Chapter 5

Lack of strain-related differences in drug metabolism and efflux transporter characteristics between CD-1 and athymic nude mice

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Abstract

CD-1 mice are commonly used in oncology metabolism and toxicity to support drug discovery and development to examine drug metabolism and toxicity properties of new chemical entities. On the other hand, athymic nude mice are the preferred animals to investigate tumour growth inhibition. Therefore, a frequently asked question is: are the metabolic and pharmacokinetic characteristics of xenobiotics in these two mice strains comparable or not? To address this issue, we characterized drug metabolism and efflux transporter properties in both strains and in different organs. For this, the metabolic stability of a set of twenty compounds and metabolite formation of cytochrome P450 (CYP) marker substrates (testosterone, ethoxyresorufin and pentoxyresorufin) were measured in liver microsomes. Drug conjugation was studied by following the disappearance of 7-hydroxycoumarin and the formation of its glucuronide and sulphate conjugates in freshly prepared liver slices. In addition, mRNA expression levels of the main cyp genes and drug efflux transporters were investigated by real-time RT-PCR in the liver, kidney, intestine and adrenal glands. No significant differences in enzymatic activities and metabolite formation were observed between the two strains. Also mRNA expression profiles of cyp and drug transporter genes were similar between CD-1 and nude mice.

Introduction

Among the animals used in research, mice comprise the majority of all experimental mammals. The remarkable genetic similarity of mice to humans, combined with their small size, makes the mouse a preferred experimental animal model within cancer drug discovery and development. Specially bred mice, with natural or introduced genetic deficiencies have provided a wide range of genetic mouse models of human diseases [1]. An example is the athymic nude mouse (nu/nu), which has an unusually low immune response. These mice are born without thymus gland and therefore can not generate mature T lymphocytes and are unable to mount most types of immune responses. Their immuno deficient status allows a variety of human tumours to be grafted without rejection which serve to investigate tumour growth inhibition of potential anti-tumour agents [2].

Parallel to in vivo screening for identifying pharmacological active and potent anti-tumour compounds, in vivo studies are performed to evaluate the safety and pharmacokinetic properties of drug candidates [3]. The pharmacokinetic information of potential anti-tumour compounds obtained from studies in CD-1 mice is often used to design appropriate dose regimes for these compounds in tumour bearing nude mice. However, both strains are not well characterized with respect to their drug metabolism and drug transporter characteristics. Therefore, information on possible strain differences between CD-1 and athymic nude mice which may affect the drug disposition of new chemical entities would be extremely useful. Both drug metabolism and drug transporters are important features that determine the disposition of drugs. The liver is the primary site for drug metabolism and contains the necessary enzymes for metabolism of drugs and other xenobiotics. These enzymes induce two metabolism pathways: phase I (functionalization reactions) and phase II (biosynthetic reactions)
metabolism [4]. Some typical examples of phase I metabolism include cytochrome P450 (CYP) mediated oxidation and hydrolysis. Phase II metabolism involves the introduction of a hydrophilic endogenous species, such as glucuronic acid or sulfate, to the drug molecule. The elimination of a drug by transport proteins, referred to as drug transporter enzymes, as phase III metabolism [5], have gained increasing interest recently. Those enzymes are expressed in many tissues such as intestine, liver, kidney, and brain, and play key roles in drug absorption, distribution, and excretion [6,7]. Modulation of drug metabolism, but also of drug transporters may increase the risk of toxicity and other adverse drug reactions. To gain an insight into possible strain-related differences in drug metabolism and drug transporters between CD-1 and athymic nude mice, we characterized both phase I- and phase II- mediated hepatic drug metabolism as well as the major drug efflux transporters (phase III). Phase I activities were investigated by studying the metabolic stability at a low concentration of a set of 20 compounds and by measuring metabolite formation of well known marker substrates of phase I metabolism (ethoxyresorufin, pentoxyresorufin, and testosterone) using liver microsomes. Phase II metabolism, in freshly prepared mouse liver slices, was evaluated using 7-hydroxycoumarin (7-HC) as a marker substrate for sulfation and glucuronidation. In addition, expression profiles of major mouse cyp isoforms and efflux transporters were determined in several organs, by measuring quantitatively mRNA levels using real-time RT-PCR. The mouse cyp genes included in this study were cyp1a1, cyp1a2, cyp2b10 and cyp3a11. The efflux transporters mdr1a, mdr1b, mrp1 and mrp2 were included in the current study because of their important protective and secretory role in affecting the hepatobiliary, renal or intestinal elimination of several drugs [8-10].

