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Brain permeability of inhaled corticosteroids

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Abstract

The aim of this study was to evaluate if the permeability of inhaled corticosteroids entering the brain is reduced and if P-glycoprotein (P-gp) transporters are involved. Currently employed inhaled corticosteroids were given intravenously and intratracheally to rats at a dose of 100 μg kg^{-1}. An ex-vivo receptor binding assay was used to monitor over 12 h the glucocorticoid receptor occupancy in the brain and a systemic reference organ (kidney). The involvement of P-gp in the brain permeability of triamcinolone acetonide was assessed in wild-type mice and mdr1a(−/−) knockout mice (mice lacking the gene for expressing P-gp). After both forms of administration, the average brain receptor occupancies were 20–56% of those of the reference organ, with the more lipophilic drugs showing a more pronounced receptor occupation. While the receptor occupancies in the liver of wild-type and mdr1a(−/−) mice were similar after administration of triamcinolone acetonide, brain receptor occupancies in mdr1a(−/−) mice were significantly greater (mdr1a(−/−): 47.6%, 40.2–55.0%, n = 2; wild-type: 11.5 ± 33.0%, n = 3). Penetration into the brain for inhaled corticosteroids (especially those of lower lipophilicity) is reduced. Experiments in mdr1a(−/−) mice confirmed the involvement of P-gp transporters. Further studies are needed to assess whether potential drug interactions at the transporter level are of pharmacological significance.

Introduction

The blood–brain barrier restricts the entry of a variety of therapeutically active agents from the systemic circulation into the central nervous system. The endothelial cells of the brain capillaries, connected via tight junctions, form a physical barrier and limit the penetration of hydrophilic substrates. In addition, the efflux transporters present on the blood–brain barrier actively remove a wide variety of structurally unrelated substrates (Schinkel et al 1995, 1996). One of these transporters, P-glycoprotein (mdr1a or P-gp), is highly concentrated on the apical membrane of the endothelial cells of brain capillaries.

The ability of P-gp to efficiently protect the brain from dexamethasone, prednisolone and cortisol has been demonstrated (Meijer et al 1998; Karssen et al 2001, 2002). This finding may be of clinical relevance as the reduced brain uptake might be responsible for a reduced modulation of the adrenocortical axis at the level of the hypothalamus. Yates et al (2003) studied the structure–affinity relationships for P-gp mediated transport of systemic glucocorticoids in cell culture and reported that non-polar bulky substituents in position C-16 reduced transporter affinity. Data on inhaled corticosteroids are currently not available. Considering the work by Yates et al (2003), inhaled corticosteroids with bulky substituents in position 16 may be only weak substrates for P-gp, which would lead to higher brain concentrations as compared with steroids that lack substituents in this region. The aim of this study was to assess in an animal model if the brain permeability of inhaled corticosteroids is reduced and if P-gp is involved in determining brain permeability.

We used a previously developed ex-vivo receptor binding assay (Hochhaus et al 1995) to determine the glucocorticoid receptor occupancies in the brain and kidney of

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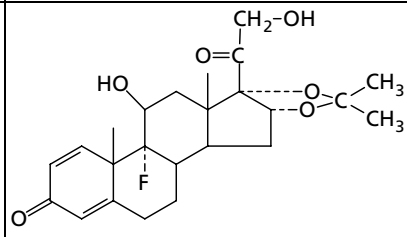
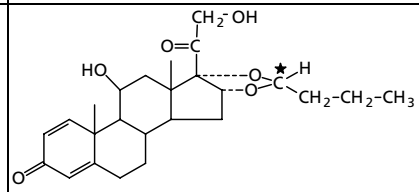
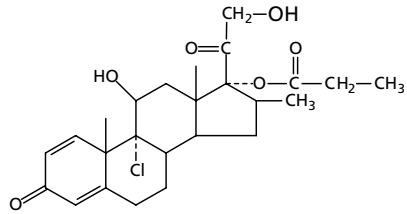
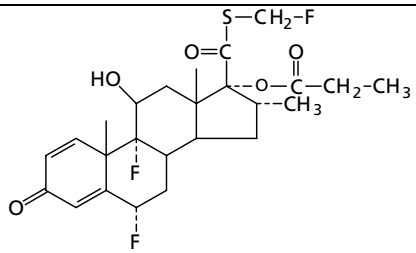
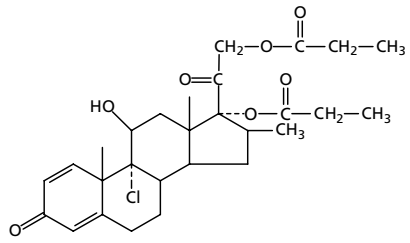
Table 1 Structure and logP values of tested glucocorticoids

