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Complete *in vivo* reversal of P-glycoprotein pump function in the blood-brain barrier visualized with positron emission tomography

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1 Homozygously *mdr1a* gene disrupted mice (mdr1a(−/−) mice) and wild type mice (mdr1a(+/+) mice) were used to develop a method for P-glycoprotein (P-gp) function imaging non-invasively and to study the effect of a P-gp reversal agent on its function *in vivo*.

2 [^{11}C]verapamil (0.1 mg/kg) was administered and the changes in tissue concentrations were determined *ex vivo* by organ extirpation and *in vivo* with PET. To block P-gp function, cyclosporin A was administered.

3 Biodistribution studies revealed 9.5-fold ($P<0.001$) and 3.4-fold ($P<0.001$) higher [^{11}C]verapamil in the brain and testes of mdr1a(−/−) mice than in mdr1a(+/+) mice. Cyclosporin A (25 mg/kg) increased [^{11}C]verapamil levels in the brain and testes of mdr1a(+/+) mice in both cases 3.3-fold ($P<0.01$ (brain); $P<0.001$ (testes)). Fifty mg/kg cyclosporin A increased [^{11}C]verapamil in the brain 10.6-fold ($P<0.01$) and in the testes 4.1-fold ($P<0.001$). No increases were found in the mdr1a(−/−) mice. This indicates complete inhibition of P-gp mediated [^{11}C]verapamil efflux.

4 Positron camera data showed lower [^{11}C]verapamil levels in the brain of mdr1a(+/+) mice compared to those in mdr1a(−/−) mice. [^{11}C]verapamil accumulation in the brain of mdr1a(+/+) mice was increased by cyclosporin A to levels comparable with those in mdr1a(−/−) mice, indicating that reversal of P-gp mediated efflux can be monitored by PET.

5 We conclude that cyclosporin A can fully block the P-gp function in the blood brain barrier and the testes and that PET enables the *in vivo* measurement of P-gp function and reversal of its function non-invasively.

Keywords: Multidrug resistance; P-glycoprotein; efflux; *in vivo*; blood-brain barrier; positron emission tomography

Introduction

Resistance of tumours to chemotherapeutic drugs is a major problem in the treatment of cancer patients. Important anti-cancer drugs, such as anthracyclines, vinca alkaloids, epipodophyllotoxins and taxanes are affected by multidrug resistance (MDR) (Endicott & Ling, 1989; Gottesman & Pastan, 1993). Several mechanisms are responsible for MDR. One of them is the overexpression of the mdr1 gene, resulting in increased levels of the ATP-dependent 170 kDa P-glycoprotein (P-gp) drug efflux pump. Apart from the above mentioned anti-cancer drugs, also several other drugs, such as cyclosporin A (Sonneveld *et al*., 1992, 1994) and verapamil (Wilson *et al*., 1995a,b; Abe *et al*., 1995) are substrates for this pump. In humans, P-gp is normally present in several tissues in the body, such as the renal proximal tubulus, the biliary membrane of hepatocytes, the capillary endothelial cells of the brain and the testes, the adrenal gland and the apical membrane of intestinal epithelial cells (Cordon-Cardo *et al*., 1989).

In mice two genes are known which encode drug-transporting P-gps (Gros *et al*., 1991). The mouse mdr1a gene is expressed in the intestine, the liver and the blood capillaries of the brain and the testes, while the mdr1b gene is expressed in the adrenals, placenta, ovary and (pregnant) uterus (Croop *et al*., 1989; Arceci *et al*., 1988; Schinkel *et al*., 1994). In addition, in the liver it has been demonstrated that disruption of the mdr1a gene results in up-regulation of the mdr1b gene (Schinkel *et al*., 1995). It was shown previously in mdr1a(−/−) knock out mice that the tissue distribution of P-gp can be a major determinant for the pharmacokinetics (and hence pharmacodynamics) of different P-gp substrates, especially in the blood brain barrier (Schinkel *et al*., 1994, 1995, 1996; Van Asperen *et al*., 1996).

