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Nonlinear accumulation in the brain of the new taxoid TXD258 following saturation of P-glycoprotein at the blood–brain barrier in mice and rats

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1 TXD258, a new taxoid antitumor agent, is a poor substrate for the P-glycoprotein (P-gp) in Caco-2 cells. In this study, we investigated the amount of drug accumulating in the brains of rats and mice under a variety of conditions (dose and infusion time, species and plasma concentration) using conventional *in vivo* pharmacokinetic techniques and *in situ* brain perfusion.

2 Mice were infused with radiolabeled TXD258 at 15, 30, 45 and 90 mg m^{-2} for 45 s or 1 h and rats were infused with 15 and 60 mg m^{-2} over 2.3 min. The radioactivity in the plasma and brains was measured. The brain concentrations of TXD258 in mice and rats were maximal from 2 min to 1 h postinfusion and radioactivity was still detectable at 168 h. While the plasma concentration of TXD258 increased linearly in mice with the infused dose, the brain content increased more than proportionally with the dose between 15 and 90 mg m^{-2}. This nonlinear uptake of TXD258 also occurred in the plasma and brain of the rat.

3 These findings suggest that the protein-mediated efflux across the blood–brain barrier (BBB) becomes saturated. *In situ* brain perfusion studies confirmed that TXD258 is a P-gp substrate at the BBB of mice and rats. The P-gp of both species was saturated at the half-inhibitory concentration ($\sim$13 μM) produced by i.v. infusion.

4 Thus, the observed nonlinear accumulation of TXD258 in the brain seems to occur by saturation of the P-gp at the rodent BBB. This saturation could have several advantages, such as overcoming a P-gp-mediated efflux, but the nonlinear pharmacokinetics could increase the risk of toxicity.
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Keywords: Blood–brain barrier; *in situ* brain perfusion; multidrug resistance; P-glycoprotein; taxanes

Abbreviations: AUC, area under the curve; BBB, blood–brain barrier; CNS, central nervous system; K_p, brain-to-plasma area under the curve ratio; MDR, multidrug resistance; P-gp, P-glycoprotein; PS80, polysorbate 80

Introduction

TXD258 is a new taxoid that has an *in vivo* spectrum of antitumor action similar to that of docetaxel; it stabilizes microtubules against cold-induced depolymerization (Bissery *et al*., 2000). TXD258 also inhibits the growth of tumor cells expressing the mdr1 gene *in vitro*. The pharmacological treatment of brain diseases is often complicated by the inability of potent drugs to pass across the blood–brain barrier (BBB), which is formed by the tight endothelial cell junctions of the brain capillaries. *In vivo*, intravenous TXD258 suppresses implanted B16/TXT-resistant melanomas and intracerebral glioblastoma, suggesting that the drug is able to cross the BBB and/or the blood–brain tumor barrier (Bissery *et al*., 2000; Dykes *et al*., 2000). Other pharmacokinetic studies indicate that TXD258 is active when given orally to mice and is recognized as a marginal substrate of P-glycoprotein (P-gp) efflux pump in human Caco-2 colon carcinoma cells, largely used to predict *in vivo* oral absorption (Bissery *et al*., 2000). These properties have prompted the development of TXD258 for clinical use, with potential to treat brain metastases. Therefore, more detailed pharmacokinetic studies are needed to investigate the amount of drug given systemically that reaches the brain, as this can be influenced by the dose and duration of the drug infusion. Our pharmacokinetic studies were performed in mice and rats, using two methods. In the first conventional method, we measured the drug concentrations in the plasma and brain over time. TXD258 was given intravenously at different doses and over various infusion times. In the second method, we used *in situ* brain perfusion to determine the parameters of TXD258 uptake across the BBB in rats and mdr1a-deficient or control mice. We showed that the transport of TXD258 across the BBB is mediated by P-gp. This transport could be saturated by vascular concentrations of TXD258 greater than 13 μM. These data explain why uptake by the brain was nonlinear in the conventional pharmacokinetic dose range and emphasize how a delivery rate above or below the plasma TXD258 concentration that saturates P-gp at BBB could be critical for controlling the amount of TXD258 in the brain parenchyma and consequently available to treat

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can be saturated and that this saturation is responsible for the difference between the concentration of a drug in the brain and its systemic concentration.

