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European Journal of Pharmacology 460 (2003) 85–91
www.elsevier.com/locate/ejphar

MDR1-deficient genotype in Collie dogs hypersensitive to the P-glycoprotein substrate ivermectin ☆

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Received 5 September 2002; received in revised form 18 December 2002; accepted 20 December 2002

Abstract

Multidrug resistance (MDR) phenotypes in cancer cells are associated with overexpression of the drug carrier P-glycoprotein. The antiparasitic drug ivermectin, one of its substrates, abnormally accumulates in the brain of transgenic mice lacking the P-glycoprotein, resulting in neurotoxicity. Similarly, an enhanced sensitivity to ivermectin has been reported in certain dogs of the Collie breed. To explore the basis of this phenotype, we analyzed the canine P-glycoprotein-encoding MDR1 gene, and we report the first characterization of the cDNA for wild-type (Beagle) P-glycoprotein. The corresponding transcripts from ivermectin-sensitive Collies revealed a homozygous 4-bp exonic deletion. We established, by genetic testings, that the MDR1 frame shift is predictable. Accordingly, no P-glycoprotein was detected in the homozygote-deficient dogs. In conclusion, we characterized a unique case of naturally occurring gene invalidation. This provides a putative novel model that remains to be exploited in the field of human therapeutics and that might significantly affect tissue distribution and drug bioavailability studies.

Keywords: Ivermectin; (Dog); (Collie); Hypersensitivity; P-glycoprotein; MDR (multidrug resistance)

1. Introduction

The acquired ability of tumors to evade the effects of therapeutic cytotoxic agents is well documented. One mechanism that leads to multidrug resistance (MDR) in mammalian cells is a substantial increase in the activity of a xenobiotic efflux system resulting from overexpression of the transmembrane P-glycoprotein encoded by the MDR1 gene (Leveille-Webster and Arias, 1995). P-glycoprotein is primarily responsible for the transport of a variety of structurally dissimilar chemicals from the cell, including drugs that are actively extruded from the inner leaflet of the cellular membrane (Eytan and Kuchel, 1999). This decreases intracellular drug bioavailability, leading to impaired treatment efficacy in the case of cytotoxic agents. In addition, P-glycoprotein, which is constitutively expressed in various epithelial cells, protects some organs (e.g. central nervous system (CNS) and testicle) from exposure to certain compounds, thus, minimizing the risk of a putative toxic impact (Tsuji, 1998).

Ivermectin is a widely used antiparasiticide and an excellent P-glycoprotein substrate. Indeed, the interference of ivermectin with P-glycoprotein was established in several investigations showing that mice exposed to P-glycoprotein blockers displayed severe signs of acute toxicity when challenged with ivermectin (Didier and Loor, 1996), or that exposure of a highly drug-resistant human cancer cell line to ivermectin elicited a marked decrease in its P-glycoprotein-associated MDR phenotype (Pouliot et al., 1997).

Ivermectin, a semisynthetic macrocyclic lactone that originates from *Streptomyces avermitilis*, potentiates the activity of glutamate-gated or γ-aminobutyric acid (GABA)-gated chloride ion channels (de Silva et al., 1997). In mammals, GABA-sensitive neurons, which are restricted to the CNS, are protected from exposure to ivermectin by the P-glycoprotein-dependent blood–brain barrier, rendering it safe to use in a wide range of mammals (Schinkel et al., 1994, 1996). In invertebrate parasites such

☆ Part of this work was presented at the Third FEBS Advance Lecture Course, March 3–10, 2001, Gosau, Austria.

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0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(02)02955-2

as nematodes and arthropods, however, ivermectin-sensitive neurons are more widely distributed. In the absence of any organized P-glycoprotein-dependent protection, ivermectin exposure results in tonic paralysis and ultimately death of the organism (Martin, 1997). This selective effect on invertebrate parasites has led to extensive use of the drug in veterinary and human medicine as an endectocide (Campbell, 1993; Campbell and Benz, 1984).