**Materials and methods**

**Chemicals.** The following compounds were obtained from the sources indicated: 7-hydroxycoumarin, 7-hydroxycoumarin glucuronide, dimethylsulfoxide (DMSO), D-glucose, gentamicin sulphate, ethoxyresorufin, pentoxyresorufin, midazolam, ketoconazole, quinidine, sulfaphenazole, chloropromazine, lansoprazole, orphenadrine, salmeterol, fluoxetine, nifedipine, warfarin, dextromethorphan, fluphenazine, erythromycin, tolbutamide, verapamil, carbamazepine and resorufin were from Sigma-Aldrich (St. Louis, MO, USA). Testosterone was from Fluka (Buchs, Switzerland). 7-hydroxycoumarin sulphate and testosterone metabolites were from Ultrafine Chemicals (Manchester, UK). Indinavir, delavirdine and triazolam were from Pfizer (Kalamazoo, USA). Williams' Medium E, 5 x first strand buffer, RnaseOUT, Superscript, dATP, dGTP, dCTP, dTTP, random hexamer primers, DTT and BSA were obtained from Gibco (Paisley, Scotland, UK). RNA 6000 Nano Assay was from Agilent Technologies (Palo Alto, California, USA). RiboGreen™ RNA Quantitation kit was from Molecular Probes (Eugene, OR, USA). Qiagen RNAeasy® mini kit was from Qiagen Ltd (Crawley, UK). RNAlater™ was from Ambion (Austin, TX, USA). TaqMan® Universal PCR Master Mix Reagents, SYBR® Green PCR Master Mix, assays-on-Demand™ Gene Expression product and TaqMan® probes were obtained from Applied Biosystems (Foster City, CA, USA). The oligonucleotide primers were synthesized by Nerviano Medical Sciences Labs (Nerviano, Milano, Italy).

**Animals.** Male CD-1 mouse and male nude mice were obtained from Charles River (Como, Italy; aged 8-10 weeks) and were maintained under a 12-h light/dark cycle, with free access to drinking water. Nude mice were fed with 4RFN food pellets that are richer in protein and lipid content and that were sterilized by γ-irradiation, while CD-1 mice received standard 4RF21 pellets (Mucedola, Settimo Milanese, MI, Italy). Mice were housed in the standard cages and bedding but for nude mice the air supply was filtered using EPA filters to protect the nude mice against infections.
Lack of strain differences between CD-1 and nude mice

Liver microsome preparation. Three CD-1 and three nude mice were killed by cervical dislocation and the livers immediately excised. Small samples of liver were snap-frozen in liquid nitrogen and stored at -80°C before homogenisation in 25 mM phosphate buffered saline, pH 7.4, using a Potter homogeniser. Microsomes were prepared by ultracentrifugation (100000 g for 1 hour) of the post-mitochondrial supernatant (9000 g for 20 min). The microsomal pellet was resuspended in homogenisation buffer and centrifuged twice at 100000 g for 60 min. The washed microsomes were suspended in 0.1 M Tris Buffer (pH 7.4), containing 20% (w/v) glycerol, so that 1 ml of microsomal suspension was equivalent to approximately 1 g original wet weight of thawed liver. Microsomes were aliquoted and stored at -80°C. The protein concentration was determined using the method by Bradford [11].

