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Review

Cellular arsenic transport pathways in mammals☆

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ABSTRACT

Natural contamination of drinking water with arsenic results in the exposure of millions of people world-wide to unacceptable levels of this metalloid. This is a serious global health problem because arsenic is a Group 1 (proven) human carcinogen and chronic exposure is known to cause skin, lung, and bladder tumors. Furthermore, arsenic exposure can result in a myriad of other adverse health effects including diseases of the cardiovascular, respiratory, neurological, reproductive, and endocrine systems. In addition to chronic environmental exposure to arsenic, arsenic trioxide is approved for the clinical treatment of acute promyelocytic leukemia, and is in clinical trials for other hematological malignancies as well as solid tumors. Considerable inter-individual variability in susceptibility to arsenic-induced disease and toxicity exists, and the reasons for such differences are incompletely understood. Transport pathways that influence the cellular uptake and export of arsenic contribute to regulating its cellular, tissue, and ultimately body levels. In the current review, membrane proteins (including phosphate transporters, aquaglyceroporin channels, solute carrier proteins, and ATP-binding cassette transporters) shown experimentally to contribute to the passage of inorganic, methylated, and/or glutathionylated arsenic species across cellular membranes are discussed. Furthermore, what is known about arsenic transporters in organs involved in absorption, distribution, and metabolism and how transport pathways contribute to arsenic elimination are described.

Introduction

The world health organization (WHO) estimates that more than 200 million people world-wide are exposed to unacceptable levels of naturally occurring inorganic arsenic (iAs) (in the forms arsenite (As^{III}) and arsenate (As^{V})) in their drinking water (IARC, 2012; Naujokas et al., 2013). The WHO defines the acceptable level of arsenic in drinking water at <10 μg/L (or 10 ppb). iAs exposure can also occur through the ingestion

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of food crops irrigated or prepared with contaminated water (Hu et al., 2006; Mabuchi et al., 1980; Mondal et al., 2010; Paiva et al., 2008). Other dietary sources of arsenic include fish and seafood, although these sources are generally low in iAs and high in arsenobetaine, arsenosugars, and arsenolipids. These latter species of arsenic are generally thought to be less harmful than iAs, however, potentially this might not always be the case (Molin et al., 2015). Anthropogenic sources of iAs include pharmaceuticals, pesticides, and preservatives (IARC, 2012). Occupational sources of iAs include but are not limited to mining, smelting, coal burning, and glass manufacturing (IARC, 2012).

Chronic exposure to iAs is a serious public health concern because As^{V} and As^{III} are Group 1 (proven) human carcinogens, causing tumors of the skin, lung, and urinary bladder (IARC, 2012). iAs exposure has also been associated with kidney, prostate, and liver tumors (IARC, 2012). In addition to cancer, chronic iAs exposure has the ability to affect almost all organ systems resulting in diseases of the vascular, respiratory, neurological, reproductive, and endocrine systems with symptoms often being trademark skin lesions (Hunt et al., 2014).

Many parts of the world are of concern for chronic iAs exposure through drinking water including regions of Asia (e.g., China, India, Taiwan, Bangladesh, and Vietnam), South America (e.g., Brazil, Chile and Argentina), and North America (Mexico, USA, and Canada) (Flanagan et al., 2012; Naujokas et al., 2013; Shankar et al., 2014). World-wide there are so called arsenic "hot spots" with Bangladesh being the most famous example. The contamination of groundwater in Bangladesh has been described as the "largest mass poisoning" in history with ground water levels higher than 50 μg/L in 35% of the wells tested and over 8% with levels higher than 300 μg/L (Smith et al., 2000).

In addition to environmental exposures, arsenic based anti-cancer drugs are used clinically. Arsenic trioxide (As_2O_3) is used for treating both newly diagnosed and relapsed acute promyelocytic leukemia, and its use results in high remission rates (Cicconi and Lo-Coco, 2016). Furthermore, As_2O_3 and another arsenical, S-dimethylarsino-glutathione (DMA(GS)), are in clinical trials for the treatment of multiple hematological and solid tumors (Emadi and Gore, 2010; Mann et al., 2009) (www.clinicaltrials.gov).

Transporters are critical for regulating the body burden of arsenic and potentially play both protective (through preventing cellular and tissue accumulation as well as facilitating elimination) and harmful (allowing the entry of arsenic across epithelial layers) roles. Studies of environmentally arsenic-exposed human populations show large inter-individual differences in susceptibility to carcinogenesis, however, the genetic basis for this variation is not completely understood (Ghosh et al., 2008; Hernandez and Marcos, 2008). Furthermore, variable adverse effects (including hepatotoxicity and prolonged cardiac QT interval) have been reported during the clinical use of As_2O_3 (Cicconi and Lo-Coco, 2016). To prevent and treat arsenic-induced toxicity it is critical to understand underlying molecular mechanisms that render a person susceptible. Arsenic metabolism and excretion pathways are complex, and multiple pathways are likely responsible for inter-individual differences in susceptibility, however, the relevance of transport proteins has become increasingly evident (Drobna et al., 2010b; Kala et al., 2004; Rosen and Liu, 2009). Despite this, the relationship between the genetic variability of arsenic transport pathways and inter-individual susceptibility of humans to arsenic-induced disease remains largely unstudied (with the exception of Banerjee et al., 2016; Gribble et al., 2013; Karagas et al., 2012; Kaya-Akyuzlu et al., 2016). This review will discuss the current knowledge about the cellular uptake and efflux pathways of iAs and its metabolites in mammalian cells. Furthermore, we will describe what is known about transporters in organs involved in the absorption, distribution, and metabolism of arsenic and how transport pathways contribute to arsenic elimination.

1. Biotransformation and transport pathways of arsenic

Speciation of arsenic can profoundly influence its toxicity, and the cellular transport pathways it utilizes. Thus the methylation, thiolation, and glutathionylation of arsenic will be considered briefly prior to discussion of specific transport pathways.

1.1. *Methylation of arsenic*

iAs undergoes extensive methylation in most mammalian cells, with the known exceptions of marmoset, tamarin and squirrel monkeys, chimpanzees, and guinea pigs (Drobna et al., 2010a; Vahter, 1999). In humans, the four major methylation products are monomethylarsonic acid (MMA^{V}), monomethylarsinous acid (MMA^{III}), dimethylarsinous acid (DMA^{V}), and dimethylarsinic acid (DMA^{III}) (Thomas et al., 2007). Further methylation can result in the formation of trimethylarsine oxide ($TMA^{V}O$) and trimethylarsine (TMA^{III}). The original pathway of arsenic methylation proposed by Challenger et al., involved the oxidative methylation of trivalent arsenic forms (Challenger, 1951). More recently, alternative pathways involving the direct methylation of trivalent arsenic forms (without oxidation) have been proposed (Hayakawa et al., 2005; Naranmandura et al., 2006), although their chemical plausibility has been questioned (Cullen, 2014). Arsenic methylation is catalyzed predominantly by the enzyme arsenic (+3 oxidation state) methyltransferase (As3MT) using S-adenosyl-L-methionine (SAM) as the methyl donor (Thomas et al., 2004, 2007). Despite the controversies over the mechanism of arsenic methylation, there is clear consensus that this process has a significant impact on the toxicity, tissue distribution, and retention of this metalloid (Wang et al., 2015). Comparison of species that do and do not methylate arsenic, suggests that arsenic methylation results in an increased rate of arsenic whole body clearance (Drobna et al., 2010a). Consistent with this, studies of *As3mt(–/–)* mice show that compared to their wild-type C57BL/6 littermates, whole body arsenic retention is significantly higher (Drobna et al., 2009; Hughes et al., 2010). In addition, *As3mt(–/–)* mice were found to be extremely susceptible to acute arsenic toxicity, and had more severe urinary bladder hyperplasia than WT-mice (Yokohira et al., 2010, 2011). Although these data, taken in isolation, make a strong case for methylation of arsenic being a detoxification pathway, other evidence strongly suggests methylation acts as a "toxification" or "bioactivation" step. Thus, the trivalent

methylated forms of arsenic (MMA^{III} and DMA^{III}) have been shown, using cell culture models, to be even more potent toxicants than As^{III} (Kligerman et al., 2003; Mass et al., 2001; Petrick et al., 2000; Styblo et al., 2000).

