Xenobiotic Metabolizing Cytochrome P450 in Pig, a Promising Animal Model

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Abstract: The pig has been used as an important animal model for human studies because of its similarity in size, physiology and disease development. However, in contrast to the extensive data available on the cytochrome P450 (CYP) system for humans and rodents, the data related to pig are limited because of, among others, the presence of intra-species differences (domestic pigs and minipigs). The knowledge of the CYP superfamily in a given experimental animal is crucial for pharmacological and toxicological tests in developing drugs and for understanding the metabolic pathways of toxicants and carcinogens. In addition, information on the CYP system in pigs is important since it plays a dominant role in the metabolism of veterinary drugs, whose residues remain in the porcine tissues which are food for humans.

The aim of the present review is to examine - in the liver and extrahepatic tissues of pig - our current knowledge of the xenobiotic-metabolizing CYPs belonging to families 1-4, in terms of drug metabolism, substrate specificity, inhibition, gene expression and receptor-driven regulation, in comparison with human data. It is hoped, furthermore, that this review may stimulate research on the porcine drug-metabolizing enzymes in order to evaluate the hypothesis whereby pig data may better reflect human drug metabolism and toxicity than those obtained from the traditional non-rodent models.

Keywords: Pig, minipig, cytochrome P450 (CYP), liver, extrahepatic tissues, animal model, xenobiotics, porcine nuclear receptors.

INTRODUCTION

The extrapolation of biological animal data to humans is often difficult, especially if no human data are available. An increasing number of studies suggests the use of the pig (or minipig) as a new animal model for humans, as this species offers many advantages. The pig is considered a good model in biomedical research because of its anatomical, physiological, and biochemical similarity to humans. Many organs and systems - including heart, nasal cavity, liver, kidneys, brain, reproductive and gastrointestinal system - show analogies with humans and advantages compared to other experimental models [1]. The complete pig genome will be available soon (www.sanger.ac.uk/Projects/S_scrofa/), and the comparative maps obtained so far have shown an extensive conserved homology with the human genome. The pig is also recognized as a model for several major human diseases, such as cardiovascular diseases (e.g. atherosclerosis), metabolic diseases (e.g. hypercholesterolemia) and neural diseases (e.g. Parkinson's and Alzheimer's) [2,3,4]. Some pig breeds (e.g. Göttingen minipig) are predisposed to obesity, and this feature could provide ideal families for the identification of genes involved in this pathology [5]. In addition, the pig is the only non-rodent species in which the generation of transgenic animals is well established [5]. Another field in which the pig represents a turning point is that of xenotransplantation. The high similarity found in heart (including coronary arteries) and liver makes the pig the best candidate for xenotransplantation to humans, thus eliminating the major problem of organ scarcity [6]. Bioartificial livers (BALs) have also been proposed and developed for the extracorporeal circulation of patients with severe liver failure [7]. Pigs may be introduced as animal model in the safety assessment of pharmaceutical or chemical products and this could have a positive impact on the so called 3R's: Replacement (i.e. substitution of dog and monkey - the non-rodent species typically used in toxicology), Refinement (i.e. use of more suitable species) and Reduction (i.e. minor animal number) [1]. For all these important potential applications, the characterization of the porcine metabolic and toxicogenomic profile is an essential prerequisite. However, the area of toxicogenomics remains largely unexploited so far.

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Cytochrome P450 (CYP) is a complex and very wide superfamily of enzymes which play a major role in xenobiotics metabolism as well as in endogenous compounds oxidation. It represents the main phase-1 metabolic system, and it could be involved in drug-drug interactions, toxicity, and bioactivation of carcinogens [8]. Nevertheless, the amount of porcine CYPs data is still scarce compared to that of humans or rodents, and only in the last two decades some efforts have been focused on these enzymes [9-11]. One of the main hurdles for the metabolic characterization of the pig is the significant difference occurring in the various animal breeds. Many breeds of both conventional pigs (e.g. Large White, Landrace or Duroc) and minipigs (e.g. Göttingen, Bama or Yucatan) are used for metabolism studies, creating data misunderstanding and ambiguous interpretations. Even a variety of microminipigs (still uncharacterized) has been recently developed with the specific aim of non-clinical pharmacological/toxicological use [12]. However it is pertinent to point out that primary structure of CYP enzymes in conventional pigs and minipigs is not expected to differ significantly. Taking in consideration only the CYPs belonging to the 1-4 families, which are the most involved in the xenobiotics biotransformation, it is relevant to note that: i) there is a high homology between humans and conventional pigs CYPs; ii) the differences between conventional pigs and minipigs CYP sequences (at least the only few available so far) are less than 1 % and they should be regarded as allelic variants (Table **1**). Nevertheless, significantly different data are in many case expected to result from CYP expression levels, substrate specificity and CYP-dependent drug metabolism profiles derived from convetional pigs and minipigs. In view of this, minipigs present some advantages with respect to commercial pigs. For instance, the Göttingen minipig is a genetically defined model (unlike routinely used dogs and monkeys) since the entire population history is well documented from the early development up to the present [13]. In addition, the smaller size of minipigs (which is not due to defective genes) makes the animals easily manageable.

Thus far, only a few porcine CYPs have been cloned and expressed in a recombinant system to characterize the enzymatic functions (for 1-4 CYP families, see Table **2**). Even the number of the CYP (mini)pig isoforms is still not complete. Furthermore, very little is known about the regulation of these genes, and – unlike humans – there is a lack of studies aimed at identifying the regulatory response elements in the promoter of CYP genes.

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Table 1. Comparison of the Nucleotide and Amino Acid Sequences of Porcine and Human CYPs.

Porcine CYP Isoform	Accession Number	Human CYP Isoform	% of identity	
			Nucleotide	Amino Acid
CYP1A1	NM_214412	CYP1A1	85.4	81.2
CYP1A2	NM_001159614	CYP1A2	85.0	81.0
CYP1B1	/	CYP1B1	85.0	84.0
CYP2A19	AB052255	CYP2A6	87.5	87.5
CYP2B22	AB052256	CYP2B6	81.1	74.0
CYP2C33	NM_214414	CYP2C8	71.0	62.0
		CYP2C9	76.0	64.0
		CYP2C19	74.0	63.0
CYP2C42	NM_001167835	CYP2C8	84.0	78.0
		CYP2C9	85.0	80.0
		CYP2C19	85.0	81.0
CYP2C49	NM_214420	CYP2C8	82.0	75.0
		CYP2C9	83.0	77.0
		CYP2C19	84.0	77.0
CYP2D21*	D89502	CYP2D6	83.5	78.3
CYP2D25	NM_214394	CYP2D6	83.2	78.1
CYP2E1	AB052259	CYP2E1	82.5	79.2
CYP2E1*	NM_214421	CYP2E1	82.5	79.2
CYP3A22*	AB006010	CYP3A4	81.5	75.0
CYP3A29	Z93099	CYP3A4	82.8	76.5
CYP3A29*	AF424780	CYP3A4	82.5	75.3
CYP3A39	NM_214422	CYP3A4	82.1	75.9
CYP3A46	NM_001134824	CYP3A4	83.5	77.8
CYP4A21	NM_214425	CYP4A11	75.0	74.0
CYP4A24/25	NM_214424	CYP4A11	73.0	74.0

* Sequence isolated from minipigs.

This review gives a comprehensive knowledge on the members of the first four (mini)pigs CYP families and the related key nuclear receptors (aryl hydrocarbon receptor, AhR, constitutive androstane receptor, CAR, pregnane X receptor, PXR, peroxisome proliferator-activated receptor alpha, PPARα, and hepatic nuclear factor 4 alpha, HNF4α), which represent the centrepiece in the overall metabolism and disposition of drugs and xenobiotics. When available, the pig data are compared to those of humans. In Table 3, some drugs known to be metabolized in pigs are reported, together with the CYP isoforms which are presumably involved in these reactions.

Table 2. Recombinant Porcine CYPs.

CYP Isoform	Expression System	Tested Substrates	References
CYP1A1	HEK-293	Chlorzoxazone	[90]
CYP1A2	E. coli	2-Aminofluorene, acetanilide, aniline, caffeine, ethoxyresorufin, methoxyresorufin, testosterone	[16]
CYP2A19	HEK-293	Chlorzoxazone	[90]
CYP2C33	HEK-293	Chlorzoxazone	[90]
CYP2C49	HEK-293	Chlorzoxazone	[90]
CYP2D21	S. cerevisiae	Bufuralol	[77]
CYP2D25	S. cerevisiae	Tolterodine, vitamin D3	[75-76]
CYP2E1	E. coli, HEK-293	Chlorzoxazone, p-nitrophenol	[86,90]
CYP3A29	Sf9 insect cells	Nifedipine, testosterone	[91,102]
CYP4A21	COS-1 cells	Lauric acid, taurochenodeoxycholic acid	[117]
CYP4A24/25	S. cerevisiae	Lauric acid, palmitic acid, taurochenodeoxycholic acid	[118]

TOTAL CYTOCHROME P450

The average content of total cytochrome P450 in conventional pig liver microsomes (0.57, 0.22 and 0.46 nmol/mg prot. for three different pig crossbreeds) is comparable to that of humans (0.43 and 0.26 nmol/mg prot. for Caucasian and Japanese, respectively), whereas Göttingen minipigs total CYP is 2 to 3-fold higher [10]. Furthermore, minipigs show more sex differences in the expression and activity of CYPs compared to humans or conventional pigs [14].

CYP1A SUBFAMILY

General Features

In mammals, the CYP1A subfamily includes CYP1A1 and CYP1A2, two enzymes involved in the bioactivation of many carcinogens such as aromatic amines, mycotoxins, and xanthines as well as in the metabolism of several drugs. Ethoxyresorufin O-deethylase (EROD) and methoxyresorufin O-demethylase (MROD) are usually used as marker activities of these isoforms, typically inducible by dioxins and polyaromatic hydrocarbons (PAHs).

Porcine CYP1A1 has been definitively isolated and sequenced, revealing a high similarity to human CYP1A1 (85.4%) [15]. Porcine CYP1A2 has been recently cloned from domestic pig (Large White x Landrace hybrid) liver [16]. The nucleotide and deduced amino acid sequences revealed 85% and 81% identities to those of human CYP1A2, respectively, and a high degree of similarity was also reported for SRSs (substrate recognition sites) of the porcine and human CYP1A2.

