Absence of Both Cytochrome P450 3A and P-glycoprotein Dramatically Increases Docetaxel Oral Bioavailability and Risk of Intestinal Toxicity

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Abstract

Docetaxel is one of the most widely used anticancer drugs. A major problem with docetaxel treatment, however, is the considerable interpatient variability in docetaxel exposure. Another disadvantage of the drug is that it has a very low oral bioavailability and can therefore only be administered i.v. The drug-metabolizing enzyme cytochrome P450 3A (CYP3A) and the drug transporter P-glycoprotein (P-gp; MDR1) are considered to be major determinants of docetaxel pharmacokinetics. It has been hypothesized that CYP3A and P-gp work synergistically in limiting the systemic exposure to many orally ingested drugs. However, it has been difficult to examine this interplay in vivo. We therefore generated mice lacking all CYP3A and P-gp genes. Although missing two primary detoxification systems, Cyp3a/Mdr1a/1b−/− mice are viable, fertile, and without spontaneous abnormalities. When orally challenged with docetaxel, a disproportionate (>70-fold) increase in systemic exposure was observed compared with the increases in single Cyp3a−/− (12-fold) or Mdr1a/1b−/− (3-fold) mice. Unexpectedly, although CYP3A and P-gp collaborated extremely efficiently in lowering docetaxel exposure, their individual efficacy was not dependent on activity of the other protein. On reflection, this absence of functional synergism makes biological sense, as synergism would conflict with a robust detoxification defense. Importantly, the disproportionate increase in docetaxel exposure in Cyp3a/Mdr1a/1b−/− mice resulted in dramatically altered and lethal toxicity, with severe intestinal lesions as a major cause of death. Simultaneous inhibition of CYP3A/P-gp might thus be a highly effective strategy to improve oral drug bioavailability but with serious risks when applied to drugs with narrow therapeutic windows. [Cancer Res 2009;69(23):8996–9002]

Introduction

Docetaxel is a widely used anticancer drug and applied against a variety of cancer types including breast, lung, and prostate. A major problem with docetaxel treatment is the considerable interpa-

tient variability in docetaxel exposure (1). Several studies have indicated that the drug-metabolizing enzyme system cytochrome P450 3A (CYP3A) is a major determinant of this variability (1). CYP3A enzymes metabolize ~50% of the currently marketed drugs, including many anticancer drugs. It is known that there are considerable interindividual but also intra-individual differences in expression and activity levels of CYP3A enzymes. Docetaxel is metabolized by CYP3A to several metabolites that are all considered to be therapeutically less effective (2, 3). Like the majority of anticancer drugs, docetaxel has a narrow therapeutic window resulting in a significant risk of underdosing or overdosing of patients. It is therefore of importance to have a good understanding of the drug-handling systems that determine the docetaxel pharmacokinetics such as CYP3A.

In addition to metabolism by CYP3A, docetaxel is also subject to active efflux by the drug transporter MDR1 [P-glycoprotein (P-gp); ABCB1; ref. 4]. It is well established that MDR1 in the intestine can markedly restrict the intestinal uptake of various substrates, whereas, in the liver, MDR1 facilitates the excretion of substrates into the bile. Docetaxel has a very low oral bioavailability and is therefore only administered i.v. in the clinic. This low oral bioavailability might, for a large part, be attributable to the fact that the drug is such a good substrate for CYP3A and MDR1. As there are many advantages of the oral administration route, enhancing docetaxel oral bioavailability by inhibiting CYP3A or MDR1 has received considerable interest in recent years (5–7). For example, in a recent clinical proof-of-concept study, it was shown that simultaneous oral coadministration of docetaxel with the CYP3A inhibitor ritonavir resulted in a docetaxel exposure that was in the same range as that achieved after i.v. administration (without ritonavir; ref. 7).

