Effect of Inflammation on Molecular Targets and Drug Transporters

Sherif Hanafy, Ayman O.S. El-Kadi; Fakhreddin Jamali
Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada

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Abstract Inflammation, the host's response to infection and injury, is associated with altered expression of genes such as metabolizing enzymes, transporters, receptors and plasma proteins. The purpose of the present work was to characterize the effect of inflammation on selected molecular targets and transporters that affect drugs' action and disposition. We have used rats with adjuvant arthritis (AA), an animal model of chronic inflammation. The AA group received 0.2 ml of 50 mg ml^{-1} *Mycobacterium butyricum* suspended in squalene into the tail base. On day 12, the rats were euthanized and their organs (heart, liver, kidneys and intestine) excised. Expression of Cav1.2, β1-AR, β2-AR, α1A-AR, Nav1.2, Nav1.6, Kv1.5, Kv2.1, Kv3.1, oatp1a1, oatp1a5, oatp1b2, oatp2b1, oatp4a1, oat2, oat3, oct1, mdr1a, bsep, mrp1, mrp3, mrp6, IL-1α, IFN-γ, iNOS, MCP-1, IL-10, Cox-1 and Cox-2 were determined by real time polymerase chain reaction (RT-PCR). Inflammation resulted in a significant reduction of oct1, oatp4a1 and mrp1 gene expression in the liver and oatp2b1, mrp6 and bsep gene expression in the kidney. Oatp4a1 and mdr1a were found to be significantly upregulated in rat heart. In conclusion, inflammation alters the gene expression of some mediators and drug transporters that can influence the behavior of drugs in the body and contribute to therapeutic failure.

INTRODUCTION

Inflammation is the host's response to infection, injury or irritation. The development of the inflammatory response is a complex process that involves increased expression of chemicals such as cytokines and chemokines in a paracrine, autocrine, and/or endocrine fashion. This enhances the host's defense through immune cell recruitment and activation, antibody production and complement system activation [1]. The process has been found to be associated with altered expression of other genes either as a part of the host's defense mechanism or because of shifting priorities in the cellular expression machinery. Examples of the affected genes are metabolizing enzymes [2-4], transporters [5], receptors [6-10] and plasma proteins [11-13]. Inflammation-induced alterations of gene expression may be responsible, at least in part, for disease pathogenesis. For example, atrial fibrillation developed in septic shock patients [14,15] has been attributed to inflammation-induced L-type calcium channel dysfunction [16]. In addition, animal and human studies have shown that inflammation is an important factor that may alter drug action and/or disposition [6,8,17-22]. Recently, we have reported that inflammation significantly downregulated angiotensin-converting enzyme-2 in rat heart, an observation that may contribute to the cardiovascular complications seen in inflammatory diseases [23]. The purpose of the present work was to investigate whether the effect of inflammation on target proteins goes beyond what has been reported earlier. We investigated the effect of inflammation on selected molecular targets and transporters that are known to be involved in the pharmacokinetics and pharmacodynamics of some drugs (Table 1). We have used rats with adjuvant arthritis (AA), an animal model of chronic inflammation that is known to mimic human rheumatoid arthritis [24].

Abbreviations. Cav1.2, α1c subunit of L-type calcium channels; β1-AR, β1 adrenergic receptor, β2-AR, β2 adrenergic receptor; α1A-AR, α1A adrenergic receptor, Nav, voltage-gated sodium channels; Kv, voltage-gated potassium channels; 18s rRNA, 18s ribosomal RNA; oatp, organic anion transporter polypeptide; oat2, organic anion transporter; oct, organic cation transporter; mdr, multidrug resistance transporter; bsep, bile salts export pump; mrp, multidrug resistance-associated protein; IL, interleukin; IFN-γ, interferon γ; iNOS, inducible nitric oxide synthetase; MCP-1, monocyte chemoattractant protein 1; COX, cyclooxygenase; ACE, angiotensin converting enzyme.

Corresponding Author: Fakhreddin Jamali, Faculty of Pharmacy & Pharmaceutical Sciences, 11361 – 87 Avenue, Edmonton, Alberta, Canada T6G 2E1; fjamali@ualberta.ca

Table 1. The biological/clinical relevance of the tested drug transporters, adrenergic receptors, ion channels and select mediators

