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Isolation and Characterization of Polyspecific Mouse Organic Solute Carrier Protein 1 (mOscp1)[§]

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A running title: Isolation and functional characterization of mOscp1

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Abbreviations: ABC, ATP-binding cassette; CT2, carnitine transporter 2; dCTP, deoxycytidine [5'-α-^{32}P]triphosphate; DHEA-S, dehydroepiandrosterone sulfate; DMSO, dimethyl sulfoxide; ES, estron-3-sulfate; 5-FU, 5-fluorouracil; GST, gonad-specific transporter; Mdrs, multiple drug resistance proteins; mOat, mouse organic anion transporter; mOatp, organic anion transporting polypeptide; mOct, mouse organic cation transporter; mOCTN, mouse organic cation transporter novel; mOscp1, mouse organic solute carrier protein 1; mNaDC, mouse sodium dicarboxylate cotransporter; 6-MP, 6-mercaptopurine; mPGT, mouse prostaglandin transporter; Mrps, multidrug resistance proteins; OAT/Oat, organic anion transporter; OATP/Oatp, organic anion transporting polypeptide; OCTs, organic cation transporters; OR2, oocyte Ringer 2; PAH, *p*-aminohippurate; PG, prostaglandin; SLC, solute carrier; SSC, standard saline citrate; TEA, tetraethylammonium; TMD, transmembrane domains.

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ABSTRACT:

We succeeded in isolating the cDNA encoding mouse organic solute carrier protein 1 (mOscp1) from a mouse testis cDNA library. mOscp1 consisted of 1137 base pairs that encoded a 379-amino acid protein, and the amino acid sequence was 85% identical to that of human OSCP1 (hOSCP1). Northern blot analysis revealed that the *gene* coding for mOscp1 is highly expressed in the testis, but not in other tissues. When expressed in *Xenopus laevis* oocytes, mOscp1 mediated the high affinity transport of *p*-aminohippurate (PAH) (K_m = 18.8 ± 4.1 μM) with a Na^+-independency. mOscp1 transported various kinds of structurally dissimilar drugs and chemicals such as probenecid, dehydroepiandrosterone sulfate (DHEA-S), glutarate with some differences in substrate specificity compared with hOSCP1. Cyclophosphamide inhibited the mOscp1-mediated PAH uptake. Immunohistochemical analysis revealed that the mOscp1 protein is localized in the plasma membrane side of Sertoli cells in the testis. Our results indicate that isolated mOscp1 is a polyspecific organic solute carrier protein and may be a key molecule for the testicular handling of organic solutes.

Introduction

Molecular cloning technology has led to the identification of the functional characterization of various polyspesific organic solute transporters (Hagenbuch and Meier, 2003; Hediger et al., 2004; Enomoto and Endou, 2005; Wright, 2005). Such transporters have been divided into two major categories, the solute carrier (*SLC*) series and the ATP-binding cassette (*ABC*) series (Burckhardt and Wolff, 2000; Dresser et al., 2000; Inui et al., 2000; Sekine et al., 2000; Trauner and Boyer, 2003; Burckhardt B and Burckhardt G, 2003; van Montfoort et al., 2003; Miyazaki et al., 2004). At present, the transporters of the *SLC* series are considered to be involved in the elimination and excretion of a broad range of endogenous and exogenous ionic compounds including environmental toxicants and their metabolites from the body (Hagenbuch and Meier, 2003; Hediger et al., 2004; Enomoto and Endou, 2005; Wright, 2005). For example, the organic anion transporter (OAT/Oat[*SLC22A*]) and organic anion transporting polypeptide (OATP/Oatp[*SLC21/SLCO*]) families mediate the transport of a wide spectrum of amphipathic organic compounds such as methotrexate (MTX), DHEA-S and ES (estron-3-sulfate) (Hagenbuch and Meier, 2004; Enomoto and Endou, 2005). Each transporter of a particular family may have overlapping substrate specificity (Cherrington et al., 2002; Hagenbuch and Meier, 2003).

Several barrier systems have been found in the body. In the testis, a physiological barrier separating the adluminal compartment of the seminiferous tubules is created by the formation of a tight junction near the base of Sertoli cells (Russell, 1977). Developing germ cells are protected from xenobiotic and immunological effects. It has been known that drugs and chemicals pass through Sertoli cells by either passive diffusion or a facilitated transporter. Selective movement of compounds across

Sertoli cells comprises the physiological or functional role of the blood-testis barrier. Because of its important role in regulating the exchange of compounds between blood and adluminal spaces and protecting the developing germ cells from various toxicants, Sertoli cells play an important role in the protection of the male reproductive system.

Recently, we isolated the novel cDNA encoding human organic solute carrier protein 1 (hOSCP1) (Kobayashi et al., 2005). When expressed in *X.* oocytes, hOSCP1 transported PAH, tetraethylammonium (TEA), prostaglandin E_2 (PGE_2), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in a Na^+-independent manner. Immunohistochemical analysis revealed that the hOSCP1 protein is shown to be localized in the basal membrane of the syncytiotrophoblast in the placenta; therefore, we proposed that hOSCP1 plays a critical role in the placental handling of organic solutes from the human body. By Northern blot analysis, we also revealed that hOSCP1 cDNA is hybridized with mouse testis mRNA, indicating that the murine homolog of hOSCP1 exists in mice testis.

Here, we describe the isolation and functional characterization of the mouse homolog of hOSCP1, mOscp1, from a mouse testis cDNA library. Our results indicate that mOscp1, as well as hOSCP1, is a polyspecific organic solute carrier protein with some differences in substrate specificity compared with hOSCP1.

