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NOTE

Cytochrome P450 3A mRNA expression along goat and rat gastrointestinal tracts

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Abstract

The cytochrome P450 (CYP) 3A family is involved in the elimination processes of almost 50% of commonly used drugs. CYP3A mRNA expressions in goat and rat gastrointestinal tracts in comparison to the liver were investigated using real-time PCR. In goats, the expression of CYP3A-like mRNAs was comparatively higher in the liver than in the gastrointestinal tract. The intestinal expression of CYP3A-like mRNA showed a gradual decrease from the duodenum to the ileum. In rats, the highest CYP3A62 mRNA expression was found in the duodenum followed by the liver. This study provides insights into the contribution of CYP3A enzymes to xenobiotic metabolism, especially in small ruminants such as goats.

Key words: Cytochrome P450 3A, Goat, Small intestine

The cytochrome P450 (CYP) superfamily comprises a large number of enzymes that catalyze oxidative biotransformation of a broad range of external compounds as well as endogenous substances[4)]. Members of cytochrome P450 are found in diverse extra-hepatic tissues although they are primarily expressed in the liver[18)]. CYP3A, which includes the isoforms CYP3A1, CYP3A2, CYP3A9, CYP3A18 and CYP3A62 in rats and CYP3A4, CYP3A5, CYP3A7, CYP3A43 in humans, accounts for up to 30% of total P450 content in the liver. CYP3A isoforms have important roles in the oxidative, peroxidative and reductive metabolism of endogenous steroids, many procarcinogens and at least 50% of all drugs[2,13)]. The most abundant CYP3A isoform expressed in the liver and gut is CYP3A4. Several lines of evidence indicate that mammalian CYP3A genes are regulated by the pregnane X receptor (PXR), a member of the nuclear receptor family of ligand-activated transcription factors[2)]. They are therefore

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likely to be induced by glucocorticoids (e.g. dexamethasone), pregnane compounds (e.g. pregnenolone 16α-carbonitrile) and macrolide antibiotics (e.g. rifampicin)[1].

During their production cycles, food-producing animals are exposed to a wide range of xenobiotic agents such as veterinary drugs, food additives, and environmental pollutants. Most of these compounds enter the animal's body through oral administration and may undergo oxidative biotransformation by drug-metabolizing enzymes. Xenobiotic metabolism takes place predominantly in the liver, although extra-hepatic tissues, such as the intestine display numerous phase I and phase II metabolic reactions. The retention time, retention level, and therapeutic and/or toxic responses of xenobiotics are determined to a great extent by the composition and abundance of CYP enzymes in tissues involved in xenobiotic metabolism. Bioaccumulation of illegally used compounds in edible tissues of food-producing animals may pose a risk to consumers[3]. This fact is a major concern for public health and consumer safety. Examination of the tissue distribution of P450 in mammals is therefore an important aspect of drug metabolism research. Moreover, investigation of the composition and abundance of drug-metabolizing enzymes in the liver and intestines of food-producing animals is also likely to facilitate the understanding of potential species similarities between species in xenobiotic biotransformation, providing data to support extension of the use of veterinary medicine licenses to different food-producing species[5].

Recent studies have underlined the necessity for obtaining further knowledge of the expression of the P450 enzyme system in food-producing animals[8]. Special attention has been paid to the contribution of the gastrointestinal tract to the metabolic fate of different xenobiotics in large ruminant species such as cattle[12,19].

Goats have recently gained economically importance as a valuable source of meat and milk in many parts of the world; however, little is known about the expression of P450, especially CYP3A in goats. In addition, the expression level of different CYP isoforms and phase II enzymes is considered as a good biomarker for pre-slaughter exposure to different xenobiotics because the concentration of these enzymes tends to increase on chemical exposure[6]. This study was therefore performed to investigate the mRNA expression pattern of CYP3A in different goat tissues in comparison with rat CYP3A62 which is the major intestinal CYP3A form[13].