Tested gene	Biological/clinical relevance
INFLUX TRANSPORTERS:	
Oatp1a1	Substrates: enalapril, indomethacin, PG E2 methotrexate, dexamethasone, cortisol, aldosterone, digoxin, T3, T4, Bile salts and pravastatin.
Oatp1a5	Substrates: Bile salts, methotrexate, digoxin, T3, T4 and fexofendine [66].
Oatp1b2	Substrates: T3, T4, digoxin, methotrexate and pravastatin [67]; The use of oatp1b2 knockout mice resulted in 4 and 8-fold decrease of the liver to plasma ratio of pravastatin and rifampicin, respectively and a 43% reduction of rifampicin clearance [68]; Knockout animals are less sensitive to the hepatotoxic effects of some toxins due to their decreased uptake [67].
Oatp2b1	Substrates: PG F2α, PG E2 and digoxin [32].
Oatp4a1	One of the thyroid hormone transporters [34].
Oat2 and oat3	Oat2 Substrates: PG F2α, salicylate, PG E2, methotrexate, acetyl salicylate, cimetidine, and indomethacin; Oat3 substrates: Pravastatin, penicillin G, cimetidine; Downregulation of OATs or their inhibition has been found to result in a reduction of the renal clearance of their substrates. For example probencid administration, an OAT inhibitor, inhibits the renal excretion of penicillin G and ACE inhibitors and prolongs their half-lives[69]; The renal uptake of xenobiotics mediated by OATs has also been described as a means for nephrotoxins accumulation in the kidney and contribution to kidney failure [70].
Oct1	Substrates: cytarabine, metformin, amantadine, quinine, quinidine, dopamine, pnacuronium, cimetidine, saquinavir and pramipexole.
EFFLUX TRANSPORTERS:	
Mdr1a	Example substrates: quinidine, verapamil and octreotide.
Bsep	BSEP is an efflux transporter located at the canalicular side of hepatocytes and is involved mainly in the hepatic secretion of bile salts[71]; It is involved in pravastatin[72] and vinblastine[73] transport.
Mrp1	MRP1 has been associated with the resistance of tumor cells to anticancer chemotherapy by reducing their cellular accumulation[74]; MRP1 plays a role in the pathogenesis of inflammation. Glutathione and its conjugates are transported by MRP1, as a part in the detoxification pathway of toxins [75]; Substrates: Vincristine, etoposide, doxorubicin, daunorubicin and 17β-estradiole conjugate.
Mrp3	Substrates: 17β estradiole conjugate and methotrexate; Both MRP1 and MRP3 are localized on the basolateral side of hepatocytes and so they are responsible for the efflux of chemicals to the blood stream [76].
Mrp6	MRP6 serves a housekeeping function in solute transport into bile [77]; MRP6 dysfunction has been implicated in the development of pseudoxanthoma elasticum, a genetic condition characterized by elastic fiber fragmentation and premature atherosclerosis [74]; Substrates: anthracyclines
ION CHANNELS:	
$Ca_v1.2$	L-type calcium channels are essential for coupling the membrane excitation to muscle contraction especially in cardiac muscles. In addition, calcium entry through those channels is responsible for neurotransmitter release, synaptic plasticity and gene expression [78,79].
$Na_v1.2$ and 1.6	They function in the action potential initiation and conduction. Both channels are actual and potential targets for antiepileptic drugs [80].
$K_v1.5$, 2.1 and 3.1	$Ka_v1.5$, an ultra rapid delayed rectifier channel, plays a role in maintaining the membrane potential and excitability of neurons and cardiac muscles [81]. It is considered a potential target for treatment of atrial fibrillation; Potassium channels alterations have been implicated in human diseases. While, $K_v1.5$ is downregulated in patients with atrial fibrillation [115], both $K_v1.5$ and $K_v2.1$ are downregulated in chronic pulmonary hypertension [116].

Table 1 continues:

Table 1 continued…..

Adrenergic Receptors	Each receptor provides important physiological functions mediated by the sympathetic nervous system. They are considered the main site of action of many drugs that are clinically in use such as β1 adrenoceptor and α1-blockers.
OTHERS:	
COX-1 and 2	Cyclooxygenases are important enzymes involved in the production of essential prostaglandins in all parts of the body.
IL-1α	An in inflammatory mediator; Activates T-cells and macrophages; Implicated in the pathogenesis of RA, septic shock and atherosclerosis[82].
IFN-γ	An inflammatory mediator; The main macrophage activator[82].
iNOS	An inducible form of nitric oxide synthetase; Promotes inflammatory reactions[82].
MCP-1	A chemokine that Promotes monocytes and T cells chemotaxis to site of inflammation[82]
IL-10	An Anti-inflammatory cytokine; Inhibits cell mediated inflammatory responses[82].

MATERIALS AND METHODS

Experimental Protocol

The study protocol was approved by the Health Sciences Animal Policy and Welfare Committee of the University of Alberta. Experiments were carried out on male Sprague-Dawley rats (220-280 g). They were housed in a temperature- controlled room with a 12-h dark/light cycle. Two animal groups (n=4/group), inflamed (AA) and healthy (Control) were used.

The inflamed group received 0.2 ml of 50 mg/ml *Mycobacterium butyricum* suspended in squalene into the tail base. Control animals received an equal volume of normal saline into the tail base. On day 12, when inflammation emerged, i.e., increased paw diameter and elevated pro-inflammatory mediators [19], rats were euthanized and their organs (heart, liver, kidneys and upper intestine) were excised and quickly frozen in liquid nitrogen. The excised organs were stored at -80^{o}.