Both CYP3A and P-gp can have a strong effect in reducing the oral bioavailability and in mediating the elimination of numerous drugs, and it is of notice that there is a very extensive overlap between their substrates. In addition to the liver, the intestine is increasingly recognized as an important drug-metabolizing organ (8). Although the total amount of intestinal CYP3A is relatively low compared with that in the liver, its strategic colocalization with P-gp in the villous tip of the enterocyte may provide a highly effective barrier against drug absorption. In view of the extensive overlap in their substrates, it has been hypothesized that, for many drugs, it is the combination of back-transport by P-gp in the intestinal epithelial cell and the presence of CYP3A-mediated metabolism within the same cell that makes for efficient first-pass metabolism of orally administered drugs (9–14). The idea is that, by lowering the intracellular drug concentration, P-gp might help to prevent saturation of enterocyte CYP3A. P-gp activity would

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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thus improve the efficacy of CYP3A, and this synergistic interaction would lead to highly efficient and extensive intestinal metabolism. For clarity, in this study, we define synergistic collaboration as meaning that one protein (e.g., CYP3A) functions more efficiently when another protein (e.g., P-gp) is also active than when the other protein is not active.

Although the individual contributions of CYP3A and P-gp to pharmacokinetics can be assessed reasonably well in model systems, it has been difficult to examine the effect of both systems functioning together. Although informative in vitro studies have been done, which support a collaborative function, (15, 16), unambiguous in vivo evidence is lacking. In case of a true synergistic collaboration between CYP3A and P-gp, the absence of either CYP3A or P-gp alone should already result in a profound increase in systemic drug exposure. An additional further increase when both systems are absent would, however, be expected to be relatively modest. Because so many drugs are affected, basic insight into how CYP3A and P-gp work together can be of great value in developing clinical strategies to improve the oral bioavailability of drugs, for example, by individual or simultaneous inhibition of CYP3A and/or P-gp. In this study, we have generated mice that lack all murine orthologs of both CYP3A and P-gp. This novel mouse model enabled us to investigate the functional interplay between these two important drug-handling systems in vivo.

Materials and Methods

Animals. Mice were housed and handled according to institutional guidelines complying with Dutch legislation. Mdr1a/1b−/− (17) and Cyp3a−/− (18) were crossed to obtain Cyp3a/Mdr1a/1b−/− double knockout mice. Genotypes of mice were evaluated by PCR. All mice used in this study were male, had >99% FVB genetic background, and were 8 to 14 weeks old. Animals were kept in a temperature-controlled environment with a 12 h light/12 h dark cycle and received a standard diet (AM-III; Hope Farms) and acidified water ad libitum.

Docetaxel plasma pharmacokinetics. Docetaxel (10 mg/mL) formulated in polysorbate 80/ethanol/water [20:13:67 (v/v/v); Taxotere; Aventis] was diluted with saline (0.9% NaCl) and administered by oral gavage or by injection into the tail vein. To minimize variation in absorption, mice were fasted for 2 h before docetaxel was administered by gavage into the stomach using a blunt-end needle. Multiple blood samples (~40 μL) were collected from the tail vein at 15 and 30 min and 1, 2, 4, and 8 h after administration. The original data and detailed protocols for RNA isolation, amplification, labeling, hybridization, and gene ID list are available at http://microarrays.nki.nl and are deposited at ArrayExpress, EBI.

Drug analysis. Docetaxel concentrations in plasma samples were determined using a previously described sensitive and specific liquid chromatography-tandem mass spectrometry assay (19). Feces and urine samples were processed using liquid-liquid and solid-phase extraction followed by reverse-phase high-performance liquid chromatography with UV detection (4, 18).

Clinicochemical analysis of serum. Standard clinical chemistry analyses on serum of wild-type and Cyp3a/Mdr1a/1b−/− mice were done on a Roche Hitachi 917 analyzer (Roche Diagnostics) to determine levels of bilirubin, alkaline phosphatase, aspartate aminotransaminase, γ-glutamyltransferase, lactate dehydrogenase, creatinine, urea, Na+, K+, Ca2+, phosphate, total protein, albumin, and cholesterol.

Hematologic analysis. Hematologic analysis was done as described (18).

Histologic analysis. Whole-body necropsy was done and tissues and organs were fixed in acidified formalin [ethanol/acidic acid/formaldehyde/saline at 40:5:10:45 (v/v/v)] and embedded in paraffin. Sections were cut at 2 μm from the paraffin blocks and stained with H&E according to standard procedures. Images were captured with a Zeiss AxiosCam HR digital camera and processed with Axiovision 4 software (both from Carl Zeiss Vision).