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Experimental Procedures

Materials. [^{14}C]Glutarate (55.0 mCi/mmol), α-ketoglutarate (54.5 mCi/mmol), [^{3}H]PGF$_{2\alpha}$ (214.7 Ci/mmol), [^{14}C]L-ascorbic acid (vitamin C) (4 mCi/mmol), [^{14}C]androst-4-ene-3,17-dione (androstenedione) (53.6 mCi/mmol), [^{3}H]ES (57.3 Ci/mmol), [^{14}C]salicylic acid (55.5 mCi/mmol), [^{14}C]PAH (52.7 mCi/mmol) and [^{3}H]DHEA-S (74 Ci/mmol) were purchased from PerkinElmer Life Sciences, Inc (Boston, MA, U.S.A.). [^{3}H]Valproate (1.0 mCi/mmol), [^{14}C]6-mercaptopurine (6-MP) (53 mCi/mmol), [^{3}H]5-fluorouracil (5-FU) (1.0 mCi/mmol) and [^{14}C]allopurinol (51 GBq/mmol) were purchased from Moravek (Brea, CA, U.S.A.). [^{14}C]TEA (55.4 mCi/mmol), [^{3}H]L-carnitine (80 Ci/mmol), [^{14}C]L-leucine (55 mCi/mmol), [^{3}H]PGE$_2$ (193.5 Ci/mmol), [^{14}C]progesterone (55 mCi/mmol), [^{14}C]erythromycin (55 mCi/mmol), [^{3}H]bumetanide (20 Ci/mmol), [^{3}H]hexanoic acid (1.0 mCi/mmol), [^{3}H]taxol (paclitaxel) (20 Ci/mmol), [^{14}C]testosterone (50 mCi/mmol), [^{3}H]L-tryptophan (20 Ci/mmol) and [^{14}C]theophylline (52 mCi/mmol), [^{3}H]tetracycline (1 Ci/mmol) were purchased from ARC Inc. (Saint Louis, MO, U.S.A). [^{3}H]Probenecid was kindly provided by Fuji BioMedix Inc. (Saitama, Japan). Deoxycytidine [5'-α-^{32}P]triphosphate (dCTP) (111 TBq/mmol) was obtained from Muromachi Yakuhin Kaisha, LTD (Tokyo, Japan). All other chemicals were of the highest grade commercially available.

Construction of cDNA library and isolation of mOscp1. A nondirectional cDNA library for screening was prepared from mouse testis poly (A)$^+$ RNA using Superscript Choice System (Invitrogen^{TM}, Carlsbad, CA, U.S.A.) and was ligated into phage vector λZipLox EcoRI arms (Invitrogen^{TM}, Carlsbad, CA, U.S.A.). The library was screened by homology using full-length hOSCP1 cDNA labeled with [α-^{32}P]dCTP by random priming (T7Quick Prime Kit, Amersham Pharmacia Biotech, Uppsala, Sweden) as a probe. Replicated filters of the phage library were hybridized

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overnight in a hybridization solution (50% formamide; 5x standard saline citrate (SSC); 3x Denhardt's solution; 0.2% SDS; 10% dextran sulfate; 0.3 μg/ml denatured salmon sperm DNA; 2.5 mM sodium pyrophosphate; 25 mM 2-morpholinoethanesulfonic acid (MES); 0.03% Antifoam A; pH 6.5) at 37°C overnight. The filters were finally washed in 0.1x SSC and 0.1% SDS at 37°C. cDNA inserts in positive λZipLox phage were recovered in plasmid pZL1 vector by in vitro excision.

DNA Sequence and Hydropathy Analysis. Double-stranded cDNA of isolated clones were sequenced in both directions. Specially synthesized oligonucleotide primers were used for sequencing of mOscp1 cDNA, which was sequenced by the dye terminator method using a dye primer cycle sequencing kit (Applied Biosystems, Foster City, CA, U.S.A.) and an automated Applied Biosystems 310 DNA sequencer. The sequence, the presence of possible signal peptides and the Kyte and Doolittle hydropathy plot were analyzed using DNASIS-Pro. (HITACHI Software Engineering, Yokohama, Japan).

***Xenopus Laevis* Oocyte Preparation, cRNA Synthesis and Transport Activity.** Isolation of *Xenopus* oocytes was performed as described elsewhere (Kobayashi et al., 2005). Stage V and VI defolliculated oocytes were selected throughout this experiment. To remove the follicular layer from *Xenopus* oocytes, collagenase A (Boehringer Mannheim, Mannheim, Germany) was used at a final concentration of 2.0 mg/ml in an OR2 solution (83 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.5) and slowly shaken for at least 1 to 2 h at room temperature. The isolated cDNA, mOscp1, was linearized with *Bam*HI, and the capped cRNA was transcribed in vitro by Sp6 RNA polymerase (Ambion, Austin, TX, U.S.A.). Defolliculated oocytes were microinjected with 50 ng of in vitro transcribed cRNA and incubated for two days in a modified Barth's solution containing gentamicin (50 μg/ml) at 18°C. Uptake

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experiments of radiolabeled substrates, as indicated in each experiment, were performed in an ND 96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.4) at room temperature. Oocytes were incubated in 450 μl of the same solution containing radiolabeled substrates for 1 h at room temperature. The uptake was terminated by the addition of 2 ml of ice-cold ND 96 solution, and the oocytes were washed with the same solution at least five times. The oocytes were solubilized with 10% SDS, and accumulated radioactivity was determined with a liquid scintillation counter. The experiments were repeated with oocytes from at least five frogs.

Kinetic study. Concentration-dependent uptake experiments of [^{14}C]PAH via mOscp1 were performed with each compound at final concentrations ranging from 1, 5, 10, 20, 50 and 100 μM. The compounds were incubated with mOscp1-expressing oocytes for 1 h at room temperature, stopped with ice-cold ND96 solution and washed 5 times as described above. Individual oocytes were transferred to scintillation vials and dissolved on 250 μl 10% SDS. A scintillation cocktail was added, and radioactivity was counted. Counts in uninjected- oocytes were subtracted from the counts in cRNA-injected oocytes. Data are presented as mean $\pm$ S.E.M., except for kinetic constants for which the error represents the error of the fit. K_m indicates the Michaelis-Menten constant (μM).

Inhibition study. For inhibition experiments, oocytes expressing mOscp1 were incubated for 1 h in ND96 solution containing 20 μM [^{14}C]PAH in the presence or absence of various inhibitors at a final concentration of 1 mM. Cyclosporin A, cyclophosphamide, and doxorubicin (adriamycin) were directly dissolved in ND96 solution from the stock solution. These stock solutions of the inhibitor were dissolved in dimethyl sulfoxide (DMSO) and diluted to a final concentration as described above.