The critical protective function of P-glycoprotein in mammals has been formally established. Schinkel et al., 1994 generated a P-glycoprotein-deficient mouse line by targeting the *mdr*1a gene. When colonies of the resulting mice were infested with mites and subsequently sprayed with ivermectin for treatment, homozygous *mdr*1a-deficient mice selectively exhibited an enhanced sensitivity to the drug that resulted in toxicity and reactions including mortality (Schinkel et al., 1994). The symptoms of neurotoxicity were associated with an 80- to 90-fold increase in ivermectin brain concentration. Invalidation of the P-glycoprotein function in this mouse model resulted in an impaired blood–brain barrier and a subsequently increased CNS permeability to xenobiotics. These results were similar to other observations, in which 25% of CF1 strain mice displayed signs of neurotoxicity when treated with ivermectin (Umbenhauer et al., 1997). The affected CF1 mice do not express P-glycoprotein in their CNS endothelial cells (Lankas et al., 1997), and this sensitive trait is associated with a restriction fragment length polymorphism that affects the *mdr*1a gene.

It has been noted that certain dogs of the Collie breed display enhanced sensitivity to the effects of ivermectin (Paul et al., 1987; Pulliam et al., 1985). These sensitive animals display toxic signs at doses as low as 100 μg/kg, while doses over 2000 μg/kg are required to elicit toxicity in Beagle dogs. Concordant data suggest an impaired P-glycoprotein function in these dogs. In control Beagle dogs, the ivermectin concentration ratios between plasma and brain and liver and brain are 10 and 100, respectively. In sensitive Collies, brain ivermectin concentrations may exceed those in plasma and liver. Consequently, therapeutic administration of ivermectin to dogs has been restricted to heartworm prophylaxis. In these circumstances, ivermectin is effective at a low dosage (6 μg/kg) which has been shown to have no adverse effects even in sensitive dogs (Fassler et al., 1991; Paul et al., 1991).

Consistent with the apparent lack of P-glycoprotein function in some Collies, two molecular causes can be hypothesized: (1) the decreased expression of a functional gene, or (2) a gene mutation that impairs protein concentration and/or activity. After cloning and sequencing of the full-length cDNA P-glycoprotein from control Beagles and ivermectin-sensitive Collies, we established that the latter display a frame shift-generating mutation in the fourth coding exon of the MDR1 gene. Although transcribed, this mutated messenger is not translated into a detectable protein in membrane preparations of sensitive dogs, representing a unique case of naturally occurring homozygous gene invalidation. This genotype, which may be identified by genomic DNA amplification, leads to a P-glycoprotein abrogation phenotype in a non-rodent model that is of broad interest for evaluating drug bioavailability and toxicity.

2. Material and methods

2.1. Animal protocols

Animal housing and experiments involving Beagle dogs (Harlan, Gannat, France) were according to E.U. institutional guidelines for animal care and use. Beagle tissues were obtained from two adult males (4 years old).

Animal housing and handling of the 11-year-old Collie dogs of our study (male and female) (Trail Lane, USA) were according to USDA guidelines for animal care and use and in accordance with the local Institutional Animal Care and Use Committee.

All tissues (liver, colon, heart) were obtained following veterinarian-supervised euthanasia. Organ fragments were snap-frozen in liquid nitrogen upon collection and stored at − 80 °C until DNA or RNA preparation.

Collection of mouth cells from Collie and Shetland dogs of the general population was achieved during an international canine exhibition under the supervision of certified experts to assess the pedigree of the animals. Consent from each animal owner was obtained prior to sampling.