Real Time Polymerase Chain Reaction (RT-PCR)

To determine the potential genetic changes, RT-PCR was carried out. Total RNA was isolated from the frozen organs using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. This was followed by spectrophotometric quantitation of the isolated RNA by measuring the absorbance at 260 nm. cDNA was synthesized from 1.5 µg total RNA samples with the random primers scheme using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Real-Time PCR was performed on an ABI 7500 Real-Time PCR system (Foster City, CA, USA). Forward and reverse primers used for the experiments are depicted in Table 2. The housekeeping gene tested was the rat 18s rRNA. Melting curves were carried out to confirm amplification of single sequences and absence of primer dimers. Primers were purchased from Integrated DNA technologies (Coralville, IA, USA). PCR products were produced and detected quantitatively using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). PCR data were analyzed using the delta delta C_T method ($\Delta\Delta C_T$) as described by Livak and Schmittgen [25]. The data are expressed as fold change relative to a calibrator (Control) and normalized to the housekeeping gene.

Data Analysis

Data are expressed as mean ± SD. Statistical significance between the control and AA groups was analyzed using a two-tailed Student's t-test at $p<0.05$. Statistics were analyzed using Prism software v. 4.03 (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

Tested Genes in organs

The constitutive expression of drug transporters, adrenergic receptors, voltage-gated ion channels and COX 1 and 2 genes was found to be tissue specific (Figures 1 and 2). Tissue distribution of drug transporters in the liver, kidney and intestine was found to be similar to what has been previously reported (Table 3). In addition, MCP-1 was detected in the organs of Control rats.

Effect of Inflammation on the Tested Genes' mRNA

The influence of inflammation on the examined genes' mRNA in various organs is depicted in Figure 3.

Liver – Inflammation resulted in a significant reduction of oct1, oatp4a1 and mrp1 gene expression in the liver. There was also a general trend towards drug transporter downregulation in the liver except for mrp3 and mdr1a. The changes, however, were not statistically significant. There was no difference between AA and control animals with respect to COX-1, COX-2, ion channels and adrenergic receptors gene expression.

Kidney – Inflammation resulted in a significant reduction of oatp2b1, mrp6 and bsep gene expression in the kidney. On the other hand, oatp4a1 demonstrated a trend towards upregulation. $Na_v1.2$ gene expression was significantly induced (up to four-fold) in inflamed animals. There was a general trend towards renal cytokine gene upregulation in AA animals. However, only the elevation of IFN-γ was statistically significant. There was no difference between AA and control animals with respect to COX-1, COX-2, other ion channels and adrenergic receptors gene expression.

Table 2. List of primers used in real-time PCR

Target	Forward Primer	Reverse Primer	Ref
$Ca_v1.2$	AGCAACTTCCCTCAGACGTTTG	GCTTCTCATGGGACGGTGAT	[83,84]
β1-AR	CTGCTACAACGACCCCAAGTG	AACACCCGGAGGTACACGAA	[85]
β2-AR	GAGCCACACGGGAATGACA	CCAGGACGATAACCGACATGA	[85]
α_{1A}-AR	CGAATCCAGTGTCTTCGCAG	ACCATGTCTCTGTGCTGTCCC	[86]
$Na_v1.2$	TCCGGTTTCGTCACGCTATC	TCCAGAGAAGACTGATGTGACACC	[87]
$Na_v1.6$	CAAGCTGGAGAATGGAGGCA	TAAGAGGGGAGGGAGGCTGT	[87]
$K_v1.5$	CGCACAACGCTCGAGGAT	CCTCAGCAGATAGCCTTCTAGGTT	[88]
$K_v2.1$	ACGAGGGTCAGCTGCTCTACAG	TGCTGAACTTGGGACTGGTACTC	[88]
$K_v3.1$	CACGCATCTGGGCACTGTT	TGAAGAAGAGGGAGGCAAAGG	[88]
18s rRNA	GGGAGGTAGTGACGAAAAATAACAAT	TTGCCCTCCAATGGATCCT	[89]
Oatp1a1	ACCTGGAACAGCAGTATGGAAAA	ACCGATAGGCAAAATGCTAGGTAT	[90]
Oatp1a5	TGATGTGGATGGAACTAACAATGAC	TGCATTTATCTGGAGCACACTTG	[90]
Oatp1b2	CCTGTTCAAGTTCATAGAGCAGCA	TGCCATAGTAGGTATGGTTATAATTCCTAA	[90,91]
Oatp2b1	ACGACTTTGCCCACCATAGC	CCACGTAAAGGCGTAGCATGA	[90]
Oatp4a1	AGAACGTCAAGTCGAGCTATTCG	GGCCCACTTCTGTGTAAACATTT	[90]
Oat2	CGTGTGTCCCAGGCATCA	TCCACACGACCCTGGGTTAG	[90]
Oat3	GAGGACCTGTGATTGGAGAACTG	CTGGCTGCCAGCATGAGATA	[92]
Oct1	TGGTGTTCAGGCTGATGGAA	GCCCAAAACCCCAAACAAA	[93]
Mdr1a	GTGGGAAAAGCACAACTGTCC	CCATGGTGACGTTTTCTCGG	[90]
Bsep	CACTGGGTACATGTGGTGTCTCAT	ATGGCCAATATTCATAGCTGCTAAT	[90]
Mrp1	CGAATGTCCTCTGAGATGGAGAC	CTCTACACGGCCTGAATGGG	[90]
Mrp3	TCTTTGTGATGGCCTTGAGGATA	TTGACCATTTCTCCCACAGTGT	[90]
Mrp6	GAAGACTCCAAGCCATTGAATCC	TCTCTCAAGTGACCAGAGGTCTTTT	[90]
IL-1α	AGGCATCCTCAGCAGCAGAA	AGCTCCACGGATGTGGAAAC	[94]
IFN-γ	GCCAAGTTCGAGGTGAACAAC	TAGATTCTGGTGACAGCTGGTGAA	[95]
iNOS	TGGTCCAACCTGCAGGTCTT	CAGTAATGGCCGACCTGATGT	[96]
MCP-1	CTGTCTCAGCCAGATGCAGTTAA	TGGGATCATCTTGCCAGTGA	[95]
IL-10	GAAGCTGAAGACCCTCTGGATACA	CCTTTGTCTTGGAGCTTATTAAAATCA	[96]
COX-1	TCCTGTTCCGAGCCCAGTT	CTTGGAAGGAATCAGGCATGA	[97]
COX-2	CCCCAAGGCACAAATATGATG	CCTCGCTTCTGATCTGTCTTGA	[97]