1.2. Thiolation of arsenic

Relative to methylated arsenic species, the understanding of thioarsenic species formation and influence on human health is in its infancy, however, progress has been made and has recently been reviewed (Wang et al., 2015). Urinary elimination of thioarsenicals has been reported after oral administration of As^{III} to hamsters and rats (Chen et al., 2016; Naranmandura et al., 2007b; Suzuki et al., 2010) and after consumption of arsenosugars by sheep (Hansen et al., 2004a, 2004b). Urinary elimination of thioarsenic species has also been reported for humans after exposure to iAs in drinking water and after consumption of arsenosugars (Raml et al., 2005, 2007). The relative toxicity of thioarsenicals has been characterized in different mammalian cell lines (Hinrichsen et al., 2014; Naranmandura et al., 2007a, 2009, 2011; Raml et al., 2007). While most thiolated arsenic species have comparable cellular toxicity to pentavalent methylated species, dimethylmonothioarsinic acid ($DMMTA^{V}$) was found to be highly toxic and comparable with trivalent inorganic and methylated species (Bartel et al., 2011; Ebert et al., 2014; Leffers et al., 2013a; Naranmandura et al., 2007a, 2011; Ochi et al., 2008). The toxicity of different thiolated arsenic species is highly correlated with the rate of cellular accumulation, suggesting that as of yet unidentified uptake pathways may play important roles in toxicity (Naranmandura et al., 2011).

1.3. Formation of arsenic-glutathione conjugates

A large body of literature describes the importance of GSH in the detoxification and elimination of arsenic (reviewed in Leslie, 2012; Thomas, 2009). In the presence of excess GSH, As^{III}, MMA^{III}, and DMA^{III} will react spontaneously to form $As(GS)_3$, $MMA(GS)_2$, and DMA(GS). The detection of arsenic-glutathione conjugates in biological samples has been difficult because of their chemical instability (Kala et al., 2000; Raab et al., 2004; Yehiayan et al., 2009). However, $As(GS)_3$ and $MMA(GS)_2$ have been isolated from rat bile and mouse urine, providing the first in vivo evidence that at least these two As-GSH complexes are formed under physiological conditions (Bu et al., 2011; Cui et al., 2004; Kala et al., 2000, 2004; Suzuki et al., 2001). Despite being the most chemically stable As-GSH complex (Kala et al., 2000, 2004; Yehiayan et al., 2009), DMA(GS) has not been identified in biological samples (Kala et al., 2000, 2004). Consistent with rodent models, biliary and sinusoidal transports of arsenic measured in sandwich cultured primary human hepatocytes were GSH-dependent (Roggenbeck et al., 2015). In mice deficient in γ-glutamyl transpeptidase, an enzyme responsible for GSH and GSH conjugate catabolism, approximately 60%–70% of urinary arsenic was present as $As(GS)_3$ and $MMA(GS)_2$ (Kala et al., 2004), suggesting that As-GSH conjugates account for a major fraction of eliminated arsenic. Evidence for glutathionylation of thiomethylated arsenic species has also recently been reported (Yehiayan et al., 2014).

1.4. Cellular uptake pathways for inorganic and methylated arsenic species

1.4.1. Arsenate uptake by phosphate transporters

As^{V} and inorganic phosphate (P_i) are both members of group Va of the periodic table and have similar physicochemical properties. At physiological pH, As^{V} (pK_a values of 2.3, 6.8, and 11.3) is a mixture predominantly of $H_2AsO_4^-$ and $HAsO_4^{2-}$, and similarly P_i (pK_a values of 2.2, 7.2, and 12.3), is predominantly $H_2PO_4^-$ and HPO_4^{2-}. For decades it has been known that As^{V} competes with P_i for cellular uptake as discussed in (Villa-Bellosta and Sorribas, 2008). In mammals, five different transporters with a clearly defined physiological role in Na^+-dependent P_i uptake are known (Forster et al., 2013). These are secondary active co-transporters utilizing the energy of the inwardly directed Na^+ gradient to power the transport of P_i uphill (plasma levels ~1 mmol/L with cellular levels ~40 mmol/L) (Virkki et al., 2007). Of these five Na^+-dependent co-transporters there are three type II proteins (Na^+/P_i-IIa, b, and c; gene names *SLC34A1, 2,* and *3*) and two type III proteins (PiT-1 and PiT-2; *SLC20A1* and *SLC20A2*) that control phosphate homeostasis in various tissues (Forster et al., 2013).

When ectopically expressed in *Xenopus laevis* oocytes, all five rat Na^+-dependent P_i transporters were found capable of transporting As^{V} (Villa-Bellosta and Sorribas, 2010). However, due to the high extracellular concentrations of P_i, kinetic analysis of transport suggested that only Na^+/P_i-IIb (*SLC34A2*) would be capable of transporting As^{V}, and only under certain physiological conditions (Villa-Bellosta and Sorribas, 2010). Thus, while the K_m (defined as the concentration of substrate at which the enzyme is at half its maximal velocity; the K_m value is inversely related to affinity) for all five rat transporters for P_i was ≤0.1 mmol/L, the K_m for As^{V} was ≥1.1 mmol/L (range 1.1 to 4.1 mmol/L), with the exception of rat Na^+/P_i-IIb, which had a much higher affinity for As^{V} with a K_m value of 50 μmol/L (Villa-Bellosta and Sorribas, 2010). Similar results were found for the human Na^+/P_i-IIb (K_m for As^{V} 10 μmol/L; K_m for P_i 29 μmol/L) (Villa-Bellosta and Sorribas, 2010) (Table 1). Even with the three-fold higher affinity of human Na^+/P_i-IIb for As^{V} than P_i, it is unlikely that this transporter would be responsible for any As^{V} uptake in tissues other than the intestine, because under homeostatic conditions the extracellular concentration of P_i is approximately 1 mmol/L and As^{V} would likely exist in the nmol/L range (Villa-Bellosta and Sorribas, 2008, 2010). However, Na^+/P_i-IIb is expressed at the apical surface of the enterocyte and could be important for the uptake of As^{V} from drinking water (as calculated in (Villa-Bellosta and Sorribas, 2008) average P_i concentration of 5 μmol/L in the intestine from drinking water, with an average exposure to iAs contaminated drinking water resulting in 0.3–1.5 μmol/L As^{V} in the intestine). Even if the P_i concentration consumed is high, as soon as the initial P_i has been transported across the intestine, As^{V} transport could likely occur (Villa-Bellosta and Sorribas, 2008).

1.4.2. Cellular uptake of As^{III}, MMA^{III}, MMA^{V}, and DMA^{V} by aquaglyceroporins

Thirteen aquaporin channel proteins have been identified in mammals, of which four (AQP3, AQP7, AQP9, and AQP10) are subclassified as aquaglyceroporins (Agre et al., 2002; Laforenza et al., 2016). In addition to water, aquaglyceroporins

Table 1 – Human transporters with experimental evidence for involvement in cellular passage of arsenic.

Transporter family	Member		Uptake/ efflux	Arsenical tested	K_m (μmol/L)	V_{max}(pmol/mg protein/min)	Model system/result
	Gene	Protein					
Aquaglyceroporins (AQP)	*AQP3*	AQP3	Uptake	As^{III}	–	–	Ectopic expression in HEK293 cells/Increased accumulation and toxicity (Lee et al., 2006). Arsenic resistant human lung adenocarcinoma/ Knockdown resulted in lower accumulation and resistance (Lee et al., 2006).
	AQP7	AQP7	Uptake	As^{III}	–	–	Ectopic expression *Xenopus laevis* oocyte/Increased accumulation (Liu et al., 2002b, 2004b).
	AQP9	AQP9	Uptake	As^{III}, MMA^{III}, MMA^{V}, DMA^{V}	–	–	Ectopic expression *Xenopus laevis* oocyte/Increased accumulation (Liu et al., 2002b, 2004b; McDermott et al., 2010).
	AQP10	AQP10	Uptake	As^{III}	–	–	Caco-2 cells/Knockdown resulted in lower accumulation (Calatayud et al., 2012a).
Glucose transporters (GLUT/*SLC2A*)	*SLC2A1*	GLUT1	Uptake	MMA^{III}	–	–	Ectopic expression *Xenopus laevis* oocyte/Increased accumulation (Liu et al., 2006a).
	SLC2A5	GLUT5	Uptake	As^{III}	–	–	Caco-2 cells/Knockdown resulted in lower cellular accumulation (Calatayud et al., 2012a).
Multidrug resistance proteins (MRPs/*ABCCs*)	*ABCC1*	MRP1	Efflux				Vesicular transport assay/MRP1 expression resulted in ATP-dependent transport.
				$As(GS)_3$	0.32	17	H69AR cells (Leslie et al., 2004).
					0.32	42	Ectopic expression in HeLa cells (Shukalek et al., 2016).
					3.8	307	Ectopic expression in HEK293 cells (Shukalek et al., 2016).
				$MMA(GS)_2$	11	11,000	Ectopic expression in HEK293 cells (Carew et al., 2011).
	ABCC2	MRP2	Efflux	$As(GS)_3$,	4.2	134	Vesicular transport assay/ectopic expression in HEK293 cells resulted
				$[(GS)_2AsSe]^-$	1.7	45	in ATP-dependent transport (Carew and Leslie, 2010).
	ABCC4	MRP4	Efflux	$MMA(GS)_2$,	0.7	112	Vesicular transport assay/ectopic expression in HEK293 cells resulted
				DMA^{V}	0.22	32	in ATP-dependent transport (Banerjee et al., 2014).
Phosphate transporter	*SLC34A2*	Na^+/P_i-IIb	Uptake	As^{V}	9.7	–	Ectopic expression *Xenopus laevis* oocyte/Increased accumulation (Villa-Bellosta and Sorribas, 2010).
Organic anion transporting polypeptides (OATP)	*SLCO1B1*	OATP1B1	Uptake	As^{III}, As^{V}	–	–	Ectopic expression in HEK293 cells/Slightly increased accumulation (Lu et al., 2006).
	SLCO2B1	OATP2B1	Uptake	As^{III}	–	–	Caco-2 cells/Knockdown resulted in lower cellular accumulation (Calatayud et al., 2012a).
Cystine transporters	*SLC7A11*	xCT	Uptake	DMA-Cys	–	–	NB4 cells/Knockdown resulted in lower cellular accumulation (Garnier et al., 2014).
	SLC1A1	xAG	Uptake	DMA-Cys	–	–	NB4 cells/Knockdown resulted in lower cellular accumulation (Garnier et al., 2014).

