.

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