Primers were purchased from Integrated DNA Technologies (Coralville, IA, USA). Cav1.2, α1c subunit of L-type calcium channels; β1-AR, β1 adrenergic receptor, β2-AR, β2 adrenergic receptor; α1A-AR, α1A adrenergic receptor, Nav, voltage-gated sodium channels; Kv, voltage-gated potassium channels; 18s rRNA, 18s ribosomal RNA; oatp, organic anion transporter polypeptide; oat2, organic anion transporter; oct, organic cation transporter; mdr, multidrug resistance transporter; bsep, bile salts export pump; mrp, multidrug resistance-associated protein; IL, interleukin; IFN-γ, interferon γ; iNOS, inducible nitric oxide synthetase; MCP-1, monocyte chemoattractant protein 1; COX, cyclooxygenase.

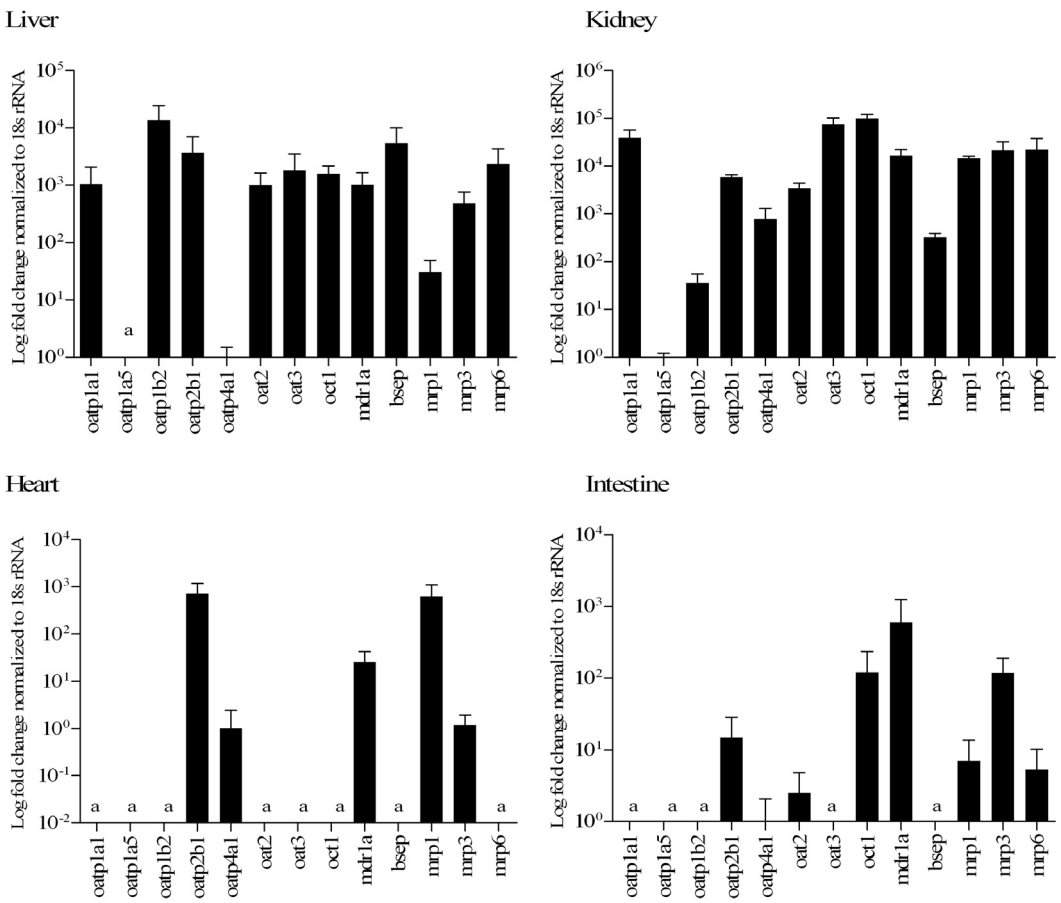

Figure 1. Constitutive expression of drug transporter genes in different rat organs (n=4/group). Expression was determined using real time PCR in rat liver, kidney, heart and intestine. Gene expression was normalized to 18s rRNA. Relative expression was determined relative to a calibrator, the gene with the lowest expression in each organ. a, was not detected in the organ.