Methods

Chemicals and reagents

TXD258 was manufactured by Aventis (Vitry, France). [^{14}C]-TXD258 was provided by NEN™ Life Science Products (Boston, MA, U.S.A.). Its molecular weight of 835.95 g mol^{-1} and its structure is shown in Figure 1.

The tracer had a specific activity of 1.99 GBq mmol^{-1}, and a radiochemical purity of 97.7% determined by h.p.l.c. [^{3}H]-sucrose (377.4 GBq mmol^{-1}) was obtained from NEN™ Life Science Products (Paris, France). Polysorbate 80 (PS80) and ethanol were purchased from Prolabo (Paris, France). Glucose 5%, (±)-verapamil hydrochloride and dimethyl sulfoxide were purchased from Meram (Melun, France), Sigma (St. Quentin Fallavier, France) and Merck Eurolab (Strasbourg, France), respectively. Liquid scintillation cocktails were purchased from Packard (Rungis, France). All other chemicals were commercial products of analytical grade.

Animals

The studies were performed on female CD_2F_1/Crl BR mice (18–23 g, 6–8 weeks old) and female Crl:CD®-(SD)BR rats (180–220 g, 6–8 weeks old), provided by Charles River Laboratories (Elbeuf, France). Adult female CF-1 mice (mdr1a(+/+) and (−/−), 30–40 g, 6–8 weeks old) were bred in-house from progenitors genotyped for mdr1a P-gp that were initially obtained from Charles River Laboratories (Wilmington, MA, U.S.A.). Tap water and diet were provided *ad libitum*. The animals used in these studies were handled and maintained in accordance with the requirements of the E.E.C. Guideline (1986) and U.S. Federal Guidelines (1985). Compliance with the above legislation was ensured by adhering to the standards set forth in the Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 86–23, revised 1985.

Pharmacokinetic studies

Formulation, dose and administration The poor TXD258 solubility prompted us to use a similar administration protocol used for preclinical taxoid drug docetaxel studies that involved PS80, ethanol and relative high amount of glucose 5% (Bissery *et al.*, 1995). [^{14}C]-TXD258 was diluted in PS80/ethanol/glucose 5% and was administered *via* the femoral vein using an infusion pump (Harvard PHD 2000, Harvard apparatus, Holliston, MA, U.S.A.), at 25 ml kg^{-1} for female mice to give doses of 15, 30, 45 or 90 mg m^{-2} and at 10 ml kg^{-1} for female rats to give doses of 15 and 60 mg m^{-2}. The proportion of each vehicle PS80/ethanol/glucose 5% for TXD258 administration at 15 mg m^{-2} was 0.3/1.0/98.7% and 0.4/1.4/98.2% for mice and rats, respectively. Each dose was perfused at a constant rate of 1 ml min^{-1} and the duration of the perfusion was approximately 45 s for mice and 2.3 min for rats. TXD258 was also given to female mice at doses of 30 and 90 mg m^{-2} using a perfusion rate of 8.3 μl min^{-1} for approximately 1 h.

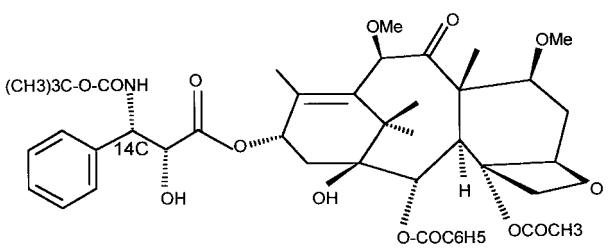

Figure 1 Chemical structure of [^{14}C]-TXD258.