Metabolic stability. The compounds (testosterone, midazolam, chlorpromazine, nifedipine, verapamil, lanosoprazole, delavirdine, dextromethorphan, indinavir, ketoconazole, orphenadrine, triazolam, fluphenazine, quinidine, salmeterol, warfarin, sulfaphenazole, erythromycin, tolbutamide and fluoxetine) were dissolved in DMSO at 10 mM. These stock solutions were diluted with acetonitrile to lower the DMSO concentration because it is known that DMSO inhibits microsomal enzyme activities [12]. Incubations were performed in 20 mM phosphate buffer at pH 7.4 for 45 min in the presence of microsomes and 2 µM of substrate at 37°C. The total concentration of organic solvent was 0.75%, of which 0.13% was DMSO. As a co-factor NADPH was added at 1 mM. Total incubation volume was 300 µl. The reactions were started by the addition of microsomes (final concentration: 0.5 mg protein/ml) and terminated by the addition of 100 µl ice-cold acetonitrile containing 7 µM carbamazepine as internal standard. All incubations were performed in duplicate using an automated liquid handling system (MultiProbe, Packard). Analysis was performed using LC-MS/MS, as described earlier [13]. Quantification was performed by comparing the peak areas with authentic standards of each metabolite. The percentage remaining of the substrate was calculated relative to control (heat inactivated) microsomes.

Metabolite formation incubation. Testosterone, ethoxyresorufin and pentoxyresorufin were dissolved in DMSO. Incubations were performed in 20 mM phosphate buffer, pH 7.4 for either 10 min (ethoxyresorufin and pentoxyresorufin) or 30 min (testosterone) in the presence of microsomes (1 mg protein/ml) at 37°C. Substrate concentrations were 0.25 mM for testosterone and 5 µM for 7-ethoxyresorufin-O-deethylase (EROD) and for 7-pentoxyresorufin-O-depenthylase (PROD). Final DMSO concentration was 0.5%. As a co-factor NADPH was added at 1 mM. Total incubation volume was 1 ml. All incubations were performed in triplicate. The testosterone metabolites were analyzed by HPLC with UV detection [13] while the formation of resorufin from ethoxyresorufin and pentoxyresorufin was detected by fluorimetry at 530 nm/excitation and 590 nm/emission, using authentic standards.

Liver slice preparation. After intraperitoneal anaesthesia with a cocktail of ketamine (67 mg/kg), xylazine (15 mg/kg) and acepromazine (1 mg/kg), livers were removed and stored in cold Williams’ Medium E until use (max. 30 min). Liver cores and slices (diameter 8 mm, wet weight about 15 mg) were prepared in ice-cold Williams’ Medium E that was oxygenated with 95% O₂ / 5% CO₂ and supplemented with extra glucose (final conc. 25 mM), using a Krumdieck tissue slicer as described earlier [13]. The slices obtained were subsequently stored in ice-cold Williams’ Medium E until use (within 1 h of preparation).

Culture of liver slices. Slices were individually incubated in 6 well culture plates (Falcon, France) in 2.0 ml Williams’ Medium E under 95% O₂ / 5% CO₂ (one slice per well) at 37°C while shaking horizontally at 90-100 times min⁻¹. The culture medium consisted of Williams’ Medium E containing glucose (25 mM) and gentamicin (50 µg/ml). CD-1 and nude mouse liver slices were incubated in triplicate with either 1 µM or 50 µM 7-hydroxycoumarin for 3 h. Prior to the immersion of the slices, 7-HC was added to the incubation medium as a 1000-times concentrated solution in methanol. At different time points (0, 30, 60, 90, and 180 min) an aliquot of media was removed and the reaction stopped by adding an equal volume of acetonitrile and stored at -20°C. The samples were analysed using LC-MS/MS as described earlier [13]. The slices were disrupted using a MSE Ultrasonic disintegrator (Fisons, Loughborough, UK) in their own incubation medium for the determination of the protein content using the method of Bradford [11]. CL₄₀₉ was determined from the disappearance of the parent compound as described earlier [14].
Lack of strain differences between CD-1 and nude mice