Heart – Out of the detected transporters in the hearts of control animals, oatp4a1 and mdr1a were found to be significantly increased by inflammation. Mrp3 was not detected in inflamed animals. Interestingly, despite being undetected in control animals, oatp1b2, oat2 and oat3 were detected in inflamed rats. There was no significant difference between AA and Control animals with respect to ion channels and adrenergic receptors gene expression. While not detectable in Control animals, COX-2 gene expression was detected in the AA group. On the other hand, inflammation significantly inhibited COX-1 gene expression.

Intestine – Due to the high variability observed in intestinal tissue, none of the tested genes were significantly different from the control. However, some transporters (such as oct1, mdr1a and mrp3) showed a strong trend towards downregulation.

DISCUSSION

We used the AA model, which mimics human rheumatoid arthritis in terms of development of systemic inflammation and arthritis [24]. Six days following administration of the adjuvant, the inflammatory mediators start to rise [19] and in approximately 12 days, animals develop arthritis. This model allowed us to study the effect of chronic inflammation on the gene expression of drug transporters, adrenergic receptors, ion channels and select mediators of biological relevance (Table 1).

MCP-1 was present in all of the examined organs of healthy and AA rats and demonstrated a trend toward increased expression in the kidney and heart in response to inflammation. MCP-1 has previously been detected in rat interstitial fibroblasts [26] and smooth muscle cells [27],

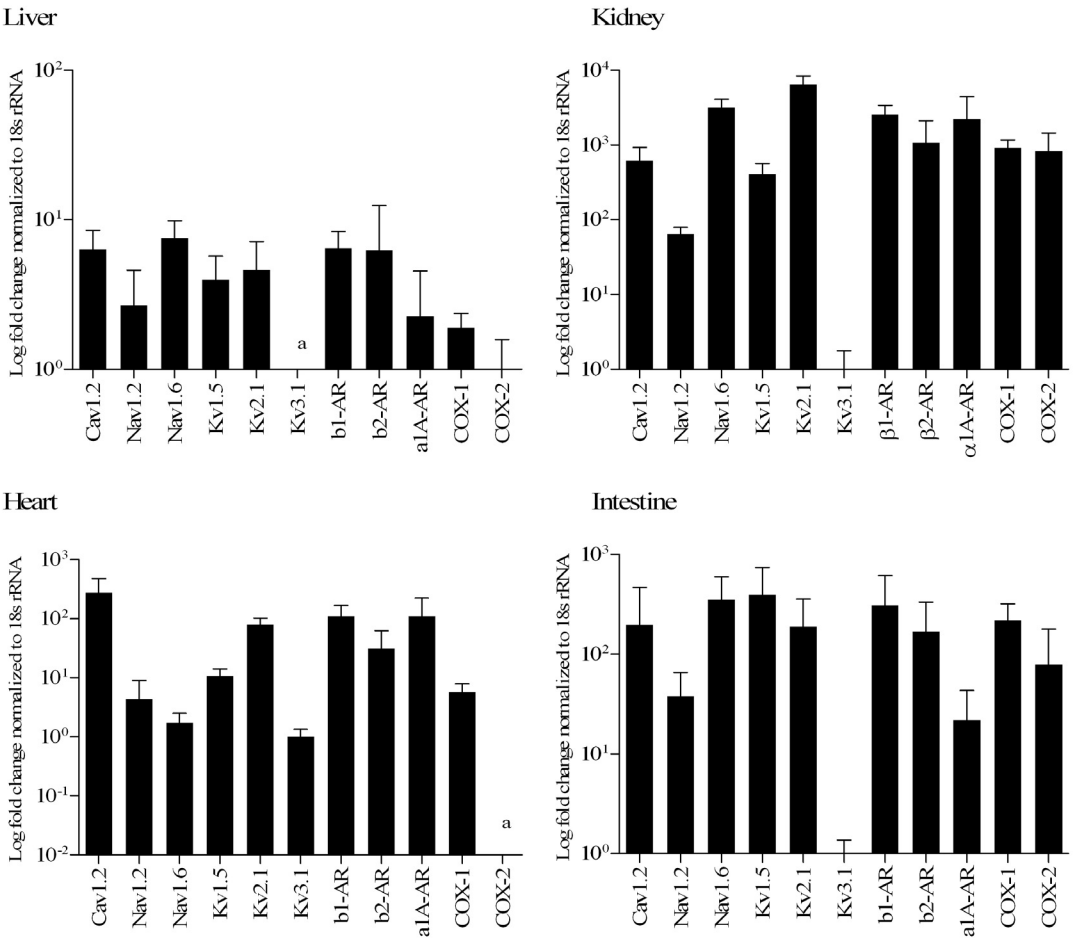

Figure 2. Constitutive expression of voltage-gated ion channels, adrenergic receptors, COX-1 and COX-2 in different rat organs (n=4/group). Expression was determined using real time PCR in rat liver, kidney, heart and intestine. Gene expression was normalized to 18s rRNA. Relative expression was determined relative to a calibrator, the gene with the lowest expression in each organ. a, was not detected in the organ.