The final concentration of DMSO in the assay medium did not exceed 1%.

Northern Blot Analysis. Total RNA was isolated from various male mouse tissues using the acid guanidine thiocyanate (GTC)-phenol-chloroform extraction method (Chomczynski and Sacchi, 1987). Two μg of poly $(A)^+$ RNA was purified from total RNA and was loaded onto 1.0% agarose/formaldehyde gel. The nucleic acids were transferred onto a nylon membrane after electrophoresis (Hybond N+, Amersham Pharmacia Biotech, Uppsala, Sweden). The filter was hybridized at 42°C overnight in a hybridization solution containing 50% formamide with a full-length cDNA of mOscp1, which was randomly labeled with ^{32}P-dCTP (Feinberg and Vogelstein, 1983). The filter was finally washed in 0.1x SSC/0.1% SDS at 42°C.

Immunohistochemical Analysis. Immunohistochemical analysis was performed as previously described (Kobayashi et al., 2005). A five-micrometer wax section of mouse testis was obtained from BioChain Institute, Inc. (San Leandro, CA, U.S.A.) and was carried out for light microscopic immunohistochemical analysis using the streptavidine-biotin-horseradish peroxidase complex technique (DAKO, Carpinteria, CA, U.S.A.). Sections were dewaxed, rehydrated, and incubated with 3% H_2O_2 to eliminate endogenous peroxidase activity. After rinsing in 0.05 M Tris-buffered saline containing 0.1% Tween-20, sections were treated with 10 μg/ml of primary rabbit polyclonal antibody (4°C, overnight). Thereafter, the sections were incubated with the secondary biotinylated goat polyclonal antibody against rabbit immunoglobulin (DAKO, Carpinteria, CA, U.S.A.) with horseradish peroxidase-labeled streptavidin. This step was followed by incubation with diaminobenzidine and hydrogen peroxide. The sections were counterstained with hematoxylin and examined under light microscopy. For the preabsorption experiment, the peptide (200 μg/ml) was added to the hOSCP1-specific antibody solution and incubated overnight at 4°C.

Statistical Analysis. Kinetic data from experiments measuring the uptake of radiolabeled substrates were fit to the Michaelis-Menten equation by nonlinear least-squares regression analysis with standard errors derived from these curves. Comparisons of data measuring initial rates of uptake of radiolabeled substrates in the presence and absence of inhibitors were performed by the unpaired Student's *t* test or one-way ANOVA (GraphPad Prism4). The values represent the mean $\pm$ S.E.M (*p<0.05).

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Results

Cloning and Structural Features of mOscp1. Using full-length hOSCP1 cDNA as a probe, a nondirectional cDNA library of mouse testis was screened as a homology screening technique. We finally purified fifteen positive plaques. mOscp1 has a single open reading frame of 1137 bp encoding a 379 amino acid sequence (M_r 43,229) (GenBank^{TM}/EMBL/DDBJ, accession number AB154826). The membrane topology of mOscp1 was analyzed by Kyte and Doolittle hydropathy analysis predicting that mOscp1 has 3 putative transmembrane domains (TMD) (Fig. 1A). There is one consensus sequence for *N*-glycosylation sites (Asn^{364}) that are predicted to be outside the membrane. The amino acid sequence alignment of mOscp1 compared with hOSCP1 is also shown in Fig. 1A. The two sequences are 85% identical. The high structural similarity to hOSCP1 revealed that mOscp1 encodes a human counterpart. A phylogenetic tree analysis showed that mOscp1 can be localized differently from the Oatp, the Oat, the Oct, the NaDC/NaCT, and the Ost families (Fig. 1B). These data suggest that hOSCP1/mOscp1 can be categorized as a new member of organic solute carrier protein. The nucleotide sequence of mOscp1 is 100% identical to a sequence from the mouse olfactory epithelium with an unknown function (BC045150). We also identified several proteins of unknown function such as 1810007P19Rik protein (GenBank^{TM}/EMBL/DDBJ accession number AAH45150, *Mus musculus*) and an unnamed protein product (similar to RIKEN cDNA 1810007P19) (GenBank^{TM}/EMBL/DDBJ accession number BC098048, *Rattus norvegicus*) that exhibited approximately 93% and 96%, respectively, predicted amino acid identity.

Tissue Distribution of the gene coding for mOscp1. To elucidate the distribution of the *mOscp1* gene, Northern blot analysis was subsequently performed.

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As shown in Fig. 2, a single mRNA band (1.4 kb) was predominant in the testis. Hybridization signals could not be detected in mRNAs isolated from other tissues, such as the brain, eye, lung, heart, liver, kidney, pancreas, and skeletal muscle.

Pharmacological and Functional Characterization of mOscp1. To elucidate the pharmacological and functional characterization of mOscp1, *Xenopus laevis* oocytes injected with mOscp1 cRNA were used. As listed in Table 1, mOscp1 mediated the transport of [^{3}H]probenecid, [^{3}H]ES, [^{3}H]DHEA-S, [^{3}H]allopurinol, [^{14}C]glutarate, [^{14}C]L-leucin, [^{3}H]5-FU, [^{14}C]L-ascorbic acid, [^{14}C]6-MP, [^{3}H]taxol (paclitaxel), [^{14}C]erythromycin, [^{3}H]bumetanide, [^{3}H]hexanoic acid, [^{3}H]tryptophan, [^{3}H]tetracycline, [^{3}H]PGE_2, [^{3}H]$PGF_{2\alpha}$, [^{14}C]PAH, [^{14}C]testosterone, and [^{14}C]TEA. Of interest was that mOscp1-expressing oocytes mediate the transport of [^{14}C]testosterone, [^{14}C]6-MP, [^{3}H]5-FU and [^{14}C]erythromycin, despite the fact that these compounds are not a substrate of hOSCP1 (Kobayashi et al., 2005). Our findings indicate that mOscp1 is a polyspecific organic solute carrier protein with some differences in substrate specificity compared with the human ortholog. We did not observe the mOscp1-mediated transport of [^{14}C]androstenedione, [^{14}C]progesterone, [^{3}H]L-carnitine, [^{14}C]α-ketoglutarate, [^{14}C]salicylate, [^{14}C]theophylline and [^{14}C]valproate.