Drug	Structure	LogP
Triamcinolone acetonide		2.2
Budesonide		2.8
Beclomethasone monopropionate		3.14
Fluticasone propionate		3.8
Beclomethasone dipropionate		4.1

adult rats after intravenous and intratracheal administration of budesonide, fluticasone propionate (FP), beclomethasone dipropionate (BDP), beclomethasone monopropionate (BMP; the active species of the prodrug BDP) and triamcinolone acetonide (TA). These steroids were selected because they are used in therapy and also show differences in lipophilicity (Table 1). The model is able to assess the degree of occupied receptors (as a measure of pharmacologically relevant free drug concentrations) in different tissues over time. Differences in receptor occupancies between brain and other organs such as liver and kidney were used to quantify the effect of the blood–brain barrier. Experiments were performed after intravenous injections and intratracheal administration to probe for potential effects of the form of administration. In addition, knockout mice (mice which lack the expression of P-gp transporters; Schinkel et al 1994, 1996; Mayer et al 1996) were used to clearly show the involvement of P-gp in modulating the permeability of one of these inhaled glucocorticoids across the blood–brain barrier.

Materials and Methods

Study design

Most of the ex-vivo receptor binding studies were performed in rats, as the animal model was originally established for this species (Hochhaus et al 1995). In order to evaluate the role of P-gp on brain permeability, experiments were performed on wild-type and mdr1a(−/−) knockout mice. Experiments after intravenous and intratracheal administration were included to determine potential differences between different forms of administration. These experiments were part of a larger ongoing study evaluating the pulmonary selectivity of inhaled corticosteroids.

Materials

Micronized budesonide was obtained from Sicor (Milan, Italy). Micronized FP was obtained from Glaxo-Wellcome (Research Triangle Park, NC, USA). Micronized BDP was kindly provided by 3M (St Paul, MN, USA). 17-BMP was purchased from the European Directorate for the Quality of Medicine (URL: http://www.pheur.org). Phosphate-buffered saline (pH 7.4) was obtained from Cellegro (Mediatech, Herndon, VA, USA). TA phosphate solution (54.4 mg mL^{-1}) was obtained from Bristol Myers Squibb (Munich, Germany). Micronized TA was obtained from Sigma (St Louis, MO, USA). ^{3}H-labelled TA (38 Ci mmol^{-1}) was obtained from New England Nuclear (Wilmington, DE, USA). All other unlabelled chemicals were obtained from Sigma or equivalent sources.

Preparation of drug formulations

For tail vein injections, inhaled corticosteroids (200 μg mL^{-1}) were dissolved in a mixture of polyethylene glycol 300 and saline (2:1 v/v). BDP (200 μg mL^{-1}) and BMP (180 μg mL^{-1}) were prepared in a mixture of polyethylene glycol 300 and saline (3:1 v/v). TA phosphate solution (200 μg mL^{-1}) used for the mouse experiments was prepared in phosphate-buffered saline. In order to give the drugs intratracheally, drug powders of BDP, BMP, TA, FP and budesonide were diluted with lactose to obtain a final concentration of 4 μg drug (mg of lactose)$^{-1}$.

Animal procedures

All animal procedures were approved by the Institutional Animal Care and Use Committee, University of Florida, an Association for the Assessment and Accreditation of Laboratory Animal Care approved facility. For the rat experiments, F-344 rats (220–250 g) were housed in a constant temperature environment with a 12-h light/dark cycle. The rats were anaesthetized with an anaesthetic mixture (1.5 mL of 10% v/v ketamine, 1.5 mL of 2% v/v xylazine and 0.5 mL of 1% v/v acepromazine) at a subcutaneous dose of 1 mL kg^{-1}. Once the animal was under complete anaesthesia, either 100 μL of glucocorticoid (budesonide, FP, BDP or BMP) solution or 100 μL of saline (for placebo rats) was slowly injected into the tail vein. The rats were decapitated at 0.5, 1, 2, 4, 6 and 12 h (0.5, 1, 2, 4, 7 and 12 h for BDP and BMP) after tail vein injection. The brain and kidneys (for TA phosphate, the liver) were removed and immediately processed for receptor binding studies. Table 2 shows the number of independent experiments (with the number of animals ranging from three to six) per time point. The selected

Table 2 Receptor occupancy and ratios of brain versus kidney data after intravenous and intratracheal administration of various inhaled corticosteroids (100 μg kg^{-1}) in rats

