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MINIREVIEW

Porcine cytochrome 2A19 and 2E1

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Abstract

Cytochrome P450 (CYP) is a major group of enzymes, which conduct Phase I metabolism. Among commonly used animal models, the pig has been suggested as the most suitable model for investigating drug metabolism in human beings. Moreover, porcine CYP2A19 and CYP2E1 are responsible for the biotransformation of both endogenous and exogenous compounds such as 3-methylindole (skatole), sex hormones and food compounds. However, little is known about the regulation of porcine CYP2A19 and CYP2E1. In this MiniReview, we summarise the current knowledge about the regulation of porcine CYP2A19 and CYP2E1 by environmental, biological and dietary factors. Finally, we reflect on the need for further research, to clarify the interaction between active feed components and the porcine CYP system.

KEYWORDS

bioactive compounds, phase I enzymes, pig, skatole

1 | INTRODUCTION

Cytochrome P450 (CYP) constitutes a ubiquitous enzyme family, maintaining diverse functions in the organism including steroidogenesis, metabolism of fatty acids, vitamins and xenobiotic compounds. Since the discovery of the CYP family, their role in the metabolism of xenobiotics in human beings has been well documented.[1] Various

attempts have been made to identify suitable animal models for use in pharmacological and toxicological studies, as well as in studies on human physiology and pathology.[2] Even though rodents are extensively used as models, pigs are often suggested as the most suitable model due to their anatomical similarities with human beings with respect to, for example, cardiovascular system and gastrointestinal tract. Moreover, the CYP expression profile and metabolic processes in pigs display many similarities with human beings. The differences and similarities between human being, mice and porcine CYP have been reviewed earlier.[3] The human and porcine CYP superfamilies contain 57 and 59 genes (including pseudogenes), respectively. They are grouped according to similarities in their amino acid sequence into 18 families, where CYP families 1, 2 and 3 are associated with xenobiotic metabolism. Additionally, porcine CYP2A19 and CYP2E1 are of interest for food scientists because of their indirect impact on meat quality. Specifically, CYP2A19 and CYP2E1 are involved in the metabolism of skatole (3-methylindole), one of the major contributors to "boar taint" in meat from uncastrated male pigs.[4] Accumulation of high concentrations of skatole in adipose tissue is often explained by impaired CYP2A19 and CYP2E1 metabolism. Intestinal bacterial flora is also important for skatole concentrations. Skatole is produced from the tryptophan metabolite, 3-indoleacetic acid, by *Lactobacillius* species, and the rate of skatole production is affected by both the intestinal microflora and the intestinal transit time.

Notable progress has been made in the understanding of the biochemical significance of CYPs for the accumulation of skatole in porcine tissues. Skatole is metabolized in the liver to produce seven metabolites, namely: (3-hydroxy-3-methylindolenine [HMI], 3-hydroxy-3-methyloxindole [HMOI], 5-hydroxy-3-methylindole [5-OH-3MI], 6-hydroxy-3-methylindole [6-OH-3MI], 3-methyloxindole [3MOI or UV3[4]], indole-3-carbinol [I3C] and 2-aminoacetophenone [2-AAP]).[5,6] CYP2A19 and CYP2E1 play a major role in the production of five known skatole metabolites, 3MOI, 6-OH-3MI, I3C, 5-OH-3MI and 2 AAP.[7] It is also important to note that 5-OH-3MI and 6-OH-3MI metabolite formation occurs more intensively by CYP2A19 rather than CYP2E1. High activities of CYP2A19 and CYP2E1 are usually associated with low skatole accumulation in fat due to their enhanced metabolism.[8] Additionally, other enzymes may be responsible for skatole metabolism, such as CYP1A,[9] CYP2C and CYP3A,[10] but their role is likely to be of minor importance.

In the present MiniReview, we focus on the two major porcine CYPs involved in skatole metabolism, CYP2A19 and CYP2E1, with special focus on regulation of their expression and activity by environmental factors.