Table 3. Tissue distribution and human orthologs of rat transporters

Transporter	Human ortholog	Constitutive gene distribution (in the present study)[a]	Tissue distribution (reported elsewhere)
Influx transporters:			
oatp1a1	OATP1B1, OATP1B3	liver, kidney	liver [76,98], kidney [98], intestine [99], brain [100]
oatp1a5	[b]	kidney	intestine [99,101], brain [99], lung [99]
oatp1b2	OATP1B1, OATP1B3	liver, kidney	liver [102]
oatp2b1	OATP2B1	liver, heart, kidney, intestine	liver, intestine [32], placenta [103]
oatp4a1	OATP4A	kidney, intestine, liver, heart	ubiquitous [103]
oat2	OAT2	liver, kidney, intestine	liver [104], kidney [105]
oat3	OAT3	kidney, liver	liver [105], kidney [105], brain [106]
oct1	OCT1	kidney, liver, intestine	kidney [107], intestine [107]
Efflux transporters:			
mdr1a	MDR1	liver, intestine, kidney, heart	ubiquitous [108,109]
bsep	BSEP	liver, kidney	liver [110]
mrp1	MRP1	kidney, heart, intestine, liver	ubiquitous [76,111]
mrp3	MRP3	intestine, liver, kidney	liver[76], kidney[112], intestine[113], brain[114]
mrp6	MRP6	liver, intestine, kidney	liver[76,77]

[a]mentioned in order of relative abundance; Oatp, organic anion transporter polypeptide; oat2, organic anion transporter; oct, organic cation transporter; mdr, multidrug resistance transporter; bsep, bile salts export pump; mrp, multidrug resistance-associated protein; [b]uncertain.