Inhaled corticosteroid	Administration route	No. of independent experiments		Receptor occupancy (%)		Ratio (brain/kidney)
		Brain	Kidney	Brain	Kidney	
Beclomethasone monopropionate	Intravenous	3	3	15.4 ± 6.1	75.0 ± 3.4	0.21 ± 0.09
	Intratracheal	3	3	11.5 ± 9.3	60.5 ± 13.6	0.20 ± 0.16
						0.20 ± 0.11
Triamcinolone acetonide	Intratracheal	3	3	11.3 ± 6.7	53.1 ± 2.7	**0.22 ± 0.14**
Budesonide	Intravenous	3	3	20.7 ± 10.7	55.6 ± 10.8	0.36 ± 0.11
	Intratracheal	6	6	12.0 ± 10.2	34.0 ± 9.5	0.33 ± 0.18
						0.34 ± 0.16
Fluticasone propionate	Intravenous	3	3	18.0 ± 3.3	42.9 ± 10.3	0.42 ± 0.05
	Intratracheal	6	6	14.0 ± 10.1	28.0 ± 8.8	0.41 ± 0.29
						0.41 ± 0.23
Beclomethasone dipropionate	Intravenous	3	3	32.7 ± 21.2	64.0 ± 5.8	0.49 ± 0.28
	Intratracheal	3	3	35.7 ± 11.9	58.3 ± 12.8	0.63 ± 0.28
						0.56 ± 0.26

The mean ± s.d. is given. Numbers in bold are the mean ± s.d. of the combined intravenous and intratracheal experiments for a given glucocorticoid. An independent experiment is defined as one receptor occupancy time profile obtained during a single day. Beclomethasone monopropionate is the active metabolite of beclomethasone dipropionate. Beclomethasone monopropionate occupancy ratios were significantly different compared with those for beclomethasone dipropionate; no other significant differences were found.

dose, although greater than that used therapeutically in man, was chosen because it was the same as that used in previous studies (Hochhaus et al 1995; Suarez et al 1998).

For the intratracheal administration of the glucocorticoid powders (TA, FP, BMP, BDP, budesonide), the neck of the completely anaesthetized animal was shaved and aseptically cleaned with isopropyl alcohol 70%. A 1-cm midline vertical incision was made originating above the sternal notch. The neck muscles and glands were carefully dissected midline until the trachea was exposed and a tracheotomy was performed between the third and fourth tracheal rings. One inch of a 14-gauge Novalon catheter sheath attached to a delivery device for intratracheal administration of dry powders (Penn-Century, Philadelphia, PA, USA) was introduced into the trachea. A mixture of 5 ± 0.5 mg (calibrated with lactose, $n = 16$) of extra fine monohydrate lactose and TA, BDP, BMP, budesonide or FP formulations in lactose (0.4%, 100 $\mu g\,kg^{-1}$) was placed in the chamber of the device and instilled in the lungs with insufflation of 3 mL of air. A control rat, which received 5 mg of the vehicle (lactose), was included in each set of experiments. The animals were further processed as described above for intravenous injections.

For the mouse experiments, wild-type and mdr1a(−/−) knockout mice (30 ± 5 g) were obtained from Taconic (Germantown, NY, USA) and housed in a sterile pathogen-free environment. On the day of the experiment, the mice were anaesthetized with the same anaesthetic mixture used for the rat experiments (1 $mL\,kg^{-1}$), injected with either saline (for placebo) or TA phosphate (100 $\mu g\,kg^{-1}$) through the tail vein, and decapitated at 1, 2.5 and 6 h to obtain brain and liver tissue. Because of the cost of the animals, two animals per time point (eight animals in total) were used for the knockout mice. Although the statistical power is reduced by this approach, the general effect of the lack of the P-gp on brain uptake was visible.

Ex-vivo receptor binding assay

A previously developed ex-vivo receptor binding assay was used to process brain, liver and kidney samples (Hochhaus et al 1995; Suarez et al 1998). Immediately after removal, the brain and kidney (or liver) were weighed and placed on ice. The weighed tissue was homogenized in 10 vols (for liver) or 4 vols (for brain and kidney) of ice-cold incubation buffer (10 mM Tris/HCl, 10 mM sodium molybdate and 2 mM 1,4-dithioerythritol). Then, 2 mL of the homogenate was incubated with 5% charcoal (in distilled water) for 10 min to remove unbound corticosteroids. After centrifugation (20 min at 20 000 g, 4°C), samples of the supernatant (150 μL for rat experiments, 75 μL for mouse experiments because of limited cytosol) were mixed with 25 μL of either 20 nM (10 nM for rat experiments) ^{3}H-labelled TA or a mixture of 20 nM ^{3}H-labelled TA and 20 μM of unlabelled TA (mixture of 10 nM ^{3}H labelled TA and 10 μM unlabelled TA for rat experiments) to determine both total and non-specific binding, respectively.

After 18 h, 200 μL (100 μL for mouse experiments) of activated charcoal (5% in water) was added. Tubes were centrifuged at 16 000 g for 5 min and 300 μL (150 μL for mouse experiments) of the supernatant was counted for radioactivity (dpm).