2.2. Sequencing of MDR1 genes

Total RNA was extracted from Beagle and Collie colon samples using Trizol® reagent (Invitrogen, Cergy Pontoise, France). Poly-A RNA was purified from 75 μg of total RNA using oligo-dT-coated magnetic beads (Dynal, Compiègne, France). Reverse transcription was carried out on 400 ng of messenger RNA using oligo-dT primers (Invitrogen). The resulting Beagle and Collie cDNAs were subsequently used as templates for polymerase chain reaction (PCR) amplification. For the Beagle, the following primers (cf. Fig. 1) were designed (OligoExpress, Grenoble, France): 1F 5′-CTAAGTCGGAGTATCTTCTTCC-3′ and 1R 5′-GTGCAGCTCCATGGATGATGG-3′; 2F 5′-GTTGTACATGCTGGTGGGAACTCTGGC-3′ and 2R 5′-GTAGACAAGCGATGAGCTATCACAATGGTGG-3′; 3F 5′-GCCTATGACTTCATCATGAAACTGCC-3′ and 3R 5′-GCGCTTTGCTCCAGCCTGCACAC-3′; 4F 5′-CTCTTGTTAGACAGCCTCATATTTTG-3′ and 4R 5′-GCGGCGCGGACTAGTGGCGCTTTTTTTTTTTT-3′.

For the Collie breed, three pairs of primers were designed using the Beagle sequence as a reference: 5F 5′-GTGACGATGGATCCTGAAGGAGGCCGTAAG-3′ and 5R 5′-CCATTCAGTTGAGTTCAGCTTCAGAATCCTCCAG-3′; 6F 5′-CCGGCTGAGTGGTGGACAGAAACAGAGAAT-3′ and 6R 5′-CAAGGGGTCATAGAAGCGCTCTAG-

GAGCTG-3′; 7F 5′-GTTCAGCTCCTAGAGCGCTTCTATGACCCCTTGGC-3′ and 7R 5′-GGTGCAGCGGCCGCGGCCACAGTTCACTAGCGTTTTGCTCCAGCCTGGACACTGATCATGG-3′.

All PCR products were cloned into plasmids using a T–A cloning strategy (PCR 2.1 Topo Cloning, Invitrogen). Following restriction characterization, Beagle and Collie cDNA clones were double-sequenced on both strands (Genome Express, Grenoble, France) using M13 and M13-reverse oligonucleotides as primers. Overlapping fragments allowed us to reconstruct full-length cDNAs, which were deposited in the GenBank database (GenBank accession numbers: AF045019, AF269224, AJ419568).

2.3. Western blots

Hepatic tissue (200 mg) or cell (10^6 cells) samples were homogenized on ice in Tris–HCl buffer (0.1 M; pH 7.6) supplemented with a protease inhibitor mix (aprotinin, leupeptin, antipaïn, pepstatin A), as previously described (Conrad et al., 2001). Following centrifugation (6500*g*, 20 min, 4 °C), supernatants were ultracentrifuged (100,000*g*; 1 h; 4 °C). Pellets, which represent membrane preparations, were resuspended in Tris–HCl buffer (0.1 M; pH 7.6). Sodium dodecylsulfate (SDS)-solubilized proteins (150 μg for tissue samples, 40 μg for cell culture samples) were separated by electrophoresis on 6.5% SDS-polyacrylamide gels and transferred to nitrocellulose membranes (Optitran BA-S 85, Schleicher & Schuell, Dassel, Germany). Membranes saturated with skim milk proteins were incubated (16 h; 4 °C) with a primary anti-P-glycoprotein antibody (#PC03, Oncogene, Boston, MA, USA) and washed three times before incubation (1 h; 20 °C) with a horseradish peroxidase-conjugated anti-rabbit-IgG sheep antibody (#A9169, Sigma, St. Quentin Fallavier, France) and final washing. Incubation and washing buffer was Tris–HCl (0.1 M; pH 7.4) + 0.1% Triton X-100 (Sigma). Immunoreactive proteins were visualized with enhanced chemiluminescent substrate (#34080, Pierce, Rockford, IL, USA) on Hyperfilm ECL™ (Amersham Biosciences, Saclay, France). Doxorubicin-resistant MES-SA/Dx5 human cells (EACC, Salisbury, UK) were cultured and used as a reliable source of human P-glycoprotein for positive control in Western blotting experiments.