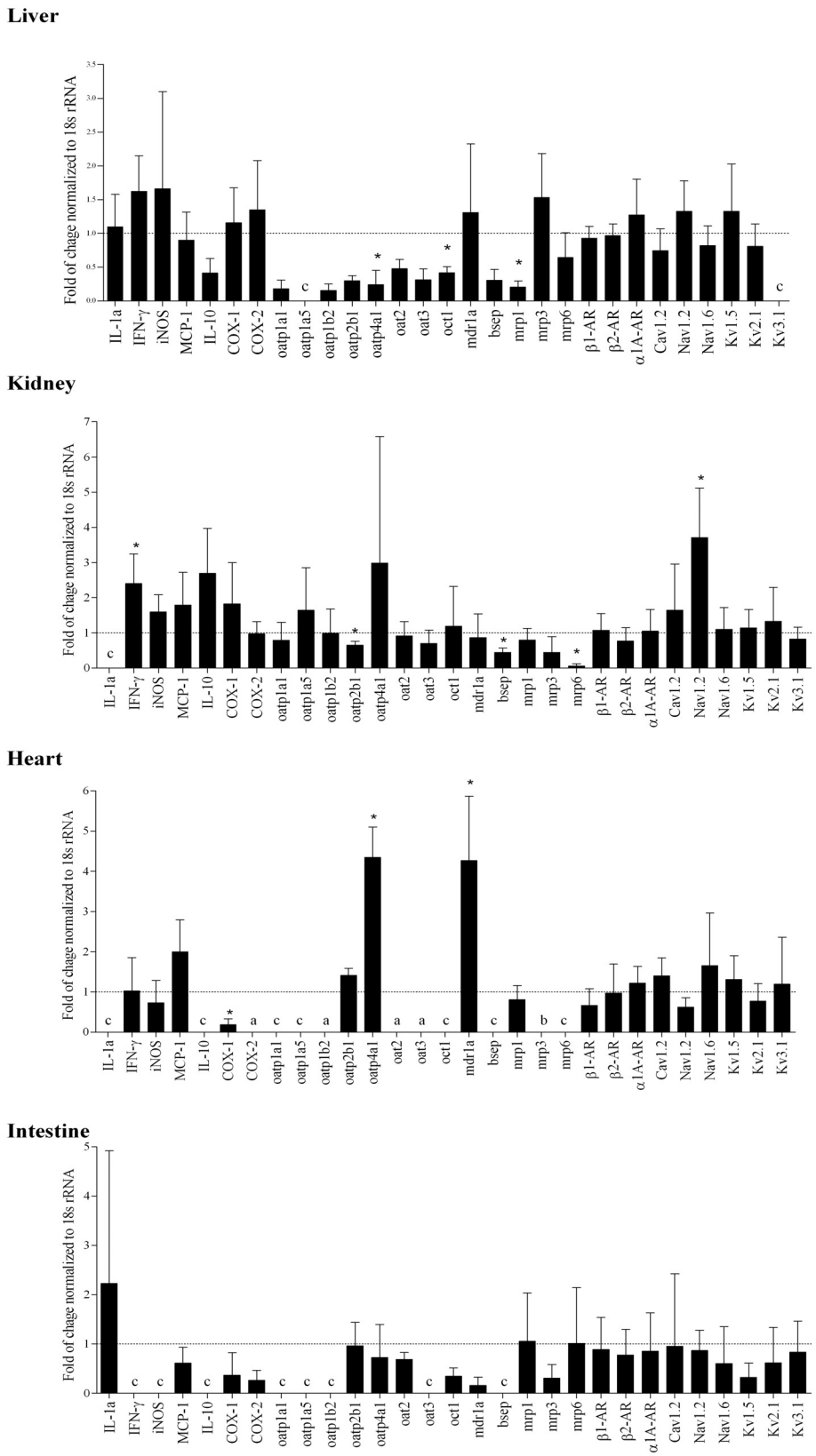

Figure 3. Effect of Adjuvant arthritis (AA) on the tested molecular targets and transporters' gene expression in different rat organs as determined using real time polymerase chain reaction (RT-PCR). Gene expression changes are represented by fold of changes of target genes in AA rats compared to Control (dotted line) (n=4/group). Gene expression was normalized to 18s rRNA. *, $p<0.05$ vs. control rats. a, was detectable in AA only; b, was detectable in Control group only; c, absent in in both healthy and AA.

which is suggestive of a basal housekeeping function of the chemokine.

With regard to other examined inflammatory mediators (iNOS, IL-1a, INF-γ, IL-10), their presence was organ-dependant. Other observed changes remained insignificant, except for IFN-γ in the kidney, which was significantly increased by AA. Pronounced alterations of these markers have previously been reported in the rat joints and lymphoid organs such as spleen and lymph nodes, the main sites ofinflammation in adjuvant arthritis [28]. Lack of statistically significant alterations of the examined expressions can, in part, be attributed to the variability which depends on the disease severity [20, 118].

COX-2 is an important enzyme responsible for the development of inflammation in the AA model. COX-2 (but not COX-1) expression is increased in AA rat paws and inhibition of COX-2 reverses the inflammatory response [28]. Determination of COX-1 and COX-2 genes expression in other AA rat organs has not been reported. COX-1 expression was significantly and substantially (by 80%) reduced by inflammation only in the heart. There was a similar strong trend for intestine that did not reach significance. For the other examined organs, the present data do not suggest significant changes in either COX-1 or COX-2 gene expression in response to inflammation. Reduced expression of COX-1 has previously been observed in the hearts and lungs of rats treated with lipopolysaccharides [29,30]. The observed down-regulation of cardiac COX-1 may have therapeutic significance as prostaglandins are important regulatory mediators of cardiovascular function [29,30]. It has been found that COX-1- deficient mice have renal vasoconstriction and lower blood pressure reduction during sleep (non-dippers), a condition associated with increased risk of cardiovascular diseases in humans [31].

Oatp2b1 gene was detected in the rat heart. Cardiac oatp2b1 expression may imply its role in the cardiac uptake of digoxin, its substrate [32]. The expression of oatp2b1 was significantly reduced in the kidney of AA rats, an observation that may explain, at least in part, the previously reported reduced digoxin clearance in TNF-α-treated mice [33].

Oatp4a1 is a thyroid hormone transporter [34]. Thyroid hormones T3 and T4 are substrates of several uptake transporters including L-amino acid transporters (LAT1, LAT2), monocarboxylate transporters (MCT8) and several OATPs [34]. Euthyroid sick syndrome is a condition characterized by altered thyroid hormone levels with normal thyroid gland function [35]. Inflammation has been implicated in the development of euthyroid sick syndrome. For example, lipopolysaccharide administration inhibited the hypothalamic pituitary-thyroid axis causing decreased TSH, T3 and T4 [36,37]. This has been explained, in part, as being due to an inflammation-induced upregulation of thyroid hormone transporters leading to increased cellular uptake of thyroid hormones. For example, MCT8 in the liver and skeletal muscles has been found to be significantly upregulated in patients with critical illness [38]. These observations are in agreement with what we have found in the AA rat hearts and the upregulation trend in the kidney. In addition, oatp4a1 was significantly downregulated in the liver of AA rats, a process that if coupled with changes to other thyroid hormone transporters, may significantly alter the hepatic uptake of thyroid hormones.

The lack of effect of inflammation on the renal oat and oct may indicate that the renal excretion of drugs that are substrates of these transporters may not be affected by inflammation. This may explain, at least in part, the lack of influence of inflammatory conditions on the clearance of renally excreted drugs such as sotalol, valsartan and losartan [8,39,40]. In contrast to what was observed in the kidney, oct1 is significantly downregulated in the liver of AA rats suggesting the potential reduction of the hepatic uptake of its substrates. TNF-α and IL-6 have been implicated in inflammation-induced downregulation of hepatic OATs and OCTs in humans. Incubation of human hepatocytes with TNF-α or IL-6 has resulted in reduced expression of OCT1 and OAT2 [41]. On the other hand, lipopolysaccharide administration in rats does not seem to alter oat2 mRNA [42], which is suggestive of a model-dependency of the changes.