Oligoarray analysis of Cyp3a/Mdr1a/1b−/− mice. 32K murine oligo microarrays were hybridized with Cy-dye-labeled pooled liver and intestinal amplified RNA (n = 4) of adult wild-type and Cyp3a/Mdr1a/1b−/− males using the TECAN HS4800 hybridization station. The original data and detailed protocols for RNA isolation, amplification, labeling, hybridization, and gene ID list are available at http://microarrays.nki.nl and are deposited at ArrayExpress, EBI.

Pharmacokinetic calculations and statistical analysis. Pharmacokinetic calculations and statistical analysis were done as described (20).

Results

Generation and characterization of Cyp3a/Mdr1a/1b knock-out mice. To study the interplay between CYP3A and P-gp, we generated a novel mouse model by cross-breeding Cyp3a knockout (18) with Mdr1a/1b knockout mice (17). In spite of lacking two important detoxification systems, homozygous Cyp3a/Mdr1a/1b−/− mice were viable and fertile and had normal life-spans and body weights. In addition, no macroscopic or microscopic anatomic abnormalities were evident in these mice. Most serum clinicochemical and all hematologic parameters showed no significant differences between wild-type and Cyp3a/Mdr1a/1b−/− mice. Adult males did show moderate increases in serum levels of creatinine (21.8 ± 7.5 μmol/L in knockout versus 14.7 ± 3.2 μmol/L in wild-type; P = 0.016), urea (17.8 ± 2.8 versus 10.7 ± 2.0 mmol/L; P < 0.001), and triglycerides (2.45 ± 0.82 versus 1.19 ± 0.28 mmol/L; P < 0.001). None of these differences were observed in “single” Mdr1a/1b−/− and Cyp3a−/− mice (data not shown). These modest changes seem unlikely to affect the usefulness of this mouse strain for pharmacokinetic studies.

To evaluate possible alterations in gene expression, a 23K mouse microarray analysis was done to compare RNA levels in liver and small intestine of wild-type and Cyp3a/Mdr1a/1b−/− adult males. Several genes were significantly (>2-fold; P < 0.01) upregulated (n = 99) or downregulated (n = 86) in the livers of Cyp3a/Mdr1a/1b−/− mice (data deposited at ArrayExpress, EBI). Consistent with observations in Cyp3a−/− mice (18, 21, 22), several genes involved in drug metabolism and disposition were upregulated in the livers of Cyp3a/Mdr1a/1b−/− mice, including members of the Cyp2b and Cyp2c family, Oatp1a4 and Mrp3 (Supplementary Table S1). In addition, a couple of Cyp4a genes were upregulated, whereas Cyp7b1 was downregulated. Some glutathione S-transferase (Gst) genes were upregulated in the livers of Cyp3a/Mdr1a/1b−/− mice (Supplementary Table S1). Also in the small intestine, several genes were significantly downregulated (n = 27) or upregulated (n = 48), including several Cyp2c and Gst genes (Supplementary Table S1).

Overall, however, alterations in gene expression appeared to be modest and the deletion of all Cyp3a and Mdr1 genes does not

http://www.ebi.ac.uk/arrayexpress, accession number E-NCMF-26.
appear to cause marked physiologic abnormalities. Although we realize that upregulations of other detoxifying systems can for some drugs affect the results obtained (21), we have no indications that these upregulations are detrimental for the evaluation of docetaxel pharmacokinetics (see below) and thus considered this mouse model suitable to obtain more insight into the interplay between CYP3A and P-gp.