2.4. Genomic DNA testing

Canine genomic DNA was purified using QuiAMP DNA mini kit (Qiagen, Courtabœuf, France). Samples used included colon tissue (ivermectin-sensitive Collies) or mouth cells (Beagle + wild-type Collies). Mouth cell samples were collected using Kito-brushes (Kaltek, Padova, Italy). Specific canine primers (OligoExpress) were designed to amplify, from both breeds, a PCR fragment that encompasses the deletion site identified within the ivermectin-sensitive Collie DNA (F 5′-CCTCTCATGATGCTGGT-3′; R 5′-TGAAATTCCTGCATTTGCA-3′). Genomic DNA (5 ng) or recombinant cDNA plasmids (1 ng), as control, were submitted to PCR amplification (30 cycles; 95 °C/30 s–60 °C/45 s–72 °C/30 s). DNA fragments were submitted to electrophoresis on ethidium bromide-stained polyacrylamide gels (12% in 1 × Tris–Borate–EDTA (TBE) buffer) and visualized using a Geldoc UV transluminator (Biorad, Marne-la-Coquette, France).

3. Results

When these investigations were initiated, molecular data regarding the P-glycoprotein-encoding gene in dogs were not available. Using reverse-transcribed colon messenger RNA from wild-type male Beagles, we designed a strategy to amplify overlapping fragments spanning the complete canine MDR1 cDNA sequence. We selected suitable pairs of PCR primers from data bank sequences, taking advantage of the presence in P-glycoprotein proteins of repeated motifs encoding enzymatically active sites (i.e. Walker A, Walker B) that display highly conserved nucleotide sequences between species (Fig. 1A). To amplify a full-length cDNA sequence, we used a restriction site-bearing oligo-dT (4R) at the 3′ end of the transcript. In order to access the entire canine sequence, we selected a 5′ forward primer (1F) downstream to the ATG start codon in the 5′ untranslated region of the human MDR1 gene that displays a high rate of homology with other related sequences. Overlapping fragments 2 and 3 (Fig. 1A) obtained from murine primers were amplified and sequenced first, allowing the subsequent amplification of fragments 1 and 4 with canine reverse and forward primers, respectively. Double sequencing on both strands allowed us to reconstitute the full-length Beagle dog MDR1 cDNA sequence (GenBank AF045016, 07-FEB-98), which was confirmed in a second independent experiment (GenBank AF269224, 04-JUL-00).

Elucidation of the canine P-glycoprotein nucleotide sequence allowed us to implement a canine primer-based amplification strategy to sequence the full-length MDR1 cDNA from ivermectin-sensitive Collie dogs. PCR templates were prepared from reverse-transcribed RNA extracted from enterocyte necropsy samples from one aged, ivermectin-sensitive, male dog. Overlapping fragments spanning the entire MDR1 cDNA were generated and cloned through a T–A cloning strategy. cDNA clones from this dog were sequenced on both strands; results were consistent with a unique MDR1 sequence from ivermectin-sensitive Collies (GenBank AJ419568, 06-DEC-01). This sequence displays 99.9% identity with the wild-type Beagle MDR1 sequence. The sole discrepancy lies at a single location in the fourth coding exon of the gene and consists of a 4-bp deletion. This deletion occurs within a palindromic sequence of five bases, ^{164}GATAG^{168}, rendering it impossible to discriminate which G residue (164 or 168) is retained in the ivermectin-sensitive Collie (Fig. 1B).