Sampling Mice and rats were anesthetized with isoflurane and exsanguinated *via* the abdominal aorta at times optimized for the metabolites profile: 0.03, 0.25, 1, 6, 24, 72 and 168 h after dosing. Blood and brain samples were collected from four mice and two rats at each time point. Blood samples were collected into heparinized syringes. The plasma was separated by centrifugation at 3000 × *g* for 15 min. The plasma samples obtained at each time point were pooled to obtain enough material for quantifying metabolites. Thus, the resulting data are averages. Brains were lyophilized and kept frozen at –80°C until analyzed.

Total radioactivity analysis The total radioactivity in plasma and brain samples was determined by liquid spectrometry using a Beckman LS 6000 SC spectrometer equipped with an external standard system (number H). A quench curve was generated using [^{14}C] quenched standards supplied by Beckman. Samples were counted for up to 10 min (0.5% precision). D.p.m. values of less than twice the background were considered to be insignificant. Plasma samples (0.5 – 1 ml) were added directly to the liquid scintillation cocktail Hionic-Fluor (Beckman). Duplicate samples of freeze-dried homogenized brain were processed in a sample oxidizer (Packard model 307) and the $^{14}CO_2$ formed was trapped in 9 ml Carbosorb (Packard). The carbosorb was mixed with 12 ml of Permafluor E+ (Packard) liquid scintillation cocktail for counting.

Metabolite analysis The parent drug and metabolites in plasma and brain extracts were measured at 1 and 6 h after the end of infusion by h.p.l.c. with on-line radiochemical detector. Aliquots of plasma were subject to solid phase extraction with Oasis HLB cartridges (Waters) equilibrated with methanol and demineralized water. The eluates were concentrated and aliquots (50 μl) were analyzed by h.p.l.c. A lyophilized sample of brain was weighed and sonicated for 15 min with 20.5 ml ethyl acetate, water/formic acid (0.1%), (100/2.5, v v^{-1}). The homogenate was stirred and centrifuged at 13,000 r.p.m. for 10 min. The resulting supernatant was evaporated to dryness, taken up in 0.5 ml of a mixture containing dimethyl sulfoxide, methanol/acetone (15%), (50/50, v v^{-1}), vortexed for 2 min and clarified by centrifugation at 15,000 r.p.m. for 10 min. The extraction procedure was repeated once, the supernatants were combined and a 50 μl aliquot analyzed by h.p.l.c. The Merck® h.p.l.c. analytical system consisted of a L6200A gradient elution system pump with autosampler model AS4000, a diode array detector (L7450) operating at 230 nm and a Berthold model LB507B on-line radioactivity detector equipped with a 500 μl flow cell. Samples were separated on a Symmetry C8 column (250 × 4.6 mm, 5 μm) connected to a Symmetry C8 guard column (Waters). Elution was performed under gradient

conditions using succession of six steps from 0 to 170 min with a mobile phase of water/trifluoroacetic acid 0.01% and acetonitrile, at a flow rate of 0.6 ml min^{-1} and at room temperature. Peaks of radioactivity were quantified on the radioactivity detector by integrating the area under each peak. The calculated intra- and interday coefficients of variation were below 15%.

Pharmacokinetic analysis The pharmacokinetic analysis was carried out using a noncompartmental model with WinNonlin® software (Version 1.0, Scientific Consulting Inc., U.S.A.). The following brain and plasma parameters were determined, C_{max}, T_{max}, and $AUC_{(0-t)}$. The area under the radioactivity concentration decay curves between 0 and t (h) were computed from the experimental points by the trapezoidal method. The partition coefficient K_p was calculated as the ratio of brain $AUC_{0-168\,h}$ over plasma $AUC_{0-168\,h}$.