**Disproportionate increase in oral docetaxel exposure in Cyp3a/Mdr1a/1b−/− mice.** To assess the effect of separate and combined deficiency in CYP3A and P-gp on oral bioavailability and pharmacokinetics, we administered docetaxel orally or i.v. to wild-type, Mdr1a/1b−/−, Cyp3a−/−, and Cyp3a/Mdr1a/1b−/− mice and subsequently determined docetaxel plasma levels at several time points (Fig. 1). Consistent with previous studies (4, 18), the area under the curve (AUC 0−∞) after oral administration was significantly higher in Mdr1a/1b−/− (2.8-fold) and Cyp3a−/− (11.5-fold) mice than in wild-type mice (Table 1). These results indicate that CYP3A by itself is an even more important determinant of docetaxel pharmacokinetics than Mdr1a/1b. Interestingly, however, in the Cyp3a/Mdr1a/1b−/− combination knockout strain, we found >70-fold increase in AUC after oral docetaxel administration. This clearly illustrates how the combined absence of CYP3A and Mdr1a/1b can

![Figure 1. Plasma concentration-time curves of docetaxel in male FVB wild-type, Mdr1a/1b−/−, Cyp3a−/−, and Cyp3a/Mdr1a/1b−/− mice after oral (A) and i.v. (B) administration of docetaxel (10 mg/kg). Points, mean concentration (n = 5–8 per time point); bars, SD. Insets, semilog plots of the data.](image-url)

**Table 1. Plasma pharmacokinetic parameters after oral and i.v. administration of docetaxel (10 mg/kg) to wild-type, Mdr1a/1b−/−, Cyp3a−/−, and Cyp3a/Mdr1a/1b−/− mice**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild-type</th>
<th>Mdr1a/1b−/−</th>
<th>Cyp3a−/−</th>
<th>Cyp3a/Mdr1a/1b−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral AUC0−∞ (ng h/mL)</td>
<td>228 ± 130</td>
<td>645 ± 272*</td>
<td>2,627 ± 1,011†</td>
<td>16,466 ± 2,020†</td>
</tr>
<tr>
<td>Fold vs wild-type</td>
<td>1</td>
<td>2.8</td>
<td>11.5</td>
<td>72.2</td>
</tr>
<tr>
<td>% of Cyp3a/Mdr1a/1b−/−</td>
<td>1.4</td>
<td>3.9</td>
<td>16.0</td>
<td>100</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>150 ± 108</td>
<td>677 ± 296*</td>
<td>975 ± 482*</td>
<td>2,062 ± 346*</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>0.58 ± 0.34</td>
<td>0.5 ± 0.0</td>
<td>1.0 ± 0.0</td>
<td>1.2 ± 0.5*</td>
</tr>
<tr>
<td>% of Cyp3a/Mdr1a/1b−/−</td>
<td>2.4 ± 0.6</td>
<td>2.8 ± 0.4</td>
<td>1.6 ± 0.2*</td>
<td>4.8 ± 1.0†</td>
</tr>
<tr>
<td>i.v. AUC0−∞ (ng h/mL)</td>
<td>2,166 ± 227</td>
<td>4,342 ± 820†</td>
<td>10,323 ± 1,683*</td>
<td>37,106 ± 5,777†</td>
</tr>
<tr>
<td>Fold vs wild-type</td>
<td>1</td>
<td>2.0</td>
<td>4.9</td>
<td>17.1</td>
</tr>
<tr>
<td>% of Cyp3a/Mdr1a/1b−/−</td>
<td>5.8</td>
<td>11.7</td>
<td>28.4</td>
<td>100</td>
</tr>
<tr>
<td>CL (L/h kg)</td>
<td>4.66 ± 0.48</td>
<td>2.37 ± 0.44*</td>
<td>0.97 ± 0.14*</td>
<td>0.27 ± 0.04*</td>
</tr>
<tr>
<td>% of Cyp3a/Mdr1a/1b−/−</td>
<td>2.2 ± 0.1</td>
<td>2.3 ± 0.3</td>
<td>1.6 ± 0.0</td>
<td>6.1 ± 1.1†</td>
</tr>
<tr>
<td>% of Cyp3a/Mdr1a/1b−/−</td>
<td>10.5</td>
<td>14.9</td>
<td>25.0</td>
<td>44.4</td>
</tr>
</tbody>
</table>

NOTE: Area under the plasma concentration versus time curve (AUC), maximal concentration obtained after oral administration (Cmax), and the corresponding time of maximum concentration (tmax), elimination half-life (t1/2, el; calculated from 2 to 8 h for both oral and i.v. administration), clearance (CL), and oral bioavailability (F) are indicated. Mean ± SD (n = 5–8).