Positron emission tomography (PET), using radiolabeled P-gp substrates potentially offers a unique opportunity to study the contribution of P-gp to drug pharmacokinetics *in vivo*. This idea was tested with [^{11}C]verapamil in wild type mice (mdr1a(+/+)) and mdr1a knock out mice (mdr1a(−/−)). Modulation of P-gp activity with non-toxic compounds might increase the pharmacological effects of certain anti-cancer drugs and other P-gp substrates and is therefore potentially of clinical importance. Therefore it was also analysed whether it is possible to modulate mdr1a-mediated effects on [^{11}C]verapamil tissue distribution using various dosages of cyclosporin A in wild type mice.

Methods

Chemicals

Ketamine (Ketalar^{R}, 50 mg/ml) was obtained from Parke-Davis (Munich, Germany), xylazine (Rompun^{R} solution 2%) from Bayer (Leverkusen, Germany) and cyclosporin A (50 mg/ml) in cremophore EL (650 mg/ml) (Sandimmune^{R}) from Sandoz (Basel, Switzerland). N-nor-methyl verapamil was purchased from RBI (Natick, MA, U.S.A.).

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Preparation of [^{11}C]verapamil

D,L[^{11}C]verapamil ($t_{1/2}$ = 20.4 min) was prepared as described by Elsinga *et al*. (1996). The specific activity was >150 Ci/mmol (555 GBq/mmol) at the end of the synthesis (EOS). After evaporation of the eluent [^{11}C]verapamil was dissolved in sterile saline.

Conventional biodistribution of [^{11}C]verapamil in mdr1a(+/+) and mdr1a(−/−) mice and rats (HSD Ham RNU rnu)

Male mdr1a(+/+) and mdr1a(−/−) mice (30±3 g), age 10–14 weeks and rats (300±20 g), age 10–12 weeks, were anaesthetized with ketamine/xylazine (2/1, 1 ml/kg). Subsequently 0.3 ml [^{11}C]verapamil (37 MBq in a tracer dose or 0.1 mg/kg) was injected in the penile vein. After 60 min the mice were sacrificed by extirpation of the heart and several tissues were dissected. Heparin-plasma was obtained from the collected blood by centrifugation (3 min, 1000 *g*). Radioactivity was measured with a gamma counter (LKB Wallac, Turku, Finland). Modulation of [^{11}C]verapamil efflux with the P-gp substrate cyclosporin A was tested in mdr1a(+/+) mice, mdr1a(−/−) mice and rats. First, 0.1 ml cyclosporin A was injected in the penile vein (25 mg/kg or 50 mg/kg in mice and 50 mg/kg in rats). After 30 min, [^{11}C]verapamil was administered in the penile vein. Sixty minutes after [^{11}C]verapamil injection, the biodistribution of [^{11}C] radioactivity was measured as described above.

To study modulating effects of cremophore EL alone, 0.1 ml cremophore EL (195 mg/ml) was injected in the penile vein. This dose of cremophore EL, was the same amount, that was present in the 50 mg/kg cyclosporin A formulation.

[^{11}C]verapamil clearance from plasma in the rat

The plasma kinetics of [^{11}C]verapamil (0.1 mg/kg) with and without cyclosporin A (50 mg/kg) were studied in rats. Rats were anaesthetized and injected with [^{11}C]verapamil and cyclosporin A as described above. Arterial blood samples (100–200 μl) were drawn from the carotic artery at various time points from 0–60 min after injection of [^{11}C]verapamil. Plasma and red blood cells were separated by centrifugation (3 min, 1000 *g*). Plasma samples (50 μl) were determined.