RNA preparation from liver, kidney, adrenal glands and intestinal samples. Tissue samples (about 30 mg) of liver, kidney, adrenal glands, duodenum, ileum and colon of both strains were taken from three male nude mouse and three male CD-1 mouse after anaesthesia as described above and stored in RNAlater™ at 4°C. Total RNA was extracted from the tissue using QIAGEN RNeasy® mini kits. The quality of the isolated RNA was assessed using RNA 6000 Nano Assay and the Agilent 2100 bioanalyzer. RNA concentration was determined using a RiboGreen™ RNA Quantitation kit.

Reverse transcription. The reaction mixture (final volume 40 µl) was prepared with final concentrations as follows: 1 x first strand buffer, 64 units RNaseOUT, 200 units Superscript, 0.6 mM of dNTP (dATP, dGTP, dCTP and dTTP), 0.75 µg random hexamer primers, 10 mM DTT and 16 ng BSA. To this mixture 1 µg of total RNA extracted was added. The reverse transcription reaction was performed for 10 min at 25°C, 60 min at 42°C and 30 min at 37°C.

Design of primers and probes. The cDNA sequences of mouse cyp1a1, cyp1a2, cyp3a11 and β-actin were obtained from GenBank accession numbers: NM 009992, NM 009993, NM 007818 and NM 007393. Taqman primer and probe sets for cyp2b10 were described earlier [15]. PCR primers and probe sequences were designed using PrimerExpress software (Applied Biosystems) and shown in Table 1. Nucleotide primers and probe sequences were checked against the NCBI BLAST database to ensure specificity for the selected gene. The Taqman primer and probe sets for mdr1a, mdr1b, mrp1 and mrp2 were bought from Applied Biosystems (Assays-on-Demand™ Gene Expression product: Mm 00440761m1, Mm 00440736m1, Mm 00456156m1 and Mm 00496899m1).

Real-time quantitative PCR. Real-time quantitative PCR was performed, employing an iCycler iQ™ Real time PCR detector system (Bio-Rad). The PCR reaction was performed in a 96 well plate. The reaction mixture (13.5 µl) was added in each well to give the following concentration: 1 x master mix reagents, either 200-900 nM of each primer and 200 nM probe for each cyp mRNA assay or 1 x Assay-on-Demand™ Gene Expression Assay Mix for transporter mRNA assay. cDNA (1.5 µl) was added to each well and the final volume was 15 µl. The thermal cycle condition was 50°C for 2 min, 95°C for 10 min to activate AmpliTaq Gold DNA polymerase, denaturation at 95°C for 15 sec and anneal/extension at 59°C for 1 min (40 cycles). Quantitative PCR for β-actin mRNA was also performed to normalize for RNA loading.

Statistical analysis. Differences among group mean values were assessed by Student’s t test. A difference of $P < 0.05$ was considered statistically significant.

Results

Comparison of metabolic stability in mice liver microsomes. The metabolic stability of a set of 20 compounds incubated with liver microsomes prepared from CD-1 and nude mice is presented in Figure 1. The set of compounds was selected to cover a wide range of metabolic stability, from unstable up to stable under the defined conditions. The metabolic stability experiments were performed at low substrate concentrations (2 µM). Using such a low substrate concentration ([S]) and assuming that $[S]<<K_m$, the disappearance of the substrate will follow first order kinetics and as a result the metabolic stability data is a direct indication of hepatic intrinsic metabolic clearance (CL_int) [14].