*P < 0.01, compared with wild-type mice.
†P < 0.001, compared with wild-type mice.
‡P < 0.05, compared with wild-type mice.
have a disproportionately large effect on reducing the systemic exposure of oral docetaxel.

When we administered docetaxel i.v., we also found marked increases in AUC in Mdr1a/1b−/− (2-fold) and Cyp3a−/− (4.9-fold) mice, but these changes were lower than seen after oral administration of the same dose (Table 1). Moreover, in the Cyp3a/Mdr1a/1b−/− strain, the effect of combined disruption was less striking than after oral administration, with a 17-fold increase in AUC compared with the wild-type strain (Fig. 1; Table 1). Accordingly, whereas in wild-type mice we found an oral docetaxel bioavailability (AUCoral/AUCi.v. * 100%) of 10%, this was increased up to ~45% in the Cyp3a/Mdr1a/1b−/− strain. Importantly, the absolute systemic exposure in Cyp3a/Mdr1a/1b−/− mice after oral docetaxel administration was 7.6-fold higher than that in wild-type mice after i.v. administration (Table 1). This further illustrates the pronounced effect of the simultaneous loss of CYP3A and P-gp activity on oral docetaxel. Analysis of pharmacokinetic parameters (Fig. 1; Table 1) revealed that the terminal elimination half-life (t1/2) was also substantially increased in Cyp3a/Mdr1a/1b−/− mice after both oral and i.v. administration. Note, though, that plasma levels in Cyp3a/Mdr1a/1b−/− mice were still quite high at t = 8 h, which might affect the precise estimates of the t1/2 and AUC0–∞. As the i.v. t1/2 was even slightly shorter in single Cyp3a−/− than in wild-type mice, the markedly delayed elimination (increased t1/2) in the Cyp3a/Mdr1a/1b−/−

Figure 2. Amounts of docetaxel and metabolites (M1-M4) excreted in feces by wild-type, Mdr1a/1b−/−, Cyp3a−/−, and Cyp3a/Mdr1a/1b−/− mice within 24 h after oral (A) or i.v. (B) administration of docetaxel (10 mg/kg). White columns, levels of 3H label after administration of [3H]docetaxel. Excretion is given as percentage of the dose (mean ± SD; n = 4-5). **, P < 0.01; ***, P < 0.001, compared with wild-type mice.

Figure 3. Toxic effects (weight loss) in male wild-type, Mdr1a/1b−/−, Cyp3a−/−, and Cyp3a/Mdr1a/1b−/− mice on oral docetaxel dosing. A, three oral docetaxel administrations of 10 mg/kg/d. All Cyp3a/Mdr1a/1b−/− died (†) by day 4. Also, Cyp3a−/− mice had to be euthanized (x) on day 6 as 5 of 6 animals had lost >20% of their body weights. B, single oral administration of 10 mg/kg. Cyp3a/Mdr1a/1b−/− mice had to be euthanized (x) on day 4 as all animals had lost >20% of their body weights. n = 4 to 8 mice were used for each group.
strain must primarily be caused by the absence of P-gp. Upregulation of Mdr1a/1b (and perhaps of other uptake and/or efflux transporters, or metabolizing enzymes for which docetaxel is a substrate) might explain the faster elimination in the Cyp3a−/− mice compared with wild-type. Indeed, we have shown previously that Mdr1a is up-regulated in the liver of Cyp3a−/− mice (22). Note that, because of the wide range of plasma concentrations between the strains, the i.v. clearance parameters (Table 1) may not behave linearly. For example, the clearance mechanism(s) responsible for the residual docetaxel elimination in Cyp3a/Mdr1a/1b−/− mice (0.27 L/h/kg) could be saturated and mediate a larger fraction of the clearance at lower plasma exposure levels.

