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SHORT COMMUNICATION

Sister of P-glycoprotein Expression in Different Tissues

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ABSTRACT. Sister of P-glycoprotein (spgp) is a gene that is closely related to the P-glycoprotein family (Pgps). This class of proteins belongs to the superfamily of ATP-binding cassette transporters and is known for its involvement in pharmacological drug interactions. Therefore, this study investigated the distribution of spgp expression in different tissues known for their high levels of Pgps expression such as brain, liver, kidney, small- and large-gut mucosa. Analysis was done by using the reverse transcription-polymerase chain reaction. In addition to a high expression in the liver, we were able to demonstrate a significant spgp expression in brain grey cortex, small- and large-gut mucosa. Although Pgps are expressed in the kidney and brain capillary endothelial cells, no expression of spgp was detected in these tissues, which might indicate that spgp has no function in the blood–brain barrier and is not involved in the renal excretion of drugs. BIOCHEM PHARMACOL **57**;7:833–835, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. Sister of P-glycoprotein; multidrug resistance proteins; tissue distribution; polymerase chain reaction

P-glycoproteins belong to the ATP-binding cassette superfamily of membrane transport proteins [1]. They were first identified in malignant tissues where their overexpression leads to resistance against multiple cytotoxic drugs by an ATP-consuming process. In addition, Pgps§ are also constitutively expressed in various non-malignant tissues, suggesting that they exert physiological functions specific to the respective tissues [2, 3]. In mammals, Pgps are encoded by small gene families with two members in humans (MDR1 and MDR2), three members in rodents, and five members in pigs (pgp1A–D, pgp3) [4, 5]. Four porcine genes (pgp1A–D) show similarity to the Class I Pgp isoform, which confers multidrug resistance. The porcine pgp3 gene shows similarity to the phosphatidylcholine-translocating Class III isoform [5].

Childs *et al.* [6] identified a novel member of the ATP-binding cassette superfamily with a high sequence similarity to Pgps. They coined it sister of P-glycoprotein (spgp), since it is not a member of the Pgps multigene family but represents a sister gene. Its expression was first shown in the liver of pigs, then in other mammalian hepatic tissue. Some recent findings demonstrate spgp to be a canalicular bile salt export pump of mammalian liver [7].

The aim of this study was to investigate the distribution of spgp in several porcine tissues known to express Pgps at high levels. These are, beside the liver, the brain, kidney, small- and large-gut mucosa [3, 8].

MATERIALS AND METHODS

RT-PCR

Total RNA was extracted using the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. It was prepared from freshly excised porcine brain grey cortex, liver, kidney cortex, small- and large-gut mucosa. RNA from confluent (10 days in culture) porcine brain capillary endothelial cells was also examined. Primary cultures of these endothelial cells were prepared as described earlier [9]. After DNase digestion, RNA was quantified using a GeneQuant photometer (Pharmacia). Its integrity was checked by ethidium bromide agarose gel electrophoresis. The purity of the RNA preparations was high, as demonstrated by the 260 nm/280 nm ratio (range 1.8–2.0). One μg of total RNA was reverse transcribed by Superscript II (GIBCO BRL) according to the manufacturer's protocol, i.e. using random hexamers as primer.

cDNA (25 ng total RNA) was used as a template for PCR with a set of primers. It was performed with a thermocycler (Biometra). Each sample was amplified for 40 cycles of 30-sec denaturation at 95°, 50 sec annealing (50° for Class I pig pgp and Class III pig pgp and 60° for spgp, GAPDH), and 50 sec extension at 72°. The reaction mixture contained 2.5 μL of template, 0.7 U AmpliTaq Gold DNA polymerase (Perkin Elmer), 2.5 μL 10X PCR

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§ *Abbreviations:* GAPDH, glycerinaldehyde phosphate dehydrogenase; Pgps, P-glycoproteins, RT-PCR, reverse transcription-polymerase chain reaction; and spgp, sister of P-glycoprotein.

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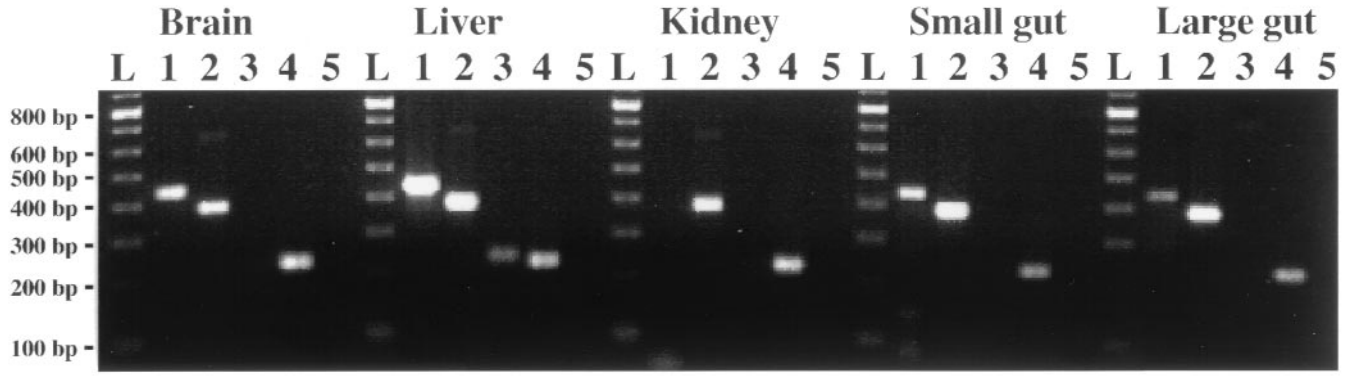