In situ *brain perfusion studies*

Surgical procedure and perfusion technique The transport of [^{14}C]-TXD258 into the brains of rats and mice was measured using the *in situ* brain perfusion method (Takasato *et al*., 1984; Smith, 1996; Dagenais *et al*., 2000). Animals were anesthetized by i.p. injection of a mixture of xylazine (Bayer, Puteaux, France) and ketamine (Panpharma, Fougères, France), at 8/140 mg kg^{-1} for mice and 4/70 mg kg^{-1} for rats.

Briefly, the right common carotid artery was catheterized with heparin-filled polyethylene tubing (0.30 mm i.d. × 0.70 mm o.d. for mice; 0.76 mm i.d. × 1.22 mm o.d. for rats, Biotrol Diagnostic, Chennevières-les–Louvre, France). The common carotid artery was ligated on the heart side before inserting the catheter. In mice, the external carotid was ligated rostral to the occipital artery at the bifurcation of the common carotid artery. In rats, the external carotid and occipital arteries were ligated. Body temperature was maintained at 37–38°C during surgery using a rectal thermistor connected to a temperature monitor. The syringe containing the perfusion fluid was placed in an infusion pump (Harvard pump PHD 2000, Harvard Apparatus) and connected to the catheter. Before perfusion, the thorax of the animal was opened, the heart was cut and perfusion immediately started with a flow rate of 2.5 ml min^{-1} for mice and 10 ml min^{-1} for rats. The perfusion fluid consisted of bicarbonate-buffered physiological saline (mM): 128 NaCl, 24 $NaHCO_3$, 4.2 KCl, 2.4 NaH_2PO_4, 1.5 $CaCl_2$, 0.9 $MgCl_2$ and 9 D-glucose. The solution was gassed with 95% O_2 and 5% CO_2 to obtain a pH of 7.4 and warmed to 37°C in a water bath. Compounds were added to the perfusate at the appropriate concentration. Ethanol and PS80 did not exceed 0.08 and 0.02% in the perfusate, respectively. Each animal was perfused with [^{14}C]-TXD258 plus [^{3}H]-sucrose (11.1 kBq ml^{-1}) to check the physical integrity of the BBB. Perfusion was terminated after 60 s by decapitating the animal. The brain was removed from the skull and dissected free on ice. The right cerebral hemisphere was placed in a tared vial and weighed. Aliquots of the perfusion fluid were also collected and weighed to determine tracer concentrations in the perfusate. Samples were digested in 2 ml of Solvable (Packard) at 50°C and mixed with 9 ml of Ultima gold XR scintillation cocktail (Packard). Both labels were counted simultaneously in a Packard Tri-Carb model 1900 TR (Packard).

Transport studies The transport of [^{14}C]-TXD258 into the brain was first measured in mice perfused with 4.5, 10, 12.5, 15, 20, 25 and 30 μg ml^{-1} drug and in rats perfused with 2.5, 4.5, 10, 12.5, 15, 25 and 30 μg ml^{-1} drug. These were the concentrations measured in previous pharmacokinetic and toxicokinetic studies. We then measured the influence of P-gp on the uptake of [^{14}C]-TXD258 by the brain using a drug concentration of 4.5 μg ml^{-1} (~5.4 μM) and 150 μM (±)-verapamil, in the perfusion fluid. These studies were conducted on rats, wild-type mice and P-gp deficient mdr1a(−/−) mice.

A 'wash-out' procedure was also used to study the trans-efflux zero and trans-inhibition of the compound (Stein, 1986). One syringe (syringe A) of a dual-syringe infusion pump (Harvard Apparatus) contained the bicarbonate-buffered physiological saline plus the radiotracer (11.1 kBq ml^{-1}; ~5.4 μM) and the other (syringe B) contained saline, no tracer, but with or without (±)-verapamil (150 μM). The carotid catheter was connected to a four-way valve (Hamilton, Bonnaduz, Switzerland). After the carotid cannulation was completed and the appropriate connections were made, syringe A was discharged at 2.5 ml min^{-1} (mice) or 10 ml min^{-1} (rats) for 60 s. Syringe A was switched off and syringe B was switched on simultaneously to wash-out the capillary space for 30 s. The animal was decapitated and the brain removed to measure its radioactivity.