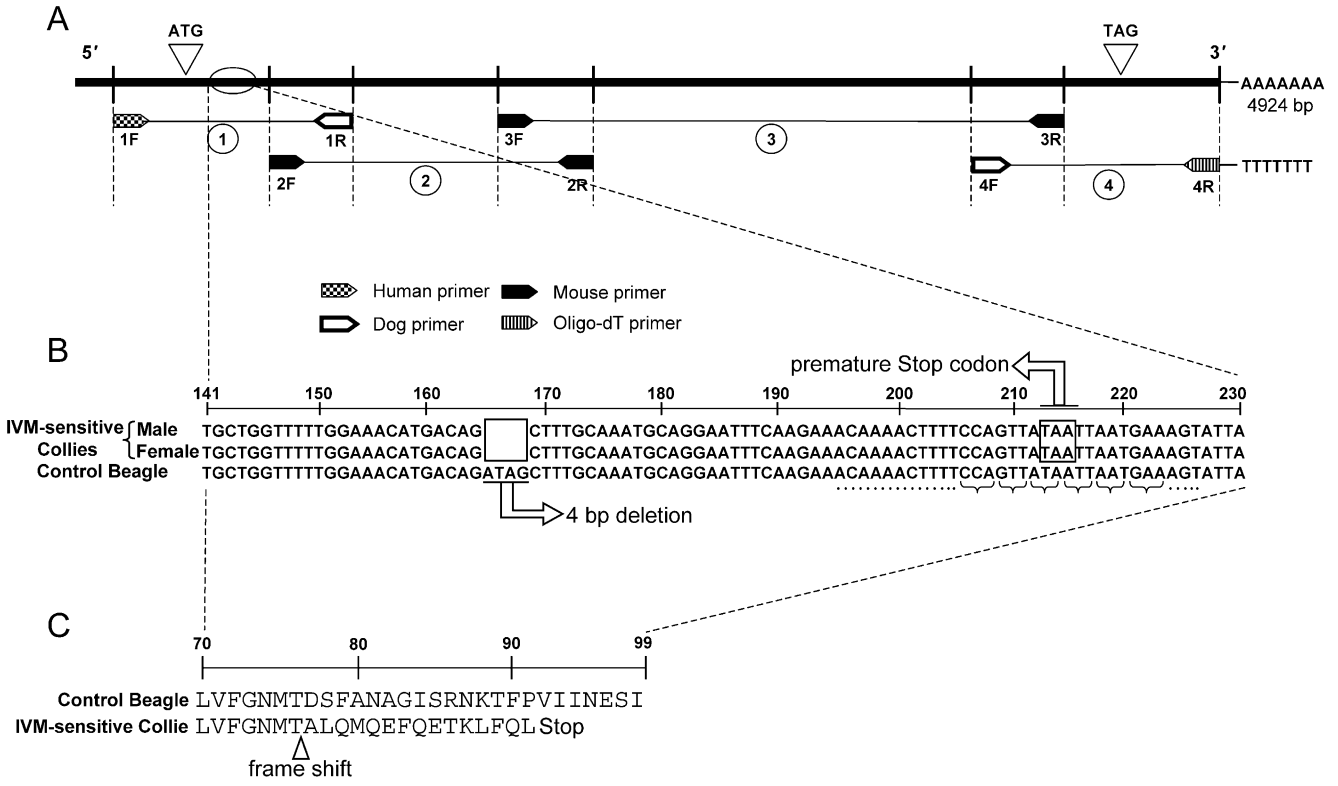

Fig. 1. (A) Strategy for canine MDR1 gene fragment amplification. Four overlapping cDNA fragments were amplified spanning the entire coding sequence of the gene. The nature of the PCR primers is indicated. (B) Comparison of cDNA sequences showing a 4-bp deletion in the ivermectin-sensitive Collie gene (GenBank accession nos.: AF045016 and AF269224 for wild-type Beagle, AJ419568 for ivermectin-sensitive Collies). (C) Peptide-translated sequence. Frame shift in the Collie gene, which results from the 4-bp deletion. Premature Stop codon at position 91 of the P-glycoprotein peptide sequence in ivermectin-sensitive Collies.

^{164}G was used in our figure (Fig. 1B). The frame shift that results from the deletion is translated into a premature Stop codon located at position 91 of the translated peptide sequence of the protein. The resulting protein is restricted to its amino-terminal segment, representing only 7.1% (91/1281) of its fully mature sequence (Fig. 1C). No enzymatically active domains were present in this translated polypeptide. The cDNA sequence and the location of the 4-bp deletion were confirmed by sequencing the P-glycoprotein cDNA of an ivermectin-sensitive Collie female, which was genetically unrelated to the male evaluated initially (Fig. 1B).