Because of the high sequence identity between h- and m-Oscp1, we next examined whether the uptake of organic solutes mediated by mOscp1 is also sodium-independent. As shown in Fig. 3A, the uptake of [^{14}C]PAH via mOscp1 was not affected by the replacement of extracellular sodium with lithium, choline, and mannitol, indicating that mOscp1, as well as hOSCP1, is a sodium-independent organic solute carrier protein. Figure 3 also shows the time- and concentration-related transport of [^{14}C]PAH in oocyte which express mOscp1. The

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oocyte-associated count of [^{14}C]PAH increased to 1.5 h of incubation. The result suggests that mOscp1 not only binds but also translocates organic solutes to the inside of the oocytes (Fig. 3B). The concentration dependence of the uptake of [^{14}C]PAH via mOscp1 is shown in Fig. 3C. The mOscp1-mediated uptake of PAH showed saturable kinetics and could be modeled by the Michaelis-Menten equation. Nonlinear regression analyses yielded K_m values of 18.8 $\pm$ 4.1 μM for PAH uptake.

Inhibition Experiment. The inhibition of mOscp1-mediated [^{14}C]PAH uptake by cyclosporin A, cyclophosphamide, and doxorubicin (adriamycin) was subsequently investigated to elucidate further the substrate specificity of mOscp1. As shown in Fig. 4, the mOscp1-mediated transport of [^{14}C]PAH was inhibited by cyclophosphamide and weakly inhibited by cyclosporine A. No inhibitory effect was observed by doxorubicin (adriamycin) in oocytes which express mOscp1.

Immunohistochemical Localization of mOscp1 in Mouse Testis. We subsequently carried out an immunohistochemical analysis in order to elucidate the membrane localization of mOscp1 in mouse testis (Fig. 5). As shown in Fig. 5A, there was immunostaining of mOscp1 in mouse testis. Under high magnification, mOscp1 was located on the plasma membrane side of Sertoli cells (Fig. 5B). By preincubation of the antibody with hOSCP1 peptide, the immunoreactivity was diminished (Fig. 5C).

Discussion

The present study describes the molecular cloning and functional characterization of a polyspecific mouse organic solute carrier protein 1, mOscp1.

An important pharmacological function performed by Sertoli cells is the formation of the functional barrier that separates developing germ cells and adluminal space. Tight junctions in the seminiferous tubules of the testis are predicted to be near the base of Sertoli cells, resulting in separation from the adluminal room. This barrier function, blood-testis barrier, mediates the absorption and excretion of essential nutrients and organic solutes and provides protection from toxic compounds (Russell, 1977). Recently, expression of several organic ion transporters such as multiple drug resistance proteins (Mdrs), multidrug resistance proteins (Mrps), Oatps, organic cation transporters (OCTs), carnitine transporter 2 (CT2), and gonad-specific transporter 1 and 2 (GST-1/2) have been demonstrated in the testis (Enomoto et al., 2002; Suzuki et al., 2003; Augustine et al., 2005). The role of these transporters is to facilitate the supply of water-soluble compounds and to protect the reproductive system. Therefore, these transporters are considered to be major xenobiotic transporters in the testis (Augustine et al., 2005). We have recently reported that hOSCP1 mRNA is strongly expressed in the mouse testis (Kobayashi et al., 2005). We attempted, therefore, to isolate the mouse homolog of hOSCP1, mOscp1, from a mouse testis cDNA library.

Using a *X.* oocytes expression system, mOscp1-cRNA-injected oocytes were used for functional and pharmacological characterization. When expressed in *X.* oocytes, mOscp1 mediated the transport of anionic compound (PAH) and cationic compound (TEA), but not of L-carnitine (zwitterions), in a Na^+-independent manner. Further, we observed that the uptake of anionic compounds such as DHEA-S mediated by

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mOscp1 is sensitive to pH (data not shown). Such similar functions have been observed in oocytes expressing hOSCP1 (Kobayashi et al., 2005). We also found that several structurally dissimilar drugs such as steroids (testosterone, ES and DHEA-S), prostaglandins (PGE_2 and $PGF_{2\alpha}$), antigout agents (allopurinol and probenecid), antibiotics (erythromycin and tetracycline), antineoplastic drugs (5-FU, 6-MP and taxol), amino acids (L-leucine and L-tryptophan) and diuretics (bumetanide) were transported via mOscp1, indicating that mOscp1 is a polyspecific organic solute carrier protein, and either anionic or cationic moieties with organic solutes would be necessary for the transport recognition of mOscp1. However, mOscp1 transports glutarate, but not α-ketoglutarate even though the chemical structure between these two compounds is very similar. At present, however, we have no definite explanation for the differential substrate recognition of mOscp1; it is possible that the binding interaction between the substrate and this carrier protein may vary. Comparison of the transport profile of glutarate and α-ketoglutarate mediated by hOSCP1 would lead to further information of substrate specificity.

To clarify whether mOscp1 is an exchanger or a facilitated transporter, the efflux of radioactivity from oocytes preloaded with 10 μM [^{14}C]PAH was measured in the absence or presence of extracellular PAH (1 mM). However, the efflux was not *trans*-stimulated by the extracellular PAH (data not shown), indicating that mOscp1-mediated transport of organic solutes could occur by facilitated diffusion but not by an exchange mechanism.

DHEA-S is a principal C-19 steroid produced by the adrenal gland and secreted from the adrenal as a sulfate-conjugated DHEA (Longcope, 1996). It is well known that DHEA is metabolized by sulfotransferase and may be a source of precursors of androgen biosynthesis in the reproductive system (Comer et al., 1993; Parker CR Jr.,

1999). In serum, DHEA-S is carried and loosely bound to albumin; however, it has a low transport rate across the membrane (Parker CR Jr., 1999). We found that DHEA-S is a substrate of mOscp1; therefore, mOscp1 as well as other gonad-specific transporters such as GST1/2 might be one of the key molecules for transporting DHEA-S in the testis.