allow the passage of other small neutral molecules including glycerol, urea, and certain species of arsenic across cellular membranes down their concentration gradients (Rojek et al., 2008). In solution at physiological pH, As^{III} exists almost exclusively in the undissociated neutral form $As(OH)_3$ (pK_a 9.2) (Ramirez-Solis et al., 2004). Studies of As^{III} permeability in oocytes of *Xenopus laevis* injected with water, or cRNA of human AQP3 (hAQP3), hAQP7, hAQP9, or hAQP10 revealed that hAQP3, hAQP7, and hAQP9 can conduct As^{III} across cell membranes, while hAQP10 could not (Liu et al., 2004b) (Table 1). In contrast with these findings, knockdown of hAQP10 in the human colorectal cell line Caco-2 resulted in a reduced As^{III} accumulation, suggesting under certain conditions hAQP10 might conduct As^{III}, although this awaits further study (Calatayud et al., 2012a) (Table 1).

Using the *Xenopus laevis* expression system, the efficiency of As^{III} permeation varied considerably for the different hAQPs with hAQP9, hAQP7, and hAQP3 conducting As^{III} at a 25-, 5-, and 2-fold higher rate than the H_2O control, respectively (Liu, 2010; Liu et al., 2004b). The authors concluded that AQP3 had a very limited ability to conduct As^{III}, however, ectopic expression of hAQP3 in HEK293 cells and knockdown of AQP3 in an As^{III} resistant human lung adenocarcinoma cell line suggest that hAQP3 can increase cell permeation of As^{III} (Lee et al., 2006). Other studies have also provided support for an important role for AQP3 in conducting As^{III} into cells and tissues, especially in the absence of AQP7 and AQP9 expression (Naranmandura et al., 2009; Sumi et al., 2015). Consistent with their human counter parts, rat (r) AQP9, mouse (m) AQP7, and mAQP9 also conducted As^{III} (Carbrey et al., 2009; Liu et al., 2002b).

Similar to As^{III}, the predicted predominant form of MMA^{III} in solution at physiological pH is also neutral, methanearsonous acid ($CH_3As(OH)_2$) (Liu et al., 2006b). MMA^{III} has been found to permeate rAQP9, hAQP9, and mAQP7, but not hAQP7 (Liu et al., 2006b; McDermott et al., 2010). Conductance of MMA^{III} by these aquaglyceroporins was 3- to 5-fold more efficient than for As^{III} (Liu et al., 2006b; McDermott et al., 2010). hAQP9 also has the ability to conduct MMA^{V} (pK_a values 3.6 and 8.2) and DMA^{V} (pK_a 6.3), although not efficiently at physiological pH due to the predominant negative charge for both of these arsenic species at pH 7.4 (McDermott et al., 2010). Consistent with this, the membrane passage of the negatively charged As^{V}, was not increased by overexpression of hAQP9.

1.4.3. *Uptake of As^{III} and MMA^{III} by facilitative glucose transporters (GLUTs/SLC2A)*

GLUTs are members of the solute carrier (SLC) family of membrane proteins, encoded by *SLC2A* genes, and are best known for the transport of monosaccharides (e.g., glucose, galactose, fructose, glucosamine, and mannose) across cell membranes (Mueckler and Thorens, 2013). There are 14 human GLUT isoforms, and with the exception of HMIT/*SLC2A13*, all are facilitative transporters, moving permeants down their concentration gradients (Mueckler and Thorens, 2013). Of the 14 GLUTs, three have been shown to have some association with arsenic uptake: GLUT1 (*SLC2A1*), GLUT2 (*SLC2A2*), and GLUT5 (*SLC2A5*). The best studied of these, in regard to arsenic transport, is GLUT1. Rat GLUT1 expressed in yeast, was found to modestly (~1.7-fold) increase the accumulation of As^{III} relative to the empty vector control (Liu et al., 2006a). Transport of MMA^{III} by rGLUT1 was ~4-fold higher levels than As^{III} (~7-fold higher than empty vector) (Liu et al., 2006a). Human GLUT1 expressed in *Xenopus laevis* oocytes was found to transport MMA^{III}, but at about a 4-fold lower rate than rGLUT1 (Liu et al., 2006a) (Table 1).

Kinetic characterization of rGLUT1 MMA^{III} transport revealed a similar affinity of GLUT1 for MMA^{III} (K_m 1.2 mmol/L) as for its prototypical substrate glucose (K_m 3 mmol/L) (Liu et al., 2006a). This K_m value is much higher than expected plasma levels of MMA^{III} (likely to be in the low nmol/L range). Given that plasma glucose levels (4–8 mmol/L) are ~6-orders of magnitude higher than potential MMA^{III} levels, transport may not be physiologically relevant. That being said, MMA^{III} transport by rGLUT1 was non-competitively inhibited by glucose, suggesting differences between translocation pathways. Furthermore, GLUT1 has weak water channel activity and the water translocation pathway has been shown to be different to that of glucose (Fischbarg et al., 1990; Iserovich et al., 2002). Evidence suggests that As^{III} and MMA^{III} are being translocated through the water pathway (Jiang et al., 2010; Liu et al., 2006a). During channel permeation little binding should occur, thus, as reported in (Liu et al., 2006a), a very low affinity is expected, even when passage of solute through the channel is significant.

Support for the involvement of the related GLUT2 is extremely weak, there are no reports of GLUT2 increasing the permeation of any arsenic species, however, expression of GLUT2 in primary human hepatocytes is associated with increased cellular accumulation of inorganic and monomethylated arsenic (Drobna et al., 2010b). The ability of GLUT2 to allow the passage of different arsenic species needs to be evaluated using direct transport experiments. Lastly, knocking-down the expression of GLUT5 in human epithelial colorectal adenocarcinoma (Caco-2) cells using small interfering RNA (siRNA) reduced As^{III} accumulation by ~60% (Calatayud et al., 2012a) (Table 1). While this knockdown study provides support for the involvement of GLUT5 in As^{III} uptake, the ectopic expression of GLUT5 in a cell expression system, followed by transport characterization would be useful corroboration.

It is interesting to note that rGLUT1 and AQP9 (Section 1.4.2) both allow the permeation of MMA^{III} at a higher rate than As^{III}. This phenomenon was somewhat surprising, especially for AQP9, because not all AQPs permit the passage of MMA^{III}, even those that allow the permeation of As^{III} (Liu et al., 2006b). Furthermore, MMA^{III} is expected to be a larger molecule than As^{III} (Liu, 2010). Differences in the structures of As^{III} and MMA^{III} could make MMA^{III} interact less with the pore region of rGLUT1 and AQP9, and permeate more quickly. In support of this, MMA^{III} is less polar than As^{III} (with one less hydroxyl group for hydrogen bonding) (Liu et al., 2006b).