2 | ANALOGIES BETWEEN PORCINE AND HUMAN CYP2E1 AND CYP2A

In evolutionary terms, pigs display high homology in nucleotide and amino acid sequence with human beings, which make them a valuable and reliable model for use in pharmacological research. It is acknowledged that porcine CYP2A19 is an analogue to human CYP2A6, while CYP2E1 is equivalent in pigs and human beings.[11] Several studies have reported a high similarity between human and porcine CYPs, as shown in Table 1. The data listed in Table 1 include different breeds, showing that small differences in CYP amino acid sequences between breeds do exist and should be taken into account during evaluation of the pig as an animal model. The CYP2A19 of two conventional pig breeds has high (>85%) nucleotide and amino acid identity to at least three human CYP2A isoforms (2A6, 2A7 and 2A13). The identity for human and porcine CYP2E1 (>79%) is lower. However, even minor differences in amino acid sequence between porcine and human CYPs could result in differences in substrate specificity and chemical inhibitors.[12]

3 | TISSUE DISTRIBUTION, HEPATIC AND EXTRAHEPATIC CYP2A19 AND CYP2E1

The liver is the major expression site for most of the CYPs. However, significant expression also exists in extrahepatic tissue. Knowledge on the tissue distribution of the major CYPs is important in order to be able to fully interpret the response in CYP regulation following exposure to environmental and biological factors. The tissue distribution of CYP in pigs has been assessed[11,13-15] and reviewed by Puccinelli et al.[16] Previous studies have determined the CYP expression profile in both minipigs and conventional pigs. Relative gene expression of CYP2A19 and CYP2E1 in tissues that contribute to detoxification processes in pig is summarised in Table 2. Both CYP2A19 and CYP2E1 have the highest abundance in the hepatic tissue of adult pigs,[15] and their constitutive mRNA expression and activities are similar in the four major lobes of the porcine liver.[17] CYP2A19 mRNA expression has been demonstrated in extrahepatic tissue, including kidney and adipose tissue, but not small intestine and skeletal muscle tissue.[11,15] CYP2E1 mRNA expression has also been demonstrated in extrahepatic tissue, including the kidney, brain, lung and adipose tissue, but not the small intestine and skeletal muscle tissue.[14,15] Although

TABLE 1 Similarities between the nucleotide sequences of porcine and human CYP2A19 (CYP2A6) and CYP2E1

Porcine CYP analogue	Pig breed	Human CYP	% of identity		Reference
			Nucleotide	Amino acid	
2A19	Landrace × Large White × Duroc	2A6	87.5	87.5	11
		2A7	86.5	85.1	11
		2A13	88.6	90.3	11
	Suffolk White	2A6	87.2	-	75
		2A13	90.1	-	75
2A	Goettingen minipig	2A6	-	70	76
2E1	Landrace × Large White × Duroc	2E1	82.5	79.2	11
	Suffolk White	2E1	79.2	-	75

TABLE 2 Comparison of CYP2A19 and CYP2E1 gene expression levels in hepatic and extrahepatic tissues of pig

Tissue	Pig breed	CYP2A19	CYP2E1	Reference
Liver	LLW, LLWD	+++	+++	11,14,15
Kidney	LLWD	++	+	11
Lung	M	N.A.	+	14
Gut	M	N.A.	+	14
Small intestine	LLW	N.D.	N.D.	15
Adipose tissue	LLW	+	+	15
Brain	M	N.A.	+	14
Heart	M	N.A.	N.D.	14
Skeletal muscle	LLW	+	N.D.	15

The expression level in extrahepatic tissue present in comparison with expression level of mRNA in hepatic tissue.
+, low-level mRNA expression; ++, medium-level mRNA expression; +++, high-level mRNA expression; LLW, Landrace × Large White; LLWD, Landrace × Large White × Duroc; M, Göttingen minipig; N.A., no data available; N.D., not detected.