Testosterone is the intermediate product in androsterone. Testosterone is biosynthesized from cholesterol in the Leydig cells, the adrenal cortex, and the theca cells of the ovary; testosterone also influences Sertoli cell function. Circulating endogenous testosterone is bound tightly to a serum glycoprotein (sex hormone binding-globulin, SHBG), and less than 1 to 2% of the circulating testosterone is believed to be unbound in the blood stream. It is known that free and weakly bound testosterone can enter target cells. Our transport experiments revealed that testosterone is transported via mOscp1 despite the fact that testosterone is not a substrate of hOSCP1 (Kobayashi et al., 2005). These findings indicate that mOscp1 functions as an organic solute carrier protein with some differences in substrate specificity compared with the human ortholog. Although the physiological functions of mOscp1 are still unknown, one possible function of this clone may be involved in the regulation of gonadotropin secretion or the testicular responsiveness to gonadotropin releasing hormone (GnRH).

The physicochemical properties of the blood-testis barrier mainly prevent entry of various organic solutes into the testis (Bart et al., 2002). It is known that smaller or ionic molecules may enter the testicular tissues by transcellular transport mechanisms across cellular membranes. Although many cytotoxic compounds are small and lipophilic, these compounds do not all have the same concentration in the testis. In this respect, Bart et al. have revealed that P-gp is expressed at the capillary

endothelium of the human testis and is also expressed at the myroid-cell layer around the seminiferous tubules (Bart et al., 2002). To elucidate a physiological role of mOscp1 in the testis, immunohistochemical analysis was subsequently performed to determine the membrane localization of mOscp1. mOscp1 was localized in the plasma membrane side of Sertoli cells, suggesting that mOscp1 acts as the entry of organic solutes from blood circulation into the adluminal compartment.

Cyclophosphamide and doxorubicin (adriamycin) have been widely used as anticancer drugs in acute regimens for the treatment of various neoplastic disorders. However, the use of cyclophosphamide causes severe testicular toxicity such as the reduction of testicular weight and oligospermia in mice (Anderson et al., 1995; Elangovan et al., 2006). Howell and Shalet reported that treatment of cyclophosphamide in male patients with cancer increases the incidence of oligo- and azoospermia and results in male infertility; however, the precise mechanism by which cyclophosphamide causes testicular toxicity is still unknown (Howell and Shalet, 1998). Interestingly, mOscp1-mediated transport of [^{14}C]PAH is inhibited by cyclophosphamide, suggesting that cyclophosphamide is a candidate for the substrate of mOscp1. Taking these published papers, our inhibition experiment and immunohistochemical study into consideration, mOscp1 is involved in the reproductive toxicity caused by various organic solutes such as cyclophosphamide.

Several membrane transporters are predicted to have twelve membrane spanning domains (Sekine et al., 2000; Miyazaki et al., 2004). In contrast to these observations, Kyte-Doolittle hydropathy analysis has predicted that mOscp1 is likely to have three membrane spanning domains. This finding suggests that mOscp1 may not have a similar membrane topology as OATs and Ostα/β and may have a different evolutionary origin (Dawson et al., 2005). An analysis of mOscp1 with the SignalP

3.0 program (http://www.cds.dtu.dk/services/SignalP/) predicted that the first 28 amino acids (amino acid 1-28) form a signal peptide. Because this region contains a putative transmembrane domain, therefore, the mature mOscp1 protein would have at least a single transmembrane domain. In this respect, further detailed study is needed.

In summary, we describe the molecular cloning and functional characterization of polyspecific mouse organic solute carrier protein 1, mOscp1. Our results, therefore, are expected to facilitate research on drug discovery and will serve as a useful reference concerning the testicular handling of organic solutes. The present findings are also expected to provide new insights into a novel mechanism regarding the toxic effect of drugs and chemicals in the reproduction system and testis-conserving therapy in patients given chemotherapy.

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Footnotes

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Legend for figures

Figure 1. A, Deduced amino acid sequence of the mouse organic anion carrier protein 1, mOscp1. Putative *N*-glycosylation sites are indicated by closed circles (●). B, Phylogenetic relationship between mOscp1 and several murine transporters. The phylogenetic tree was constructed using DNAsis-Pro. (HITACHI Software Engineering). Branch length is drawn to scale. mOat, mouse organic anion transporter; mOct, mouse organic cation transporter; mOCTN, mouse organic cation transporter novel; mOatp, mouse organic anion transporting polypeptide; mNaDC, mouse sodium dicarboxylate cotransporter; mOst, mouse organic solute transporter; mOscp1, mouse organic solute carrier protein 1; hOSCP1, human organic solute carrier protein 1; mNaDC-1, mouse sodium dicarboxylate cotransporter 1; TMD, transmembrane domain.

Figure 2. Tissue distribution of mOscp1 mRNA by Northern blot analysis. A high-stringency Northern blot analysis of poly(A)$^+$ RNA from various tissues of the male mouse probed with ^{32}P-labeled mOscp1 cDNA. Poly(A)$^+$ RNA (2 μg) was loaded onto 1.0% agarose/formamide gel and transferred to a nylon membrane. A highly expressed 1.4-kb transcript was predominantly detected in the testis. Hybridization signals could not be detected in mRNA isolated from other tissues, such as the brain, eye, lung, heart, liver, kidney, pancreas, and skeletal muscle. Other experimental conditions and details are described in *Experimental Procedures*. Kb, kilobase pairs.