As shown in Figure 1, there is a remarkable similarity in metabolic stability between the two strains.
Table 1. Taqman® primer and probe sequences of mouse cyp mRNA

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers/Probes</th>
<th>5'→3' Sequence</th>
<th>Amplicon Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cyp1a1</td>
<td>Forward</td>
<td>ATAAGGTCACTCAGATTGTTTGG</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GTACATGAGGCTCCACGAGAT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>ACAGTCACAACGTGC</td>
<td></td>
</tr>
<tr>
<td>cyp1a2</td>
<td>Forward</td>
<td>CGTCAGCAAGCTTCAGAAGG</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GATGTCAGCATCTCCTCG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>CAAAAGCAGATGGACACCAATGAC</td>
<td></td>
</tr>
<tr>
<td>cyp2b10</td>
<td>Forward</td>
<td>CAGGTGATCGGCTCACACC</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GACTGCACTCAGATGAGCATT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>CAAACCCATTGAGACCCGACCA</td>
<td></td>
</tr>
<tr>
<td>cyp3a11</td>
<td>Forward</td>
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</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>TCCATCCTCGTGTGGTTGTC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>CAGAGAAGTAAATTGC</td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>Forward</td>
<td>TTCTTTTCAGCTCCTCTGC</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>ACCAGCGACCGATATC</td>
<td></td>
</tr>
</tbody>
</table>

Despite the fact that ketoconazole, quinidine and chloropromazine were found to be slightly more stable in liver microsomes prepared from CD-1 mice, in general the rank-order of compounds from unstable to stable was very similar between the two mice strains studied.

Figure 1. Metabolic stability, expressed as % remaining, of 20 drugs after incubation with liver microsomes obtained from CD-1 and nude mice. Results are the mean (n=3) ± SEM.
Lack of strain differences between CD-1 and nude mice

Comparisons of metabolic capacity in mice liver microsomes. Testosterone hydroxylase (TOH), EROD and PROD activities in liver microsomes from CD-1 and nude mice are shown in Figure 2 and Table 2, respectively. In these experiments, enzymatic activities were measured using relatively high concentrations ([S] >> Km) and therefore these data are assumed to be indicative of the metabolic capacity. No significant differences between the strains were observed. Not only the overall hydroxylation rate of testosterone, but also the hydroxylation at specific positions of testosterone was very similar.

Figure 2. Testosterone hydroxylase activity in liver microsomes of CD-1 and nude mice. Results are the mean (n=3) ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>CD-1 mouse</th>
<th>Nude mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>EROD activity (pmol/min/mg)</td>
<td>125.5 ± 4.2</td>
<td>113.5 ± 2.0</td>
</tr>
<tr>
<td>PROD activity (pmol/min/mg)</td>
<td>16.8 ± 0.5</td>
<td>13.6 ± 0.3</td>
</tr>
</tbody>
</table>

Table 2. Ethoxyresorufin-O-deethylase and pentoxyresorufin-O-depenthylase activity in liver microsomes of CD-1 and nude mice. Results are the mean (n=3) ± SEM.

Conjugation of 7-hydroxycoumarin by mouse liver slices. The CLint of 7-HC (1 µM) and the formation of 7-HC metabolites (50 µM) are shown in Table 3. Incubation with 7-HC (50 µM) gave rise to both sulphate and glucuronic acid conjugates. 7-HC was preferentially conjugated with glucuronide, which is in agreement with the results of Steensma et al. [16]. There were no significant differences observed in the depletion rate or CLint of 7-HC, and in the formation of 7-hydroxycoumarin conjugates using CD-1 and nude mouse liver slices.
Lack of strain differences between CD-1 and nude mice

<table>
<thead>
<tr>
<th></th>
<th>CD-1 mouse</th>
<th>Nude mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLint (ml/min/mg)</td>
<td>5.7 ± 0.7</td>
<td>7.9 ± 0.8</td>
</tr>
<tr>
<td>7-HC sulphate (pmol/min/mg)</td>
<td>64.0 ± 15.6</td>
<td>91.7 ± 9.6</td>
</tr>
<tr>
<td>7-HC glucuronide (pmol/min/mg)</td>
<td>128.0 ± 17.0</td>
<td>111.0 ± 23.0</td>
</tr>
</tbody>
</table>

Table 3. Metabolism of 7-hydroxycoumarin by CD-1 and nude liver slices. Results are the mean ± SEM (n=6 slices) from two separate experiments.