For a given rat or mouse tissue (liver, kidney or brain), the radioactivity counts corresponded to the total binding (specific and non-specific) of the tracer. The non-specific binding was subtracted from the total binding to obtain estimates of the specific binding. The specific binding obtained in rats administered saline (placebo) corresponded to 100% free receptors. The percentage free receptors present in the brain or kidney/liver was calculated as:

$$\%\ \text{free receptors} = (\text{specific tissue binding after drug} / \text{specific tissue binding after saline}) \times 100$$

For each tissue, the area under the free receptor–time profile $AUC_{0\text{–}6\ h}$ ($AUC_{0\text{–}7\ h}$ for BDP and BMP) was calculated over a 6-h (7-h for BDP and BMP) time period by the trapezoidal rule. A 6-h (7-h for BDP and BMP) time window was used as the data density between 6 h (7 h for BDP and BMP) and 12 h was too small, but would have a significant effect on the resulting AUC. $AUC_{0\text{–}6\ h}$ could range from 0%*h (all receptors are occupied) to 600%*h (no receptors are occupied by the drug). To obtain the area under the bound receptor–time curve for the 6-h time period, $AUC_{0\text{–}6\ h}$ was subtracted from 600%*h (6 h*100% free receptors). The mean receptor occupancy in the organs was obtained by dividing the area under the bound receptor–time curve by 6 h (the relevant time range). Accordingly, the $AUC_{0\text{–}7\,h}$ was calculated for BDP and BMP experiments (6-h time point was missing) and the area under the bound receptor–time curve and the mean receptor occupancy was calculated accordingly. To compensate for differences in receptor binding affinities and other factors, we also computed the ratios (average brain receptor occupancy/average kidney receptor occupancy) in order to determine the degree of brain uptake. These data were compared for different glucocorticoids using a one-way analysis of variance (GraphPad Prism, version 3; GraphPad Software, San Diego, CA, USA) followed by the t-test, using the combined data for intravenous and intratracheal administration routes, since calculated results were very similar for the two administration routes (Table 2).

LogP calculations

Log octanol–water partition coefficients (logP) were calculated as described previously (Buchwald & Bodor 2004) by averaging three computed values: CLOGP (from ChemDraw Ultra 7.0), ACD/LogP and QlogP. Values calculated by this procedure were found to be in good agreement with experimental logP values, where available.

Statistical analysis

The number of independent rat experiments (number of animals per time point) ranged from three to six (Table 2). Considering a standard deviation of 50% in the outcome parameter (ratio between brain and liver receptor occupancy)

and assuming no statistical difference between intravenous and intratracheal administration, the experiments should have a power of 0.9 to detect a difference of 0.2 in the outcome parameter. This was judged as sufficient for these types of experiments.

Statistical analysis was performed with GraphPad Prism version 4.00 for Windows, (GraphPad Software). The effect of route of administration on the occupancy ratios was statistically analysed for individual drugs using the Mann–Whitney *U*-test. The non-parametric Kruskal–Wallis test was used to test for differences in the occupancy ratios among the tested glucocorticoids. Intravenous and intratracheal administration route data were combined for this purpose. Dunn's post-hoc test was used to discern between individual differences. A Wilcoxon matched-pair test across all treatments was performed for comparing differences between kidney and brain average receptor occupancies.

Because of the lower number of experiments, mean receptor occupancies were not analysed for mouse experiments. Occupancy ratios observed in knockout and wild-type mice for all time points (except time 0) were calculated and compared by the Mann–Whitney *U*-test. Data collected over the 1–6-h time period were pooled. In addition, a Kruskal–Wallis test was performed to compare differences in liver and brain occupancy in the investigated mice (number of free receptors measured at defined time points). Post-hoc comparison was performed using Dunn's post-hoc test.

A significance level of $P < 0.05$ was used to define significant differences. All data are expressed as mean $\pm$ s.d.

Results

Preliminary experiments in mouse cytosol indicated that the receptor density and affinity were similar for the brain (IC50 of 12.5 nM and B_{max} of 38 fmol per 100 μL of cytosol) and kidney (IC50 of 9.4 nM and B_{max} of 49 fmol per 100 μL of cytosol). This, together with literature data for the rat (Ballard et al 1974), ensured that a direct comparison of occupied receptors in brain and kidney reflected differences in the free drug concentrations present in the organs and are not affected by differences in the receptor number and affinity. Preliminary ex-vivo experiments for TA at a dose of 100 μg showed that time profiles of occupied receptors in kidney and liver were similar for this glucocorticoid after intratracheal administration (average liver occupancy: 55.3 $\pm$ 7.8%; average kidney receptor occupancy: 53.1 $\pm$ 2.7, n = 3). This good agreement was of relevance for the subsequent mouse experiments for TA, as liver tissues were used for these studies.