1.4.4. *Evidence for transport of arsenic by organic anion transporting polypeptides (OATPs/SLCOs)*

OATPs are members of the SLC superfamily of transporters and encoded by the *SLCO* (formerly *SLC21A*) genes (Hagenbuch and Stieger, 2013). There are 11 OATPs in humans and they are responsible for the transport of numerous endogenous (e.g.,

bilirubin, bile salts, thyroid hormones, prostaglandins, steroids, etc.) and exogenous (including drugs and toxicants) solutes down their concentration gradients, across membranes in an ATP- and Na^+-independent manner (Roth et al., 2012).

Relative to the AQPs, GLUTs, and Na^+/P_i-IIb, little experimental work has been done to study the involvement of OATPs in arsenic transport. HEK293 cells transfected with the liver specific OATP1B1 (*SLCO1B1*) were moderately more sensitive to As^V and As^{III} than empty vector transfected control cells (Lu et al., 2006). Consistent with this, a very modest increase in the cellular accumulation of As^{III} and As^V was observed in HEK293 cells expressing OATP1B1 compared with empty vector (Lu et al., 2006) (Table 1). A study on the association between human *SLC01B1* variants and urinary arsenic metabolite patterns identified two single nucleotide polymorphisms of this gene that were significantly associated with altered arsenic metabolite percentage in urine (Gribble et al., 2013). Lastly, knocking-down the expression of the related *SLCO2B1* (OATP2B1) in Caco-2 cells using siRNA, reduced As^{III} accumulation by ~70% (Calatayud et al., 2012a). Although these data provide some evidence that OATP1B1 and OATP2B1 might have a role in arsenic transport, more extensive transport studies are required to corroborate the current evidence.

1.4.5. Other uptake pathways for iAs and metabolites

S-dimethylarsino-glutathione (DMA(GS)), is in clinical trials for the treatment of multiple hematological and solid tumors (Emadi and Gore, 2010; Lo-Coco et al., 2016; Mann et al., 2009). Evidence suggests that DMA(GS) enters cells via cystine/glutamate exchangers after being enzymatically degraded to DMA-Cys (Garnier et al., 2014) (Table 1). With the exception of DMA-Cys uptake by this pathway, and MMA^{III} uptake by AQPs and GLUTs, very little is known about how arsenic metabolites are taken up by cells. Evidence from octanol–water and liposome-water partition coefficients suggests that certain thio-pentavalent and trivalent arsenic species can cross cell membranes through simple diffusion (Chávez-Capilla et al., 2016), however, the contribution of simple diffusion compared with transport to membrane passage is not known. Further research is needed to understand how methylated, thiomethylated, and glutathionylated species of arsenic gain entry into cells. This is especially important for understanding the tissue distribution and ultimately the toxicity of arsenic hepatic metabolites. It is likely that in addition to the already (at least partially) characterized pathways described above, other transporters are involved in arsenic uptake.

1.5. Cellular efflux pathways of inorganic and methylated and/or glutathionylated arsenic species

Many of the Na^+-independent transport proteins involved in cellular arsenic uptake described in Section 1.4 are also candidates for arsenic efflux because they are bidirectional transporters/channels. For example, although AQPs are well characterized as channels that allow permeation of neutral arsenic species into cells, they are bidirectional channels, theoretically allowing permeants to move down their concentration gradients out of cells. It has been proposed that hAQP9 allows the cellular uptake of As^{III} followed by methylation of arsenic and outward flow of the methylated form down its concentration gradient (Liu et al., 2006b; McDermott et al., 2010). In support of this, *AQP9* knock-out mice have a reduced total arsenic clearance and fecal elimination as well as reduced urinary elimination of As^{III}, As^V, and DMA^V, and are more susceptible to As^{III}-induced toxicity (lower survival rate and higher cardiotoxicity) (Carbrey et al., 2009). GLUT1 has only been shown capable of the cellular uptake of As^{III} and MMA^{III}, and its ability to efflux these species is unknown. However, GLUTs are well characterized as bidirectional transporters of their prototypical permeants (e.g., glucose), allowing their passive movement across membranes (from high to low concentrations) (Mueckler and Thorens, 2013). Lastly, although OATPs are well characterized uptake transporters there is also evidence that they can function in a bidirectional manner (Li et al., 2000; Mahagita et al., 2007).

Thus, it is theoretically possible that if enough free As^{III} or MMA^{III} (and other neutral arsenic species, in the case of AQPs) is accumulated in the cell, these "uptake" transport pathways will facilitate passive movement out of the cell. However, to our knowledge nobody has directly demonstrated efflux of arsenic by these transporters in cell models. This is likely because it is technically difficult to measure efflux from an intact cell due to the propensity of trivalent arsenic species to rapidly bind to cellular components. In fact, it has been estimated that 99% of cellular As^{III} is thiol bound at As^{III} levels below 1 mmol/L (Kitchin and Wallace, 2005), and based on chemical reactivity, this is very likely also the case for MMA^{III}. Considering that GSH conjugates account for a major fraction of eliminated arsenic (Section 1.3), and that GSH conjugates (large and negatively charged) are highly unlikely to be substrates for AQPs and GLUTs, additional pathways must be in place for cellular export of As-GSH conjugates. Furthermore, although AQP9 allows the permeation of the major human urinary metabolite DMA^V it is not efficient at physiological pH, suggesting additional export pathways for this arsenic species as well. To date, the best characterized arsenic-GSH and DMA^V efflux transporters are members of the ATP-binding cassette (ABC) transporter superfamily, subfamily C (ABCC). What is known about these proteins and their role in arsenic efflux will be discussed in more detail below.

1.5.1. ATP-binding cassette transporters and cellular arsenic export

In humans there are 48 ABC transporter proteins divided into 7 subfamilies (A through G) based on domain structure and sequence identity. They are collectively responsible for the transport of a huge number of molecules including lipids, peptides, bile salts, bilirubin, hormones, signaling molecules, therapeutic agents and toxicants (George and Jones, 2012). ABC transporters are primary active transporters that directly hydrolyze ATP in their cytosolic nucleotide binding domains (NBDs) to induce conformational changes in their membrane spanning domains (MSDs) to allow molecules to translocate across cellular membranes (Locher, 2016). At the plasma membrane, mammalian ABC transporters are mono-directional export pumps, moving solutes from the cytosol to the extracellular space.

1.5.1.1. Cellular export of arsenic by ABCC transporters. In mammals there are twelve ABCC proteins, nine of which are export pumps and commonly referred to as multidrug resistance proteins (MRP1 through 9). The founding member MRP, MRP1 (encoded by *ABCC1*), was identified based on its ability to confer resistance to a structurally diverse array of anti-cancer agents through an ATP-dependent decrease in cellular drug accumulation (Cole, 2014b). In addition to its role in drug resistance, MRP1 and other MRPs are important for the transport of a long list of physiological and xenobiotic metabolites often conjugated to GSH, glucuronate, or sulfate (Cole, 2014a; Leslie et al., 2005; Slot et al., 2011). The substrate specificities of MRP1–MRP9 have been recently reviewed (Slot et al., 2011; van der Schoor et al., 2015; Wen et al., 2015). MRP1, MRP2 (*ABCC2*), and MRP4 (*ABCC4*) are established arsenic efflux pumps and will be discussed in more detail below (Banerjee et al., 2014; Carew and Leslie, 2010; Kala et al., 2000; Leslie, 2012; Leslie et al., 2004) (Table 1). Please note that rodent forms of MRPs are referred to in lower case (i.e., Mrp) while human forms are referred to in capitals (MRP).

1.5.1.1.1. Cellular export of arsenic by MRP1 (ABCC1). The overexpression of human MRP1 decreases the cytotoxicity of As^{III}, As^{V} and MMA^{III} in cultured cells in a GSH-dependent manner (Carew et al., 2011; Cole et al., 1994; Vernhet et al., 2001a). Consistent with the efflux of an arsenic-GSH conjugate, MRP1-overexpressing cell lines were shown to efflux twice as much GSH upon treatment with As^{III}, compared to controls (Zaman et al., 1995). Furthermore, heterologous expression of mouse Mrp1 decreases the cytotoxicity of As^{III} and As^{V}, and *Mrp1(–/–)* cell lines have an increased sensitivity to As^{III} (Allen et al., 2000; Rappa et al., 1997; Stride et al., 1997). MRP1 protein levels are also increased in a variety of As^{III}, MMA^{V}, and DMA^{V} selected cell line types including human myelogenous leukemia, rat liver epithelial, human hepatoma, human epidermoid carcinoma, and acute promyelocytic leukemia cell lines (Chen et al., 2009; Kojima et al., 2006; Liu et al., 2001; Seo et al., 2007; Tachiwada et al., 2007).