Detection of cyp and efflux transporter mRNA levels. In both strains, the major cyp isoforms present in liver tissues were cyp1a2 and cyp3a11, whereas cyp1a1 and cyp2b10 were predominantly expressed in the upper part of the intestinal tract (Table 4). The expression levels of cyp1a1, cyp1a2, cyp2b10 and cyp3a11 by kidney, colon and adrenal glands were low or even absent (Table 4). The efflux transporter mdr1a was highly expressed in adrenal glands and in the intestinal tract of both strains and levels increased from duodenum towards colon (Table 4). In the kidney the major transporters present were mrp1 and mrp2, whereas in the liver, mrp2 was expressed at significant levels. Mdr1b is highly detected in the adrenal glands (Table 5).

The cyp and transporter mRNA expression profiles for both strains in all organs evaluated were very similar. The only significant difference between species was observed with respect to cyp1a1, which was found to be expressed 3 times higher in the duodenum of nude mice.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>CD-1 mouse</th>
<th>Nude mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cyp1a1 cyp1a2 cyp2b10 cyp3a11</td>
<td>cyp1a1 cyp1a2 cyp2b10 cyp3a11</td>
</tr>
<tr>
<td>Liver</td>
<td>+* ++++ ++ ++++</td>
<td>+ ++++ ++ ++++</td>
</tr>
<tr>
<td>Kidney</td>
<td>- + * +</td>
<td>- + + +</td>
</tr>
<tr>
<td>Duodenum</td>
<td>++* - ++++ +</td>
<td>++++ - ++++ +</td>
</tr>
<tr>
<td>Ileum</td>
<td>++ - ++ +</td>
<td>++ - ++ +</td>
</tr>
<tr>
<td>Colon</td>
<td>- - - -</td>
<td>- - - -</td>
</tr>
<tr>
<td>Adrenal Glands</td>
<td>- - - -</td>
<td>- - - -</td>
</tr>
</tbody>
</table>

Table 4. Relative mRNA expression level of cyp1a1, cyp1a2, cyp2b10 and cyp3a11 in liver, kidney, duodenum, ileum, colon and adrenal glands of CD-1 and nude mouse. Total RNA (3.5 ng) was loaded for one-step real-time RT-PCR assay and the levels of cyp mRNA were expressed as ratios of levels of β-actin mRNA, which was used to normalize RNA loading. Results are the mean from three animals.

+ to ++++ indicate increasing levels of mRNA expression.
- indicates undetectable or signal after more than 35 PCR cycles. * P<0.05
Lack of strain differences between CD-1 and nude mice

<table>
<thead>
<tr>
<th>Tissue</th>
<th>CD-1 mouse</th>
<th></th>
<th>Nude mouse</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mdr1a</td>
<td>mdr1b</td>
<td>mrp1</td>
<td>mrp2</td>
</tr>
<tr>
<td>Liver</td>
<td>+*</td>
<td>-</td>
<td>+</td>
<td>++++</td>
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<td>Kidney</td>
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<td>+*</td>
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<td>Duodenum</td>
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<td>-</td>
<td>+*</td>
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<td>Ileum</td>
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<td>Colon</td>
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<tr>
<td>Adrenal Glands</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5. Relative mRNA expression level of mdr1a1, mdr1b, mrp1 and mrp2 in liver, kidney, duodenum, ileum, colon and adrenal glands of CD-1 and nude mouse. Total RNA (3.5 ng) was loaded for one-step real-time RT-PCR assay and the levels of cyp mRNA were expressed as ratios of levels of β-actin mRNA, which was used to normalize RNA loading. Results are the mean from three animals.