To investigate how the individual and combined activity of CYP3A and P-gp determines the excretion pattern of docetaxel, we collected feces and urine for 24 h after oral and i.v. administration of [3H]docetaxel (10 mg/kg) to the mouse strains. In wild-type mice, substantial amounts of docetaxel and its CYP3A-generated metabolites M1-M4 (4) were primarily excreted via the feces with only minor quantities in urine (data not shown) after both oral and i.v. administration (Fig. 2), in accordance with previous studies (4, 18). Whereas in Mdr1a/1b−/− mice virtually no unchanged docetaxel but only the docetaxel metabolites M1-M4 were found in the feces, in Cyp3a−/− mice it was primarily parent docetaxel and not the metabolites M1-M4 that could be recovered. In contrast, only low quantities of both parent docetaxel as well as the metabolites M1-M4 were excreted in the feces of Cyp3a/Mdr1a/1b−/− mice (Fig. 2). Accordingly, a reduced amount of the total radioactivity (∼50%) was recovered over 24 h in feces of Cyp3a/Mdr1a/1b−/− mice after oral or i.v. administration (Fig. 2). The radioactivity levels found in urine were <3% for all strains (data not shown). Overall, these data show that whereas docetaxel and/or its primary metabolites are still the main vehicle of excretion and elimination in the absence of either CYP3A or P-gp, this excretion is severely impaired when both detoxifying systems are absent.

Lethal and qualitatively altered toxicity of oral docetaxel in Cyp3a/Mdr1a/1b−/− mice. Combined CYP3A and Mdr1a/1b deficiency resulted in a strikingly increased sensitivity to docetaxel toxicity, as we noted in a pilot chemotherapy experiment, where we gave the panel of mouse strains an oral docetaxel administration of 10 mg/kg on 3 consecutive days. Although the initial body weight loss in Cyp3a/Mdr1a/1b−/− mice was comparable with that of Cyp3a−/− mice, the condition of Cyp3a/Mdr1a/1b−/− mice deteriorated quickly and they all died by day 4 (Fig. 3A). Cyp3a−/− mice, while looking comparatively better on day 3, continued to lose weight and had to be euthanized on day 6, as most animals had lost >20% of their body weights. In contrast, wild-type and Mdr1a/1b−/− mice hardly lost any weight, or even gained some, during the study (Fig. 3A).

Pathologic examination done 72 h after the first docetaxel administration revealed severe intestinal toxicity in Cyp3a/Mdr1a/1b−/− mice that was not apparent at all in wild-type mice (Fig. 4A and B). Degeneration and necrosis of the intestinal mucosa took place throughout the entire intestinal tract from duodenum to distal colon and was accompanied by edematous changes in the submucosa (Fig. 4B). The lesions in colon and ileum were even more severe than those in jejunum and duodenum. However, there was no indication for perforation of the intestinal wall. Moderate toxicity was apparent in the small intestine of Cyp3a−/− mice, whereas this was only mild in Mdr1a/1b−/− mice. In striking contrast to the Cyp3a/Mdr1a/1b−/− mice, however, no pathologic alterations were seen in the colon of these single knockout strains.

In addition to the intestinal toxicity, massive depletion of hematopoietic cells of all three cell lineages was observed in bone marrow of sternum (Fig. 4C and D) and hind limbs as well as in the red pulp of the spleen of Cyp3a/Mdr1a/1b−/− but not wild-type mice (Supplementary Fig. S1). Also in Cyp3a−/− mice, some diminishment of the hematopoietic cells was observed, but this was not encountered in Mdr1a/1b−/− mice (data not shown). Furthermore,
highly increased numbers of degenerative and necrotic spermatogenic cells were present in the testis of Cyp3a/Mdr1a/1b−/− mice, which was accompanied by the formation of many multinucleated giant cells and a reduced number of germinall epithelial cells in seminiferous tubules (Supplementary Fig. S1). Wild-type testis appeared completely normal (Supplementary Fig. S1) as was the case for the testis of Cyp3a−/− and Mdr1a/1b−/− mice (data not shown). None of the other major organs of wild-type or Cyp3a/Mdr1a/1b−/− mice revealed indications for localized pathologic changes. Cyp3a/Mdr1a/1b−/− mice thus displayed a major qualitative shift in toxicity, with severe damage to colon and testis that was not observed in either wild-type or the single Cyp3a and Mdr1a/1b knockout strains.