Mdr1a and mdr1b are the rodent orthologs of human MDR1 (p-glycoprotein, P-gp). In the Sprague Dawley rats, mdr1a is the predominant transporter [43]. P-gp is the most studied efflux

transporter owing to its ubiquitous distribution in most body tissues in humans and animals, its broad substrate specificity and its association with numerous clinically relevant drug interactions. Mdr1a is strategically located in different organs to facilitate specific functions. It is localized in the apical side of hepatocytes, renal tubular and intestinal cells and is responsible for the biliary secretion, tubular secretion and resisting absorption of drugs and their respective metabolites. The clinical significance of mdr1a has been investigated by the use of mdr1a inhibitors and mdr1a knockout animals. For example, injection of radiolabeled digoxin and cyclosporine in mdr1a knockout animals resulted in 20- to 50-fold increase of drug exposure [44]. The effect of inflammation on mdr1a expression has been found to be model, species and organ dependent. Acute inflammatory rats models (turpentine-induced [45] and endotoxin-induced [46]) are associated with reduced expression and activity of mdr1a in the rat liver, uppergastrointestinal tract and kidney. On the other hand, endotoxin-induced inflammation in mice has been associated with upregulation of renal mdr1a and increased doxorubicin renal clearance [47].

Other rodent models such as renal failure [48] and colitis [49] have been found to be associated with reduced expression of P-gp in intestinal tissues. However, no change has been found in experimental cholestasis [50]. The reason for these discrepancies has been explained, in part, by the variation in inflammatory mediator profiles in different diseases and models of inflammation [51]. For example, while in vitro incubation of IL-6 with hepatocytes results in mdr1a downregulation [52,53], incubation with IFN-γ yields increased mRNA and protein expression with no change in the activity in Caco-2 cells [54]. In the present study, mdr1a gene expression was not altered in the rat liver and kidney (the two major elimination organs), suggestive of unaltered biliary clearance and tubular secretion of mdr1a substrates. Interestingly, mdr1a mRNA was significantly increased more than 4-fold in the hearts of AA rats, in contrast to what has been previously reported in a rat model of acute inflammation [55]. This may, at least in part, explain the observed reduced response of cardiovascular drugs in AA rats [6,8,9,17,18,20]. Furthermore, altered cardiac P-gp function can have a crucial role in determining drug cardiotoxicities as in the case of idarubicin [57]. However, inflammation-induced pharmacodynamic alterations caused by target channel or receptor downregulation [9], seem to have higher impact than the reduced cardiac uptake. For example, the reduced verapamil response in rats with endotoxemia has been found to be independent of its reduced uptake [56].

Generally, bsep is exclusively expressed in the liver. However, tissue distribution studies have detected its presence in the rat gut [58]. Herein, we report the expression of bsep in the rat kidney as well. However, the biological function of bsep in the kidney and intestine is not known. Lipopolysaccharide administration in rats resulted in a decline in bsep expression, an effect that can explain LPS-induced intrahepatic cholestasis [42]. In the present study, bsep is downregulated in the liver and kidney by AA. This alteration may affect bile salt transport in AA rat liver and may lead to cholestasis similar to those produced in rats by estrogen and troglitazone, which are bsep inhibitors [59,60]. The consequences of the reduced function of bsep are species dependent. While bsep gene mutation or inhibition in humans can lead to progressive familial intrahepatic cholestasis or drug-induced cholestasis, respectively, bsep knockout mice experienced only mild cholestasis [61,62]. The latter has been explained by the compensatory effects of p-glycoprotein in bile salt transport in mice [61].

Changes at the gene level may not be reflected at the level of protein or protein function. Therefore, further confirmatory experiments may be instituted to test the significance of the changes reported herein. For example, in post-myocardial injury rats, despite its protein downregulation, $Ca_v1.2$ mRNA was not significantly different suggesting that inflammation-induced alterations are at the post-transcriptional (translational) level [63]. Similarly, the absence of a significant change in expression of $Ka_v1.5$ may not be extrapolated at the protein level. For example, Brundel et al have found a discrepancy in $Ka_v1.5$ gene and protein expression in the hearts of patients with atrial fibrillation. While $K_v1.5$ mRNA did not change, its protein is downregulated [64]. Inflammation did not affect the mRNA expression of adrenergic receptors. However, it has been found that potency of propranolol (a β1 adrenoceptor blocker) is reduced in inflammation [17,18] and this has been attributed to reduced expression of β1 receptor proteins [65]. The reason for this discrepancy can

be explained at the level of protein rather than the geneexpression; in other words, the effect is post-transcriptional as in the case of L-type calcium channels.

Previous reports are suggestive of the involvement of inflammatory conditions (20, 117-120) and the effect of various drugs with anti-inflammatory effects (9, 17, 121) in the outcome of pharmacotherapy at least for cardiovascular drugs. The exact mechanisms behind these involvements remain to be explored. Nevertheless, the overall influence of inflammation on the target receptors must be kept in mind.

In conclusion, inflammation, the host defense mechanism against exogenous pathogens, alters the gene expression of some mediators and drug transporters that can influence the behavior of drugs in the body and contribute to therapeutic failure. The observed expression changes needs to be confirmed at the protein and functional level. Although preliminary, this is an exploratory investigation into the general pattern of the effect of inflammatory conditions on the status of some important proteins.

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