Using MRP1-enriched membrane vesicles prepared from the small cell lung cancer cell line H69AR, we have shown that MRP1 transports As^{III} as $As(GS)_3$ with high affinity (low K_m) but low capacity (low maximal rate of transport or V_{max}) (K_m of 0.32 μmol/L and V_{max} of 17 pmol/mg/min) (Leslie et al., 2004). We obtained similar kinetic results using vesicles isolated from HeLa cells stably transfected with MRP1; however, a marked difference in $As(GS)_3$ transport kinetics using MRP1-enriched membrane vesicles from HEK293 cells with a 12-fold higher K_m and 7-fold higher V_{max} (K_m of 3.8 μmol/L and V_{max} of 307 pmol/mg protein/min) (Shukalek et al., 2016). Evidence suggests that the substantial differences in the kinetics of $As(GS)_3$ transport by WT-MRP1-enriched membrane vesicles isolated from HEK293 and HeLa cells are caused by cell line differences in MRP1 post-translational modification. Specifically, MRP1 affinity and capacity for $As(GS)_3$ are influenced by *N*-glycosylation of Asn19 and Asn23 and phosphorylation of Tyr920 and Ser921 (Shukalek et al., 2016). These data also indicated that cross-talk occurs between MRP1 *N*-glycosylation and phosphorylation, with phosphorylation dictating the affinity and capacity for $As(GS)_3$, and *N*-glycosylation influencing the phosphorylation status. We have proposed that phosphorylation of MRP1 at Tyr920 and Ser921 allows a switch from high affinity low capacity to low affinity high capacity transport of $As(GS)_3$ (Shukalek et al., 2016). Considering that cellular arsenic exposure can result in a general increase in protein phosphorylation (Alp et al., 2010; Wen et al., 2010), and that arsenic can inhibit certain phosphatases (Rehman et al., 2012), exposure of cells to arsenic is likely to result in a shift to a pro-phosphorylation state. This would in turn promote MRP1 to switch from a high affinity low capacity to a low affinity high capacity $As(GS)_3$ transporter. Thus, when Tyr920/Ser921 are dephosphorylated, MRP1 is capable of exporting arsenic with high affinity, making it an efficient transporter at low (nmol/L) arsenic levels. When the cell is exposed to more arsenic and shifted to a pro-phosphorylation state MRP1-Tyr920/Ser921 phosphorylation would result in MRP1 becoming an efficient transporter of $As(GS)_3$ at higher (μmol/L) arsenic levels. Thus, dynamic phosphorylation of Tyr920/Ser921 allows MRP1 to detoxify arsenic over a broad concentration range.

In addition to $As(GS)_3$ we have also shown, using MRP1-enriched membrane vesicles, that MRP1 transports MMA^{III} as $MMA(GS)_2$. Transport of $MMA(GS)_2$ was found to be of high affinity and capacity (K_m of 11 μmol/L and V_{max} of 11,000 pmol/mg/min) (Carew et al., 2011). In tissues with sufficient GSH levels, as well as methylation capacity MRP1 is likely an important detoxification pathway for the highly toxic MMA^{III}.

MRP1 expressed stably in HeLa cells did not confer cellular protection against the toxicity of DMA^{III}, MMA^{V}, and DMA^{V}, providing indirect evidence that these methylated arsenicals are not transported by MRP1 (at least not in HeLa cells) (Carew et al., 2011).

1.5.1.1.2. Cellular export of arsenic by MRP2 (ABCC2). The strongest evidence for the involvement of MRP2 in the detoxification of arsenic comes not from cell line work, but from studies using the Mrp2-deficient TR^- Wistar rat strain (Kala et al., 2000). This is reviewed in Section 2.2.2 below. The in vivo TR^- studies showed that rat Mrp2 transports $As(GS)_3$ and $MMA(GS)_2$ (Kala et al., 2000). We have shown using vesicular transport assays that human MRP2 transports $As(GS)_3$ with high affinity and capacity (K_m of 4.2 μmol/L and V_{max} of 134 pmol/mg/min) (Carew and Leslie, 2010) and that $MMA(GS)_2$ is also transported (Roggenbeck, Kaur, and Leslie, unpublished data). Furthermore, we have shown that human MRP2 can transport the seleno-bis(*S*-glutathionyl) arsinium ion $[(GS)_2AsSe]^-$ with higher affinity and somewhat lower capacity (K_m of 1.7 μmol/L and V_{max} of 45 pmol/mg/min) (Carew and Leslie, 2010). The formation and biliary excretion of $[(GS)_2AsSe]^-$ is thought to be responsible (at least in part) for the remarkable mutual detoxification of arsenic and selenium observed in vivo (Gailer, 2009) and will be discussed in more detail in Section 2.2.2.

MRP2 protein levels are increased in arsenic selected cell lines including human lung adenocarcinoma and rat liver epithelial cell lines (Lee et al., 2006; Liu et al., 2001). Furthermore, short term exposure to low levels of arsenic also increases protein levels in primary rat and human hepatocytes (Roggenbeck et al., 2015; Vernhet et al., 2001b).

MRP2 is thought to play an important role in the enterohepatic circulation of arsenic-GSH conjugates and this will be discussed in more detail in Section 2.2.2, below

(Bu et al., 2011; Carew and Leslie, 2010; Kala et al., 2000; Leslie, 2012; Roggenbeck et al., 2015).

1.5.1.1.3. *Cellular export of arsenic by MRP4 (ABCC4).* Initial studies of the involvement of MRP4 in protection of cells from arsenic were done with a NIH/3T3 cell line stably expressing MRP4, and revealed that MRP4 did not confer resistance to As^{III} (Lee et al., 2000). We similarly observed that two independently derived HEK293 cell clones stably expressing MRP4, did not confer resistance to As^{III}, but did confer resistance to the other arsenicals tested As^{V}, MMA^{III}, MMA^{V}, DMA^{III}, and DMA^{V} (Banerjee et al., 2014). Consistent with the cytotoxicity data, HEK-MRP4 cells had a lower accumulation of As^{V}, MMA^{III}, MMA^{V}, DMA^{III}, and DMA^{V} (but not As^{III}) relative to empty vector transfected control cells (Banerjee et al., 2014). Using MRP4-enriched membrane vesicles we identified $MMA(GS)_2$ and DMA^{V} as the species of arsenic transported (As^{III}, As^{V}, MMA^{V}, and DMA^{III} were not transported by MRP4 under various conditions in the presence and absence of GSH) (Banerjee et al., 2014). In contrast with the findings by Banerjee et al., and Lee et al., it was reported that MRP4 stably expressed in HEK293 cells conferred resistance to As^{III} (Yuan et al., 2016). In Yuan et al., the authors did not report the species of arsenic present in culture media or complete vesicular transport assays, therefore the reasons for the differences are not clear. One possible explanation is that the MRP4 stable clone used in (Yuan et al., 2016), more efficiently methylates As^{III} than our HEK293 MRP4 stable cell clones (Banerjee et al., 2014) and the MRP4 NIH/3T3 stable clone (Lee et al., 2000), therefore more rapidly producing arsenic species that are effluxed by MRP4 and preventing As^{III}-induced cytotoxicity.

MRP4 protein levels were increased in HEK293 cells upon exposure to As^{III}, DMA^{III}, and DMA^{V} (Banerjee et al., 2014). Similarly, MRP4 levels were dose dependently increased in sandwich cultured human hepatocytes by As^{III} (Roggenbeck et al., 2015) and we have observed increased MRP4 levels in HepG2 cells after exposure to As^{III} and MMA^{III} (Fig. 1).

We reported in Banerjee et al. (2014) that ATP-dependent transport of $MMA(GS)_2$ and DMA^{V} by MRP4-enriched membrane vesicles was osmotically sensitive and co-operative (Hill coefficients of 1.4 ± 0.2 and 2.9 ± 1.2, respectively). Furthermore, $MMA(GS)_2$ and DMA^{V} are transported by MRP4 with high affinity ($K_{0.5}$ values of 0.7 ± 0.16 and 0.22 ± 0.15 μmol/L, respectively) (Banerjee et al., 2014). Considering that tissue levels of arsenic during chronic exposure are estimated to be in the 10 to 100 nmol/L range, this level of affinity is of high physiological relevance (Banerjee et al., 2014; Kritharis et al., 2013). As will be discussed further in Sections 2.2.3 and 2.3.3, MRP4 is localized to the basolateral surface of the hepatocyte and the apical surface of the renal proximal tubule (Hoque et al., 2009; Rius et al., 2003; Slot et al., 2011; van Aubel et al., 2002). This differential tissue specific membrane localization makes MRP4 an ideal candidate for the urinary elimination of hepatic arsenic metabolites and MRP4 could be a major player in arsenic elimination. We have recently found that single nucleotide polymorphic (SNP) variants of MRP4 have differing abilities to transport arsenic through both altered function and membrane localization (Banerjee et al., 2016). Further investigation is warranted to determine if genetic variations in *ABCC4* contribute to inter-individual differences in susceptibility to arsenic-induced diseases (Banerjee et al., 2016).