Given the dramatic outcome with the triple docetaxel administration, we repeated the docetaxel exposure experiment under milder conditions and gave only a single oral docetaxel dose of 10 mg/kg and monitored weight loss. Again, Cyp3a/Mdr1a/1b−/− mice rapidly lost weight and had to be euthanized on day 4 as their weight loss exceeded 20% (Fig. 3B). Pathologic examination of Cyp3a/Mdr1a/1b−/− mice (72 h after docetaxel administration) revealed a very similar and equally severe toxicity profile as described above for the triple administration. Interestingly, in this experimental setup, not only the wild-type and Mdr1a/1b−/− mice but also the Cyp3a−/− mice stayed around their initial body weight (Fig. 3B) and did not display any particular pathologic alterations (data not shown). Also, when we gave a single i.v. docetaxel administration (5 mg/kg) to Cyp3a/Mdr1a/1b−/− mice, we encountered a similar toxicity profile as described above for the oral administration, including the severe intestinal toxicity, whereas no pathologic alterations were found in Mdr1a/1b−/− and Cyp3a−/− mice (data not shown). Collectively, these experiments show that the combined absence of CYP3A and Mdr1a/1b severely increases the susceptibility to docetaxel toxicity and leads to a qualitative shift in toxicity in colon and testis. The severe intestinal toxicity, which would greatly impair effective food and water uptake, is the most likely reason for early weight loss and death of the Cyp3a/Mdr1a/1b−/− mice.

Discussion

To study the functional interplay between CYP3A and P-gp, we generated Cyp3a/Mdr1a/1b−/− mice. In addition to the two Mdr1 genes, these mice are lacking all (eight) Cyp3a genes, representing a human situation in which all CYP3A activity (including that of CYP3A4 and CYP3A5) is absent. Although these mice are missing two important broad-specificity detoxification systems, Cyp3a/Mdr1a/1b−/− mice are viable and fertile and do not show marked spontaneous abnormalities. However, challenging Cyp3a/Mdr1a/1b−/− mice with the anticancer drug docetaxel resulted in >70-fold increase in systemic exposure after oral administration compared with wild-type mice. This effect of combined ablation was disproportionate when compared with the single Mdr1a/1b−/− and Cyp3a−/− mice, illustrating the significance of both systems working together in lowering oral bioavailability. It is worth noting that the fold increase in systemic exposure seen in Cyp3a/Mdr1a/1b−/− versus wild-type mice was much more pronounced after oral than after i.v. administration (72-fold versus 17-fold), in line with the importance of intestinal metabolism in limiting docetaxel oral bioavailability (18). Importantly, the disproportionate increase in docetaxel exposure resulted in qualitatively altered and lethal toxicity of a modest single oral dose of docetaxel in Cyp3a/Mdr1a/1b−/− mice.

Although potentially clinically relevant, it has been difficult to assess the combined contribution of CYP3A and P-gp to reducing bioavailability. Whereas several in vitro studies have addressed the question whether CYP3A and P-gp can work synergistically to lower the bioavailability of drugs, strong in vivo evidence is lacking. The present study shows that the combined absence of both CYP3A and P-gp has a disproportionate effect on the systemic exposure of docetaxel, which could have important clinical implications. From a fundamental point of view, however, it is important to realize that this disproportionate increase shows effective collaboration but not necessarily true functional synergism between CYP3A and P-gp. A basic implication of synergism is that CYP3A and/or P-gp would function more efficiently in the presence of the other system than in its absence. Although seemingly counterintuitive, a fair way of assessing this is to compare the effect on docetaxel AUC of P-gp or CYP3A alone or in combination relative to a fully deficient situation. When compared with the fully deficient Cyp3a/Mdr1a/1b−/− situation, either the activity of CYP3A alone (in Mdr1a/1b−/− mice) or P-gp alone (in Cyp3a−/− mice) can already reduce the oral docetaxel systemic exposure by 96.1% (to 3.9%) or 84% (to 16%), respectively (Table 1). Based on these percentages, the theoretically predicted combined effect of CYP3A and P-gp together on the systemic exposure is a reduction to 0.62% (i.e., 3.9% of 16%). This is close to the 1.4% of the AUC of the Cyp3a/Mdr1a/1b−/− we experimentally observed for the wild-type. Clearly, in case of true functional synergism, this experimental percentage for the wild-type mice should have been (much) lower than 0.62%. Our data therefore show that each of the two systems functions with equal efficiency independent of the presence or absence of the other system.