Analysis of [^{11}C]verapamil metabolism

For analysis of [^{11}C]verapamil, 1 h after injection of [^{11}C]verapamil (0.1 mg/kg) in the penile vein, the mice were sacrificed by extirpation of the heart. In the organs where a higher accumulation of radioactivity was measured in mdr1a(−/−) than in mdr1a(+/+) mice, metabolism of [^{11}C]verapamil was studied. This was the case in the brain and the testes. The brain and the testes were quickly removed. These tissues were homogenized in acetonitrile (2 ml), using a Heidolph diax 600 apparatus (Salm & Kipp b.v., Breukelen, The Netherlands). Thereafter, the extracts were centrifuged (3 min, 3000 *g*) and acetonitrile was evaporated under reduced pressure at 50°C. To the extracts of the brain and the testes, 180 μl sterile water, 20 μl sodium hydroxide (0.1 M) and 1 ml diethyl-ether was added. The mixtures were vortexed for 1 min and centrifuged (5 min, 3000 *g*). The organic phase was separated and acidified with 150 μl sulphuric acid (0.5 M). Subsequently, the mixtures were vortexed for 1 min and centrifuged (5 min, 3000 *g*). To the supernatant 350 μl sterile water was added and this was applied onto a HPLC column (Novapak C18 4 μm, 150 × 3.9 mm; mobile phase: KH_2PO_4 (0.1 M): acetonitrile = 85 : 15; pH 2.5 by addition of ortho-phosphoric acid; flow rate 1.5 ml/min). Fractions (0.75 ml) of the eluate were collected during 10 min and counted in a calibrated gamma counter (LKB Wallac, Turku, Finland). Ten minutes after injection of the supernatant, the mobile phase was changed to collect lipophilic compounds (KH_2PO_4 (0.1 M): acetonitrile = 70 : 30; pH 2.5 by addition of ortho-phosphoric acid; flow rate 2 ml/min). Fractions (1 ml) were treated as described above. The recovery of applied radioactivity in the eluate was >95%.

PET studies in mdr1a(+/+) and mdr1a(−/−) mice

The P-gp mediated efflux of [^{11}C]verapamil in mdr1a(+/+) and mdr1a(−/−) mice was studied with a positron camera. After anaesthesia as described above, the long axis of the mouse was positioned in the PET camera parallel to the transaxial plane of the tomograph, in order to obtain sagittal sections. A transmission scan to correct for attenuation of photons by the body tissues was obtained immediately before the emission scan. Subsequently, [^{15}O]H_2O (37 MBq) dissolved in 0.3 ml saline was injected in the penile vein. The following frames were acquired: 4 consecutive frames of 5 s, 1 frame of 10 s, 2 consecutive frames of 30 s and 1 frame of 120 s. Twenty minutes after intravenous injection of [^{15}O]H_2O, [^{11}C]verapamil (37 MBq, 0.1 mg/kg) was injected in the penile vein. The following frames were acquired: 12 consecutive frames of 10 s, 6 of 30 s, 5 of 60 s, 5 of 120 s and 8 of 300 s. Measurements were performed with an ECAT 951/31 positron camera (Siemens/CTI, Knoxville, TN, U.S.A.). Radioactive decay was corrected automatically and data analysis was performed using the Siemens ECAT software (V6.3 D) on a Sun workstation. All experiments were carried out in compliance with the Law of Animal Experimentation of The Netherlands.

Statistical analysis

Differences between [^{11}C]verapamil accumulation in mdr1a(−/−) mice and mdr1a(+/+) mice were analysed using unpaired Student's *t*-test. Only P values <0.05 were considered significant.

Results

Conventional biodistribution of [^{11}C]verapamil in tissues of mdr1a(+/+) and mdr1a(−/−) mice

Table 1 summarizes the tissue levels obtained 1 h after intravenous injection of 0.1 mg/kg [^{11}C]verapamil in mdr1a(−/−) mice and mdr1a(+/+) mice. In mdr1a(−/−) mice [^{11}C]verapamil content was 9.5-fold increased in the brain and 3.4-fold increased in the testes compared to mdr1a(+/+) mice. In contrast, no significant differences in [^{11}C]verapamil levels in plasma and other tissues were measured between the two types of mice. With a tracer dose of [^{11}C]verapamil (1 μg/kg), accumulation was increased 13.8-fold in the brain and 3.2-fold in the testes of mdr1a(−/−) mice compared to mdr1a(+/+) mice. There was no significant difference of [^{11}C]verapamil accumulation in plasma or any other tissues between mdr1a(+/+) mice and mdr1a(−/−) mice (data not shown).