Tissue Distribution

Pig CYP1A1 and 1A2 mRNAs have been found in liver, lung, heart, and kidney of domestic Large White x Landrace hybrid pigs, although at different levels [16, 17]. In the porcine liver, the level of CYP1A2 expression has been found to be 3-9% of the total CYP content, similar to the expression of CYP1A2 in human liver (about 10%) [16]. With regard to CYP1A1 mRNA expression, no quantitative data are available, but it appears to be lower than CYP1A2 expression [16], as reported in humans [18]. In Table 4 the tissue distribution of porcine CYPs and nuclear receptors is summarized. Another study deepened the presence of pig CYP1A1/1A2 in the brain [19]. Both the isoforms were found expressed at mRNA level in various brain regions including cortex, cerebellum, midbrain, hippocampus and blood-brain interfaces (meninges and cortex capillaries), with a particularly high expression in blood-brain interfaces. A protein immunorelated to CYP1A has been also found in porcine olfactory nasal epithelium [20], and this finding has been confirmed and extended by a recent study [21] which demonstrated the presence of CYP1A1 and 1A2 mRNA and related activities in both respiratory and (at a much higher extent) olfactory nasal mucosa.

Substrates and Reactions

Already in the 1990s, different groups detected EROD and MROD activities in liver microsomes from various pig and minipig breeds, although at lower level than in human ones [22-26]. Indeed, EROD activity in pigs ranged from 5 to 95 pmol/min · mg protein whereas the same activity in humans ranged from 25 to 190 pmol/min mg protein. MROD activity ranged from 2 to 13 pmol/min · mg protein in pigs and from 24 to 32 pmol/min · mg protein in humans. However, some differences with the human orthologous were pointed out: i) besides EROD and MROD, β-naphthoflavone (βNF) was able to induce also 7-pentoxyresorufin *O*-depenthylation (PROD), 7-ethoxy-4-trifluoromethylcou-marin O-dealkylase (EFCOD) and 7-benzyloxyresorufin *O*-debenzylase (BROD) activities which represent known markers of human CYP2B subfamilies; ii) all-*trans*-retinal oxidation and 17β-estradiol 2-hydroxylation, both linked to human CYP1As and 3A4, were not increased indicating a difference in substrate specificity between porcine and human CYP1As [17].

Table 3. Drugs Metabolized by Pig Liver

CYP Subfamilies	Drug	Clinical Use	References
2B ?	Benzphetamine	Anorectic drug	[137]
2B, 2D	Bufuralol	β-adrenergic receptor blocker	[78]
2B, 2D	Dextromethorphan	Cough suppressant	[78,81]
2C	Omeprazole	Gastric acid blocker	[71]
2C ?	Diclofenac	NSAID	[58]
2C ?	Tolbutamide	Hypoglycemic drug	[22,58]
2C ?, 2B ?	*S*-mephenytoin	Anticonvulsant	[14,58]
2C ?, 3A ?	Taxol	Chemoterapic drug	[72,58]
2D	Dapsone	Antibacterial agent	[145]
2D	Sulfamethoxazole	Antibiotic	[145]
2D	Tolterodine	Antimuscarinic muscle relaxant	[79]
2E	Bupropion	Antidepressant	[92]
2E, 1A, 2A	Chlorzoxazone	Muscle relaxant	[90]
3A	17α-Ethynylestradiol	Synthetic estrogen	[110]
3A	Erythromycin	Antibiotic	[137,59]
3A	Lovastatin	Hypolipidemic drug	[108]
3A	Midazolam	Benzodiazepine	[113]
3A	Nifedipine	Antihypertensive	[22,24,14]
3A	Tacrolimus	Immunosuppressant	[114]
3A ?	Ethylmorphine	Cough suppressant	[137]
3A ?	Triacetyloleandomycin	Antibiotic	[137]
?	Ampicillin	Antibiotic	[140]
?	Antipyrine	Analgesic and antipyretic	[138,139]
?	Azidothymidine	Antiretroviral drug	[144]
?	Hexobarbital	Barbiturate anaesthetic	[143]
?	Naproxen	NSAID	[140]
?	Norfloxacin	Antibiotic	[142]
?	Paracetamol	Analgesic and antipyretic	[139]
?	Sulfadimidine	Antibacterial agent	[141]
?	Vancomycin	Antibiotic	[139]

Note: fields marked by a question mark indicate no further information available.

Recently, pig CYP1A2 has been cloned, expressed in a heterologous system and characterized [16]. It was confirmed to possess a good catalytic activity towards caffeine, acetanilide, and methoxyresorufin (the pig CYP1A2 Vmax for this substrate is even higher than that of the human enzyme), all known markers of human CYP1A2. In addition, the purified pig enzyme, unlike rat CYP1A2 [27] but in keeping with human CYP1A2 [28, 29], failed to oxidize aniline or testosterone (in the 6β position).

Pig CYP1A2 is also involved, along with CYP2A19, in the metabolism of skatole, whose accumulation in the adipose tissue is responsible for boar taint [30].

Table 4. Relative Expression of Porcine CYPs and Nuclear Transcriptional Factors at mRNA Level in Comparison with the Liver.

CYPs and Receptors Genes	Liver	Kidney	Lung	Intestine	Respiratory Nasal Mucosa	Olfactory Nasal Mucosa	Brain	Heart	References
CYP1A1	+++	+	+	+/-	+	++	+/-	+/-	[16-17,19-21]
CYP1A2	+++	+/-	-	-	+	+++	+	+/-	[16-17,19-21]
CYP1B1	+	+	+	+	+	++	+	+	[17,19,21]
CYP2A19	++	+	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	[15]
CYP2B22	+++	+	++	+/-	+	+	+/-	+/-	[15,21,58-60]
CYP2C33	+++	++	+	+	+	+	+	+	[58,67]
CYP2C42	+++	-	-	+	+/-	-	-	-	[58]
CYP2C49	+++	-	+/-	+	+/-	+	+/-	+	[15,58]
CYP2D25	+++	++	+	+	n.a.	n.a.	+/-	+	[75,79]
CYP2E1	+++	+	+	+	n.a.	n.a.	+	+/-	[15,88-89]
CYP3A22	+++	+	+	++	+	+	+	+	[21,58-60,102]
CYP3A29	+++	+	+	++	+	+	+	+	[21,58-60,102-103,105]
CYP3A46	+++	+	+	++	+	+	+	+	[21,58-60,102]
CYP4A21	+++	+++	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	[117-118]
CYP4A24/25	+++	+++	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	[117-118]
AhR	+++	+	++	+	+	++	+	+	[17,19,21,129-130]
CAR	+++	+	+	+	+/-	+	+	+/-	[21,58-60,125-126]
HNF4α	+++	++	+	+	+/-	+/-	n.a.	n.a.	[21,58-59,132]
PPARα	+++	++	+/-	+	n.a.	n.a.	+/-	+	[123,133]
PXR	+++	++	+	+	++	++	+	+/-	[21,58-60,125-126,128]

+++ = high expression; ++ = moderate expression; + = low expression; +/- = barely detectable expression; - = undetectable expression; n.a. = data not available.

Inhibitors

The known porcine CYP inducers and inhibitors are summarized in Table **5**. As in humans, α-naphthoflavone and anti-rat CYP1A2 are able to strongly inhibit EROD activity in liver microsomes from Yucatan minipig [31]. In addition, ellipticine is reported to be a potent inhibitor of EROD activity performed by recombinant pig CYP1A2, as the human counterpart [16].

Inducibility and Regulation

EROD and MROD activities have been found increased in primary cultures of pig hepatocytes after a treatment with the AhR agonists βNF and 3-methylcholanthrene (3-MC) - but not with phenobarbital (PB), rifampicin (RIF) or dexametazone (DEX), in agreement with human data - in association with an increase in mRNA levels [32-33].

Fenbendazole, an anthelmintics widely used in veterinary medicine, determined a significant and concentration-dependent increase of EROD activity in primary pig hepatocytes [34]. This finding should be taken into account in the treatment of pigs with this drug. Besides hepatocytes, primary porcine enterocytes also showed EROD activity, although not inducible by βNF nor by 3-MC [35]. On the other hand, Roos *et al.* [36] reported that CYP1A1 was strongly induced in Göttingen minipig duodenum besides liver,

Table 5. Known Porcine CYP Inducers and Inhibitors.

CYP Subfamily	Inducers	References	Inhibitors	References
1A	β-Naphthoflavone	[17-19,21,32]	Ellipticine	[31]
	3-Methylcholanthrene	[33]	α-Naphthoflavone	[16]
	Benzo(a)pyrene	[36-37]		
	Omeprazole	[38]		
1B	β-Naphthoflavone	[19,21]		
2A	Phenobarbital	[50]	8-Methoxypsoralen	[46]
			Diethyldithiocarbamate	[46,86]
			Androgens	[50-57]
2B	Phenobarbital	[24,33,58]		
	Rifampicin	[59]		
2C	Rifampicin	[32]	Quercetin	[58]
	Phenobarbital	[32,58]	Sulfaphenazole	[58]
	Cortisol	[60]	Ticlopidine	[58]
			Tranylcypromine	[10]
			β-Naphthoflavone	[32]
2D			Tolterodine	[79]
			Quinine	[81]
			Quinidine	[81]
			Phenobarbital	[82]
2E	Ethanol	[89]	Propofol	[93]
	Isoniazide	[89]	Diethyldithiocarbamate	[46,86]
	Phenobarbital	[24]	Androstenone	[55]
	Skatole	[94]	17β-Estradiol	[55]
			S-adenosylmethionine	[146]
			4-Methylpyrazole	[46]
3A	Dexametazone	[32,35,38]	Ketoconazole	[31,102]
	Rifampicin	[32,38,21,59,60]	Triacetyloleandomycin	[22,102,108]
	Phenobarbital	[32,35,58]	Tiamulin	[147]

lung, kidney, and spleen after the oral administration of soils contaminated with different kinds of PAHs. The induction was particularly powerful in the duodenum, which represents a key organ for contaminant absorption and consequent distribution to tissues, reaching activity levels even higher than in the liver. Benzo(a) pyrene, a PAH found in tobacco smoke, is able to strongly increase the expression of CYP1A1 in porcine urinary bladder epithelial cells, indicating this enzyme as both indicator and contributor for benzo(a)pyrene toxicity [37]. This finding is particularly interesting as the consumption of tobacco products is the most relevant risk factor for the development of bladder cancer.