Distribution in brain microvascular and parenchymal compartments To distinguish molecules that are trapped in endothelial cells from those that reach the brain parenchyma by transcytosis, distribution of [^{14}C]-TXD258 in the brain microvascular and parenchymal compartments was assessed in rats using the capillary depletion method of Triguero *et al*. (1990) as modified by Rousselle *et al.* (2000). We used the wash-out procedure described above with syringe B containing compound-free bicarbonate-buffered saline in order to remove nonspecific adsorption and free circulating labeled compounds from the vascular space (Triguero *et al*., 1990). At the end of the wash-out, the right cerebral hemisphere was rapidly removed, weighed and homogenized (15 pestel strokes) in 3.5 ml buffer (10 mM HEPES, 141 mM NaCl, 4 mM KCl, 1 mM NaH_2PO_4, 2.8 mM $CaCl_2$, 1 mM $MgSO_4$ and 10 mM D-glucose, pH 7.4) on ice. Chilled 37% dextran solution (4 ml) was added to obtain a final dextran concentration of 18.5%. All homogenizations were performed rapidly at 4°C. An aliquot of homogenate was removed and the remainder was centrifuged at 5400 × g for 15 min at 4°C in a swinging-bucket rotor. The pellet and supernatant were carefully separated and counted in the liquid scintillation counter. The pellet was composed mainly of brain capillaries and the supernatant reflected the parenchyma.

Calculation of BBB transport parameters All calculations were made as described by Smith (1996). Brain vascular volume (V_{vasc}; μl g^{-1}) was estimated from the tissue distribution of [^{3}H]-sucrose, which diffuses very slowly across the BBB, using the following equation:

$$V = \frac{X^*}{C^*_{perf}} \quad (1)$$

where X^* (d.p.m. g^{-1}) is the amount of sucrose measured in the right brain hemisphere and C^*_{perf} (d.p.m. μl^{-1}) is the concentration of labeled sucrose in the perfusion fluid. Transport across

the BBB was expressed in terms of the apparent distribution volume (V_{brain}) and the transport coefficient (K_{in}). The apparent distribution volume was calculated from the radioactivity in the right brain hemisphere using the following equation:

$$V_{brain} = \frac{X_{brain}}{C_{perf}} \quad (2)$$

where X_{brain} (d.p.m. g^{-1}) is the calculated amount of [^{14}C]-TXD258 in the right cerebral hemisphere and C_{perf} (d.p.m. μl^{-1}) is the labeled TXD258 tracer concentration in the perfusion fluid. Brain tissue radioactivity was corrected for vascular contamination with the following equation:

$$X_{brain} = X_{tot} - V_{vasc} C_{perf} \quad (3)$$

where X_{tot} (d.p.m. g^{-1}) is the total quantity of tracer measured in the tissue sample (vascular + extravascular).

Brain uptake, expressed as K_{in} ($\mu l\, s^{-1}\, g^{-1}$), was calculated from

$$K_{in} = \frac{V_{brain}}{T} \quad (4)$$

where T is the perfusion time (s).

The perfusion time used in single-time uptake studies was long enough to ensure that at least 40% of the total radioactivity in the tissue was outside the vascular space ($X_{brain} \geqslant 0.4 X_{tot}$; Takasato *et al*., 1984).