Since homozygosity in ivermectin-sensitive animals could not be inferred from our sequencing results, which were obtained from a limited number of samples, we therefore designed a selective strategy to discriminate wild-type and invalidated alleles of the MDR1 gene in canine genomic DNA. The aim was to develop a reliable diagnostic tool to assess the homozygous mutant status of some dogs and to predict their drug-sensitive phenotype. Analysis of DNA fragments that differ by only 4 bp required the amplification of reasonably short fragments (i.e. 75 and 79 bp) and their analysis under discriminating conditions. Following amplification with appropriate primers, fragments that encompass the deletion site were analyzed in ethidium bromide-stained 12% polyacrylamide gels. Our results showed that the sensitive animals of our canine population were indeed homozygous for the deleted allele. Conversely, individuals of both breeds, Beagle and Collie, that were not sensitive to ivermectin displayed solely the wild-type allele (Fig. 2A and B).

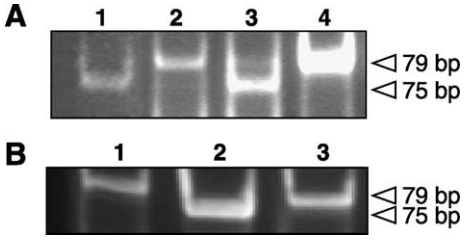

Fig. 2. PCR amplification of genomic DNA fragments of the canine MDR1 gene (see Material and methods for primer sequences). PCR-amplified fragments were analyzed on an UV transluminator in an ethidium bromide-stained 12% polyacrylamide gel. (A) Lane 1: recombinant plasmid with the deletion-bearing allele of ivermectin-sensitive Collies, lane 2: recombinant plasmid with wild-type allele, lane 3: colon genomic DNA from ivermectin-sensitive Collie, lane 4: mouth cells genomic DNA from wild-type Beagle. (B) Lane 1: mouth cells genomic DNA from wild-type Beagle, lane 2: recombinant plasmid with the deletion-bearing allele of ivermectin-sensitive Collies, lane 3: mouth cells genomic DNA from wild-type (nonsensitive) Collie. Wild-type alleles generated a 79-bp-long fragment while alleles that harbored the 4-bp deletion displayed a 75-bp-long fragment.

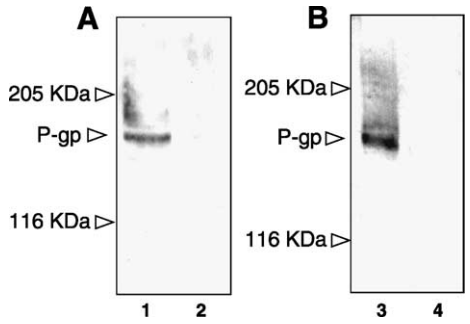

Fig. 3. Western blot analysis of cellular membrane preparations. The primary antibody was raised against a peptide derived from the human P-glycoprotein. (A) Liver samples (150 μg of proteins/lane). Lane 3: wild-type Beagle. Lane 4: ivermectin-sensitive Collie. (B) Cell culture samples (40 μg of proteins/lane). Lane 1: human, doxorubicin-resistant MES-SA/Dx5 cells. Lane 2: negative control (LLC PK1, porcine renal epithelial cell line).