The inducibility of AhR-regulated CYP genes by βNF has been investigated in different extrahepatic tissues of conventional Large White x Landrace hybrid pigs. A study in lung, heart and kidney, along with liver, showed a transcriptional and activity induction of CYP1A1 but not CYP1A2 in all the analysed extrahepatic tissues, although to a different extent [17]. The treatment with βNF determined the induction of CYP1A1, but not of CYP1A2, in pig cortex, cerebellum, midbrain, hippocampus, and cortex microvessels. In parallel, EROD but not MROD activity was increased by βNF in all the brain regions [19]. A recent study [21] has revealed the inducibility by βNF of CYP1A1 but not of CYP1A2 in respiratory and olfactory nasal mucosa, at both transcriptional and activity levels. Omeprazole is a potent inducer of CYP1A2 activity - interestingly not through the action of AhR - (measured by EROD) in both human and minipig hepatocytes, but not in rat hepatocytes indicating pig as a more appropriate model system for the evaluation of CYP drug induction in humans [38].

EROD activity, related to CYP1A1 and 1A2, has shown marked sex differences (with females having a much higher activity

than males) in Göttingen minipigs and Meishan conventional pigs (but not in Landrace or Landrace x Yorkshire x Duroc domestic pigs) [14, 39], indicating an important role of hormones in the regulation of these enzymes.

In general, it can be said that CYP1A1 is poorly expressed at constitutive level even in the liver but it is very well inducible in both hepatic and extrahepatic tissues. Differently, CYP1A2 is essentially expressed at a high level and inducible only in the liver. This restriction in the presence and inducibility of CYP1A2, which also reflects what is found in humans [40], could be due to the need for HNF1-4 factors in its regulation.

Polymorphisms

So far, no important mutations of pig CYP1A1 or CYP1A2 has been reported. Two amino acid changes (R71C and D110N) have been observed in porcine CYP1A2 and R71 is shared with humans, but the influence of this substitution still has to be examined [16].

CYP1B SUBFAMILY

General Features

Cytochrome P450 1B1 (CYP1B1), the only member of the CYP1B subfamily present in mammals, is often found in tumour tissue and is suspected to play a role in oncogenesis and drug resistance. Unlike other P450s, CYP1B1 is known to be a predominantly extrahepatic isoform [41].

CYP1B1 has not been fully characterized in pig yet, and the swine sequence for CYP1B1 is not available in GenBank. Chirulli *et al.* [17] isolated and sequenced a fragment of 448 bp of pig CYP1B1 from Large White x Landrace hybrid pigs. Alignment analysis showed 87% similarity (of both aa and nu sequences) compared with the human orthologous. Very recently, pig CYP1B1 has been isolated and sequenced, revealing an open reading frame (ORF) of 1635 bp encoding a protein of 543 aa, as the CYP1B1 of other mammals including humans reported in the GeneBank [Messina A., private communication]. The SRSs of porcine CYP1B1 were totally shared (100%) with those of bovine orthologous and, except for SRS1, also with that of the canine counterpart. However, the SRSs of human CYP1B1 showed a higher homology with those of pig CYP1B1 than with those of rat CYP1B1.

Tissue Distribution

Messina *et al.* [21] performed real time RT-PCR (reverse transcriptase-polymerase chain reaction) experiments on liver, olfactory nasal mucosa and respiratory nasal mucosa samples from Large White x Landrace hybrid pigs. The results showed a low constitutive expression of this isoform in the analysed tissues, and in particular in the hepatic samples. Immunoblotting experiments performed using anti-rat CYP1B1 antibodies have revealed a protein band in hepatic and pulmonary microsomes but not in renal or cardiac ones [17]. Nannelli *et al.* [19] demonstrated the expression of CYP1B1 mRNA in various brain regions of the same pig breed - including cortex, cerebellum, midbrain, hippocampus, meninges and cerebral capillaries - with similar levels compared to humans. The high expression noticed in the blood-brain interfaces (meninges and cortex microvessels) is in agreement with mouse and human data and suggested a role of CYP1B1 in the protection of brain from xenobiotics. Further experiments confirmed the constitutive presence of CYP1B1 in several pig tissues such as liver, kidney, small intestine, lung, nasal mucosa, heart, coronary arteries, adrenal gland, and spleen, with the higher expression in adrenal gland and the lesser mRNA expression in liver [Messina A., private communication]. This expression pattern reflects what found in humans, where CYP1B1 is considered mostly extrahepatic and well expressed in steroidogenic organs.

Substrates and Reactions

No 17β-estradiol-4-hydroxylase activity (a marker reaction for human CYP1B1) has been detected in porcine liver, kidney, lung, and heart, indicating either a low assay sensitivity or a different substrate specificity of porcine CYP1B1 compared to the human isoform [17].

Inducibility and Regulation

A treatment with βNF – an agonist of the nuclear receptor AhR which regulates CYP isoforms of 1 family – enhanced CYP1B1 mRNA in pig liver but not in respiratory and olfactory nasal mucosa. Since AhR mRNA has been found also in pig nasal tissues, these data indicate an involvement of additional tissue-specific factors in the transcriptional regulation of this gene [21]. The treatment with βNF was able to increase the transcription of this isoform in pig midbrain and, especially, in capillaries [19], although the enzymatic induction needs to be confirmed at activity level because of the possibility of a post-transcriptional regulation.

CYP2A SUBFAMILY

General Features

Up to now, the only porcine member of the CYP2A subfamily identified and cloned is CYP2A19, which shares 87.5% of similarity with the human orthologous CYP2A6 [15]. CYP2A19 was isolated from liver of Landrace x Large White x Duroc crossbred conventional pigs and it revealed an ORF of 1485 bp encoding 494 amino acids. A study of Soucek *et al.* [42] revealed that the sequence of the first 20 amino acids at the N-terminus of Göttingen minipig CYP2A are highly similar to human CYP2A6 (70% identity). In addition, six substrate recognition sites have been identified in the human CYP2A sequences, and they are all present in the porcine protein [43], suggesting similar properties to the human orthologous.

Tissue Distribution

Pig CYP2A19 mRNA has been found in liver and, to a lower extent, in kidney of Landrace x Large White x Duroc crossbred pigs [15]. However, CYP2A19 mRNA has not been found in spleen, thymus, lung, muscle, small intestine, heart or ovaries from many conventional pig breeds [44].

Substrates and Reactions

Coumarin 7-hydroxylation is the most utilized marker activity for human CYP2A6. Although in human liver this activity level is higher than in pig liver (300-1114 pmol/min · mg protein versus 20-310 pmol/min · mg protein) [22,23,31,45], it is also possible to use this reaction as a marker for pig CYP2A19, as a good correlation has been shown between the activity and the immunochemical level, or the mRNA expression [14,33,44]. Furthermore, this activity was strongly inhibited by anti-human CYP2A6 antibodies. Also the formation of cotinine from nicotine, another human CYP2A6 marker reaction, is catalyzed by porcine CYP2A. At high nicotine concentrations, other isoenzymes probably contribute to the reaction, as the inhibitory anti-human CYP2A6 inhibition rate decreases from about 90% to 50%. This is in accordance with human data: CYP2A6 is the predominant enzyme with 50 μM substrate, whereas with 500 μM nicotine CYP2B6 and CYP2D6 also play an important role [43]. Besides liver and kidney, CYP2A-dependent activity has been found in pig (Large White x Landrace) nasal mucosa, and in particular in the olfactory mucosa where the activity levels were even higher than those in liver [20].

A study performed with primary porcine hepatocyte cultures using two CYP2A inhibitors suggested that CYP2A – unlike CYP2E1 – has a minor role in 3-methylindole (3-MI or skatole) metabolism, whose accumulation in adipose tissue is responsible

for the boar taint in 10-15% of non-castrated male pigs [46]. However, other results obtained by Diaz and Squires [47] revealed that the production of 3-MI metabolites was affected by the presence of inhibitors of CYP2A6 and CYP2E1 in the microsomal incubations and a significant negative correlation was found between the CYP2A19 content/activity and 3-MI levels in fat, thus demonstrating that CYP2A19 is critical for an adequate clearance of 3-MI. Furthermore, a particular single base deletion of CYP2A19, resulting in a frame shift in the coding region that produces a non-functional enzyme, was associated with high levels of skatole in fat tissue [44]. This finding further pointed out the significant role of CYP2A19 in the skatole biodisposition. Also, the oral administration of dried chicory root determined a decrease of the concentration of skatole in the adipose tissue of entire male pigs (multiple domestic pig crossbreeds) through an increase of transcription and translation of CYP2A [48,49]. In addition, Matal *et al.* [30] have recently demonstrated in a reconstituted system a role of porcine CYP2A19 in the formation of 3-methyloxyindole and indole-3-carbinol from skatole.

Over the past years, the important role of human CYP2A6 in the bioactivation of some industrial compounds (e.g. tert-butyl methyl ether and 1,3-butadiene) and procarcinogens (e.g. N-nitrosodimethylamine and N-nitrosobenzylmethylamine) has been ascertained [40]. However, in pig the metabolism of these substrates and the role of CYP2A19 remain to be investigated.

Inhibitors

Pig CYP2A19 can be inhibited by 8-methoxypsoralen and diethyldithiocarbamate, two typical inhibitors of human CYP2A6 [47,46]. Furthermore, menthofuran, a potent, mechanism-based inactivator of CYP2A6, can be utilized as CYP2A inhibitor in pigs [47].

Inducibility and Regulation

Little is known about CYP2A19 regulatory mechanisms. In primary porcine hepatocyte cultures, CYP2A-dependent activity is significantly increased by PB, an indirect CAR activator, and by CITCO (6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde-*O*-(3,4-dichlorobenzyl)oxime), a human CAR ligand [50]. These results show that the induction profile of CYP2A *in vitro* shares similarity with that of human CAR-regulated CYPs, indicating an involvement of pig CAR in the regulation of this subfamily. A study of Myers *et al.* [25] demonstrated that a co-treatment with βNF, PB, and DEX is able to increase CYP2A19 marker activity, but not to increase the expression of the enzyme, as assessed with Western blot analysis. In porcine hepatocyte cultures, unlike human hepatocytes [51], CYP2A was not induced by pyrazole [52]. This finding indicated a different regulation of CYP2As in these species.