Figures 1 and 2 show the percentage free receptors versus time profiles after intravenous and intratracheal administration of the tested glucocorticoids (100 μg kg^{-1}). These experiments showed that the average receptor occupancies observed for the five glucocorticoids in the brain were significantly lower than in the reference organ.

Table 2 shows the resulting average receptor occupancies over the 6-h observation period in the brain and kidney as well as the resulting brain/kidney ratios of data shown in Figures 1 and 2. The Wilcoxon matched-pair test indicated that across all investigated glucocorticoids, significantly fewer receptors were occupied in the brain than in the kidney (Table 2). The Mann–Whitney *U*-test performed for all glucocorticoids separately showed that the ratios of

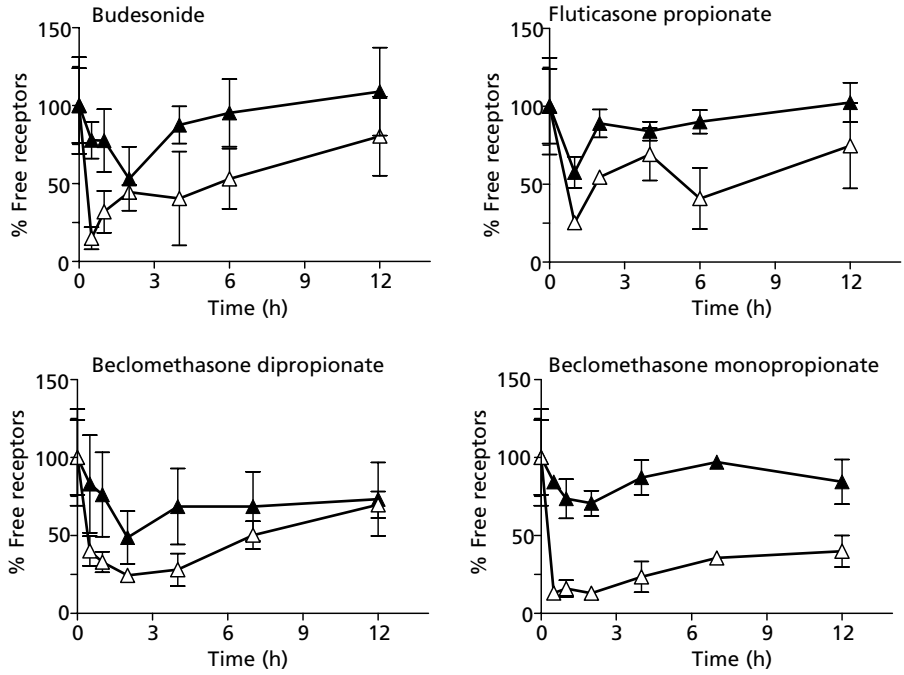


Figure 1 Brain (▲) and kidney (△) receptor occupancy profiles in rats after intravenous administration of inhaled corticosteroids (100 μg kg^{-1}). The mean $\pm$ s.d. is given. For number of animals per time point see Table 2.