It was then established that ivermectin-sensitive Collies display a homozygous mutation in an MDR1 exonic sequence. However, the null phenotype of these animals is best demonstrated through the study of the tissue expression of P-glycoprotein. Although unlikely, it is possible that a short polypeptide is expressed. To test for this, we were able to locate an antibody raised against a human peptide (21 residues) of P-glycoprotein that matched the canine sequence completely (^{1204}SALDTESEKVVQEALDKAR-EGRT^{1225}). Membrane preparations of dog hepatocytes were then analyzed by Western blotting. A human tumor cell line overexpressing the P-glycoprotein was used as a positive control (Fig. 3B). This antibody, which showed an immuno-detected band in preparations from Beagle dogs, did not display any signal in samples from ivermectin-sensitive Collies (Fig. 3A), indicating that the antihuman P-glycoprotein peptide antibody cross-reacted with the canine mature protein while it failed to detect any polypeptide in membrane preparations from dogs homozygous for the MDR1-deleted allele. Subsequently, the occurrence of gene invalidation affecting MDR1 P-glycoprotein in some dogs was established.

4. Discussion

In conjunction, several investigations are highly evocative of the involvement of the drug carrier P-glycoprotein in the occurrence of a reported ivermectin-sensitive phenotype in dog (Pulliam et al., 1985). Ivermectin was shown to strongly interfere with P-glycoprotein-dependent drug resistance profiles of tumor cells (Pouliot et al., 1997). Additionally, ivermectin was shown to be a very potent substrate for P-glycoprotein (Didier and Loor, 1996). When it was later shown that ivermectin accumulates at toxic levels in the brain tissue of mdr1a-deficient mice, a functional link was established between this ABC transporter and the abnormal therapeutic index of this drug in some dogs (Schinkel et al., 1994). It was then possible to speculate that the canine phenotype of ivermectin sensitivity could be associated with a genotype of natural invalidation of both alleles of the orthologous MDR1 canine gene. Nonetheless, spontaneous gene invalidation in non-rodent species is a very infrequent event that deserves to be fully substantiated when suspected. As an example, the abrogation of the myostatin gene in cattle leads to the "double-muscled" phenotype, which provides obvious benefits (Grobet et al., 1997).

Although the hereditary nature of ivermectin sensitivity is recognized, its precise mode of inheritance has remained elusive since no genetic data were available in dogs to initiate molecular investigations. We, therefore, started our investigation by documenting, for the first time, the MDR1 cDNA wild-type canine sequence. Analysis of the 1282-residue-translated sequence revealed a prototypical P-glycoprotein, which harbors all structurally and chemically active segments of these membrane-anchored proteins (i.e. 12 transmembrane domains, 2 nucleotide-binding domains (NBD) and 1 linker region). The product of the human MDR1 gene is, among all species documented in data banks, the most similar to that of the canine gene. Human and dog proteins display 91% overall homology, with non-consensus residues being located outside the functional segments. Indeed, Walkers A and B motifs of the NBD domains, which are essential for P-glycoprotein functionality, are 100% conserved. Conversely, the rate of homology decreases to 70.8% in the less critical linker region. This elevated rate of identity in peptide sequences might subsequently strengthen interest in using a canine model to predict in vivo human P-glycoprotein behavior in the field of cellular drug transport. Further ongoing studies should delineate the substrate specificity of the canine P-glycoprotein and compare it to that of human and rodent orthologous proteins (Tang-Wai et al., 1995; Yamazaki et al., 2001).

Using canine-specific primers, we were able to document the first full-length sequence of the MDR1 cDNA originating from an ivermectin-sensitive Collie (GenBank AJ419568, 06-DEC-01). This animal was from a line of ivermectin-sensitive Collies and had been determined to be sensitive to ivermectin phenotypically by administration of a single oral dose of ivermectin (150 μg/kg), followed by careful evaluation for clinical signs of ivermectin toxicity (e.g. ataxia, depression, salivation and mydriasis). The finding that the sole difference between wild-type and drug-sensitive dogs was represented by a 4-bp deletion in the fourth exon of the MDR1 cDNA was initially obtained from a male individual and reproduced in a sensitive female from a different genetic line. The mutation, which maintains a ^{164}G nucleotide in both cases, could either be a "GATA" or a "ATAG" deletion. This result is supported by a report published by Mealey et al. (2001). With respect to the Beagle MDR1 sequence (AF045016), Mealey et al. (2001) amplified overlapping PCR fragments spanning most, but not all, of the MDR1 normal transcript and excluding a 3′

segment. In several male and female ivermectin-sensitive Collies from a defined origin, this deletion was identified in the MDR1 gene. A sequence of 433 bp was documented; no other sequences related to this work are available from data sources. We documented the complete P-glycoprotein cDNA sequence (4924 bp) of ivermectin-sensitive Collies (AJ419568) and we feel confident that this deletion represents the sole genetic mutation found in the MDR1 gene of the tested dogs.