In Tables 2 and 3, the effect of cyclosporin A on the [^{11}C]verapamil levels are presented. Co-administration of intravenous cyclosporin A (25 mg/kg) increased the

[^{11}C]vera-pamil accumulation in mdr1a(+/+) mice 3.3-fold ($P<0.01$) in the brain and 3.3-fold ($P<0.001$) in the testes compared to mdr1a(+/+) mice without cyclosporin A modulation (compare Tables 1 and 2). Modulation with intravenous cyclosporin A at 50 mg/kg increased the [^{11}C]verapamil concentration 10.6-fold ($P<0.001$) in the brain and 4.1-fold ($P<0.001$) in the testes in mdr1a(+/+) mice compared to mdr1a(+/+) mice without cyclosporin A modulation (compare Tables 1 and 3). In contrast, 25 mg/kg and 50 mg/kg cyclosporin A did not affect [^{11}C]verapamil levels in mdr1a(−/−) mice. In addition, in plasma, no significant modulating effects on [^{11}C]verapamil concentrations were observed after administration of cyclosporin A (25 or 50 mg/kg) in either mdr1a(+/+) or mdr1a(−/−) mice. Control experiments were performed with cremophore EL, as a well-known P-gp substrate (Levin *et al*., 1980; Jette *et al*., 1985; Schuurhuis *et al*., 1990), at the solvent concentration used for cyclosporin A administration. No modulating effects of cremophore EL on [^{11}C]verapamil accumulation in the brain of mdr1a(+/+) mice were measured (data not shown). This indicates that the observed reversal effects were caused by cyclosporin A.

Increased brain uptake of [^{11}C]verapamil (0.1 mg/kg) after cyclosporin A treatment was also observed in the rat. In this case, the level of radioactivity in the brain was 11.6±2.6 ng/g tissue ($n=3$), 1 h after injection. After modulation with cyclosporin A the level was increased 12.8 fold ($P<0.0025$) to 148.0±30.2 ng/g tissue ($n=3$). These results are comparable to the results measured in the brain of mdr1a(+/+) mice (see Tables 1 and 3).

High pressure liquid chromatography (HPLC) analysis demonstrated that more than 90% of the [^{11}C]radioactivity was present as unchanged [^{11}C]verapamil compound in plasma, brain and testes of both strains of mice and the rats (data not shown) at 1 h after injection of [^{11}C]verapamil.

Table 1 Tissue levels of radioactivity in mdr1a(−/−) and mdr1a(+/+) mice 1 h after intravenous (i.v.) injection of 0.1 mg/kg [^{11}C]verapamil. Results are expressed as mean±s.d. in nanograms per gram tissue

Tissue	*mdr1a(−/−)* ($n=3$)	*mdrla(+/+)* ($n=3$)	*Ratio*
Brain	142±16	15±2	9.5*
Testes	81±11	24±7	3.4*
Surrounding head tissue	88±6	86±3	1.0
Gallbladder	726±387	449±113	1.6
Kidney	267±73	215±25	1.2
Spleen	166±44	130±30	1.3
Pancreas	171±34	153±22	1.1
Bladder	203±143	155±35	1.3
Heart	106±32	77±25	1.4
Epididymis	83±68	38±20	2.2
Small intestine	156±58	173±73	0.9
Colon	123±20	119±70	1.0
Red blood cells	37±23	20±6	1.9
Lung	349±125	376±127	0.9
Stomach	119±58	186±35	0.6
Muscle	53±12	118±161	0.5
Liver	265±87	243±45	1.1
Plasma	43±20	35±7	1.2

*$P<0.001$.

Table 2 Tissue levels of radioactivity in mdr1a(−/−) and mdra(+/+) mice 1 h after intravenous (i.v.) injection of 0.1 mg/kg [^{11}C]verapamil and 25 mg/kg cyclosporin A. Results are expressed as mean±s.d. in nanograms per gram tissue

Tissue	*mdr1a(−/−)* ($n=4$)	*mdrla(+/+)* ($n=5$)	*Ratio*
Brain	135±28	49±17	2.8*
Testes	97±20	78±17	1.2
Surrounding head tissue	66±11	54±9	1.2
Gallbladder	666±582	426±295	1.6
Kidney	367±81	423±103	0.9
Spleen	191±33	197±29	0.9
Pancreas	196±41	180±31	1.1
Bladder	79±31	87±38	0.9
Heart	136±43	110±19	1.2
Epididymis	36±3	33±10	1.1
Small intestine	290±89	323±122	0.9
Colon	115±10	97±20	1.2
Red blood cells	20±4	19±4	1.1
Lung	455±52	451±74	1.0
Stomach	69±17	59±7	1.2
Muscle	43±9	36±7	1.2
Liver	268±59	238±47	1.1
Plasma	34±7	35±8	1.0

*$P<0.005$.