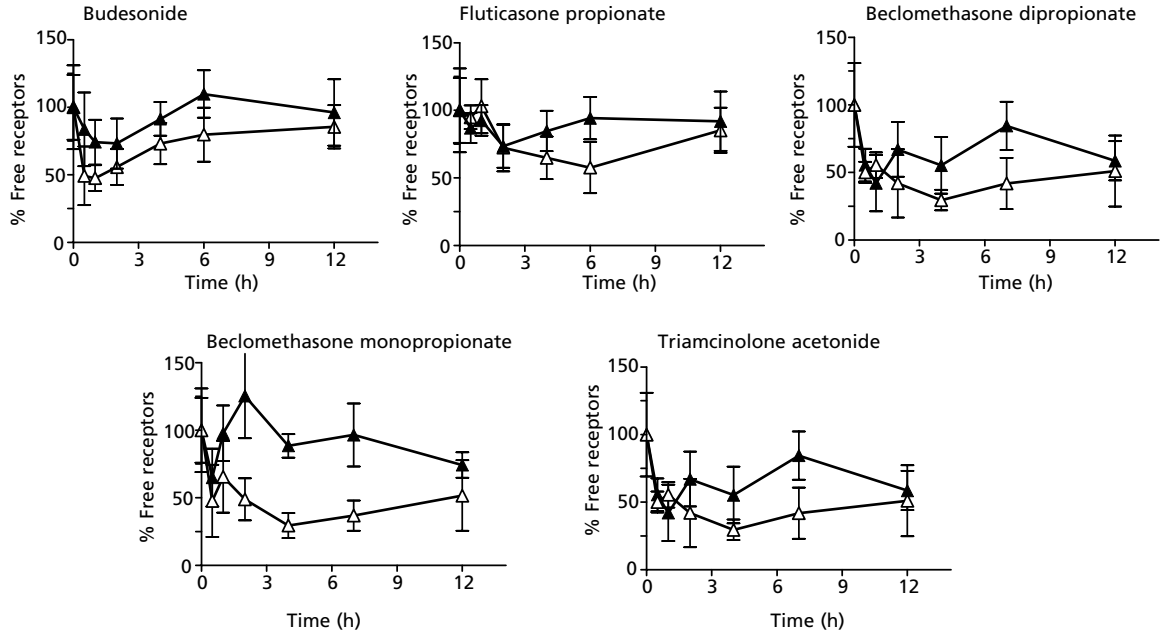


Figure 2 Brain (▲) and kidney (△) receptor occupancy profiles in rats after intratracheal administration of inhaled corticosteroids ($100\,\mu g\,kg^{-1}$). The mean ± s.d. is given. For number of animals per time point see Table 2.

average receptor occupancy for intravenous and intratracheal administration were similar (Table 2). Thus, combined data were used for the statistical analysis. Kruskal–Wallis statistics suggested that ratios of brain/kidney receptor occupancies differed significantly for the investigated glucocorticoids, ranging from 0.20 to 0.56 (Table 2), being most pronounced for BDP and BMP ($P < 0.05$, Dunn's post-hoc test). All other post-hoc comparisons were not significant. A graphical representation of the relationship between logP and occupancy ratios is given in Figure 3 ($r^2 = 0.7$).

Figure 4 and Table 3 summarize the percentage free receptors versus time profiles in the liver and brain, respectively, after intravenous administration ($100\,\mu g\,kg^{-1}$) of TA phosphate to wild-type and knockout mice. Kruskal–Wallis statistics of individual receptor occupancies over the 1–6-h time period (not average receptor occupancies, see above) with post-hoc Dunn's comparison showed that wild-type and knockout mice had similar liver receptor occupancies ($P > 0.5$), indicating that P-gp does not effectively control free levels in the liver, while brain receptor occupancies differed significantly ($P < 0.05$). Contrary to these results, brain receptor occupancies differed between wild-type and knockout mice ($P < 0.01$), indicating that P-gp is responsible for the decreased brain receptor occupancy in wild-type mice. Receptor occupancies in liver and brain differed in wild-type mice, but they were not statistically different in knockout mice. As a result, the Mann–Whitney U-test of brain to liver occupancy ratios differed between wild-type and knockout mice.

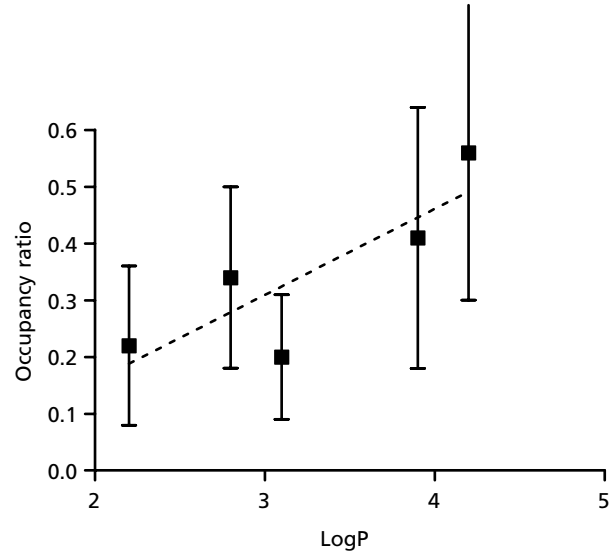


Figure 3 Relationship between logP and the brain to kidney receptor occupancy ratio in rats. The mean ± s.d. is given.

Discussion

This study demonstrated a reduced brain permeability for inhaled corticosteroids using an ex-vivo receptor binding assay. There are a number of in-vitro cell culture methods available to measure transport of drugs by P-gp (Yates et al 2003). We used an animal model (Hochhaus et al 1995) that monitors the number of occupied glucocorticoid receptors in brain and other organs not controlled by the blood–brain barrier. Such a model does not represent an over-simplified system but will include all factors controlling the blood–brain barrier for glucocorticoids. In addition, using the receptor occupancy as an endpoint