All experiments using animals were performed according to the guidelines of the Hokkaido University Institutional Animal Care and Use Committee. Samples from 5 female healthy Japanese goats (*Capra hircus*) aged four-six months (4.67 ± 0.58 months) were collected following their slaughter at Hokkaido University. Goats had been reared on grass feed and had no medical history for at least one month prior to sacrifice. Tissue samples from tongue, esophagus, rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, cecum, colon, rectum and liver were collected within 20–40 min of slaughter, snap-frozen in liquid nitrogen and subsequently stored at −80°C until use. Nine-week-old female Wistar rats (SLC) were housed at 24 ± 1°C with a 12 h light and 12 h dark cycle and given laboratory feed and water ad libitum. Rats were anaesthetized and sacrificed with carbon dioxide, and the liver, tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon and rectum were removed, perfused with cold 1.15% KCl to remove the blood, and used for comparison in this study.

Total RNA was extracted from the different tissue samples using TRI reagent (Sigma) according to the manufacturer's instructions. Concentration and purity of the RNA fraction was determined spectrophotometrically at 260 and 280 nm respectively. RNA integrity was checked though running a non-denaturing 1.5% agarose gel electrophoresis using 3 μg of total RNA for each sample.

Total RNA was reverse transcribed using oligo (dT) primers and RT-ReverTra Ace

reverse transcriptase (Toyobo) according to the manufacturer's instructions. The cDNA generated were qPCR-amplified to determine the expression levels of goat CYP3A using β-actin as an endogenous control. Although the available sequence for goat CYP3A is a partial fragment (X76503) but it locates a well-described cysteine-containing heme-binding region. A more extensive comparison of this region between different species is reported[14]. It can be suggested that the heme-binding region of CYP3A is highly conserved within the different species. Thus, this region has been regarded as an unconverted region of CYP3A in mammals and suggested to be appropriate region for the analysis of CYP3A expression. CYP3A primer sequences were set as sense 5'-ACTGGACCCCGAAACTGTGTT-3' and antisense 5'-AGGTTGTTCTGGCTGCGTAA-3' (X76503). For β-actin the sense primer was set as 5'-TTTCAACACTCCTGCCATGT-3' and antisense primer as 5'-CCACGCTCCGTGAGAATCTT-3' (AF481159). The PCR reaction mixture was prepared with SYBR® qPCR Mix (Toyobo), 10 μM of each primer, 600 ng cDNA and 50x ROX reference dye and RNase-free water, and made up to a final volume of 10 μl. The reaction cycle comprised an initial holding stage at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 1 min and extension at 72°C for 30 s. Melting curve analysis and agarose gel electrophoresis confirmed the amplification of a single amplicon of the expected size as well as the absence of primer dimers and genomic DNA amplification. Each measurement was performed in duplicate and repeated three times. The expression of each gene was normalized to the expression of β-actin and was calculated relative to the corresponding gene expression in the tongue for each animal. Quantitative real-time PCR for rat CYP3A62 mRNA levels was performed using SYBR® qPCR Mix (Toyobo). Sequences of the CYP3A62 primers were set as sense 5'-GGAGATAAAGAGTCTCA CC-3' and antisense 5'-AGTTCTGCAGGACTCAG AC-3' (NM_001024232), for β-actin the sense primer was set as 5'-ATGTACGTAGCCATCCAG GC-3' and antisense 5'-TCCACACAGAGTACTTG CGC-3' (V01217). The reaction cycle used was identical to that described above. Measurements of CYP3A62 and β-actin were performed in duplicate and repeated three times. The expression of each gene was normalized to the expression of β-actin and was calculated relative to that of the tongue of each animal using the relative comparative threshold cycle (Ct) method following Schmittgen and Livak[17]. Cycling was performed with the StepOne Plus Real-Time PCR system (Applied Biosystems) according to the manufacturer's instructions. Statistical significance was evaluated using the Tukey-Kramer HSD test (JMP statistical package, SAS) with a significance threshold of 0.05.

The expression of CYP3A-like mRNAs exhibited a gradually decreasing trend along the goat small intestine from the duodenum to ileum (Fig. 1A). Similar attributes were observed in case of rat CYP3A62 mRNA expression (Fig. 1B). Studies in horses, pigs, rats and human have shown similar patterns[18,20]. However, our results differ in that CYP3A-like mRNAs expression in the cecum and colon was slightly (though not significant) higher than in the small intestine.