Discussion

In oncology drug discovery and development, the athymic nude mouse is a very useful investigation model due to its ability to support the growth tumours of human origin. On the other hand, and at the same time, pharmaceutical industries are using extensively CD-1 mice to investigate the toxicity and pharmacokinetic behaviour of new chemical entities. Linking the efficacy data obtained in nude mouse to the pharmacokinetic parameters obtained in CD-1 mice, raises the question of whether the metabolic and pharmacokinetic characteristics of xenobiotics in these strains are comparable or not. Moreover, pharmacokinetic data from CD-1 mice may be used to build a pharmacokinetic/pharmacodynamic model in order to understand better the relationship between drug exposure and tumour growth inhibition in nude mice. Therefore, the purpose of this study was to characterize these two mouse strains with respect to their drug metabolism and drug transporter properties. Only the male mouse was investigated because it is predominantly used in toxicological studies. Hepatic phase I and phase II metabolism of a set of well-known drugs and the enzymatic activities towards testosterone, ethoxyresorufin and pentoxyresorufin were investigated. Results clearly demonstrate close similarity between the two strains.

In addition, mRNA levels of major cyp isoforms and drug efflux transporters were investigated, by real-time RT-PCR, in the liver, kidney, intestine and adrenal glands of both strains. Cyp1a2 and cyp3a11 were significantly expressed in the liver, but no, or only a weak expression was observed in other tissues. Similar findings were described previously for BALB/c mice [17]. On the other hand, cyp1a1 and cyp2b10 were found to be expressed extra-hepatically, mainly in duodenum and ileum. In a recent study, constitutive mRNA levels of cyp1a1 and cyp2b10 have also been detected in small intestines of B6 mice, and could be induced by β-naphthoflavone and phenobarbital, respectively [18]. In this study, no remarkable differences were detected between the two strains, except for cyp1a1, the expression of which was significantly higher (3-fold) in
Lack of strain differences between CD-1 and nude mice
nude mouse duodenum. However, it should be mentioned that a 3-fold increase in
cyp1a1 mRNA expression is relatively small and, in general, reflects an even smaller
change in protein activity. For example, in rats treated with Aroclor, induction of CYP1A1
gave rise to ~23000-fold induction of mRNA level, but only to 127-fold difference in
protein activity [19]. It can be hypothesized that the difference in duodenal cyp1a1
mRNA expression is due to the fact that nude mice are not exposed to bacteria, and
bacteria derived factors such as lipopolysaccharide (LPS). Both bacteria and LPS are well
known for their ability to down-regulate CYP isoforms [15]. Regarding the drug
transporters genes, mdr1a was highly expressed in the intestine and adrenal glands of
both strains. An interesting observation in this study is the increase in expression level of
mdr1a going from duodenum towards colon. Previously, several other groups have also
reported the intestinal expression of mdr1a in mice, both at the level of mRNA and
protein [10,20,21].
High expression levels of mdr1b have been detected previously in the adrenal gland,
pregnant uterus and ovaries of mice [22]. The current study confirmed the adrenal gland
specific expression of mdr1b, as weak or no mdr1b mRNA gene expression was detected
in liver, kidney and intestinal tissue.
The expression of mrp2 was high in liver and kidney and only a weak or no expression
was detected in the intestine and the adrenal glands. Mrp1 could be detected in all
tissues, with highest expression in kidney and adrenal glands. Using another mouse
strain (C57/BL6), similar profiles for mrp1 and mrp2 were earlier described [23]. Despite
the fact that the expression of drug metabolizing enzymes and drug transporters have
been studied in different tissues and in different animals, including mice, this is the first
study in which two mice strains, commonly used in oncology discovery and development,
were characterized for their hepatic drug metabolism and drug transporters properties.
In conclusion, CD-1 and athymic nude mice demonstrate remarkable similarities in
mRNA expression of major drug metabolizing enzymes and efflux transporters in all
organs. The comparison of cyp expression between CD-1 and nude mice is also reflected
a close similarity of enzymatic activities between the two strains. Therefore, the potential
error in extrapolating pharmacokinetic data obtained from CD-1 to nude mice, or vice
versa, is expected to be minimal.
References


Lack of strain differences between CD-1 and nude mice