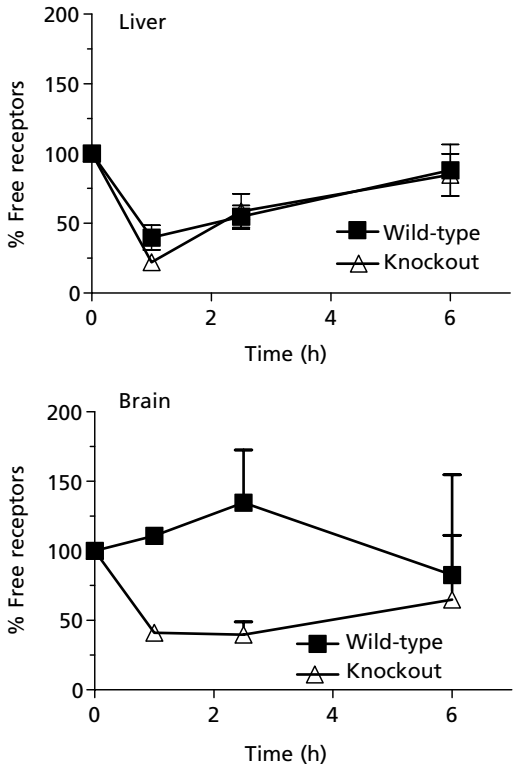


Figure 4 Liver and brain receptor occupancy in wild-type and mdr1a(−/−) knockout mice after intravenous administration of triamcinolone acetonide phosphate ($100\,\mu g\,kg^{-1}$). The standard error is given for allowing estimation of the variability but limitations (i.e. n = 2) need to be considered.

Table 3 Receptor occupancy in the brain and liver of wild-type (n = 3) and mdr1a(−/−) knockout mice (n = 2) after intravenous administration of triamcinolone acetonide phosphate ($100\,\mu g\,kg^{-1}$)

	No. of experiments	Receptor occupancy (%)	
		Brain	Liver
Wild-type mice	3	−11.5 ± 33	34.9 ± 3.0
mdr1a(−/−) mice	2	47.5 (40.2, 55.0)	38.0 (37.4, 38.5)

The mean ± s.d. is given for wild-type mice; the mean and range is given for knockout mice. Statistical evaluation on the basis of mean receptor occupancy values was not possible. A limited analysis based on non-cumulative estimates for individual animals (brain/liver receptor occupancy for a given animal under treatment) obtained after 1, 2.5 and 6 h were pooled and analysed by the Mann Whitney *U*-test. This analysis showed significant differences between wild-type and knockout mice.

allowed us to directly assess if reduced brain permeability impacts pharmacodynamic endpoints. Intravenous as well as intratracheal administration routes were included in the rat experiments, as other parts of the overall study investigated the pulmonary selectivity of inhaled corticosteroids, allowing us in the present study to test if brain permeability is dependent on the form of administration. Experiments were performed at doses that are relatively high for inhaled glucocorticoids. These doses had previously been shown to be suitable for the assessment of pulmonary targeting. Theoretically, high doses could have masked the effects of P-gp on the blood–brain barrier because it could have saturated the transporter. The fact that this study showed significant differences between brain and kidney receptors supports the fact that the doses were not too high.

Although our experiments clearly showed the importance of P-gp for the decreased brain permeability of inhaled corticosteroids, it could be argued that other factors may be responsible for affecting the results in a significant way. For example, receptor occupancy also depends on the affinity of the inhaled corticosteroids for the receptor and differences in pharmacokinetics (clearance, volume of distribution, protein binding). This was the reason why experiments included a reference organ (liver or kidney) and the results were expressed as the ratio of brain to kidney (or liver) receptor occupancy in order to adjust for the above differences (as they would affect brain and systemic organs in the same way). The suitability of using this ratio to correct for these differences was reflected in the fact that brain permeabilities for intravenous and intratracheal administration route data for a given inhaled corticosteroids were similar, despite the fact that the systemic exposure differed somewhat between the two forms of administration, probably because of a lower systemic bioavailability after intratracheal administration (Table 2). This indicated that the parameter used for describing brain permeability is relatively robust, and that differences in 'dose', binding affinity, plasma protein binding and form of administration are likely to be buffered by using this parameter. In addition, receptor occupancies in brain and liver were very similar in mdr1a(−/−) knockout mice. This experiment (Figure 4; Table 3), together with the in-vitro control experiments (similar B_{max} and K_d in both organs) showed that the receptor systems in these organs behave similarly. Further, one might argue that free levels in the control tissue might be affected by P-gp. However, liver receptor occupancy in knockout and wild-type mice were not significantly different, indicating that P-gp does not seem to affect the free levels of TA in the liver.

High intrinsic clearance drugs such as FP, but not TA (see above), are efficiently inactivated in the liver and show hepatic drug concentrations that are significantly less than those in non-metabolic organs (unpublished results). In contrast, administration of prodrugs such as BDP, which is activated in the liver to BMP, might result in free concentrations of the pharmacodynamically active species that are greater in the liver than in other organs (Wang & Hochhaus 2002). This was why the less metabolically active kidney was selected for determining the overall systemic exposure in rats. Preliminary results suggested that for drugs with a smaller intrinsic hepatic clearance, such as TA, kidney versus liver receptor occupancy–time profiles are similar. This justified experiments in wild-type and mdr1a(−/−) mice that were performed

with liver instead of kidney tissue, as it provided sufficient cytosol for the experiments.