PET of [^{15}O]H_2O in brain of mdr1a(−/−) and mdr1a(+/+) mice

Experiments were performed with [^{15}O]H_2O, an indifferent perfusion tracer. PET[^{15}O]H_2O images enabled localization of the head region. Furthermore, time activity curves of [^{15}O]H_2O demonstrated a fast distribution phase in the brain of mdr1a(−/−) and mdr1a(+/+) mice. Within 1 min, a plateau level was reached (data not shown).

PET of [^{11}C]verapamil in brain of mdr1a(−/−) and mdr1a(+/+) mice

Time activity curves in the head region of mdr1a(−/−) and mdr1a(+/+) mice after injection of [^{11}C]verapamil (0.1 mg/kg) demonstrated different tissue concentrations in both types

Table 3 Tissue levels of radioactivity in mdr1a(−/−) and mdr1a(+/+) mice 1 h after intravenous (i.v.) injection of 0.1 mg/kg [^{11}C]verapamil and 50 mg/kg cyclosporin A. Results are expressed as mean±s.d. in nanograms per gram tissue

Tissue	*mdr1a(−/−)* ($n=3$)	*mdrla(+/+)* ($n=3$)	*Ratio*
Brain	152±43	159±27	0.9
Testes	101±10	98±18	1.0
Surrounding head tissue	67±16	79±9	0.9
Gallbladder	250±282	214±127	1.2
Kidney	479±195	459±43	1.0
Spleen	179±8	188±52	0.9
Pancreas	185±42	222±29	0.8
Bladder	83±6	126±80	0.7
Heart	124±36	142±23	0.9
Epididymis	42±8	64±29	0.7
Small intestine	219±21	277±53	0.8
Colon	127±31	155±42	0.8
Red blood cells	30±13	38±14	0.8
Lung	428±98	557±109	0.8
Stomach	80±6	72±22	1.1
Muscle	48±21	48±20	1.0
Liver	233±52	283±22	0.9
Plasma	51±16	70±31	0.7

of mice (Figure 1). In mdr1a(−/−) and mdr1a(+/+) mice a plateau was reached respectively 20 and 5 min after injection of the tracer. The plateau level of the time-activity curves 1 h after injection of [^{11}C]verapamil was 62% in mdr1a(+/+) mice compared to the mdr1a(−/−) mice. The difference in [^{11}C]verapamil accumulation in the brain between the two strains of mice is lower if measured with a PET camera than with conventional biodistribution. This can be explained by the fact that the heads of the mice are quite small and thus the positron camera measures the brain plus surrounding tissues in the head. These surrounding tissues accumulate more radioactivity than the brain itself (see Table 1). Therefore, due to the higher accumulation of radioactivity in the surrounding tissues, results obtained with the positron camera underestimate the difference in the brain.

After pretreatment with 50 mg/kg cyclosporin A the [^{11}C]verapamil time activity curves in mdr1a(+/+) mice were higher than without cyclosporin A (compare Figures 1 and 2). Plasma levels were increased after 60 min, although not significantly (Tables 1 and 3). The levels of [^{11}C]verapamil detected in the head region of mdr1a(+/+) were increased to a 2.4-fold higher level compared to the level in unmodulated mice. The time activity curves of [^{11}C]verapamil accumulation in the head region of mdr1a(+/+) and mdr1a(−/−) mice after modulation with 50 mg/kg cyclosporin A shown in Figure 2 are virtually identical, suggesting a complete blockade of P-gp activity in the blood-brain barrier.