In minipigs, CYP2A expression is strongly gender-dependent, with the highest activity in females. In particular, Göttingen minipigs show marked differences (the females have 70-fold higher activity than males), but also Yucatan minipigs and conventional pigs (Landrace x Yorkshire x Duroc) show some significant differences [14, 31]. Experiments effected *in vivo* with male (castrated or non-castrated) Göttingen minipigs have shown that CYP2A is greatly - but reversibly - inhibited by androgens on a transcriptional basis, demonstrating a strong modulation by sex hormones in this species [50]. It has been proved that human chorionic gonadotropin is able to suppress hepatic CYP2A and 2E1 activities in various domestic pig breeds, probably through an increase in the levels of testicular steroids such as androstenone, dehydroepiandrosterone sulphate, oestradiol and oestrone sulphate [53, 54], thereby decreasing the metabolism of skatole and leading to its accumulation in adipose tissue. Another study revealed that only a slight decrease of CYP2A *in vitro* activity occurs incubating pig liver microsomes in presence of testicular steroids [55], whereas, using pig hepatocytes, a significant inhibitory effect of androstenone on CYP2A protein content has been demonstrated [56]. Recent microarrays experiments effected in pigs with extremely high levels of androstenone [57] have confirmed a down-regulation of CYP2A19 in Duroc but not in Norwegian Landrace pigs. This finding confirmed the relationship between CYP2A19 and androgens (the higher is androgen level, the lower is CYP2A19-dependent activity), but also highlighted some differences between various conventional pig breeds.

Polymorphisms

In humans, 7-hydroxylation of coumarin shows large inter-individual differences due to genetic polymorphisms (at least 20 different polymorphisms have been identified for CYP2A6, leading to no, decreased or unchanged enzyme activity) (http://www.cypalleles.ki.se/cyp2a6.htm).

Studies on three different polymorphisms identified in minipig CYP2A revealed that, unlike for human CYP2A6, the differences recorded on CYP2A19 activity are not due to genetic polymorphisms but rather to a different transcriptional regulation [43].

Interestingly, two different cDNAs were identified in Göttingen minipigs: one was completely homologous to conventional pig CYP2A19, whereas the other one encoded a truncated protein missing the last SRS (SRS6). The meaning of this deletion is not known, but this sequence may represent a CYP2A7 or CYP2A13 cDNA-like. Indeed, these two human isoforms are very similar to CYP2A6 (96% and 94% respectively) but they are poorly functional [40].

CYP2B SUBFAMILY

General Features

The literature about CYP2B subfamily in pig is limited. Pig CYP2B22, the only CYP2B identified in this species so far, has been isolated from a cDNA library of adult female pig (Landrace x Large White x Duroc) liver, and sequenced [15]. The isolated cDNA presented an ORF of 1482 bp and encoded a 493 amino acids protein. This isoform showed a higher similarity to human CYP2B6 (81.1% and 74% for nucleotide and amino acid sequences, respectively), rather than to rat or mouse CYP2Bs.

Tissue Distribution

The expression of CYP2B22 mRNA was confirmed in porcine liver and, at a lesser extent, in kidney by RT-PCR experiments in various domestic pig crossbreeds (Landrace x Large White x Duroc or Landrace x Large White) [15,58,59]. The constitutive presence of CYP2B22 mRNA has also been ascertained in porcine lung, bronchi, and trachea. Interestingly, in lung CYP2B22 is basically expressed at higher level than in liver, in agreement with data of other mammals [59]. Furthermore, CYP2B22 mRNA has been found at constitutive level in pig small intestine and in the olfactory and respiratory nasal mucosa [21,58]. Nannelli *et al.* [60] also demonstrated the expression of CYP2B22 in various pig brain regions including cortex, cerebellum, midbrain, hippocampus, meninges and cerebral capillaries. While in meninges and cerebral capillaries CYP2B22 mRNA levels are high and comparable to the hepatic ones, in the other compartment the basal expression is about or below 10% of the corresponding values in liver, in keeping with what has been reported for humans [61]. The high expression of CYP2B22 in blood-brain interfaces might have important implications for either pharmacological profiles of neuroactive drugs or the regulation of brain blood tension, as well as human CYP2B6 is involved in the formation of vasoactive arachidonic acid metabolites [62].

Substrates and Reactions

Dated reports failed to detect any CYP2B apoprotein or activity in primary cultures of pig hepatocytes or minipig (Göttingen) liver microsomes using an anti-rat CYP2B1 antibody or PROD as a marker reaction [32,22]. On the contrary, Donato *et al.* [23] were

able to measure another activity often used as marker of CYP2B subfamily, BROD, in both hepatocyte cultures and liver microsomes from Large White domestic pigs, although at much lower levels than in human samples. The BROD activity in porcine microsomes was about 1 pmol/min · mg protein whereas human activity was known to be about 12 pmol/min · mg protein. Bogaards *et al.* [31] tested EFCOD, a reaction catalyzed in humans mainly by CYP2B6 (but also by other P450 enzymes such as CYP1A2) on Yucatan minipig liver microsomes. The activity rate was similar in male and female minipigs, and comparable to that of humans (Km= 1.8-2.3 μM; Vmax= 286-353 pmol/min · mg protein). Furthermore, anti-rat CYP2B1 antibodies were able to moderately inhibit this activity (30-60%), suggesting that a CYP2B also takes part in this reaction in pigs. Another typical activity related to human CYP2B6, such as *S*-mephenytoin N-demethylase, has been measured in pig liver and, to a much lower extent, in kidney [58]. Turpeinen *et al.* [45] compared bupropion hydroxylase activity (a selective CYP2B marker activity in humans at 50 μM substrate concentration) in Göttingen minipigs and humans. The activity rate was higher in minipigs (435 pmol/min · mg protein on the average) than in humans (131 pmol/min · mg protein on the average).

An interesting work by Kawahigashi *et al.* [63] revealed that pig CYP2B22 is able to metabolize some herbicides including chlortoluron, amiprofos-methyl, pendimethalin, metolachlor, and esprocarb. These information could be useful for human health in order to produce herbicide-tolerant transgenic plants into which have been introduced particular CYP enzymes.

Inducibility and Regulation

Desille *et al.* [24] found CYP2B-related activity, mRNA, and protein present and inducible by PB in primary porcine hepatocytes. Also Behnia *et al.* [33] showed that PROD and BROD activities could be induced by PB in porcine hepatocytes after 8 days in culture, but not after 4 days, when a decrease of many activities was noted. Other evidence of the presence and inducibility of CYP2B in pigs have been found by treating commercial animals (Landrace x Poland China) with an induction cocktail containing PB, DEX and βNF [25]. EFCOD, PROD, and BROD activities and an apoprotein immunoreactive with anti-rat CYP2B1, were all increased in liver microsomal fractions obtained from the treated pigs. CYP2B22 has been also found markedly induced at transcriptional, protein and activity level in pig liver and kidney - but not in lung, bronchi and trachea - after an *in vivo* treatment with RIF, a known inducer of CYP3As through the nuclear receptor PXR [59]. Similarly, CYP2B22 was induced in pig liver at transcript and activity level after an *in vivo* treatment with PB, whereas in the small intestine the same treatment increased the mRNA level but not the enzymatic activity [58]. In the olfactory and respiratory porcine nasal mucosa, the lack of induction of CYP2B22 after an *in vivo* treatment with RIF, βNF, or PB has been ascertained at both transcriptional and activity level [21,58]. CYP2B22 has also been found to be resistant to induction by RIF in porcine brain [60].

In general, in humans the PB-dependent induction of CYP2B6 is mediated by the activation of the nuclear receptor CAR and its interactions with the PB-responsive enhancer modules (PBREMs) present in the 5'-flanking region of CYP2B6 [64]. Furthermore, a cross-talk of the nuclear receptor CAR with other nuclear factors (i.e. PXR, RXR, HNF4α and CCAAT/enhancer-binding protein alpha C/EBPα) has been observed [65,66]. Little information is available on the specific regulatory mechanisms of porcine CYP2B22. The lack of induction of CYP2B22 in pig airways and brain either by RIF, PB, or βNF could be due to the scarce expression of these nuclear receptors or to a more complex tissue-specific regulation [59,21,58].

CYP2C SUBFAMILY

General Features

The CYP2C subfamily is known to be very divergent amongst species and represents, perhaps, one of the largest and most complicated subfamilies, making it difficult to find a good mammalian model for the extrapolation of data to humans.

In humans, the CYP2C subfamily consists of CYP2C8, 2C9, 2C19 and the minor CYP2C18, accounting for about 20% of the total P450 hepatic content and playing a prominent role in the metabolism of 20-30% of all drugs [40].

In 1995, Zaphiropoulos *et al.* [67] used a couple of primers designed on rat CYP2C common regions to carry out some RT-PCR experiments on pig ovarian samples. Eleven sequences were isolated, eight of which were very similar to human CYP2Cs whereas the other three were more similar to rat CYP2C23. All these clones were subsequently classified as CYP2C32, CYP2C33, CYP2C34, CYP2C35, CYP2C36 and some allelic variants.

In 1998, Nissen *et al.* [68] isolated two CYP2C-like clones from a porcine small intestine cDNA library: one was the incomplete CYP2C42 (now available in the full-lenght form) and the other encoded a pseudogene. Kojima and Morozumi [15] cloned CYP2C49, the last porcine CYP2C sequence available in literature.

So far, only CYP2C33, 2C42 and 2C49 have been studied in detail, while the other sequences could be allelic or splice variants of these genes.

The comparative analysis revealed that CYP2C42 and CYP2C49 shared 80% of aa sequence identity between them, about 60% with CYP2C33, and about 77-81% with human CYP2C9 and 2C19. Furthermore, porcine CYP2C33 shared only about 62-64% of identity versus human CYP2Cs, suggesting differences in the substrate specificity of these enzymes. Diversely, CYP2C33 resulted more similar to whale CYP2C78 or cattle CYP2C86 [69], sharing an identity of 82-83% [58].

Tissue Distribution

CYP2C33 has been found expressed at mRNA level in porcine ovarian tissue [69]. Porcine CYP2C49 mRNA has been found highly expressed in pig liver and poorly expressed in pig kidney [15,58]. Pig CYP2C33, 2C42 and 2C49 mRNAs have also been found expressed, although at different levels, in small intestine and nasal mucosa (both respiratory and olfactory) [58]. A CYP2C member, not better identified, has been found expressed in porcine coronary arteries, as assessed by RT-PCR and Western blot analysis [70].