Because of the low data density between 6- and 12-h time points, the cumulative measure of receptor occupancy was based on a 6-h time period. Although a broader time range would result in different AUC estimates and the potential of slightly different brain/kidney ratios, the overall outcome of these studies should not be affected.

The results for the knockout and wild-type mice clearly showed that P-gp is involved in the brain permeability of TA in mice (Figure 4; Table 3). This suggests that the reduced brain permeability observed in the rat model for the other inhaled corticosteroids (Figure 1; Table 2) is also likely due to a P-gp related drug efflux out of the brain.

Inhaled corticosteroids are lipophilic moieties and are expected to easily permeate across the blood–brain barrier. However, recent studies have shown that the P-gp transporter is involved in the active efflux of a variety of systemic glucocorticoids, such as dexamethasone, across the blood–brain barrier (Meijer et al 1998). Active transport by a similar intestinal efflux pump was suggested as the mechanism for the glucocorticoid resistance in inflammatory bowel disease (Farrell & Kelleher 2003; Dilger et al 2004), an indication that P-gp can modulate the pharmacological effect.

Structure–affinity studies demonstrated that the affinity for P-gp differs among glucocorticoids. Although the endogenous (cortisol in humans and corticosterone in rats) and exogenous (e.g. dexamethasone) corticosteroids share the same basic four-member ring structure, closely related steroids (e.g. cortisol and corticosterone) show different affinities for P-gp (Karssen et al 2001). TA is transported by P-gp to a much lesser degree than dexamethasone (Medh et al 1998). Yates et al (2003) suggested that bulky substituents around C-16 diminish the affinity for the transporter.

Our data also provided some information on structure–activity relationships. The five inhaled corticosteroids tested in the rat model all had bulky substituents in the C-16/17 region of the molecule, with logP values ranging from 2.2 to 4.1 (Table 1). Figure 1 and Table 2 indicate that the brain to kidney receptor occupancy ratios ranged from 0.2 to 0.6, although the only significant difference was between BDP and BMP because of the variability of the data. However, the data indicate that for all the investigated inhaled corticosteroids, the efflux transport rate is still sufficient to maintain significantly lower free concentrations in the brain. As mentioned above, low activity of P-gp towards TA has been described (Medh et al 1998). Our experiments in rats and mice suggest, however, that the blood–brain barrier works efficiently for TA. Thus, although the rate with which P-gp is transporting TA might be less than that of more hydrophilic drugs lacking bulky substituents in this region (hydrocortisone, methylprednisolone, prednisolone, cortisol and dexamethasone) (deKloet 1997; Meijer et al 1998), the overall P-gp transport rate of TA is sufficient to counteract the passive diffusion into the brain, reduce the free brain concentration and, consequently, the brain receptor occupancy. Similarly, all other inhaled corticosteroids modified in this region of the molecule showed lower brain receptor occupancy, an indication that P-gp is also relevant for these steroids. It is the strength of the applied model that it is able to test for the pharmacological relevance of such transport phenomena.

BDP is a prodrug of BMP and the 21-ester needs to be cleaved by esterases. The high activity of esterases in most of the organs and blood achieves this in a very short period of time, the half-life of BDP being in the minute range. Despite this short half-life, more brain receptors were occupied after the intravenous and intratracheal administration of BDP than after BMP administration. BDP therefore seems to escape more efficiently the P-gp efflux. Whether the esterification of the 21-OH group in the molecule reduces the affinity of BDP towards P-gp or significantly facilitates passive diffusion across membranes needs further investigation. However, the results indicate that the use of lipophilic prodrugs, even if their half-life is short, might be used to modulate brain permeability.

Brain permeability increased roughly with an increase in lipophilicity (Figure 3). The most likely explanation is that increased lipophilicity facilitates passive diffusion, resulting in the pump capacity not being sufficient to maintain low brain levels. Alternatively, a reduced affinity of the more lipophilic glucocorticoids for P-gp might explain the results. Further structure–activity relationship studies in cell culture probing just for the P-gp affinity should answer this question.

The incidence of drug–drug interactions due to the competition of two drugs with the P-gp transporter have been widely reported (Greiner et al 1999; Verschraagen et al 1999; Wandel et al 2000), and changing tissue distributions based on such interactions have been described (Christians et al 2002). The results of our study suggest that the brain permeability of inhaled corticosteroids is also modulated by P-gp transporters. Consequently, concomitant administration of inhaled corticosteroids and other drugs interacting with these transporters might potentially lead to drug–drug interactions, resulting in a higher brain uptake of glucocorticoids. An increased cortisol suppression on the hippocampal levels and/or induction of other 'brain-located' side-effects needs to be evaluated.

In conclusion, the results of our study strongly suggest the brain permeability of inhaled corticosteroids is reduced and that P-gp plays a critical role in modulating the permeability of inhaled corticosteroids.

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