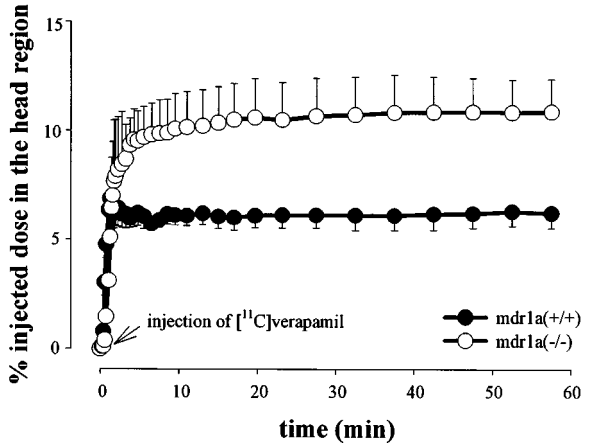

Figure 1 Time-activity curves of [^{11}C]verapamil (0.1 mg/kg) in the head region of mdr1a(−/−) and mdr1a(+/+) mice, obtained from PET images. Each point represents the mean±s.d. of three independent experiments. In each experiment, [^{11}C]verapamil pharmacokinetics were measured in one mdr1a(−/−) and one mdr1a(+/+) mouse.

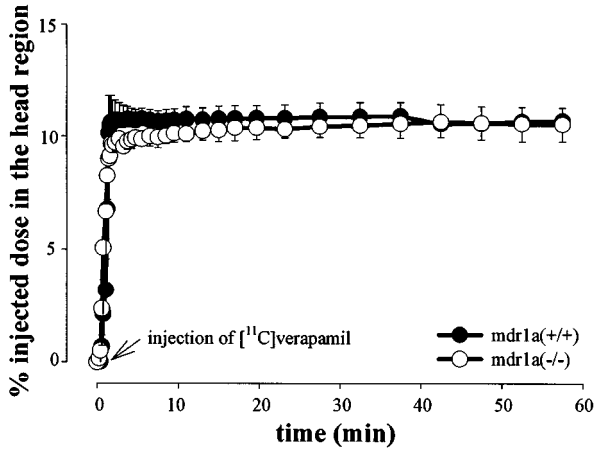

Figure 2 Time-activity curves of [^{11}C]verapamil (0.1 mg/kg) in the head region of mdr1a(−/−) and mdr1a(+/+) mice after administration of 50 mg/kg cyclosporin A, obtained from PET images. Each point represents the mean±s.d. of three independent experiments. In each experiment, [^{11}C]verapamil pharmacokinetics were measured in one mdr1a(−/−) and one mdr1a(+/+) mouse.

Plasma clearance of [^{11}C]verapamil in rats

Because of the small blood volume, mice do not allow simultaneous blood sampling for input function measurements in a single individual during a PET scan. To study potential differences in input function that may have been caused by cyclosporin A, plasma concentrations of [^{11}C]verapamil (0.1 mg/kg) were studied in rats both in the absence and presence of cyclosporin A (50 mg/kg). No significant changes in arterial plasma curves were observed with and without cyclosporin A treatment (Figure 3) over a period of 1 h after injection of [^{11}C]verapamil.

Discussion

The present study demonstrates the feasibility of non-invasively measuring P-gp function and the reversal of its function *in vivo*. Biodistribution studies of [^{11}C]verapamil and time-activity curves of [^{11}C]verapamil with PET clearly demonstrated different [^{11}C]verapamil concentrations in the brain of mdr1a(−/−) and mdr1a(+/+) mice. At the same time, no differences in [^{11}C]verapamil accumulation in the surrounding tissues of the brain of both types of mice were measured. This indicates that the different pharmacokinetics as measured by PET in mdr1a(−/−) and mdr1a(+/+) mice are caused by P-gp in the brain and not in surrounding tissues.