Figure 3. A, Effect of extracellular cation on [^{14}C]*p*-aminohippuric acid in *Xenopus laevis* oocytes expressing mOscp1. The uptake rates of [^{14}C]*p*-aminohippuric acid (10 μM) by control oocytes or mOscp1-expressing oocytes for 1 h were measured in the presence or absence of extracellular Na^+. Extracellular Na^+ was replaced with an

equimolar concentration of lithium, choline, and mannitol. Data are mean ± S.E.M. (n = 8–10 oocytes). B, Time course of *p*-aminohippuric acid uptake by mOscp1-expressed oocytes. The uptake of 10 μM *p*-aminohippuric acid in oocytes expressing mOscp1 was measured during 1.5 h of incubation. Data are mean ± S.E.M. (n = 12–19 oocytes). C, Concentration-dependence of mOscpP1-mediated uptake of [^{14}C]*p*-aminohippuric acid. The uptake rates of *p*-aminohippuric acid by mOscp1-expresing oocytes for 1 h were measured at variable concentrations. Other experimental conditions and details are described in *Experimental Procedures*. PAH, *p*-aminohippuric acid. Data are mean ± S.E.M. (n = 16–19 oocytes).

Figure 4. Inhibition of mOscp1-mediated [^{14}C]*p*-aminohippurate uptake by cyclosporin A, cyclophosphamide and doxorubicin (adriamycin). The concentration of [^{14}C]*p*-aminohippurate was 20 μM and those of inhibitors in the assay medium were 1 mM. The values are expressed as a percentage of mOscp1-mediated [^{14}C]*p*-aminohippurate uptake in the absence of inhibitors. Data are expressed as the mean ± S.E.M. for 5–24 oocytes. The significance between with or without inhibitors (control) was determined by one-way ANOVA followed by Dunnett (cyclophosphamide vs control) (*p<0.05). Other experimental conditions and details are described in *Experimental Procedures*.

Figure 5. Immunohistochemical analysis of mOscp1 in mouse testis. Five-micrometer sections were incubated with polyclonal hOSCP1 antibody. The plasma membrane side of Sertoli cells was stained (A, x200; B, x400). Immunoreactivity was completely abolished by pretreatment of antibody with hOSCP1 oligopeptide (C). Other experimental conditions and details are described in *Experimental Procedures*.

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Table 1
Uptake of various ^{14}C- and ^{3}H-labeled compounds by mOscp1-expressing oocytes

Tracer	Concentration	Non-injected	mOscp1	
[^{14}C]Androstenedione	5 μM	22.75 ± 1.05	21.88 ± 1.42	
[^{14}C]Progesterone	5 μM	56.75 ± 2.56	57.75 ± 5.81	
[^{14}C]Testosterone	2 μM	9.24 ± 0.08	18.22 ± 0.68	*
[^{3}H]Probenecid	300 nM	47.50 ± 3.27	109.30 ± 4.33	*
[^{3}H]ES	50 nM	17.20 ± 8.60	100.80 ± 9.96	*
[^{3}H]DHEA-S	50 nM	21.50 ± 1.32	95.50 ± 9.51	*
[^{3}H]Allopurinol	5 μM	0.48 ± 0.15	1.68 ± 0.29	*
[^{3}H]L-Carnitine	50 nM	47.00 ± 1.29	45.25 ± 2.66	
[^{14}C]Glutarate	10 μM	0.22 ± 0.03	1.05 ± 0.10	*
[^{14}C]α-Ketoglutarate	5 μM	0.64 ± 0.09	1.12 ± 0.08	*
[^{14}C]L-Leucine	20 μM	15.25 ± 1.11	21.01 ± 1.08	*
[^{3}H]5-FU	20 nM	4.85 ± 0.33	17.75 ± 1.55	*
[^{14}C]L-Ascorbic acid	50 μM	1.90 ± 0.13	4.40 ± 0.52	*
[^{14}C]6-Mercaptopurine	10 μM	1.96 ± 0.21	6.98 ± 0.71	*
[^{3}H]Taxol (paclitaxel)	50 nM	23.52 ± 0.60	64.08 ± 9.94	*
[^{14}C]Erythromycin	5 μM	0.16 ± 0.02	0.26 ± 0.02	*
[^{14}C]Salicylate	5 μM	1.67 ± 0.15	1.55 ± 0.10	
[^{14}C]Theophylline	5 μM	15.00 ± 1.47	17.50 ± 1.04	
[^{3}H]Valproate	20 nM	12.88 ± 0.59	12.23 ± 0.25	
[^{3}H]Bumetanide	100 nM	1.29 ± 0.05	3.34 ± 1.14	*
[^{3}H]Hexanoic acid	50 nM	91.10 ± 6.15	122.3 ± 9.50	*
[^{3}H]L-Tryptophan	100 nM	113.07 ± 5.16	159.70 ± 7.29	*
[^{3}H]Tetracycline	5 μM	0.55 ± 0.06	0.76 ± 0.03	*
[^{3}H]PGE_2	10 nM	1.05 ± 0.23	8.25 ± 0.40	*
[^{3}H]$PGF_{2\alpha}$	10 nM	1.06 ± 0.12	3.72 ± 0.49	*
[^{14}C]PAH	10 μM	0.80 ± 0.04	3.12 ± 0.13	*
[^{14}C]TEA	10 μM	0.10 ± 0.02	1.32 ± 0.07	*

After two days incubation, uptake experiments were performed in a solution containing Na^+ for 1 h. Values are mean ± S.E.M. of 15-21 oocyte determinations. The significance between control (non-injected) and mOscp1-cRNA-injected oocytes was determined by the unpaired *t* test (*p<0.05). The units of measurement were femtomoles per oocyte per hour for ^{3}H tracers and picomoles per oocyte per hour for ^{14}C tracers. Other experimental conditions and methods are described in *Experimental Procedures*. 5-FU, 5-fluorouracil; ES, estron sulfate; DHEA-S, dehydroepiandrosterone sulfate; PAH, *p*-aminohippuric acid; TEA, tetraethylammonium; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.

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Fig. 1.