CYP2A19 and CYP2E1 mRNA expressions were observed in extrahepatic tissue, corresponding specific substrate metabolism was not demonstrated.[15]

The constitutive expression of CYP2A19 and CYP2E1 is highly dependent on the weight and age of the pigs. Thus, hepatic CYP2E1 mRNA levels in the foetal state were 7.5-fold higher in foetuses with low body-weight (approximately 0.43 kg) compared to high body-weight (approximately 0.74-0.79 kg).[13] Neither CYP2A19 nor CYP2E1 protein was expressed in foetal hepatic tissue.[13] This might be explained by the absence of the transcription factors (CAR and PXR) which control CYP expression at higher level later in life. Therefore, in adult pigs, CYP2A19 and CYP2E1 mRNA and protein levels increased dramatically.[13]

4 | TRANSLATIONAL AND POST-TRANSLATIONAL REGULATION OF CYP2A19 AND CYP2E1

The transcriptional control of the CYP1-3 is dependent on the transcription factors aryl hydrocarbon receptor (AhR), pregnane X receptor (PXR) and constitutive androstane receptor (CAR). It has been suggested that porcine CYP2A19 is controlled by the transcription factor, CAR.[18] In a study using porcine primary hepatocytes, it was demonstrated that CYP2A19-dependent substrate metabolism was induced following treatment with the CAR activators phenobarbital and CITCO.[19] However, other studies have not demonstrated the induction of CYP2A19 mRNA levels in porcine primary hepatocytes following the treatment with CITCO.[20,21] The transcription regulation of the human CYP2A19 orthologue, CYP2A6, has been shown to be controlled by CAR, PXR, glucocorticoid receptor (GR), oestrogen receptor α, HNF-4α and PGC-1α.[22-28]

The level of CYP2A19 protein seems to be largely dependent on the transcription of the CYP2A19 gene, as a high positive correlation between mRNA and protein levels has been observed.[29] However, in human beings, mRNA levels of CYP2A6 were not correlated with its protein level.[30]

Activation of transcription factors using prototypical chemical activators did not affect porcine *CYP2E1* transcription,[21,31] suggesting that they are not involved in the regulation of the CYP2E1 gene. This is in accordance with previous observations in primary human hepatocytes.[32] Nevertheless, a few transcription factors have been demonstrated to be involved in regulating *CYP2E1* transcription. In pigs, it has been shown that chick ovalbumin upstream promoter transcription factor 1 (COUP-TF1) and hepatocyte nuclear factor 1 (HNF-1) binding sites were located in the promotor region of the CYP2E1 gene.[33] The later (HNF-1) has also been suggested to regulate CYP2E1 transcription in other animals [34,35] and shown to diminish

Cyp2e1 mRNA levels when this is missing in a mice knockout model.[36] Moreover, research in primary human hepatocytes has suggested that activation of the WNT/β-catenin pathway increases the mRNA expression of CYP2E1.[37] This is also supported by the observation that Cyp2e1 mRNA expression was almost completely missing in β-catenin knockout mice.[38,39]

Often, CYP2E1 protein expression and corresponding activity are regulated at the post-transcriptional level.[16,18] Thus, changes in porcine CYP2E1 mRNA expression are not always reflected in corresponding changes in CYP2E1 protein expression and activity.[29,40,41] Early studies have shown that acetone and ethanol treatment induces CYP2E1 protein expression in rats because of stabilisation of the protein by inhibiting its degradation.[42] In addition, CYP2E1 mRNA levels in human liver tissue, together with CYP2A6 and CYP2C9, showed no correlation with protein and enzymatic activity.[30,43,44] In recent years, elucidation of the involvement of microRNA (miRNA) in the post-translational regulation of the human CYP2E1 has begun. It was demonstrated that CYP2E1 mRNA levels were decreased by over-expressing miR-378 in vitro.[43] Moreover, the same study showed that in human liver samples, the CYP2E1 mRNA levels were negatively correlated with the expression levels of miR-378. Later, it was shown that more miRNAs are also involved in the regulation of CYP2E1.[45-47]