Comparison of *ex vivo* data and *in vivo* data (which consists of the brain and the surrounding head tissue) demonstrates a good correlation. For example: 1 h after injection of [^{11}C]verapamil, the *ex vivo* data demonstrated the presence of 2.53±0.23% of the injected dose in the brain and 7.94±0.55% in the surrounding head tissue of mdr1a(−/−) mice. Thus, the total amount in the brain and the surrounding head tissue is 10.47±0.60%. This is comparable to the camera data where the percentage of injected dose in the head region is

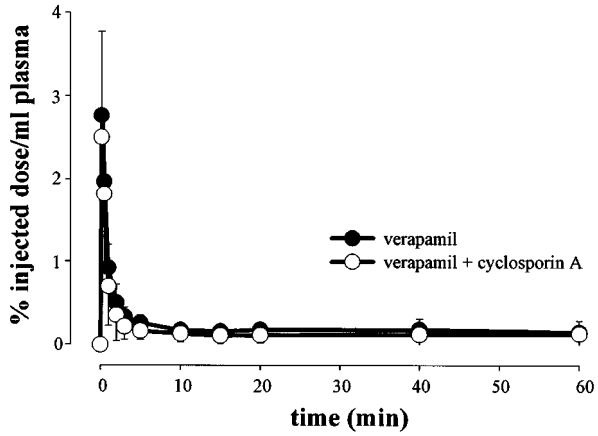

Figure 3 Plasma clearance of [^{11}C]verapamil (0.1 mg/kg) in rats with and without cyclosporin A (50 mg/kg). Each point represents the mean±s.d. of three independent experiments.

$10.88 \pm 1.48\%$. In mdr(+/+) mice, the *ex vivo* data demonstrated $0.13\% \pm 0.026\%$ of the injected dose in the brain and $7.03 \pm 0.27\%$ in the surrounding head tissue of mdr1a(+/+) mice 1 h after injection of [^{11}C]verapamil. The total amount in the brain and the surrounding head tissue is $7.16 \pm 0.27\%$. This is also comparable to the camera data where the percentage of injected dose in the head region was $6.24 \pm 0.71\%$.

Time-activity curves of [^{15}O]H_2O demonstrated a steady state level in the brain within 1 min in both types of mice. In contrast, a steady state level was reached, at the earliest, 5 min after injection of [^{11}C]verapamil. This shows that [^{11}C]verapamil is not a perfusion tracer. Therefore, the effects of [^{11}C]verapamil in the brain of mdr1a(−/−) and mdr1a(+/+) mice can not be attributed to differences in brain perfusions.

One might argue that changes in drug uptake in the brain in the presence and absence of P-gp inhibitor could have been caused by differences in systemic pharmacokinetics. Due to the small circulating blood volume in mice no input function could be determined during the PET-data acquisition. To gain more insight into the effects of cyclosporin A on the efflux of [^{11}C]verapamil, potential changes in input function were measured in rats. No significant changes of [^{11}C]verapamil concentrations in arterial plasma were observed. In contrast, the level of [^{11}C]verapamil in the rat brain was drastically increased after modulation with cyclosporin A, indicating a P-gp blockade by cyclosporin A in the blood-brain barrier, rather than systemic changes in drug supply to the brain. Biodistribution studies demonstrated also a different accumulation of [^{11}C]verapamil in the testes of both types of mice. The small size of the testes precluded discrimination from surrounding tissues, making it impossible to study [^{11}C]verapamil concentrations in the testes of the mice with the positron camera.

One h after injection of [^{11}C]verapamil into the mice, more than 90% of the total [^{11}C] radioactivity in the brain, the testes and plasma was present in the form of parent [^{11}C]verapamil. This indicates that metabolites of [^{11}C]verapamil do not play a major role in these tissue concentration measurement with PET.

The brain distribution data are in accordance with results observed by Schinkel *et al.* (1995) for other compounds, such as [^{3}H]dexamethasone, [^{3}H]digoxin and [^{3}H]cyclosporin A; especially in the brain, differences in accumulation were observed between both types of mouse (Schinkel *et al.*, 1995).

Additional proof of P-gp involvement was obtained in the present study by P-gp blockade with cyclosporin A in the wild type mice. Cyclosporin A is a potent modulator *in vitro* and *in vivo* (Sonneveld *et al.*, 1994, 1992; Boesch *et al.*, 1991). In this study, we have demonstrated *in vivo* blockade of the mdr1a drug efflux pump which was dependent on cyclosporin A dose and which was apparently complete for blood-brain barrier P-gp at a cyclosporin A dose of 50 mg/kg.