Substrates and Reactions

A study of Anzenbacher *et al.* [22] revealed the presence, in hepatic Göttingen minipig samples, of tolbutamide 4-hydroxylase activity - a human CYP2C9 marker activity - although at lower levels with respect to humans (62-136 pmol/min · mg protein versus 140-395 pmol/min · mg protein). Similarly, experiments with hepatic and renal porcine microsomes showed that pigs and minipigs were able to metabolize diclofenac (another specific substrate of human CYP2C9), but with lower rates compared to humans [31,58]. Indeed, diclofenac 4'-hydroxylase activity ranged from 20 to 60 pmol/min · mg protein in pigs and from 1.5 to 1.9 nmol/min · mg protein in humans. Other reactions usually used as CYP2C marker activities are 7-methoxy-4-(trifluoromethyl)coumarin O-demethylase (MFCOD) and omeprazole hydroxylase. The former is a very sensitive activity detected in pig (Landrace x Large White) liver, kidney, small intestine and nasal mucosa microsomes [58], whereas the latter (tested in Göttingen minipig) has been reported to be a good activity in liver microsomes [71]. On the contrary, no activity could be detected in pig or minipig liver microsomes using

S-mephenytoin, a substrate metabolized in humans by CYP2C19 [14,31,25]. However, this activity became detectable in liver microsomes after an *in vivo* pig treatment with PB suggesting the presence of a CYP2C19-like isoform also in this species [58]. The oxidation of paclitaxel and amodiaquine – two marker reactions of human CYP2C8 – did not lead to the formation of correspondent metabolites in both pig or minipig [58, 72].

So far, no porcine CYP2C has been cloned and expressed in a reconstituted system to characterize its enzymatic functions. However, the results on the expression and activities present in literature highlight some differences between human and pig CYP2Cs, suggesting that pig might not be a suitable model for the study of drugs metabolized in humans by this subfamily.

Pig CYP2C49, transgenically introduced into rice plants, has been demonstrated to be able to metabolize several herbicides including chlortoluron, norflurazon, amiprofos-methyl, alachlor, and isoxaben. This capability could have interesting consequences for human health, e.g. for the construction of herbicide-tolerant transgenic crops enriched with CYP species [63].

Inhibitors

Inhibition experiments carried out in Landrace x Large White hybrid pigs using inhibitors of human CYP2C such as quercetin, sulphaphenazole, ticlopidine and tranylcypromine have shown a different selectivity toward porcine CYP2Cs [11,58]. Tolbutamide hydroxylation could be inhibited in pigs by tranylcypromine – a CYP2C19 inhibitor – but not by sulphaphenazole – a CYP2C9 inhibitor [11,58]. Quercetin, a human CYP2C8 inhibitor, was able to significantly inhibit paclitaxel hydroxylation, tolbutamide hydroxylation (a CYP2C9-related activity) and S-mephenytoin hydroxylation (a CYP2C19-related activity), whereas ticlopidine and sulfaphenazole failed to inhibit all these reactions [58]. Anti-rat CYP2C11 was able to inhibit diclofenac 4'-hydroxylase activity - another marker of human CYP2C9 - in liver pig microsomes although at lower levels than in human microsomes [31].

Inducibility and Regulation

In primary porcine hepatocytes tolbutamide 4-hydroxylase activity has been found increased by treatment with RIF (a PXR ligand) and PB (a CAR activator), and decreased after treatment with βNF (an AhR agonist) [32]. The metabolism of tolbutamide and diclofenac in Landrace-Poland China pigs has been found slightly increased after treatment with a combination of PB, DEX and βNF [25]. By investigating in detail the inducibility by PB of CYP2C33, CYP2C42, and CYP2C49, a recent work [58] showed that in conventional pig liver and small intestine (Landrace x Large White hybrid pigs) these three isoforms are inducible at transcriptional and activity level. In addition, the treatment resulted in an up-regulation of mRNA levels of CYP2C42 and CYP2C49 (but not of CYP2C33) also in kidney, but not in respiratory and olfactory nasal mucosa, demonstrating a tissue- and isoform- differential regulation. In pig coronary endothelial tissue the expression of a CYP2C member, not better identified, resulted to be increased by cortisol in association with an endothelium-derived hyperpolarizing factor (EDHF) mediated relaxation of arteries [70]. These observations support the concept that an epoxygenase homologous to human CYP2C8/9 plays a crucial role in the generation of arachidonic acid-derived vasoactive metabolites.

With regard to the regulation mechanisms of CYP2Cs in pig very little is known. It has been suggested that the PB induction of pig CYP2Cs is primarily due to a tissue-specific activation of the nuclear receptors CAR, PXR and HNF4α, as found in humans [58]. Sex differences in the activity of CYP2Cs have been investigated using human CYP2C substrates: no sex differences were detected in conventional pigs whereas male minipigs showed higher activity than the females, in contrast to what had been seen for other CYP subfamilies in minipigs [14,71]. The regulation of the members of this subfamily by hormones, and particularly by androgens and growth hormones, has also been suggested for human CYP2Cs [73].

Polymorphisms

Human CYP2C19 is known to be strongly polymorphic, with the population divisible in poor, intermediate or extensive metabolizers depending on the functionality of the enzyme [74]. However, it is not known whether a pig CYP2C enzyme possesses a similar feature. It is possible that some of the sequences available in Genbank for porcine CYP2Cs constitute polymorphic variants. Further genomic studies are needed to ascertain the presence of relevant polymorphisms in pigs and to verify the real differences between the various breeds (domestic pigs, minipigs, microminipigs).

CYP2D SUBFAMILY

General Features

In humans, CYP2D6 is one of the most important enzymes for drug metabolism, accounting for the metabolism of about 15% of all drugs. Its genetic polymorphism is one of the most relevant clinical human polymorphisms of oxidative drug metabolism, and it is known not to be inducible at all [40].

In 1997, a cytochrome P450 which catalyzed 25-hydroxylation of vitamin D3 has been cloned from domestic pigs and expressed in simian COS cells [75]. Even if human CYP2D6 can not catalyze vitamin D3 25-hydroxylation, the cloned porcine CYP showed 70-80% identity with the mammalian members of the 2D subfamily and was subsequently named CYP2D25. Site-directed mutagenesis studies have revealed that residues in SRS3 resulted crucial for the function of the enzyme in vitamin D3 metabolism, but not for tolterodine metabolism (a substrate of human CYP2D6) [76]. In 2004, the hepatic CYP2D21 has been cloned from Göttingen minipig and expressed in yeast cells [77]. This CYP2D21 showed 97.8% identity of amino acid sequence with the domestic pig CYP2D25. There were 10 amino acid differences, and one was located in the putative SRS3: Gln204 or Leu204 for CYP2D21 or CYP2D25, respectively. It remains to ascertain if these two enzymes are allelic variants with a distinct substrate specificity.

Tissue Distribution

Initially, Skaanild and Friis [14] suggested the absence of CYP2D in pig liver by immunoblotting experiments using monoclonal anti-human CYP2D6 antibodies. Later, the lack of response in this immunoblotting test has been confirmed and indicated to be due to differences in the structure of pig and human CYP2D [78]. Northern blot analysis have shown that CYP2D25 mRNA is present in porcine liver and, to a lesser extent, in kidney [75]. RT-PCR experiments have revealed that CYP2D25 mRNA is also expressed in small amounts in porcine adrenals, brain, heart, intestine, lung, muscle, spleen, and thymus [79].

Substrates and Reactions

Pig CYP2D25 was able to catalyze 25-hydroxylation of vitamin D3 [75] and multiple hydroxylations of 25-hydroxyvitamin D3 [80]. Other reactions catalyzed by CYP2D25 are 25-hydroxylation of vitamin D2 and the conversion of tolterodine into the 5-hydroxymethyl metabolite (a reaction also catalyzed by human CYP2D6) [79]. The enzyme activity of CYP2D in pigs has been analysed using three classical human CYP2D6 test substrates (debrisoquin, bufuralol and dextromethorfan), and differences with human CYP2D6 have been pointed out. No metabolism of debrisoquin could be detected in both Göttingen minipigs or Landrace x Yorkshire x Duroc crossbred conventional pigs [14]. Bufuralol 1'-hydroxylation activity has been found higher in Göttingen or Yucatan minipig liver microsomes than in those of human origin (770 pmol/min mg prot versus 182 pmol/min · mg protein, on the average) [22,31]. However, correlation analysis between dextromethor-

fan or bufuralol microsomal oxidation rates and the protein content obtained with anti-human CYPs polyclonal antibodies indicated that these activities in pigs may be attributed primarily to CYP2B rather than to CYP2D [78]. The recombinant CYP2D21 enzyme has shown bufuralol 1'-hydroxylase activity, confirming that minipig possesses a CYP2D enzyme able to metabolize a human CYP2D6 substrate, although the contribution to this enzyme was smaller than that of other CYPs, such as CYP2B [77]. Thus, it is quite clear that pig is not the best model for the study of processes involving CYP2D in humans since pig CYP2D(s) are different from the human counterpart.

Inhibitors

Tolterodine was able to inhibit the microsomal 25-hydroxylation of vitamin D2, whereas quinidine - a human CYP2D6 inhibitor - did not markedly inhibit the reaction [79]. Jurima-Romet *et al.* [81] noticed an inhibition of dextromethorfan O-demethylation by quinine and quinidine (known human CYP2D6 inhibitors) in Yorkshire x Landrace pig liver microsomes and primary hepatocytes cultures. Orphenadrine, pilocarpine and resveratrol (CYP2B inhibitors in humans, mice and rats respectively) have been shown to inhibit dextromethorphan O-demethylation in Göttingen minipig liver microsomes [78].

Inducibility and Regulation

Monshouwer *et al.* [32] found dextromethorfan O-demethylation induced by PB and RIF although these compounds do not induce human CYP2D6. An induction cocktail containing PB, DEX and βNF had no effect on the expression of CYP2D in domestic pigs at protein and activity level [25]. It has been demonstrated that PB treatment was able to suppress the activity of CYP2D25 (25-hydroxylation of vitamin D3) in pig hepatocytes via a direct enzyme inhibition and transcriptionally down-regulating the gene promoter via a mechanism involving PXR or CAR [82]. This could explain, at least in part, the PB-induced vitamin D deficiency observed in humans.

CYP2E SUBFAMILY

General Features

The only known member of CYP2E subfamily is CYP2E1 - an isoform responsible in humans for the metabolic activation of many low molecular weight compounds suspected to act as chemical carcinogens. CYP2E1 is one of the most conserved P450 enzymes according to the primary structure, exhibiting nearly 80% sequence identity across species [83], and literature data suggest a species-conserved mechanism for oxidative biotrasformation by CYP2E1 [84]. It is a major catalyst of the oxidation of ethanol, benzene, styrene, chloroform and other chlor derivates of methane and ethane, and it is known to take part in the activation of nitrosamines [85].