FIG. 1. RT-PCR analysis of spgp tissue distribution. The amplicon of spgp is 439 bp. The Class I primer differentiates between the 389 bp product of pgp1A and the ~660 bp product of pgp1B/pgp1C/pgp1D. The Class III (pgp3) amplicon and GAPDH are 240 bp and 226 bp, respectively. (1) spgp; (2) Class I; (3) Class III; (4) constitutive expression of GAPDH; (5) negative control with not reverse-transcribed RNA and GAPDH primers; (L) 1 μg of 100 bp ladder with intenser 800 bp band (Pharmacia).

buffer (Mg^{2+}-free, Perkin Elmer), $MgCl_2$ at a final concentration of 2 mM, 2 μL of dNTP reaction mixture (2.5 mM each, Perkin Elmer), 7.5 pmol of each primer and water to a final volume of 25 μL. The reaction was preincubated for 10 min at 95° to activate AmpliTaq Gold in order to get a hot start PCR. After 40 cycles, the reaction was maintained for 10 min at 72° for terminal elongation. Negative controls were performed by omitting RNA from the cDNA synthesis and specific PCR amplification. PCR products were separated by electrophoresis in 1.5% agarose, visualized by UV in the presence of ethidium bromide, and documented by a Gel Doc 1000 system (Bio–Rad).

PCR Primers

Primers were synthesized (GIBCO BRL) for Class I pig pgp (5′AAGCGCTCATCAACTGTG3′ and 5′GGCACTTTATGCAAACATTC3′) and Class III pip pgp (5′GAAGCCCTGGACAAA3′ and 5′AACGATTGGAATTTATTTTAAA3′) according to Childs and Ling [5]. Porcine GAPDH primers (5′GAAGATGGTGATGGGATTTC3′ and 5′GATGGTGAAGGTCGGAGTG3′) were used as an internal control. The specific spgp primer pair (5′CGGTGTTGTTTGCCTGCA3′ and 5′GCTCCTTTCTTGGCCATCAG3′) was designed based on the spgp sequence (accession no. U20587) by the PrimerExpress 1.0 program (Perkin Elmer). The Class I primer pair amplicon is 389 bp for pgp1A and around 660 bp for pgp1B/pgp1C/pgp1D. The Class III amplicon (pgp3) is 240 bp. The products of spgp and GAPDH are 439 bp and 226 bp, respectively.

Confirmation of cDNA Sequence

For direct sequencing, PCR reactions were performed with brain cDNA and spgp primers as described above. The ~450 bp band was recovered from the agarose gel with the QIAquick Gel Extraction Kit (Qiagen) and sequenced in both directions by using the 377 DNA Sequencer (Perkin Elmer) with fluorescent dideoxynucleotides. Sequence comparison of the amplicon revealed identity with the spgp gene.

RESULTS AND DISCUSSION

Brain grey cortex, liver, kidney, small- and large-gut mucosa are known to express high levels of Pgps [3, 10–16]. The distribution of spgp was therefore investigated in these tissues. With RT-PCR, spgp expression could be demonstrated in brain, liver, small and large gut, whereas no spgp could be detected in the kidney (Fig. 1). The identity of the gene product with spgp was confirmed by cDNA sequence analysis. To determine which types of Pgps are expressed by these porcine tissues, PCR primers detecting pgp1A, pgp1B/pgp1C/pgp1D and pgp3 [5] were applied. All tissues used expressed pgp1A, whereas pgp3 could only be detected in the liver. In contrast to results obtained with Northern blot analysis [6, 17], analysis by RT-PCR seems to be a more sensitive method [18]. When assessed with RT-PCR, the tissue distribution of spgp does not appear to be restricted to the liver. The physiological function of spgp has not yet been defined. However, there is recent evidence that spgp may act in the liver as a bile salt exporting pump [7]. Our results show the existence of spgp in the gut. An ATP-dependent bile salt transporter exists in intestinal tissue [19]. This carrier enhanced uptake of taurocholate from the apical side of Caco-2 monolayers. Therefore, intestinal expression of the ATP-dependent spgp may point to its involvement in the enterohepatic circulation of bile salts. In addition to this hypothesized function, it may be assumed that spgp has a broader substrate specificity, in analogy to the other multidrug resistance proteins [20].

To determine whether the spgp detected in brain tissue was due to its expression at the blood–brain barrier, RT-PCR was performed with cultured porcine brain capillary endothelial cells. Surprisingly, no specific signal for spgp could be detected (data not shown), although a clear expression of pgp1A could be demonstrated in these cells.

The subcellular localization of spgp is only shown in the

liver, where it is expressed at the canalicular microvilli and at subcanalicular smooth membrane vesicles [7]. It is likely that spgp in brain, small-gut, and large-gut mucosa might contribute to the extrusion of endogenous and exogenous compounds and possibly effect drug–drug interactions.

Unlike the other multidrug resistance proteins such as Pgps and multidrug resistance-associated protein, spgp has not yet been detected in any tumour tissue. It could not be decided, therefore, whether spgp is conferred with a multidrug resistance phenotype.

We conclude that spgp expression is not restricted to the liver, but is also extended to brain grey cortex, small- and large-gut mucosa. This broader tissue distribution indicates that its function may not be limited to hepatic transport.

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