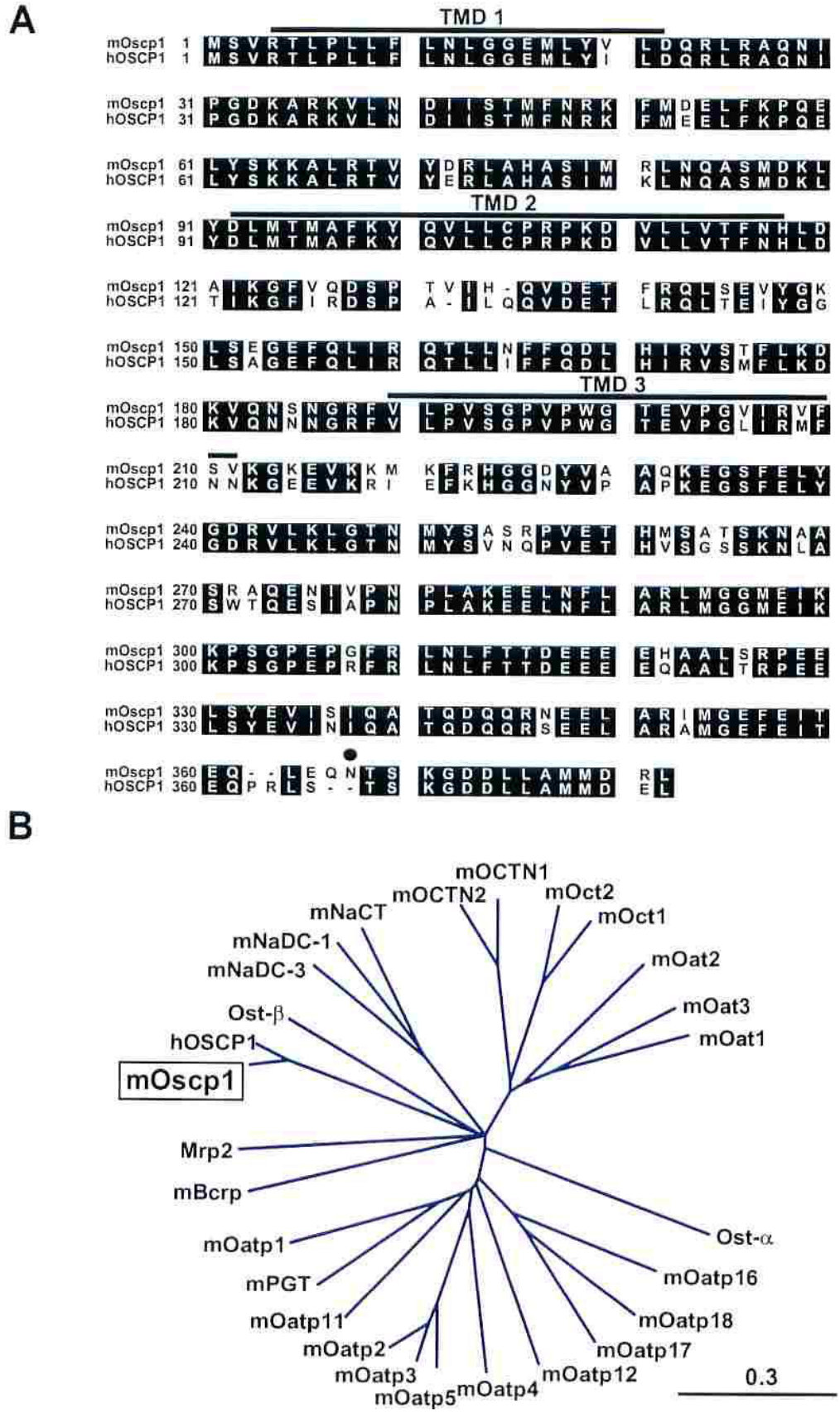

Fig. 2.

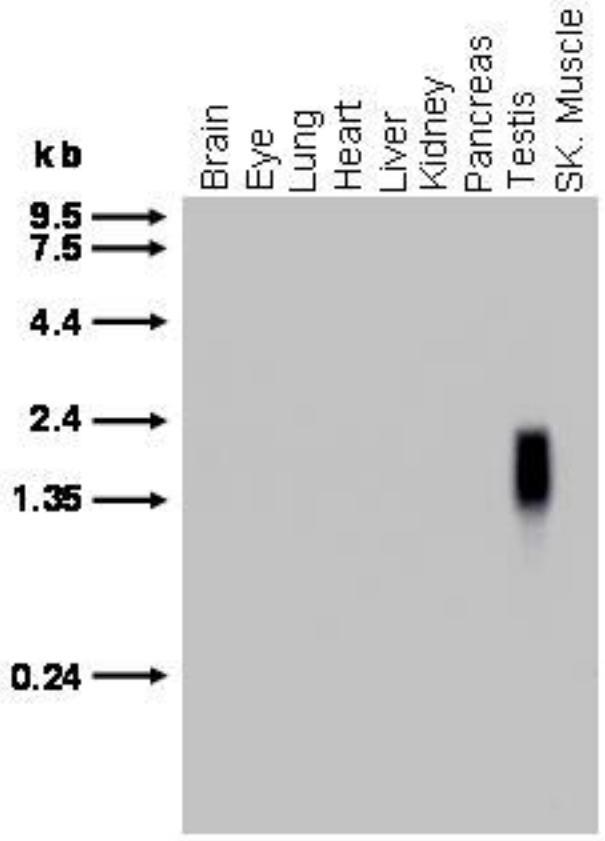
kb
9.5
7.5
4.4
2.4
1.35
0.24
Brain
Eye
Lung
Heart
Liver
Kidney
Pancreas
Testis
SK. Muscle

Fig. 3.