BBB transport modeling As the relation between K_{in} values and the TXD258 perfusate concentration was sigmoidal, an empirical Hill function was derived for assessing kinetic transport parameters. Kinetic analyses were carried out using nonlinear regression with the least-squares method of Systat 5.01 (Systat Inc., IL, U.S.A.) to fit the equation:

$$K_{in} = K_{in,min} + \frac{(K_{in,max} - K_{in,min}) C^n}{IC_{50}^n + C^n} \quad (5)$$

where C is the TXD258 concentration in the perfusate, $K_{in,min}$ is the minimal and $K_{in,max}$ the maximal brain coefficient transport (K_{in}) value measured for [^{14}C]-TXD258, n is the Hill coefficient, and IC_{50} is the concentration at which brain transport was half-maximally inhibited.

Statistical analysis Data are presented as means ± s.d. for four to eight animals, unless specified otherwise. Student's unpaired t-test was used to identify significant differences between groups when appropriate. All the tests were two-tailed and statistical significance was set at $P < 0.05$. The error values associated with the kinetic parameters (IC_{50}, $K_{in,min}$, $K_{in,max}$) are asymptotic standard errors returned by a nonlinear regression routine and are a measure of the certainty of the best-fit value.

Results

Pharmacokinetics in plasma and brain

The plasma and brain total radioactivity kinetics in rats and mice following a single short intravenous administration of [^{14}C]-TXD258 at 15 mg m^{-2} are shown in Figure 2. The other doses tested gave similar plasma and brain dispositions (data not shown). The maximal plasma concentration of TXD258 radioactivity was reached in the first sample, that is, at the end of infusion, after both short and long infusions. The concentration then rapidly decreased up to 6 h, and slowly thereafter up to 168 h, for all doses in both species. The TXD258-related radioactivity rapidly penetrated into the brain and the concentration was maximal in brain 2 min after the end of a short infusion at doses lower than 90 mg m^{-2} in both mice and rats, and 15 min after the end of short infusion of 90 mg m^{-2} in mice. The maximal brain concentration in mice occurred 15 min after the end of the 1 h infusion of TXD258 at 30 mg m^{-2} and 60 min after an infusion of 90 mg m^{-2} (Table 1). The brain radioactivity decreased slowly thereafter, but was still detectable for up to 168 h (Figure 2).

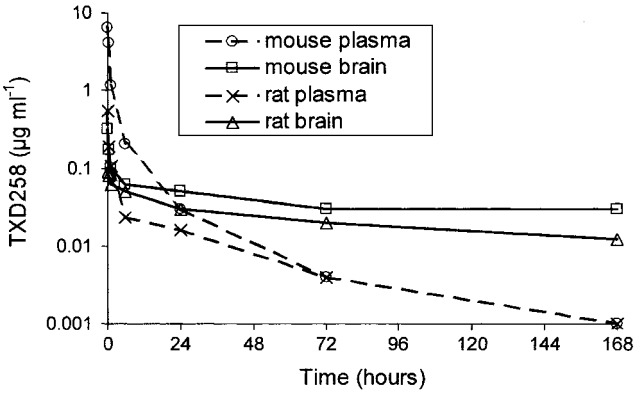

Figure 2 Average changes in the plasma and brain concentrations of TXD258 over time. [^{14}C]-TXD258 (15 mg m^{-2}) was infused into mice for 45 s and into rats for 2.3 min. Samples were taken from four mice and two rats at each time and pooled to obtain an average analytical determination.

Radio-h.p.l.c. analysis of plasma showed that unchanged drug was the major compound, accounting for at least 60% of the radioactivity at 1 h and 100% at 6 h after infusion in mice, and about 84% at 1 h and 76% at 6 h in rats, at all the perfusion times and doses studied. One of the five metabolites, docetaxel, accounted for 2–11% of the total plasma radioactivity 1 h after the end of both short or long infusions for all the doses studied in both species. Only TXD258 was detected in the brains of mice after infusion of the lowest dose. Unchanged drug represented about 90% of the total radioactivity in the brain at the end of the short infusion of TXD258 at 45 and 90 mg m^{-2}, and about 72% of the brain radioactivity after a long infusion at 90 mg m^{-2}. Docetaxel was not detected in the brains of either rats or mice at any of the doses of TXD258 studied.