The expression of CYP3A-like mRNA in goats was considerably higher in the liver than in the intestine (data not shown). The mRNA expression in the liver was about 900-fold, 2000-fold, and 3000-fold higher than that in the duodenum, jejunum, and ileum, respectively, indicating that CYP3A enzyme expression, was extremely variable in a tissue-dependent manner. It should be noted that the collected intestinal samples, including the muscle and serosal surface layer, may not express P450 genes as strongly as we expect in the epithelial cell layer. Thus, the results may underestimate the P450 gene expression in the intestinal samples when compared with the liver. However, rat duodenum showed higher CYP3A62 levels compared to the liver (Fig. 2B). Similar results have been reported in Sprague-Dawley rats and humans[7,9,13]. In

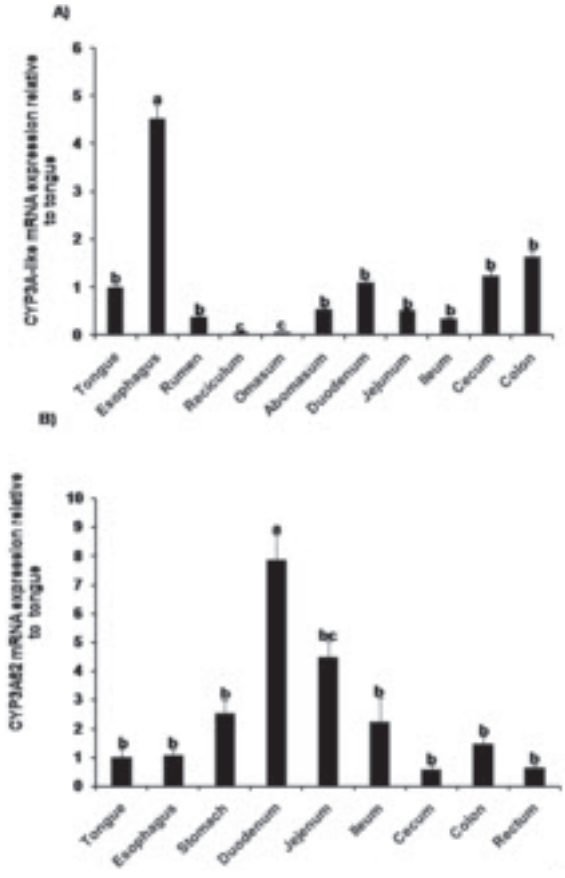

Fig. 1. Tissue distribution of CYP3A-like mRNA in goat and rat gastrointestinal tracts. A) CYP3A-like mRNA expression in the different tissues of the goat gastrointestinal tract determined using real-time RT-PCR analysis. cDNA samples were amplified as described in the text. Each measurement was performed in duplicate and repeated three times. The mRNA expression was normalized to β-actin expression and was calculated relative to that of the tongue (set to 1.0). Tissue distribution was expressed as mean ± standard deviation (SD) of five animals. Identical letters denote values that were not significantly different from each other. $P < 0.05$. B) Tissue distribution of CYP3A62 mRNA in the rat gastrointestinal tract determined using. real-time RT-PCR analysis. cDNA samples were amplified as described in the text. Each measurement was performed in duplicate and repeated three times. The mRNA-expression was normalized to the expression of β-actin expression and was calculated relative to that of the tongue (set to 1.0). Tissue distribution was expressed as mean ± standard deviation (SD) of five animals. Identical letters denote values that were not significantly different from each other. $P < 0.05$.