Assuming that the drug sensitivity originated from this mutation, only animals harboring a homozygous deletion would display a P-glycoprotein null phenotype. Similar to Mealey et al. (2001), we could only detect deleted sequences as being different in our sensitive dog. To formally document the homozygous mutant genotype, we used canine primers to amplify short genomic sequences spanning the deletion site, which were successfully separated on high concentration gels. We found a direct correlation between the observed sensitive phenotype and the double mutant-allele genotype. Thus, Collie genotyping from genomic DNA is a suitable strategy for tracing the deleted allele and could be used to select animals for breeding and to implement genetic monitoring. Results presented here were obtained with genomic DNA from organ biopsies or from mouth cells collected on nylon brushes used in forensic medicine. Although the latter approach offers a noninvasive sampling alternative, DNA samples of this kind did not display fully reliable amplification results. Contamination by bacterial DNA from mouth flora might provide templates for cross-amplification of DNA fragments.

Although the frame shift-inducing deletion in the Collie MDR1 gene was clearly established, analysis of the level of expression of P-glycoprotein was necessary to conclude that the observed phenotype is a direct consequence of the genotype. Indeed, we identified an antibody raised against a P-glycoprotein peptide of human origin that cross-hybridized with the Beagle dog protein. This molecular tool failed to detect any polypeptide in the corresponding organ preparation from sensitive Collie dog, thus establishing that the genetic deficiency translates into a lack of protein and function.

Until recently, gene knockout in mammals other than mice was not achievable due to the lack of functional embryonic stem (ES) cells in other species. Although the development of nuclear transfer technologies opens new prospects in this field, the identification of original non-rodent models of gene invalidation relies mainly on serendipitous identification. Our results provide evidence that a spontaneous gene knockout has occurred in Collie dogs which have been previously shown to display abnormal drug bioavailability and tissue distribution for a recognized P-glycoprotein substrate (Pulliam et al., 1985; Sutherland and Campbell, 1990). Following supplementary validation studies, wild-type and deficient dogs might represent a valuable model to test the in vivo role of the P-glycoprotein in influencing the tissue distribution of selected molecules. In addition to its current use in veterinary drug development (Fassler et al., 1991; Paul et al., 2000; Tranquilli et al., 1991), this model could be useful to the field of human therapeutics. Moreover, recent findings have shown in intact brain endothelial capillaries that the human therapeutic agents cyclosporin A and verapamil are potent competitors of the P-glycoprotein-mediated transport of ivermectin (Nobmann et al., 2001). Regarding P-glycoprotein drug transport, Beagle (the reference breed in toxico-pharmacology studies) and wild-type Collie dogs can be considered as equivalent since we established that they display fully identical MDR1 sequences. The marked degree of homology between canine and human P-glycoprotein that we documented strengthens interest in the canine model for drug bioavailability studies. Moreover, the MDR1 spontaneous knockout Collie line of dogs will represent a novel and useful model for evaluating the role of the P-glycoprotein function in interfering with the tissue distribution of any test compound. Applications are easily conceivable in the fields of drug distribution, neurotoxicity and efficacy of chemical cancer treatments.

Acknowledgements

We thank C. Bétoulières and F. Lasserre for excellent technical assistance, and N. Ledger for comments on this manuscript.

This work was supported by a grant from the Région Midi Pyrénées (97002502). A. Roulet is a recipient of a fellowship from the A.R.C.

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