Dose ranging effect on plasma and brain C_{max} and AUC

The values of C_{max} and $AUC_{0-168\,h}$ determined from the total plasma radioactivity increased proportionally with the dose in mice between 15 and 90 mg m^{-2}, but greater than proportionally with the dose in rats between 15 and 60 mg m^{-2} (Table 1 and Figure 3). The brain $AUC_{0-168\,h}$ increased eight-fold greater than proportionally with the dose in mice and 2.5-fold greater in rats, at the highest dose investigated compared to the lowest dose. However, higher doses were not investigated in rats because of the toxicity of TXD258, which possibly prevented a clear demonstration of the nonlinear brain uptake of TXD258. The brain-to-plasma $AUC_{0-168\,h}$ ratios also

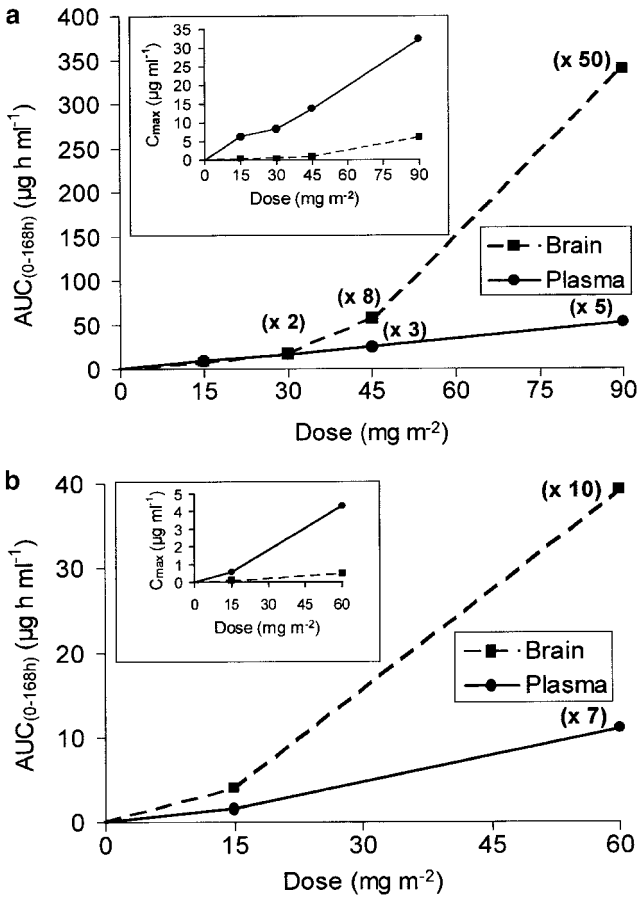

Figure 3 (**a**) Relations between the average areas under the curves ($AUC_{0-168\,h}$) or maximal concentration (C_{max}; inset) in the brains and plasma of mice infused intravenously with [^{14}C]-TXD258 at 15, 30, 45 and 90 mg m^{-2} for 45 s. Values in parentheses represent the increases in the AUC obtained at 30, 45 or 90 mg m^{-2} compared to the value measured at 15 mg m^{-2}. (**b**) Rat brain and plasma $AUC_{0-168\,h}$ or C_{max} (inset) values. The rats were given an intravenous infusion of [^{14}C]-TXD258 at 15 and 60 mg m^{-2} lasting 2.3 min.

increased more than proportionally as the doses increased (Table 1).