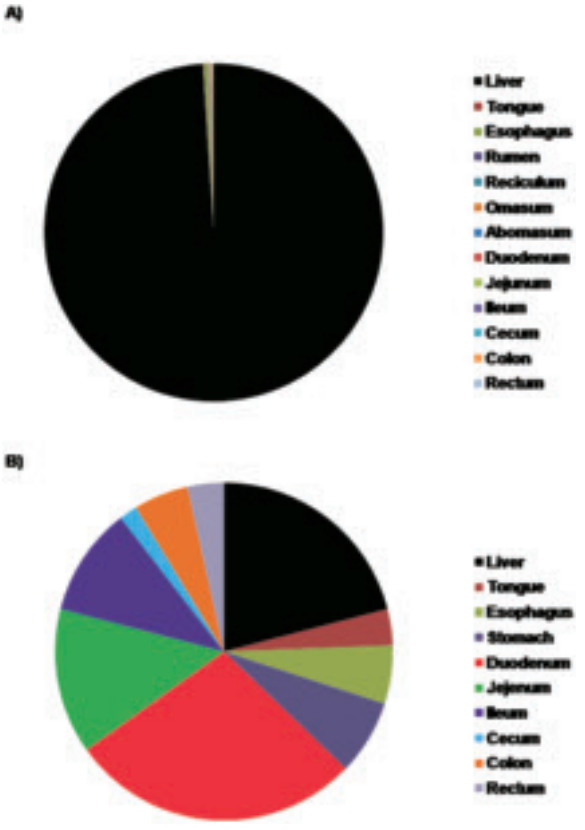

Fig. 2. Distribution of CYP3A mRNA expression in different tissues in goats (A) and rats (B). The ratio of CYP3A-like and CYP3A62 mRNA expressions in the different tissues of the rats and goat gastrointestinal tracts were analyzed.

contrast, studies in horses and trout have demonstrated higher CYP3A mRNA expression in the intestines than in the liver[11].

We also analyzed CYP3A-like mRNA expression in goat tongue, esophagus and forestomach as these act as portals of entry for foreign compounds. We found that among these tissues, CYP3A-like mRNA had significantly higher expression in the esophagus than in other tissues ($P < 0.05$), which has also been reported in humans[10]. This may suggest that several xenobiotics to which animals are exposed may be metabolized in the basal cell layer of the epithelium. In the examined rats, the duodenum showed the highest extra-hepatic expression of CYP3A62, followed by the jejunum and ileum (Fig. 1B, 2B). Figure 2A shows a summary of CYP3A-like mRNA expression in goats, which is different from that of rats, as liver constitutes the major organ for CYP3A-like mRNA expression. Figure 2B summarizes CYP3A62

distribution in the liver and gastrointestinal tissues of rats, showing that the highest levels of CYP3A62 mRNA expression were recorded in the duodenum and liver, followed by the jejunum and ileum. Minor expressions were observed in other tissues. Generally, there are linear correlations between the mRNA expression, the protein abundance and enzymatic activities of CYP3A in mammals[15,16].

In conclusion, all examined goat tissues expressed CYP3A-like mRNA with a distinct decreasing pattern of CYP3A from the duodenum to ileum while the liver and esophagus showed the highest levels. Since it is known that human CYP3A has several isoforms, including CYP3A4, CYP3A5, CYP3A7 and CYP3A43, the current work is still limited to fully address the roles of the CYP3A enzymes in the P450 3A pool. Further investigation including identifying new P450 3A enzymes, characterizing the abundance in tissues and metabolic capabilities towards their substrates in goats is still required.