5 | ENDOGENOUS SUBSTRATES FOR CYP2A19 AND CYP2E1

To date, no endogenous substrates for CYP2A19 or CYP2E1 have been identified in pigs. In human beings, CYP2A6 (the human orthologue to porcine CYP2A19) plays a major role in the metabolism of nicotine[48] and has also been suggested to have minor activity towards oestrogens,[49] but this has never been further confirmed.[50] Bilirubin, a product of haem metabolism, has also been demonstrated to be metabolized by human CYP2A6.[51]

Based on a high degree of homology between human and porcine CYP2E1 and similarities in the metabolism of probe substrates,[52] it has been suggested that porcine CYP2E1 shares endogenous substrates with human beings. In human beings, it has been demonstrated that CYP2E1 can metabolize a number of compounds of low molecular weight. In rats and mice, CYP2E1 is responsible for oxidation of acetone.[53,54] Acetone is produced during gluconeogenesis and is subsequently converted into acetol by CYP2E1. A possible role of CYP2E1 in the hydroxylation of fatty acids has also been suggested. Using human being and rat microsomes, it was demonstrated that CYP2E1 was involved in the ω-1 hydroxylation of oleic acid.[55] Moreover, CYP2E1 was also suggested to be involved in the metabolism of steroids,[56] although a later study failed to confirm this.[49]

6 | DIETARY REGULATION OF CYP2A19 AND CYP2E1

Apart from the macronutrients, feed generally contains complex mixtures of phytochemicals, which have a great potential to alter CYP2A19 and CYP2E1 expression and activity. Moreover, macronutrients in the diet (total protein, fat and carbohydrate ratios, and total energy intake) can also alter CYP expression and activity.[16,57] Specific plants and herbs have historically been used in folk medicine, especially in Eastern countries such as India, China, Japan and Korea. More recently, a tendency towards the use of particular plants and herbs for medical properties has also been observed in the European countries and USA. This use has mainly been focused on improvement of human health, although it can also be of interest for animal health. It should be emphasised that the effects of phytochemicals are concentration-dependent.

A public awareness of animal welfare of male piglets exerts additional pressure on regulatory bodies to develop methods other than surgical castration to reduce boar taint. One such method can be enhancement of skatole metabolism through induction of the activities of CYP2E1 and CYP2A19, or other skatole-metabolizing enzymes by dietary means.

Phenolic compounds have attracted considerable attention in both human nutrition and as potential feed additives in animal nutrition during the last decade. Generally, the effects of phenolic compounds are mainly studied in rats, mice and human beings. Inhibition of CYP activities, especially CYP1A and CYP3A4, by phenolic compounds, has been of special interest because of their potential positive effect in reducing the risk of cancer.[58] A number of natural compounds, such as isothiocyanates found in cruciferae, diallylsulphide found in garlic, and bergamottin found in grapefruit, can potential inhibit CYP2E1 activity.[59]

Quercetin is a natural flavonoid that occurs ubiquitously in plants such as onions and apples; and plant derivatives such as tea and red wine; and in animal feed. Quercetin inhibited CYP2E1 activity in vitro in porcine[60] and human[61] microsomes, and in vivo in rats[62] and human beings.[63] It can be speculated that the presence of high concentrations of quercetin in the diet can decrease skatole metabolism, resulting in increased skatole accumulation. However, this requires further investigations.

Addition of tannins to the diet fed to pigs is usually associated with lower feed efficiency and thus lower weight gains. However, low concentrations of hydrolysable tannins in feed can display beneficial effects on pig health.

Dietary supplement with chestnut wood (*Castanea sativa*) extract, which is a source of hydrolysable tannins, induced activities of CYP2A19 and CYP2E1 in cross-bred (Large White × Landrace) entire male pigs.[64] These results suggested that hydrolysable tannins can be used to reduce skatole levels in pigs by induction of skatole-metabolizing enzymes. However, later studies on dietary supplementation with hydrolysable tannins to conventional pig breeds did not show any effect on CYP2A19 and CYP2E1 mRNA expression.[40,65] These contradictory results might indicate breed-related differences in the response to dietary tannins. It should also be emphasised that in the study of Bee et al[65] only mRNA expression, but not enzymatic activities, was measured.