Effect of doses and infusion times on [^{14}C]-TXD258 in the brain and plasma of mice

These plasma concentrations produced AUC brain-to-plasma ratios of ~1.1 for the short infusion and 0.7 for the long infusion. Doses of 90 mg m^{-2} infused for 45 s or 1 h resulted in dramatically higher K_p values of 2.3 and 1.7, respectively (Table 1). When K_p values are compared between 30 and 90 mg m^{-2}, the values are increased 6.1- and 2.6-fold for 45 s and 1 h infusion, respectively. This increase in K_p could be attributed to the saturation of an active efflux transport at the BBB. The lower K_p values obtained following the 1 h infusions could, in part, be explained by a lower maximal peak plasma after the long infusion; it was 7 µg ml^{-1} after the long infusion and 32 µg ml^{-1} after the short infusion of 90 mg m^{-2}.

Effect of the concentration of TXD258 on transport into the brain of wild-type mice and rats

The effect of the TXD258 concentration on drug transport into the brain was investigated by *in situ* brain perfusion. More [^{14}C]-TXD258 was taken up by the brain when the perfusate concentrations were over 10 µg ml^{-1} (~11 µM) of TXD258 in both rats and mice, suggesting the saturation of an active efflux process at the BBB (Figure 4). The variation in the blood – brain transport coefficient K_{in} with the TXD258 concentration fitted a sigmoid equation with a Hill coefficient of 23 and TXD258 IC_{50} values of 12.9±0.4 µM in rats and 13.0±0.4 µM in mice. The K_{in} values at lower concentrations ($K_{in,min}$) were 0.63±0.01 and 0.83±0.01 µl s^{-1} g^{-1} and K_{in} values at higher concentrations ($K_{in,max}$) were 1.97±0.01 and 2.76±0.01 µl s^{-1} g^{-1} in mice and rats, respectively (Figure 4). The differences between the K_{in} in mice and rats were statistically significant ($P<0.001$), whereas the IC_{50} values in the two species were not different. The K_{in} ratios between the high and low concentrations were about 3. The amount of drug in the brain after infusions of concentrations from 2.5 to 30 µg ml^{-1} showed that the BBB was not damaged by TXD258 as the [^{3}H]-sucrose vascular volumes remained within the normal range (data not shown).

Involvement of P-glycoprotein in the transport of [^{14}C]-TXD258 across the BBB

We evaluated the importance of P-gp for the transport of TXD258 across the BBB using two approaches. First, brain trans-influx zero experiments were performed in rats and mice with 4.5 µg ml^{-1} [^{14}C]-TXD258, as this concentration does not saturate P-gp. The K_{in} in mdr1a(−/−) mice was about three-fold higher than in the wild-type mice (Figure 5). Perfusion of rats and wild-type mice with [^{14}C]-TXD258 (4.5 µg ml^{-1}) plus the P-gp modulator (±)-verapamil (150 µM) increased the

Table 1 Brain and plasma pharmacokinetic parameters obtained after the intravenous infusion of mice and rats with [^{14}C]-TXD258

	Mice						*Rats*	
Infusion time	*45 s*				*1 h*		*2.3 min*	
Dose (mg m^{-2})	15	30	45	90	30	90	15	60
C_{max} plasma (µg ml^{-1})	6.4	8.4	13.6	32.2	6	6.98	0.55	4.31
C_{max} brain (µg ml^{-1})	0.31	0.39	0.92	6.1	0.39	0.45	0.09	0.49
T_{max} brain (min)	2	2	2	15	15	60	2	2
$AUC_{0-168\,h}$ plasma (µg h ml^{-1})	9.7	15.4	24.7	52.1	16	26.9	1.6	11
$AUC_{0-168\,h}$ brain (µg h ml^{-1})	6.8	16.5	57	339	10.6	45.4	3.9	39.1
K_p	0.70	1.07	2.31	6.51	0.66	1.69	2.44	3.55

The parameters (C_{max}, T_{max}, $AUC_{0-168\,h}$) were calculated by a noncompartmental model using WinNonlin software. The partition coefficient (K_p) was calculated as the ratio of the brain $AUC_{0-168\,h}$ to the plasma $AUC_{0-168\,h}$.

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