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References

1) Dacasto, M., Eeckhoutte, C., Capolongo, F., Dupuy, J., Carletti, M., Calléja, C., Nebbia C., Alvinerie, M. and Galtier, P. 2005. Effect of breed and gender on bovine liver cytochromes P450 3A (CYP3A) expression and inter-species comparison with other domestic ruminants. *Vet. Res*., **36**: 179–190.
2) Dacasto, M., Montesissa C. and Nebbia, C. 2006. Illegal drug treatments and drug metabolism: Biomarkers or not? *Vet. Res. Com*., **30**: 113–119.
3) Darwish, W. S., Ikenaka, Y., El-Ghareeb, W. R. and Ishizuka, M. 2010b. High expression of the mRNA of cytochrome P450 and phase II enzymes in the lung and kidney tissues of cattle. *Animal*, **4**: 2023–2029.
4) Darwish, W. S., Morshdy, A. E., Ikenaka, Y., Ibrahim, Z. S., Fujita, S. and Ishizuka, M. 2010a. Expression and sequence of CYP1A1 in camel. *J. Vet. Med. Sci*., **72**: 221–224.
5) Fink-Gremmels, J. 2008. Implications of hepatic cytochrome P450-related biotransformation processes in veterinary sciences. *Europ. J. Pharmacol*., **585**: 502–509.
6) Fujita, S., Chiba, I., Ishizuka, M., Hoshi, H., Iwata, H., Sakakibara, A., Tanabe, S., Kazusaka, A., Masuda, M., Masuda, Y. and Nakagawa, H. 2001. P450 in wild animals as a biomarker of environmental impact. *Biomarkers*, **6**: 19–25.
7) Gonzalez, F. J. and Gelboin, H. V. 1992. Human cytochromes P450: evolution and cDNA-directed expression. *Environ. Health Perspect*., **98**: 81–85.
8) Ioannides, C. 2006. Cytochrome p450 expression in the liver of food-producing animals. *Cur. Drug Metabol*., **7**: 335–348.
9) Koch, I., Weil, R., Wolbold, R., Brockmoller, J., Hustert, E., Burk, O., Nuessler, A., Neuhaus, P., Eichelbaum, M., Zanger, U. and Wojnowski, L. 2002. Interindividual variability and tissue-specificity in the expression of cytochrome P450 3A mRNA. *Drug Metabol. Dispos*., **30**: 1108–1114.
10) Lechevrel, M., Casson, A. G., Wolf, C. R., Harde, L. J., Flinterman, M. B., Montesano, R. and Wild, C. P. 1999. Characterization of cytochromes p450 expression in human oesophageal mucosa. *Carcinogenesis*, **20**: 243–248.
11) Lee, S. J., Hedstrom, O. R., Fischer, K., Wang-Buhler, J. L., Sen, A., Cok, I. and Buhler, D. R. 2001. Immunohistochemical localization and differential expression of cytochrome P450 3A27 in the gastrointestinal tract of rainbow trout. *Toxicol. Appl. Pharmacol*., **177**: 94–102.
12) Mate, L., Virkel, G., Lifschitz, A., Sallovitz, J., Ballent, M. and Lanusse, C., 2010. Phase 1 and phase 2 metabolic activities along the small intestine in adult male sheep. *J. Vet. Pharmacol. Ther*., **33**: 537–545.
13) Matsubara, T., Matsubara, T., Kim, H. J., Miyata, M., Shimada, M., Nagata, K. and Yamazoe, Y. 2004. Isolation and characterization of a new major intestinal CYP3A form, CYP3A62 in the rat. *J.*

Pharmacol. Exp. Ther., **309**: 1282–1290.
14) Natsuhori, M., van Raak, M., Ligtenberg, M., Kleij, L., Ten Berge, D., Zweers-Zeilmaker, W. M., de Groene, E. M., van Miert, A. S., Witkamp, R. FWitkamp, R. F. and Horbach, G. J. 1997. Isolation of a bovine full length cytochrome P450 (CYP3A) cDNA sequence and its functionalexpression in V79 cells. *Environ. Toxicol. Pharmacol.*, **3**: 17–24.
15) Pérez, G., Tabares, B., Jover, R., Gómez-Lechón, M. J. and Castell, J. V. 2003. Semi-automatic quantitative RT-PCR to measure CYP induction by drugs in human hepatocytes. *Toxicol. In Vitro*. **17**: 643–649.
16) Richert, L., Tuschl, G., Abadie, C., Blanchard, N., Pekthong, D., Mantion, G., Weber, J. C. and Mueller, S. O. 2009. Use of mRNA expression to detect the induction of drug metabolizing enzymes in rat and human hepatocytes. *Toxicol. Appl. Pharmacol.*, **235**: 86–96.
17) Schmittgen, T. D. and Livak, K. J. 2008. Analyzing real-time PCR data by the comparative C (T) method. *Nat. Protoc.*, **3**: 1101–1108.
18) Tydén, E., Olsén, L., Tallkvist, J., Larsson, P. and Tjälve H. 2004. CYP3A in horse intestines. *Toxicol. Appl. Pharmacol.*, **201**: 112–119.
19) Virkel, G., Carletti, M., Cantiello, M., Della Donna, L., Gardini, G., Girolami, F. and Nebbia, C. 2009. Characterization of xenobiotic metabolizing enzymes in bovine small intestinal mucosa. *J. Vet. Pharmacol. Ther.*, **33**: 295–303.
20) Yao, M., Dai, M., Liu, Z., Cui, W., Li, D., Zhang, H., Li, J., Liu, Y. and Yuan, Z. 2011. mRNA expression profiles of P450 3A enzymes in the liver and small intestine of the domestic pig. *Res. Vet. Sci.*, In press.