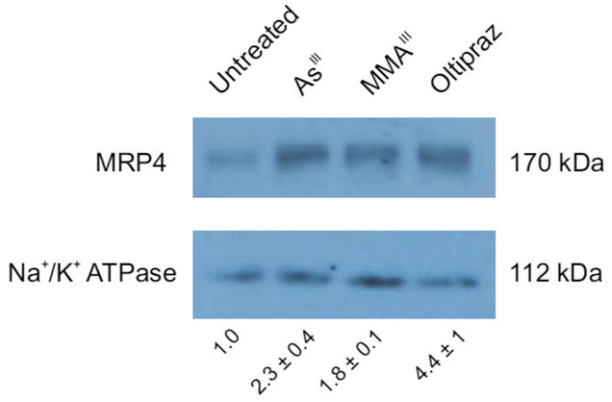

Fig. 1 – As^{III} and MMA^{III} increase MRP4 levels in HepG2 cells. HepG2 cells were seeded in 60 mm culture plates at 1×10^6 cells/plate. Twenty-four hours post-seeding cells were treated with As^{III} (1 μmol/L), MMA^{III} (1 μmol/L) or oltipraz (50 μmol/L) for 48 h. Oltipraz was used as a positive control because it is known to induce MRP4 mRNA expression and protein levels (Roggenbeck et al., 2015). Cells were harvested and crude membranes prepared as described previously (Almquist et al., 1995). MRP4 protein levels were then determined by immunoblot analysis using the rat anti-human MRP4 antibody M_4-I10 (Abcam, Cambridge, MA) (1:2000), after resolving 10 μg of protein by SDS-PAGE. The anti-Na^+/K^+-ATPase antibody (1:10,000) (H-300, Santa Cruz Biotechnology, Dallas, TX), was used to detect the Na^+/K^+-ATPase as a loading control. Densitometry was performed with ImageJ software and mean ± S.D. values from three independent experiments are shown at the bottom of the figure.

1.5.1.1.4. *Potential cellular export of arsenic by other MRPs (ABCCs).* Other than for MRP1, MRP2, and MRP4 little evidence currently exists to support a role for other MRPs in the cellular export of arsenic. MRP3 expressed in the human ovarian carcinoma cell line 2008 did not confer resistance to As^{III} or As^{V} (Kool et al., 1999). Consistent with this, we observed that HEK293 cells stably expressing MRP3 did not confer resistance to As^{III}, As^{V}, MMA^{III}, MMA^{V}, DMA^{III}, or DMA^{V} (Banerjee et al., 2014). Furthermore, no correlation between the expression of MRP3 in primary human hepatocytes and the level of total inorganic (As^{III} + As^{V}), total monomethylated (MMA^{III} + MMA^{V}), or total dimethylated (DMA^{III} + DMA^{V}) arsenicals in culture media or cells was found (Drobna et al., 2010b). Similarly, MRP5 expressed in HEK293 cells did not confer resistance to As^{III}, As^{V}, MMA^{III}, MMA^{V}, DMA^{III}, or DMA^{V} (Banerjee et al., 2014; McAleer et al., 1999; Wijnholds et al., 2000). These results suggest that MRP3 and MRP5 are not important mediators of inorganic and methylated arsenic transport. To our knowledge, the roles of MRP6 (*ABCC6*), MRP7 (*ABCC10*), MRP8 (*ABCC11*), and MRP9 (*ABCC12*) in cellular export of arsenic have not been investigated.

1.5.1.2. *Other ABC transporters and potential involvement in arsenic efflux.* P-glycoprotein (gene symbol *ABCB1*) is a member of subfamily "B" of the ABC transporter family, and

like MRP1, was originally identified based on its ability to confer resistance to natural product type anti-cancer agents (Juliano and Ling, 1976). P-glycoprotein is also known to confer cellular protection against a diverse array of toxicants (Leslie et al., 2005). Unlike MRP1, P-glycoprotein substrates are generally large, hydrophobic or amphipathic, neutrally or positively charged, aromatic ring containing structures, and do not include GSH conjugates (Eckford and Sharom, 2009). These characteristics and additional experimental evidence make it highly unlikely that P-glycoprotein transports arsenic-GSH conjugates (Leslie et al., 2005; Zaman et al., 1995). Mice lacking the two murine *Abcb1* genes (*Abcb1a/Abcb1b*) were found to be more sensitive to As^{III} and accumulated more total arsenic in certain tissues than wild-type animals (Liu et al., 2002a; Xie et al., 2004). These findings were inconsistent with previous reports that P-glycoprotein does not protect cells against As^{III} or modify its accumulation (Allen et al., 2000; Salerno et al., 2002). The reasons for these differences remain unclear. If P-glycoprotein is directly involved in arsenic efflux, the chemical species transported await further experiments for identification.

2. Contribution of transport proteins to the absorption, distribution, metabolism, and elimination of arsenic

In humans, arsenic exposure is predominantly through the oral route. After absorption across the gastrointestinal tract, arsenic is extensively methylated in the liver; however, arsenic elimination occurs predominantly (60%–80%) in urine, and of this, 10%–30% is eliminated as iAs, 10%–20% as monomethylated, and 60%–80% as dimethylated forms (Loffredo et al., 2003; Vahter, 2000).

In the preceding sections we have described the individual transport proteins shown experimentally to contribute to the passage of As^{III}, As^{V}, methylated, and glutathionylated arsenic species across cellular membranes. In the next section we will address how these transport pathways potentially work together to influence the absorption, distribution, and elimination of arsenic by facilitating its passage across epithelial layers of the gastrointestinal tract, liver, and kidney.

2.1. *Gastrointestinal tract absorption of arsenic*

Although the gastrointestinal tract is the first line of defense against arsenic after oral exposure, the study of arsenic transport pathways in this tissue has not been extensive. Oral intake of arsenic by humans occurs predominantly in the forms of As^{III} and As^{V} via drinking water (Singh et al., 2007). Experimental studies in animals and humans have shown that the gastrointestinal absorption of As^{III} and As^{V} is extensive, with urinary elimination accounting for 50%–94% (hamster and mouse) and 45%–75% (humans) of the oral dose (Ng et al., 2015). The first step in the absorption of arsenic across the gastrointestinal tract is its passage across the epithelial cells (enterocytes) of the intestine. This passage can occur through transcellular pathways, requiring arsenic species to traverse the apical and then the basolateral membrane of the enterocyte. Alternatively, absorption can occur through the paracellular route (between cells), requiring the passage of arsenic through tight junctions.

In vitro studies to investigate transepithelial transport of inorganic and methylated forms of arsenic have predominantly employed polarized Caco-2 cells, derived from a human colorectal adenocarcinoma cell line. Caco-2 cells are considered to be a good model for studying the physiology of the human intestine, however, limitations should be noted. There are differences in mRNA and protein levels for membrane transport proteins between Caco-2 cells and primary human enterocytes (Englund et al., 2006; Olander et al., 2016). For example, OATP2B1 mRNA and protein are found at higher levels in Caco-2 cells relative to the primary enterocyte (Englund et al., 2006; Olander et al., 2016). Furthermore, Caco-2 monolayers are known to be less permeable than the human intestine in vivo due to differences in tight junctions (Wikman-Larhed and Artursson, 1995), and could underestimate the contribution of the paracellular transport pathway to arsenic absorption (Calatayud et al., 2012b).

Studies using Caco-2 cells provide evidence that arsenic utilizes both transcellular and paracellular pathways; however, the extent of each is dictated by the chemical species of arsenic (Calatayud et al., 2010, 2011; Liu et al., 2016) (Table 2). The passage of pentavalent inorganic and methylated arsenic species across the Caco-2 cell monolayer have a larger paracellular component than the trivalent species (Calatayud et al., 2010, 2011, 2012b). What is known about the transcellular transport pathways are described in the following sections.