Relatively lipophilic drugs, such as verapamil and cyclosporin A are supposed to diffuse easily across lipid membranes and yet they do not enter the brain efficiently (Bradbury *et al.*, 1985; Cefalu *et al.*, 1985; Seelig *et al.*, 1994; Saeki *et al.*, 1993). The present study shows that verapamil can penetrate the brain. However, it is transported efficiently and fast back into the blood by P-gp in the blood brain barrier. Interestingly, this study shows that there should be an instantaneous release of the drug from the endothelial cells back into the blood in wild type mice. This is in agreement with previous studies in which differences in drug levels in the brain of mdr1a(+/+) mice *versus* mdr1a(−/−) mice have been observed (Schinkel *et al.*, 1995, 1996) both at 30 min and 1 h after injection. Since PET scanning allows real-time measurements of [^{11}C]-drug levels in the brain, this study demonstrates that differences in net brain uptake of drugs can be caused by P-gp alterations immediately (i.e., within minutes) after injection. Obviously, P-gp functions as a real barrier in the endothelial cells and can prevent entrance of drugs in the brain. This also supports the hypothesis that P-gp in the blood brain barrier is involved in the extrusion of drugs, such as cyclosporin A and verapamil from the endothelial cells in the brain (Cordon-Cardo *et al.*, 1989; Saeki *et al.*, 1993). In addition, after penetration of [^{11}C]verapamil in the brain in mdr1a(−/−) mice, no efflux of the radiolabeled compound was observed. Obviously, transport from the brain back to the blood is rather slow. Since no metabolites of [^{11}C]verapamil were observed, [^{11}C]verapamil may be bound in the brain, which prevents transport from the brain to the blood. For instance, it has been shown that verapamil can bind to phospholipids in membranes (Spoelstra *et al.*, 1992).

Apart from the brain and the testes, other organs, such as the liver and the kidney contain P-gp. However, differences in [^{11}C]verapamil levels were less than those in the brain and the testes. This is in agreement with other compounds tested in studies of others (Cordon-Cardo *et al.*, 1989; Saeki *et al.*, 1993). This can be explained by (1) upregulation of mdr1b P-gp in the liver and the kidneys (Schinkel *et al.*, 1994), (2) the brain and the testes have unique barriers, (3) experimental artefacts. For example, upon dissection of the liver for biodistribution studies, the bile ducts and their contents are also included, possibly obscuring the difference in mdr1a(+/+) and mdr1a(−/−) mice.

Several studies have been performed where P-gp function is measured using ^{99m}Tc-sestamibi and single photon emission tomography (SPECT) *in vivo* (Piwnica-Worms *et al.*, 1993; Luker *et al.*, 1997). It has been suggested that ^{99m}Tc-sestamibi can be used as a functional imaging agent to detect multidrug resistance non-invasively (Scopinaro *et al.*, 1994). On theoretical grounds the advantage of [^{11}C]verapamil with PET above ^{99m}Tc-sestamibi scintigraphy is that [^{11}C]verapamil with PET allows for quantification and improved sensitivity. We have recently shown that ^{99m}Tc-sestamibi is also a substrate of the multidrug resistance associated protein (MRP) (Hendrikse *et al.*, 1998), while verapamil is a relatively poor substrate for MRP. This makes verapamil a more specific tracer for P-gp imaging.

Even with a tracer dose, differences in the tissue concentrations of [^{11}C]verapamil could be demonstrated between the brain tissue in the two types of mice. This indicates that it is also possible to study P-gp mediated efflux with a sub-pharmacological dose. These data demonstrate the possibility to study P-gp function even with a non-toxic dose of [^{11}C]verapamil with PET *in vivo*. This is of major interest, because it potentially enables the non-invasive measurement in humans of the inhibition of efflux of [^{11}C]verapamil in P-gp expressing tissues, such as tumours, brain, and testes with different modulators.

Secondly, the fact that P-gp function can be completely blocked in the brain may be translated into major clinical applications. Several P-gp substrates with potential interest for treatment of diseases in the brain, such as chemotherapeutic drugs, are known to accumulate poorly in the brain. This study strongly suggests that coadministration of the drug of interest together with a non-toxic reversal agent may increase efficacy and/or toxicity of drugs at the level of the brain. This knowledge may help to design new drug delivery strategies utilizing pharmacological drug pump modulation.

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