In 2004, CYP2E1 was cloned from Landrace x Large White x Duroc crossbred domestic pig by Kojima and Morozumi [15]. A year later, CYP2E1 was also isolated from Göttingen minipig liver microsomes and the respective cDNA was cloned and sequenced [86]. Both porcine CYP2E1 were just two residues shorter than the human orthologous, and the only difference between minipig and pig sequence was the presence of Asp346 in the former instead of Val346 in the latter. However, the differences in the primary structure of the pig enzyme with respect to the human counterpart do not include the key amino acid residues predicted to interact with a human CYP2E1 typical substrate, chlorzoxazone [86]. A study revealed how the recombinant porcine CYP2E1 is more stable than the human CYP2E1, and that the human form has a greater compressibility in the heme active site than the porcine one [87].

Tissue Distribution

Porcine CYP2E1 mRNA has been found highly expressed in pig liver and, at lower levels, in kidney [15]. The presence of the protein in the hepatic tissue was confirmed immunochemically by various authors [25,26]. Chlorzoxazone 6-hydroxylase activity has also been detected in some Göttingen minipig extrahepatic tissues such as kidney, lung, small intestine, brain, and leukocytes with the highest level – besides liver – in kidney [88,89].

Substrates and Reactions

Both pig and minipig enzymes were able to convert two prototypical substrates of human CYP2E1, chlorzoxazone and *p*-nitrophenol, into the respective metabolites in liver microsomal fractions or reconstituted systems with rates similar to those obtained with the human enzyme [13,22,23,31,45,86-90,91]. Indeed, chlorzoxazone hydroxylase activity ranged across pig breeds from 0.3 to 6.0 nmol/min · mg protein whereas in humans this activity varies from 1.2 to 3.1 nmol/min · mg protein. Porcine *p*-nitrophenol hydroxylase activity varied from 0.2 to 0.7 nmol/min · mg protein while the same activity ranges from 0.1 to 0.4 in humans. Furthermore, *p*-nitrophenol hydroxylase activity has been found at comparable levels in porcine and human hepatocytes cultures [23]. Probably, pig CYP2E1 is not the only enzyme involved in the hydroxylation of chlorzoxazone and *p*-nitrophenol. Indeed, CYP2A is also demonstrated to contribute to these activities [90, 92], and bupropion (metabolized at high concentration by human CYP2E1) seems to be a more specific substrate for porcine CYP2E1 [92]. However, recent experiments by Köhler *et al.* [89] performed with diethyldithiocarbamate indicated that chlorzoxazone 6-hydroxylation was predominantly due to CYP2E1 in Göttingen minipig liver, kidney and lung microsomes. CYP2E1 has been identified as one of the main enzymes involved in the metabolism of skatole, whose accumulation in non-castrated male pigs is a major cause of boar taint, along with CYP2A19 and CYP1A2 [47,46,30].

Inhibitors

Neither α-naphthoflavone nor furafylline, inhibited chlorzoxazone 6-hydroxylation in Yucatan minipig hepatic microsomes [31]. Nevertheless, ketoconazole and troleandomycin - two CYP3A inhibitors - were able to markedly inhibit chlorzoxazone hydroxylase activity [31]. Also 4-methylpyrazole (a known CYP2E1 inhibitor) inhibited p-nitrophenol hydroxylase activity in primary cultured porcine hepatocytes [46]. Propofol (2,6-diisopropylphenol), a widely used intravenous anesthetic agent, markedly inhibited CYP2E1-dependent hydroxylation of chlorzoxazone in both human (Ki = 48 μM) and porcine (Ki = 19 μM) hepatic microsomes [93]. The inhibition of chlorzoxazone 6-hydroxylation with diethyldithiocarbamate (a typical inhibitor of human CYP2E1) gave comparable Ki values for Göttingen minipig and human enzymes, indicating a similarity between porcine and human CYP2E1 [86].

Inducibility and Regulation

An *in vivo* study in domestic pigs has shown a marked induction by PB of CYP2E1 mRNA and apoprotein levels, whereas no changes have been found in the metabolism of chlorzoxazone [24]. On the contrary, an induction cocktail containing βNF, PB and DEX had no effect on the expression of CYP2E1 in conventional pigs (Landrace x Poland China pigs) [25]. In pig hepatocytes, skatole enhanced CYP2E1 at protein level [94]. As in humans, ethanol feeding was able to induce hepatic CYP2E1, and this has been demonstrated in both castrated and non-castrated Yucatan and Göttingen minipigs [88, 89, 95,]. Ethanol also induced chlorzoxazone hydroxylase activity in minipig brain whereas it led to a repression of this activity in the lung [89]. The same effect was obtained with isoniazide, a known inducer of human CYP2E1. In particular, in

liver, CYP2E1 activity was increased by ethanol and by isoniazide whereas the protein level remained unaffected. Hence, CYP2E1 also appeared to be regulated by post-translational mechanisms. Nevertheless, a very weak correlation has been found between the capacity to metabolize chlorzoxazone and the content of protein or the mRNA expression [14], thus indicating that the regulation of CYP2E1 expression could take place at all steps in the protein biosynthesis, as found in humans [96]. In addition, a study on hnRNA (heterologous nuclear RNA) and mRNA of CYP2E1 in Göttingen minipigs has suggested that splicing and/or modulation of mRNA stability could be involved in CYP2E1 regulation [97].

Human chorionic gonadotropin stimulation has been demonstrated to suppress hepatic CYP2E1 in pubertal male pigs, probably through an increase in testicular steroid levels [54], since it has been previously observed that androstenone and 17β-oestradiol exerted an inhibitory effect on CYP2E1 enzymatic activity, although with two different mechanisms [98,55]. A functional analysis of pig CYP2E1 gene promoter has revealed two activating elements, one of which was able to bind the hepatic nuclear factor 1 (HNF-1) and the other was identified as a binding site for the chick ovalbumin upstream promoter transcription factor 1 (COUP-TF1) [99]. In addition, it has been demonstrated that androstenone was able to inhibit the binding on COUP-TF1, without affecting HNF-1 binding, thus providing an explanation for the inhibition of CYP2E1 protein expression by androstenone in pig hepatocytes.

Some differences have been pointed out between humans and pigs on CYP2E1 expression and activity [14]: humans and conventional pigs did not present sex differences in chlorzoxazone hydroxylase activity, whereas in Göttingen minipigs the same activity was much higher in females than in males.

Polymorphisms

Some functional polymorphisms of porcine CYP2E1 have been identified as a possible strategy toward the selection of genetic markers used to reduce boar taint without affecting the levels of sexual hormones [100,101].

CYP3A SUBFAMILY

General Features

CYP3A4 represents in humans the most important P450 enzyme with regard to the presence (it amounts to approximately 30% of total hepatic CYP content) and to the metabolic function (it is able to metabolize about a third of all drugs). For this reason, CYP3A is one of the most studied subfamilies in humans, and it is also necessary to characterize it in pigs.

The first porcine CYP3A sequenced and mapped was CYP3A29, isolated from a pig small intestine cDNA library [68]. The translation of the ORF of CYP3A29 resulted in a protein of 503 amino acids, which showed a very high similarity to CYPs belonging to the CYP3A subfamily, and in particular to human CYP3A4 (only monkey CYP3A8 scored better) [91]. In addition, the deduced N-terminal amino acid sequence of pig CYP3A29 matched very well with the one of human CYP3A4 sharing a 60% similarity (12 of 20 amino acids identical) [42]. CYP3A29 was also cloned from Göttingen minipig liver [91]. Pig and minipig CYP3A29 were different in just eight amino acid residues, six of which were conservatives. Besides CYP3A29, there are four other porcine CYP3A sequences available in Genbank, named CYP3A22, 3A39, 3A46, and 3A88. However, CYP3A88 is likely to be an allelic variant of CYP3A46. CYP3A22 has been isolated from a Göttingen minipig liver cDNA library and showed 81.5, 83.9, and 76.5% amino acid sequence identities with pig CYP3A29, 3A39, and 3A46, respectively [77]. Because of the high sequence similarity between CYP3A39 and CYP3A46 (93% nucleotide sequence identity) the expression of CYP3A39 has not yet been studied.

Tissue Distribution

CYP3A22, 3A29 and 3A46 mRNAs have been found in Landrace x Large White crossbred pig liver and several extrahepatic tissues, such as kidney, lung, bronchi, trachea, intestine, heart, spleen, respiratory nasal mucosa and olfactory nasal mucosa [21,58, 59,102]. These three isoforms have been also found constitutively expressed in various brain regions, although at much lower levels than in liver [60]. Interestingly, a CYP3A-related activity (benzyloxyquinoline debenzylase, BQD) was found particularly high in porcine cortex capillaries, suggesting a protective role of CYP3As towards the entrance of xenobiotics in the brain. In porcine brain, unlike liver, higher levels of CYP3A-related activity have been found in mitochondria compared to microsomes [60]. Thus, an important role of CYP3As in the metabolism of brain endogenous substrates, such as neurosteroids has been hypothesized. CYP3A29 mRNA has also been detected in adrenal gland, skin, testis, uterus and ovary of different age groups of Bama miniature pig [103]. CYP3A4 is the major form of P450 expressed in human enterocytes representing about 70% of intestinal CYPs [104], and also in porcine small intestine CYP3A22, 3A29, and 3A46 mRNAs were well expressed [58,102]. A diminishing gradient expression of porcine CYP3A29 along the intestine has been observed [102, 105], in agreement with experiments on human intestine [106].

A very recent work by Hermann and Skaanild [107] has demonstrated an interesting similarity between human CYP3A4 and porcine CYP3A29. The CYP3A4 expression is very low during foetal development and becomes the dominant CYP3A isoform in adult liver, whereas the opposite is for human CYP3A7. Their findings demonstrated that minipig CYP3A29 had a pattern of expression comparable to CYP3A4 in humans. In addition, antibodies raised against human CYP3A7 revealed the presence of an isoform (CYP3A22?) which followed the pattern of CYP3A7 in foetal and adult minipig samples. Also experiments with the recombinant porcine CYP3A29 indicated that this isoform is similar to human CYP3A4 with regard to expression and activity, and that CYP3A29 gives the greatest contribution of CYP3A activity in pig hepatic microsomes [102].