Table 2 – Summary of human transport pathways responsible for the absorption of different chemical species of arsenic across the intestinal epithelium (enterocyte). [a]

Arsenic species	Transcellular		Paracellular
	Uptake (apical/lumen)	Efflux (basolateral/blood)	
As^{III}	AQP7 AQP10	AQP3	++[b]
As^{V}	Na^+/P_i-IIb	[c]	+++[b]
MMA^{III}	[c]	[c]	+[b]
MMA^{V}	[c]	[c]	++++[b]
DMA^{III}	[c]	[c]	[d]
DMA^{V}	[c]	MRP4	++++[b]
[e]$As(GS)_3$	[c]/[d]	MRP1	[c]/[d]
[e]$MMA(GS)_2$	[c]/[d]	MRP1 MRP4	[c]/[d]
[f]$[(GS)_2AsSe]^-$	[c]/[d]	[c]/[d]	[c]/[d]

[a] Only human transporters that have been demonstrated to directly transport arsenic when overexpressed in a cell system are included.
[b] Increasing contribution of paracellular transport to total transepithelial passage (apical to basolateral direction) (Calatayud et al., 2010, 2011, 2012a).
[c] Not know.
[d] Potentially irrelevant.
[e] Chemically unstable and never isolated from blood. As^{III} and MMA^{III} likely dissociate from GSH in bile/intestinal tract (Leslie, 2012).
[f] Thought to remain stable in the intestinal tract and be eliminated in feces (Carew and Leslie, 2010; Gailer et al., 2002; Levander and Baumann, 1966).

2.1.1. *Arsenic enterocyte transcellular transport pathways*

2.1.1.1. *Transport across the apical surface of the enterocyte.* Several candidate iAs uptake transporters described in Section 1.4 are present at the apical surface of the enterocyte (Fig. 2 and Table 2). One study used Caco-2 cells to show that gene-knockdown of *SLCO2B1* (OATP2B1), *AQP10*, or *SLC2A5* (GLUT5), resulted in a decreased As^{III} accumulation while knockdown of *SLC34A2* (Na^+/P_i-IIb) resulted in a decreased As^V accumulation (Calatayud et al., 2012a). With the exception of Na^+/P_i-IIb, which has been carefully characterized for As^V uptake in *Xenopus laevis* oocytes (Section 1.4.1), no additional evidence exists to support a role for these proteins in the cellular uptake of iAs. Thus, although the gene-knockdown study by Calatayud et al., suggests a potential role for these transporters in the intestinal absorption of As^{III}, further studies using heterologous expression systems would provide necessary corroborating evidence. The result for OATP2B1 is especially surprising because OATPs generally transport large, amphipathic, organic anions (Obaidat et al., 2012). Furthermore, hAQP10 did not allow the conductance of As^{III} when overexpressed in *Xenopus laevis* (Section 1.4.2) (Liu et al., 2004b).

Other candidate As^{III} transport proteins localized to the apical surface of the enterocyte include AQP7 and GLUT2. As described in Section 1.4.2 AQP7 transport of As^{III} has been shown when this protein is ectopically expressed in *Xenopus laevis* oocytes. GLUT2 is present constitutively at the basolateral surface of small intestine enterocytes, but, can be recruited transiently to the apical surface in response to high lumen glucose levels (Kellett and Helliwell, 2000). Thus, GLUT2 could be both a candidate protein involved in uptake of As^{III} at the apical surface of the intestine and for movement of As^{III} across the basolateral surface of the intestine. However, direct transport studies need to be conducted to determine if As^{III} is a permeant of GLUT2. It is worth noting that GLUT7 (*SLC2A7*) is highly expressed in the small intestine and colon with high protein levels detected in brush border membrane preparations from the jejunum and ileum of Sprague Dawley rats (Li et al., 2004). The role of GLUT7 in arsenic absorption across the intestine may be worth investigating.

The potential pathways for the passage of iAs across the apical surface of the enterocyte have been described above. There is growing evidence that iAs can be methylated and/or thiolated by the gut microbiome during gastrointestinal transit (Rubin et al., 2014; Van de Wiele et al., 2010). This would influence the species of arsenic available for absorption across the colon. Arsenic species are also known to undergo enterohepatic circulation resulting in the presence of methylated and possibly thiolated products in the upper portion of the small intestine. iAs is known to be absorbed across the rat small intestine (Gonzalez et al., 1997; Rowland and Davies, 1982) and Caco-2 cells as a model of colon indicates that iAs, methylated and thiolated species can be absorbed across the colon (Calatayud et al., 2010, 2011; Hinrichsen et al., 2015; Leffers et al., 2013b). Nutrient absorption is largely completed in the upper portion of the small intestine, thus this is the

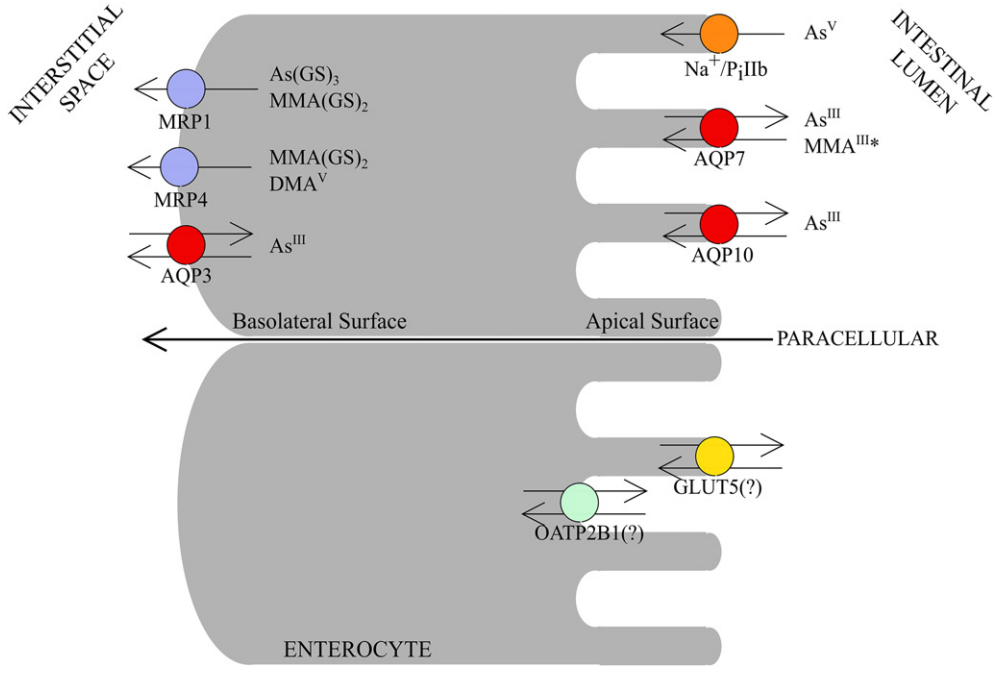

Fig. 2 – Potential arsenic transport pathways in the mammalian enterocyte. Arrows denote the direction of transport, into or out of the enterocyte across apical or basolateral surfaces. Transporters with arrows in both directions are theoretically capable of moving arsenic species into or out of the cell, down the concentration gradient. While all potential arsenic transporters are discussed in the text, only transporters with experimental evidence for their involvement in arsenic transport are included in this figure. Transporters with some experimental evidence, but that require further validation for their role in arsenic transport, are indicated with "(?)". The chemical species of arsenic transported are only indicated for pathways with previously published direct transport data. *MMA^{III} is transported by mAQP7, but not hAQP7 (McDermott et al., 2010).

predominant region of the gastrointestinal tract where GLUTs, P_i transporters, and AQP7 are expressed. While GLUTs and AQPs have the potential to transport neutral methylated forms of arsenic (Sections 1.4.2 and 1.4.3), more research is required for understanding the major enterocyte uptake pathways for thiolated and charged methylated arsenic species.

2.1.1.2. Transport of arsenic across the basolateral surface of the enterocyte. Once iAs and metabolites have been transported across the apical surface of the enterocyte they have to traverse the basolateral membrane prior to entry into the interstitial space followed by the capillaries (Fig. 2 and Table 2). Studies using Caco-2 cells have shown that arsenic methylation can occur within the enterocyte (Calatayud et al., 2012c), albeit slowly and to a lesser extent in polarized versus non-polarized cells. Transport proteins likely localize more efficiently in polarized versus non-polarized Caco-2 cells allowing for more rapid transport of iAs across the epithelial layer, and allowing less time for methylation to occur. Studies of rat small intestine perfused with As^V showed that the majority of arsenic entering the portal circulation was in the form As^{III} (with some As^V, MMA, and DMA) suggesting As^V is reduced to As^{III} within the enterocyte and some (although limited) methylation occurs (Rowland and Davies, 1982).