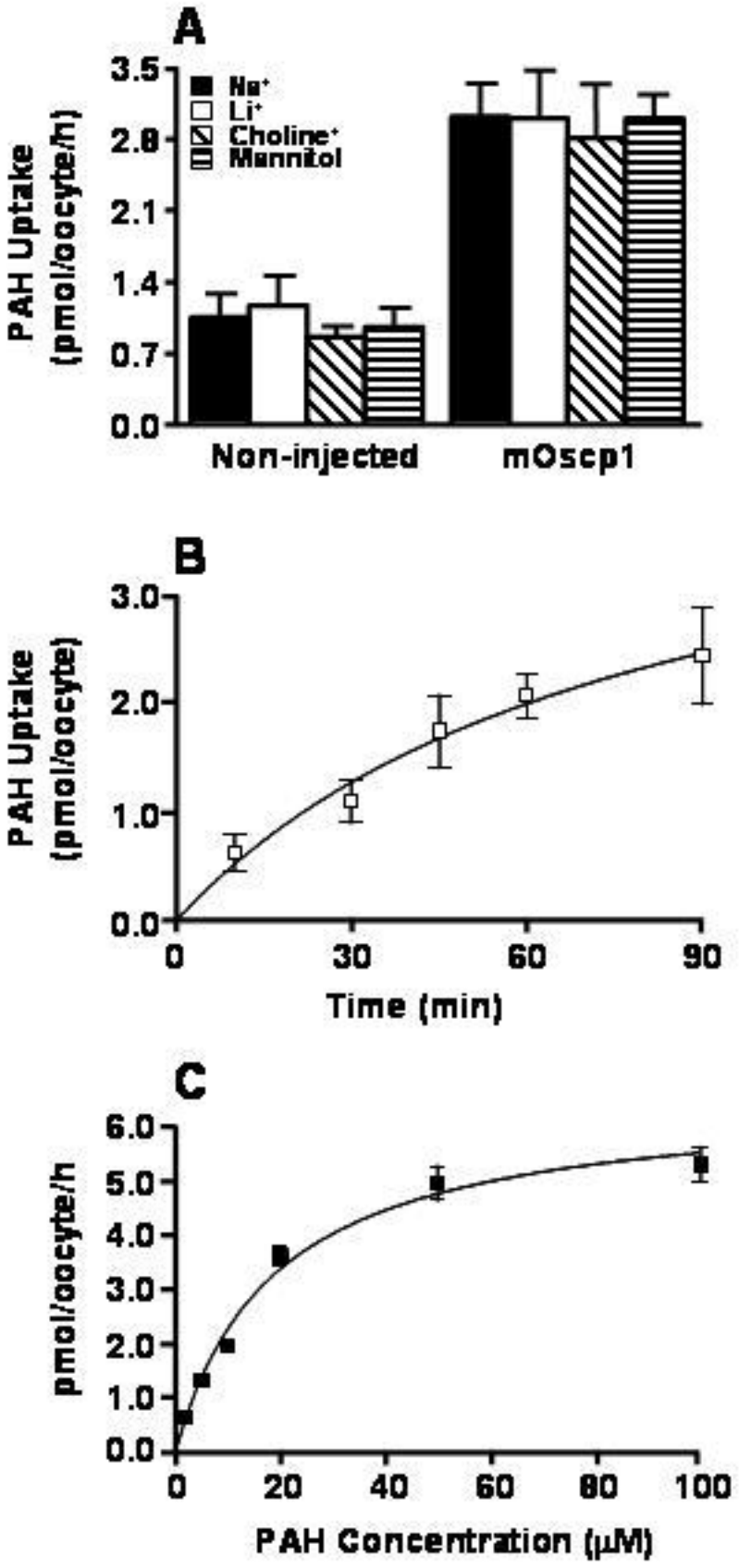
A
PAH Uptake (pmol/oocyte/h)
3.5
2.8
2.1
1.4
0.7
0.0
Na+
Li+
Choline+
Mannitol
Non-injected
mOscp1
B
PAH Uptake (pmol/oocyte)
3.0
2.0
1.0
0.0
0
30
60
90
Time (min)
C
pmol/oocyte/h
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
20
40
60
80
100
PAH Concentration (μM)

Fig. 4.

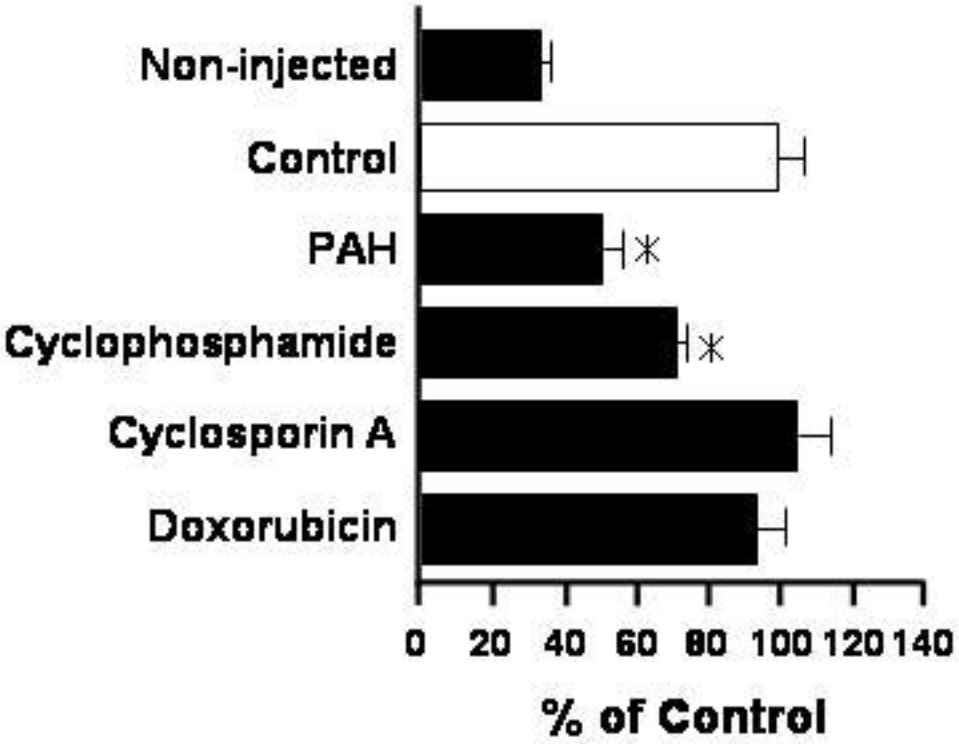
Non-injected
Control
PAH
Cyclophosphamide
Cyclosporin A
Doxorubicin
*
*
0
20
40
60
80
100
120
140
% of Control

Fig. 5.