Substrates and Reactions

Testosterone 6β-hydroxylation (the most widely used marker reaction for human CYP3A4) and nifedipine oxidation have been measured in different strains of minipigs and conventional pigs. Both the substrates were metabolized with levels of activities comparable to humans, although with some differences depending on the breeds of pigs [14,22,24,25,31]. Testosterone 6β-hydroxylase activity in pigs ranged from 0.45 to 4.45 nmol/min · mg protein, which compare well with the average activity in humans (1.7 nmol/min · mg protein). Also the average porcine nifedipine oxidation rate (1.5 nmol/min · mg protein) was comparable to that of humans (1.72 nmol/min · mg protein). These two enzyme activities were well correlated amongst themselves and with the immunochemical levels of pig CYP3A, as assessed with anti-human CYP3A4 antibodies [14]. Another drug specific substrate of human CYP3A4 – lovastatin – has been recently used to validate Bama minipigs as a model for drug evaluation in humans [108]. The results showed that the metabolites and the enzyme kinetic parameters were similar in pigs and humans. Apart from testosterone, other sexual hormones can be metabolized by CYP3A4, such as 17 alpha-ethynylestradiol (EE2) [109]. The increase in 2-hydroxylation of EE2 after the exposure of porcine primary hepatocytes to various CYP3A inducers has been found to be well correlated with the increase in 6β-hydroxylation of testosterone and with the increase in the protein level of CYP3A detected by a monoclonal anti-human CYP3A4 antibody, thus confirming the 2-hydroxylation of EE2 in pigs as being biotransformed by a CYP3A, as in humans [110]. Other reactions known to be catalyzed mainly by CYP3As in rat

and/or humans are BQD and erythromycin demethylase (ErD) [111,112]. While ErD activity was only detectable in pig liver because of its low sensitivity, BQD was also detected in extrahepatic tissues of domestic pigs [21,58-60].

Both minipig and pig CYP3A29 have been purified or expressed in Sf9 insect cells, respectively, and analysed in a reconstituted system [91,102]. The enzymes exhibited testosterone 6β-hydroxylation and nifipidine oxidation, as reported for human CYP3A4. However, domestic pig CYP3A29 exhibited a higher affinity (Km) for testosterone than minipig CYP3A29, thus suggesting that the domestic pig enzyme could be more similar to human CYP3A4 than the minipig enzyme [102].

Inhibitors

Ketoconazole and triacetyloleandomycin (TAO) (typical human CYP3A4 inhibitors) were able to inhibit 6β-hydroxylation of testosterone and N-oxidation of nifedipine, fairly indicating that one or more CYP3A-like enzymes are also responsible for these two reactions in pigs [22,31]. A very recent work [102] has confirmed the efficiency of these two inhibitors toward recombinant CYP3A29 activity, and ketoconazole has been identified to be more potent than TAO. While the inhibition of testosterone 6β-hydroxylation by ketoconazole has been found to be as strong as in humans [31], TAO inhibited nifedipine oxidation with lower potency in minipig microsomes compared to human ones, indicating structure variations in the active sites of the CYP3A enzymes [22]. TAO was also able - both in pig and minipig microsomes - to strongly decrease the formation of two metabolites of paclitaxel formed in humans by CYP3A4 [72]. TAO was well able to inhibit the metabolism of lovastatin, further confirming the efficiency and specificity of this inhibitor [108].

Inducibility and Regulation

The expression and activities of CYP3As have been found to be induced in primary porcine hepatocyte and enterocyte cultures after treatment with DEX, RIF or PB, all drugs able to induce human CYP3As [32,35,38,110,113, 114]. More recent works have revealed that CYP3A22, 3A29, and 3A46 are inducible at transcriptional and activity level by an *in vivo* pig treatment with RIF and PB in liver but not in nasal mucosa (respiratory or olfactory), lung, bronchi, trachea, and kidney [21,58-60]. In the small intestine, the treatment with PB resulted in an increase of mRNA of these three genes, but not of the related activities [58]. Interestingly, CYP3A22 and CYP3A29 (but not CYP3A46) mRNAs have been found significantly increased by RIF in pig cerebral cortex and hippocampus, but not in cerebellum, midbrain, and blood-brain interfaces, indicating a differential regulation of these CYPs in the various regions of brain. However, no induction of BQD activity has been observed in the brain regions [60].

Little is known about the regulation mechanisms of CYP3As in pigs. In humans, the induction of CYP3A genes generally occurs at transcriptional level through the activation of PXR or CAR and the contribution of other nuclear factors (e.g. HNF4α). As a good expression of these nuclear receptors has been demonstrated in porcine liver [59], probably the induction mechanisms of CYP3As in pigs are similar to those shown in humans. The lack of induction of CYP3A-dependent activities observed in the above-mentioned porcine extrahepatic tissues could be due to the scarce expression of these nuclear receptors or to the involvement of other tissue-specific transcriptional factors. As in humans, no sex differences in the expression and activity of CYP3As have been found in pigs or minipigs [14]. However, the castration of pigs has been demonstrated to determine a slight increase in CYP3A mRNA, protein, and activity [50].

CYP4A SUBFAMILY

General Features

Isoforms belonging to the CYP4A subfamily have been identified in different species including humans, rodents, monkeys and dogs [115]. Enzymes of this subfamily play a key role in bile acid biosynthesis and in the metabolism of fatty acids (mainly ω-hydroxylation and, to a lesser extent, (ω-1)-hydroxylation). Members of the CYP4A subfamily also catalyze the oxidation of arachidonic acid leading to the formation of physiologically important metabolites involved in blood flow regulation [116]. The most common marker activity of CYP4As is lauric acid 12-hydroxylase, which seems to be generally conserved amongst species [31].

So far, three CYP4A isoenzymes have been identified in pigs: CYP4A21, 4A24 and 4A25. Nevertheless, it is still unclear whether CYP4A25 constitutes a different isoform or is a variant of CYP4A24. In 2001, Lundell *et al.* [117] cloned CYP4A21 from pig liver. The cDNA encoded a protein of 504 amino acids, which shared a high similarity (74% of identity) with the human CYP4A11. Later, pig CYP4A24 and 4A25 were cloned by Lundell [118]. These enzymes shared an extensive sequence identity (approx. 99% nucleotide identity and 97% amino acid identity) and about 94% identity with CYP4A21. The amino acid differences between the two sequences were found in N- and C-terminal regions, confined to β-sheets 1 and 4, indicating a possible difference in substrate specificity or regioselectivity. The porcine CYP4A21, 4A24 and 4A25 have probably evolved from a common ancestral gene - maybe by gene duplication - in conjunction with species-specific habits, as indicated also by a study [119] in which CYP4A21 and 4A24 revealed a sequence identity that extends beyond the exons.

Tissue Distribution

The expression of CYP4A21 mRNA was found in pig kidney but not in heart, muscle, intestine, spleen, thymus, lung, or adrenal gland [117]. CYP4A24 and 4A25 mRNAs have been found in both pig liver and kidney [118]. With regard to the abundance, CYP4A21 seemed to be the major liver CYP4A, whereas CYP4A24 has been found more abundant in kidney [118]. The expression of a CYP4A protein, not better identified, has been demonstrated in porcine coronary arteries with immunoblotting experiments [121].

Interestingly, it has been demonstrated that, in pig liver, CYP4A21 has a developmental-dependent expression opposed to CYP8B1 (a sterol 12α-hydroxylase which is a key step in the formation of cholic acid, tha major bile acid in humans), as assessed by RT-PCR experiments. CYP8B1 was well expressed in the foetal samples but no signal was detected in weaned pig liver, determining the lack of cholic acid formation in adult pigs. On the contrary, the expression of CYP4A21 (an enzyme involved in the production of hyocholic acid) was much higher in weaned than in foetal pig liver [120]. Certainly, further studies are needed to clarify the mechanism underlying the shift of expression of these two enzymes and to understand its biological meaning.

Substrates and Reactions

Porcine CYP4A21 has been cloned and expressed in a recombinant system [117]. This enzyme was able to hydroxylate taurochenodeoxycholic acid in 6α position, leading to the formation of hyocholic acid (the species-specific primary bile acid in the pig). Despite the high similarity between the porcine CYP4A21 and the human CYP4A11, the former showed no ω-hydroxylase activity toward lauric acid and other fatty acids whereas the latter showed no 6α-hydroxylase activity toward taurochenodeoxycholic acid. Mutagenesis experiments indicated that three amino acid substitutions in a region around position 315, which was highly conserved

in all previously known CYP4As, could be involved in this specific catalytic activity of CYP4A21 [117]. On the contrary, CYP4A24 and 4A25, expressed in yeast cells, exhibited (ω-)- and (ω-1)-hydroxylase activities towards lauric acid and palmitic acid, but not hydroxylase activity towards taurochenodeoxycholic acid, like human CYP4A11 [118]. In particular, microsomal preparations from CYP4A24/25 transformed yeast cells revealed an average lauric acid ω-hydroxylation rate of 286 pmol/min · mg protein, whereas the average palmitic acid ω-hydroxylation rate was 113 pmol/min · mg protein.

In humans, the CYP4A subfamily plays a role in the regulation of vasal tone participating in the formation of vasoactive metabolites of arachidonic acid (in particular in the formation of the vasoconstrictor 20-HETE, 20-hydroxyeicosatetraenoic acid) [122]. In porcine coronary arteries, the formation of 20-HETE has been found to be reduced after a treatment with N-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS), an inhibitor of CYP4A, suggesting that porcine CYP4A could also be involved in the formation of 20-HETE [121].

Inducibility and Regulation

To date, very little is known about the induction and regulation of CYP4As in pigs. An induction cocktail containing βNF, PB and DEX had no effect on the protein level of liver CYP4As, as assessed with anti-rat CYP4A1/3 polyclonal antibodies, although the metabolism of lauric acid was higher in hepatic S10 preparations from treated pigs compared with controls (study performed using Landrace x Poland China crossbred pigs) [25]. Also the pig (Yorkshire x Landrace) administration of clofibrate, a drug which is strong inducer of CYP4As in mice and rats through the activation of PPARα, failed to induce the mRNA expression of hepatic CYP4As [123]. The unresponsiveness of porcine CYP4A genes to peroxisome proliferator agonists is similar to that shown for CYP4As in human hepatocytes [124].

NUCLEAR RECEPTORS

General Features

Most of CYP isoforms are mainly regulated in a transcriptional way through the action of nuclear receptors acting as transcription factors. In humans, members of CYP1, 2, 3, and 4 families are primarily regulated by AhR, CAR, PXR, and PPARα, respectively. However, the regulation mechanisms are complicated by the involvement of many other nuclear factors [e.g. retinoid X receptor (RXR), HNF4α, etc.] and by the overlap existing for the sets of target genes of these receptors.