Generally, less is known about dietary regulation of CYP2A19 activity. Some phenolic compounds are substrates for CYP2A6 (the human analogy of porcine CYP2A19). In pigs, CYP2A metabolized scoparone, a natural bioactive compound found in Chinese herbal medicines.[66] In human beings, the plant polyphenol curcumin ingested in dose of 1000 mg daily during 14 days,[67] and quercetin in dose of 500 mg daily for 13 days[68] enhanced CYP2A6 activity. Because of high similarity between the porcine and human physiology, similar effects can be expected for porcine CYP2A19, which might lead to enhanced skatole metabolism. It should be noted, however, that an in vitro study did not show any effect of quercetin on CYP2A19 in porcine microsomes.[60]

6.1 | Alternative feed ingredients in pig diet

There have been various attempts to develop proper alternative diets for pigs, which fulfil nutritional requirements while reducing skatole levels without compromising animal welfare. Feeding entire male pigs with sugar beet pulp, which is a by-product from the sugar-refining industry, resulted in increased expression of CYP2E1,[69] but the specific compound(s) which was responsible for this increase has not been identified. Sugar beet pulp has a high level of soluble fibre such as pectins and glucans. In general, high dietary fibre content leads to lower body fat content. However, the results obtained are often contradictory. It was shown that pig performance and carcass quality were not negatively affected in rations with up to 20% dried beet pulp. Moreover, an increase in dietary fibres may result in reduced ammonia absorption from the colon and lower the urinary N excretion.[70]

Chicory roots are also high in fibres, especially inulin, and it has also been suggested that it be used to prevent the development of boar taint. Feeding dried chicory root to pigs increased the mRNA expression of CYP2A19 and CYP2E1.[41] However, this induction was only reflected in higher CYP2A activity, not higher CYP2E1 activity. Similar to sugar beet, the compound(s) responsible for the changes in mRNA expression are not known. However, esculetin, a secondary metabolite present in chicory roots, increased the level of CYP2A19 and CYP2E1 mRNA in porcine primary hepatocytes.[21]

Skatole levels were repeatedly reduced by the inclusion of raw potato starch (RPS, 20%-30% of the total feed amount) in pig diet.[8,71,72] This effect, however, was mainly due to the altered intestinal microflora by resistant starch and the inhibition of colonocyte apoptosis.[73] No effect of RPS on hepatic CYP2A6 and CYP2E1 activities was observed in male pigs.[72] Interestingly, CYP2A6 activity was higher in female pigs fed RPS.[72]

7 | IMPLICATIONS AND FUTURE RESEARCH

The levels and activities of CYP2A19 and CYP2E1 are affected by numerous factors, including diet. Further research is needed to understand the complexity of dietary regulation of porcine CYP2A19 and CYP2E1. Several bioactive compounds in animal feed were identified as modifiers of the activities of porcine CYP2A19 and CYP2E1 through either inhibition or induction mechanisms. However, the investigations on the role of this modification in relation to skatole levels in fat are limited.

Interestedly, it seems to be easier to affect CYP2E1 than CYP2A19, by dietary means. However, before attempts to induce CYP2E1 activity with the aim to enhance skatole metabolism, the following issues should be considered. It is well known that CYP2E1 activity is directly related to the hepatic steatosis, and the up-regulation of CYP2E1 can enhance lipid peroxidation.[74] Thus, the effect of any dietary components with CYP2E1-inducing properties should be carefully studied before large-scale applications. Additionally, there are considerable inter-individual variations in the activities of both CYP2A19 and CYP2E1 due to genetic factors. Thus, breed-related differences should be considered when studying regulation of these enzymes.

With the present MiniReview in mind, it is striking how little is known about the molecular events regulating both CYP2A19 and CYP2E1 in pigs. The same is actually also true for the human versions. Thus, to fully utilise the pig as a model for human beings and also to develop future strategies to avoid surgical castration to prevent boar taint, more basic research on mechanism regulation CYP2A19 and CYP2E1 expression and activity must be initiated.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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