We note that, although conceptually appealing, from a biological point of view, true functional synergism between CYP3A and P-gp would create a highly vulnerable situation, as disruption or inhibition of just one of the participating systems would already lead to a functional collapse of the detoxification mechanism. This would conflict with the biological need for robust protection from xenobiotic toxins. Indeed, the results of this study show that, in the case of docetaxel, CYP3A and P-gp have independent but overlapping and efficiently cooperating functions that allow each to still "perfect" the function of the other.

Docetaxel is one of the most widely used anticancer drugs and applied against numerous cancers. Due to the fact that docetaxel is a good CYP3A and P-gp substrate, it has a very low oral bioavailability and is therefore administered i.v. in the clinic. As there are many advantages of the oral route, enhancing docetaxel oral bioavailability has received considerable interest in recent years (5–7). For example, in a recent clinical proof-of-concept study, it was shown that simultaneous oral coadministration of docetaxel with the CYP3A inhibitor ritonavir resulted in a docetaxel exposure that was in the same range (130%) as achieved after i.v. administration (100%; without ritonavir; ref. 7). In the present study, we show that when CYP3A and P-gp are both absent, the oral systemic exposure was increased to 760% when compared with CYP3A/P-gp-proficient wild-type animals that had received the drug i.v. (100%). Hence, although inhibition of either CYP3A or P-gp alone could improve oral bioavailability to some extent, the present study indicates that simultaneous inhibition of CYP3A and P-gp could be a promising strategy to really boost the oral availability of docetaxel.

The disproportionate increase in systemic exposure when both CYP3A and P-gp are absent or inactive can have far-reaching clinical
consequences. For example, in people with low or inhibited CYP3A and P-gp activity, drug plasma levels could easily increase to toxic levels, especially for orally administered drugs as illustrated in the docetaxel toxicity experiment. Although we did not observe any signs of toxicity in wild-type mice, we encountered severe toxicities in Cyp3a/Mdr1a/1b−/− mice, especially in those tissues with rapidly dividing cells (intestinal, hematopoietic, and spermatogenic), consistent with the antimitotic action of docetaxel. Apart from the increased toxicity in small intestine and bone marrow, the qualitative emergence of additional toxicities in colon and testis was especially striking. All other major organs of Cyp3a/Mdr1a/1b−/− mice, including liver, kidney, brain, and lungs, were not noticeably affected by the docetaxel treatment. Among the toxicities observed, the severe intestinal toxicity is likely the most important cause of the early death of the Cyp3a/Mdr1a/1b−/− mice. Importantly, this suggests a qualitative shift in the type of toxicity, as hematotoxicity is typically dose-limiting for docetaxel in mice, whereas intestinal toxicity has not been observed previously in safety studies with i.v. docetaxel in this species (23, 24). In contrast to mice, humans already show some intestinal toxicity (diarrhea) even with i.v. docetaxel (1). A possible further shift toward intestinal toxicity in humans should therefore be monitored very carefully and could well limit the applicability of combined CYP3A and P-gp inhibition to improve docetaxel oral bioavailability.

In conclusion, the results from this study show that the absence of both CYP3A and P-gp results in a dramatic, disproportionate increase in systemic docetaxel exposure with a concomitantly increased and altered risk of toxicity. Especially with drugs that have a narrow therapeutic window, there are serious risks when such drugs are deliberately or unintentionally coadministered with other drugs or food constituents that interfere with CYP3A and P-gp activity. The risk of such drug-drug and drug-food interactions is especially relevant given the high number and large overlap in substrates and inhibitors for CYP3A and P-gp. Variable activity of CYP3A alone can lead to lethal overdosing or subtherapeutic underdosing of orally taken drugs (25). Although extrapolation from mouse data to humans should always be done with caution, it is clear from this study that interfering with both CYP3A and P-gp activity could drastically exacerbate such consequences and should therefore be carefully considered during further drug development and application.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Absence of Both Cytochrome P450 3A and P-glycoprotein Dramatically Increases Docetaxel Oral Bioavailability and Risk of Intestinal Toxicity