Expression

The mRNA expression of both porcine CAR and PXR has been detected in liver, small intestine, kidney, lung, bronchi, trachea, nasal mucosa and various regions of pig brain by using RT-PCR, with the greatest expression in liver followed by kidney [21, 58-60, 125,126]. It is worth noticing that high levels of mRNA expression have been found in porcine blood-brain interfaces like meninges and cortex capillaries (25-150% of liver expression), indicating a possible role in the regulation of genes involved in physiological functions of the brain [60], whereas human CAR and PXR have turned out to be just detectable in human brain [127]. In addition, Northern blot analysis have shown a fair PXR expression in pig heart, colon, and stomach, while a minor expression has been observed in mandibular lymph node, thymus, adrenal gland, ovary, uterus, bladder, spleen, and mesenchymal lymph node [128].

Porcine AhR, whose gene has been only partially sequenced so far [17], has been found expressed at transcriptional level in liver, lung, heart, kidney, brain, ovary, thyrocytes, artery endothelial cells, olfactory and respiratory nasal mucosa [17, 19, 21,129-131].

Pig HNF4α mRNA has been detected in liver, kidney, lung, bronchi, trachea, nasal mucosa, and small intestine [21, 58, 59, 132]. The resulting pattern of expression was in agreement with human data [127], showing the higher expression in liver, kidney, and small intestine and a very low presence in the other tissues.

Porcine PPARα mRNA has been found highly expressed in liver and kidney, variably expressed in small and large intestine, moderately expressed in heart and skeletal muscle, and barely expressed in brain, spleen, lung and adipose tissue [133]. Furthermore, PPARα mRNA has been found expressed in vascular endothelial cells [131]. The quantification of PPARα mRNA in the liver revealed that PPARα expression was higher in pigs than in rats and mice which, in turn, were reported to have a higher expression than in humans [123]. A study performed using two different breeds of pigs (Duroc and Norwegian Landrace) revealed an age- and breed-dependent expression of PPARα in liver and heart [133]. This finding could reflect a difference between the two breeds with respect to the tissue distribution of fat and the use of fatty acids as an energy source in piglets.

Structure and Functions

Porcine CAR and PXR have been cloned from cDNA libraries and characterized [126]. The porcine CAR gene comprised a 1047 bp coding region that encodes a protein of 348 amino acids. The mRNA sequence of porcine PXR was composed of 1266 bp putative coding region encoding 421 amino acids. The porcine CAR and PXR proteins showed a high degree of sequence identity in their DNA-binding domain (DBD) and ligand-binding domain (LBD) with 80%-90% amino acid identity with respect to human ones. In particular, porcine PXR has turned out to be more similar to human PXR than mouse PXR (87% vs. 77% identity) and to contain the human residues at four locations (R203, P205, Q404, and Q407) which are keys for human PXR activity [128]. The complete sequence of pig HNF-4α mRNA, which is available in Genbank (accession n. DQ061106), encodes a protein of 474 aa with about 75% homology to that of human HNF-4α [132]. The cDNA containing the ORF of PPARα has been isolated from Duroc and Norwegian Landrace pig liver [133]. The deduced protein sequence of 468 aa showed a high identity with the human counterpart (92.3%). An alternative spliced mRNA lacking exon 5 (presumably determining a C-terminal truncated protein) was detected in several porcine tissues, but further studies are needed to ascertain its potential effect on the functionality of the wild-type form.

A study performed with Yorkshire pigs revealed that the activation response of porcine CAR by CITCO (a specific agonist of human CAR) and other nine ligands out of twelve was similar to the response of human CAR. On the other hand, for the murine CAR only five ligands were in common with human CAR [134]. Furthermore, a pig treatment with TCPOBOP (1,4-Bis-[2-(3,5-dichloropyridyloxy)]benzene,3,3′,5,5′-Tetrachloro-1,4-bis(pyridyloxy)benzene) (a specific murine CAR ligand) had no effects on CAR-regulated activities, thus suggesting that porcine CAR is more similar to human CAR than the murine orthologous [50, 125,134]. The inducibility of CAR, PXR, AhR and HNF4α transcripts, along with their target genes, has been investigated in many pig tissues by using classical P450 inducers, such as RIF, PB and βNF [17, 19, 21, 58-60]. In all these studies (performed with Landrace x Large White domestic pigs), the nuclear receptors mRNA levels were not affected by the treatment, in agreement with human data [135].

In general, in liver, the induction of CAR-, PXR-, and AhR-regulated genes after the exposure to the classical inducers reflects the abundant expression of the nuclear receptors in this organ. In the extrahepatic tissues, the regulation of the same genes is probably more complex and limited by either the lower levels of these main receptors or by the need of other tissue-specific factors. Also single nucleotide polymorphisms (SNPs) and splice variants (SVs) of nuclear receptors can alter biological function, representing a

new layer of complexity. Porcine PXR gene has been characterized in several pig breeds, and revealed multiple SVs in the ligand-binding domain, as well as human PXR [128,136]. Of the five identified porcine PXR SVs, SV1 was able to significantly increase the transactivation of the wild-type form [136]. All human and porcine PXR SVs were species-specific, suggesting that SVs might act as a species-specific mechanism for adaptation to different environmental exogenous compounds encountered by each species. Only one nonsynonymous SNP (S178L) has been found in the pig PXR ligand-binding domain, but further investigations are needed to determine its eventual effect [128]. Skatole has been recently identified as a novel inverse agonist for pig PXR, as well as for pig CAR [136]. Porcine CAR multiple SVs has also been recently identified, each of which generated a truncated protein [134]. These five variants were found present in pig liver samples from about 5 to 9% of total pig CAR, suggesting that they might play a functional role *in vivo*. In particular, SV2 has been found to significantly decrease the activity of the wild-type protein. This may represent another level of transcriptional regulation, by which SVs are generated to limit the activity of the receptor.

CONCLUSIONS

From the information collected in this review it appears that (mini)pig could actually represent a new prime large animal model for future drug metabolism and pharmacological studies. On the basis of substrates, inducers, inhibitors, tissue distribution and regulation data - as summarized in Tables **1-5** - no major qualitative differences among the CYP1A1, 1A2, 2B, 2E1 and 3As of pig compared to human orthologues have been revealed so far. However, a note of caution has to be put forward in relation to a strict functional similarity among these orthologous CYPs. This tentative conclusion must be balanced by the observation that even a simple substitution of a single amino acid, especially in the active site of these enzymes, might dramatically change the substrate specificity. It should also be kept in mind that: i) several drugs are metabolised to different products by multiple CYPs reflecting their tissue-specific expression and specific rates; ii) structurally different CYPs may catalyse the same reaction, with their own specificity and rate. Thus, with this caution, the porcine experimental data deriving from the drug biotransformation by the above mentioned CYPs may be considered useful for humans. On the contrary, drug metabolic profiles primarily due to the catalysis of CYP2A, CYP2Cs and CYP2D in pig, may not at all reflect what occurs in human and therefore pig appears to be an unsuitable model for the metabolism of these drugs in human. As to the catalytic similarity between pig and human CYP1B1 and CYP4As, too limited evidence are available to give a clear indication.

Besides the oxidation of xenobiotics, many enzymes belonging to the cytochrome P450 superfamily are also involved in the metabolism of several endogenous compounds implicated in the regulation of important physiological pathways, such as hormones, sterols, vitamins, fatty acids and vasoactive molecules. Since pig is recognized as a useful model for human pathologies, it becomes significant to extend the characterization of CYPs beyond the first four families. Furthermore, the detailed knowledge of the important enzymatic system of cytochrome P450 is not sufficient to face all the potential applications of the pig as an animal model. Further studies are needed to extend the characterization to other phase-1 enzymes (such as flavin-containing monooxygenases, monoamine oxidases, epoxide hydrolase or aldehyde dehydrogenase), for which only limited information is available in literature. Also, an in-depth examination of phase-2 enzymes (such as UDP-glucuronosyltransferases, glutathione S-transferases or sulfotransferases) and drug transporters (the so-called phase-3 of metabolism) needs to be carried out in pigs.

ABBREVIATIONS

3-MC	=	3-Methylcholanthrene
3-MI	=	3-Methylindole
20-HETE	=	20-Hydroxyeicosatetraenoic acid
aa	=	Amino acid
AhR	=	Aryl hydrocarbon receptor
BALs	=	Bio-artificial livers
βNF	=	β-naphthoflavone
BQD	=	Benzyloxyquinoline debenzylase
bp	=	Base pairs
BROD	=	7-benzyloxyresorufin *O*-debenzylase
C/EBPα	=	CCAAT/enhancer-binding protein alpha
CAR	=	Constitutive androstane receptor
cDNA	=	Complementary deoxyribonucleic acid
CITCO	=	(6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde-*O*-(3,4-dichlorobenzyl)oxime)
COUP-TF1	=	Chick ovalbumin upstream promoter transcription factor 1
CYP	=	Cytochrome P450
DBD	=	DNA-binding domain
DDMS	=	N-methylsulfonyl-12,12-dibro-mododec-11-enamide
DEX	=	Dexametazone
e.g.	=	*Exempli gratia*
EDHF	=	Endothelium-derived hyperpolarizing factor
EE2	=	17 alpha-ethynylestradiol
EFCOD	=	7-ethoxy-4-(trifluoromethyl)coumarin O-deethylase
ErD	=	Erythromycin demethylase
EROD	=	Ethoxyresorufin O-deethylase
HNF	=	Hepatic nuclear factor
hnRNA	=	Heterologous nuclear RNA
i.e.	=	*id est*
LBD	=	Ligand-binding domain
MROD	=	Methoxyresorufin O-demethylase
MFCOD	=	7-methoxy-4-(trifluoromethyl) coumarin O-demethylase
mRNA	=	Messenger ribonucleic acid
ORF	=	Open reading frame
P450	=	Cytochrome P450
PAH	=	Polyaromatic hydrocarbons
PB	=	Phenobarbital
PBREM	=	PB-responsive enhancer module
PPARα	=	Peroxisome proliferator-activated receptor alpha
PROD	=	7-pentoxyresorufin *O*-depenthylation
PXR	=	Pregnane X receptor
RIF	=	Rifampicin
RT-PCR	=	Reverse transcription polymerase chain reaction
RXR	=	Retinoid X receptor

SNP	=	Single nucleotide polymorphisms
SRS	=	Substrate recognition site
SV	=	Splice variants
TAO	=	Triacetyloleandomycin
TCPOBOP	=	1,4-Bis-[2-(3,5-dichloropyridyloxy)] benzene,3,3',5,5'-Tetrachloro-1,4-bis(pyridyloxy)benzene

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