The only candidate protein localized to the basolateral membrane of the enterocyte with direct evidence for cellular As^{III} permeation is AQP3 (Section 1.4.2, Table 2 and Fig. 2) (Lee et al., 2006). GLUT2 is also localized to this membrane and could play a role, however direct evidence for a role in arsenic transport has not been established (Section 1.4.3). MRP1 is localized to the basolateral surface of the small intestine and colon enterocyte (Peng et al., 1999), and could transport As^{III} and MMA^{III} in their GSH conjugated forms $As(GS)_3$ and $MMA(GS)_2$, respectively (Carew et al., 2011; Leslie et al., 2004). MRP4 is functionally important at the basolateral surface of enterocytes in the small intestine (de Waart et al., 2012; Ming and Thakker, 2010) and could allow passage of MMA^{III} (in the form of $MMA(GS)_2$) and DMA^V (Banerjee et al., 2014).

2.2. Arsenic hepatobiliary transport and metabolism

Once arsenic is absorbed across the intestinal epithelium it enters the portal circulation and is carried to the liver sinusoids. The liver is the major site of arsenic methylation (reviewed by Drobna et al. (2010b)). Thus, transport of arsenic into the hepatocyte facilitates the methylation process. Furthermore, transport of arsenic and its metabolites across the apical (canalicular) surface into bile or back across the basolateral (sinusoidal) surface into the sinusoidal blood influences the distribution and ultimately elimination of arsenic. The transport pathways (known and potential) involved in this arsenic "processing" by hepatocytes are described below and summarized in Fig. 3 and Table 3.

2.2.1. Arsenic uptake across the basolateral surface of the hepatocyte

The only directly demonstrated arsenic uptake pathway that has been shown definitively to be localized to the basolateral surface of (rat and mouse) hepatocytes is AQP9 (Carbrey et al., 2009; Elkjær et al., 2000) (Fig. 3 and Table 3). Primary mouse hepatocytes with AQP9 knocked-down by 50% had a 25% reduction in As^{III} accumulation compared to controls, supporting a role for AQP9 in As^{III} uptake by hepatocytes (Shinkai et al., 2009). As described in Section 1.4.2 AQP9 is permeable to neutral forms of MMA^{III}, MMA^V, and DMA^V suggesting that AQP9 is capable of facilitating the entry of arsenic metabolites either formed in the intestine or undergoing enterohepatic cycling. Conflicting reports exist about whether AQP3 and AQP7 are expressed in hepatocytes (possibly related to species differences in expression) (Gregoire et al., 2015; Zhao et al., 2016) and their subcellular localizations (i.e., basolateral or apical localization) are unclear (Laforenza et al., 2016), thus these proteins will not be discussed further in this section.

Other candidate arsenic uptake transport proteins known to be at the basolateral surface of the hepatocyte include the GLUTs (GLUT2 and GLUT9) (Keembiyehetty et al., 2006; Thorens et al., 1990). Currently there is no evidence that either GLUT2 or GLUT9 are arsenic transporters, however, their basolateral localization in hepatocytes, and relatedness to GLUT1 (Section 1.4.3) warrants further investigation regarding their potential roles in arsenic uptake. Similarly, based on their basolateral location several OATPs are candidates for arsenic uptake across the hepatocyte basolateral membrane including OATP1B1 (*SLCO1B1*), OATP2B1 (*SLCO2B1*), and OATP1B3 (*SLCO1B3*). As described in Section 1.4.4 OATP1B1 and OATP2B1 have modest evidence for roles in arsenic uptake, while no evidence exists for OATP1B3.

2.2.2. Arsenic export across the apical surface of the hepatocyte and enterohepatic cycling

Current data overwhelmingly implicates MRP2 as the major transporter of arsenic across the apical/canalicular surface of the hepatocyte into bile (Tables 1, 3, and Fig. 3). It has been known for many years that the biliary excretion (transport across the apical/canalicular hepatocyte membrane) of As^{III} and As^V is completely dependent upon the presence of GSH, and Gyurasics et al. (1991), proposed that this was due to the formation and efflux of arsenic-GSH complexes. More recently, it was shown that Mrp2-deficient TR^- rats administered As^{III} intravenously had less than 1% of total arsenic in bile compared to the wild-type (WT) Wistar rats (Kala et al., 2000). WT-Wistar rats treated with buthionine sulfoxamine (BSO), an inhibitor of GSH synthesis, prior to As^{III} administration, also resulted in less than 1% of total arsenic in bile compared to the GSH replete WT control (Kala et al., 2000). The same study showed that 80%–90% of arsenic detected in the bile of WT rats after administration of either oral (5 mg/kg) or intravenous arsenic (5 mg/kg) was in the form of $As(GS)_3$ and/or $MMA(GS)_2$, however, when lower doses of arsenic (0.5 mg/kg) were used (orally or intravenously), only $MMA(GS)_2$ was detected (Kala et al., 2000). This dose dependent difference in arsenic metabolites in bile was proposed by the authors to be due to saturation of arsenic methylation pathways. The observations of Kala et al. (2000), that $MMA(GS)_2$ and $As(GS)_3$ are the predominant arsenic species in rat bile and that biliary excretion is Mrp2-dependent have been independently reproduced with an Mrp2-deficient Sprague Dawley rat strain [Eisai hyperbilirubinemic rat (EHBR)] (Bu et al., 2011). Dimethylated arsenic species have not been identified in bile

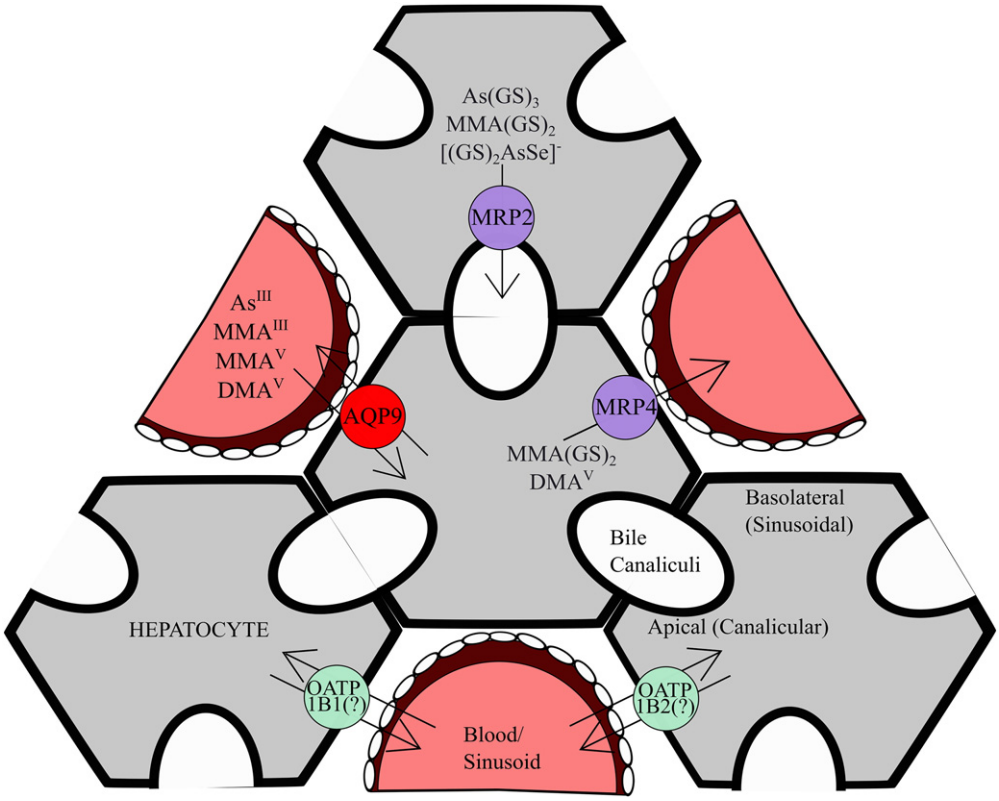

Fig. 3 – Potential arsenic transport pathways in the mammalian hepatocyte. Arrows denote the direction of transport, into or out of the hepatocyte across apical or basolateral surfaces. Transporters with arrows in both directions are theoretically capable of allowing the passage of arsenic species into or out of the cell, down the concentration gradient. While all potential arsenic transporters are discussed in the text, only transporters with experimental evidence for their involvement in arsenic transport are included in this figure. Transporters with some experimental evidence, but that require further validation for their role in arsenic transport, are indicated with "(?)". The chemical species of arsenic transported are only indicated for pathways with previously published direct transport data.

of rats (Bu et al., 2011; Gregus et al., 2000; Kala et al., 2000; Suzuki et al., 2001), with the exception of one study (Cui et al., 2004).

Our studies of arsenic hepatobiliary transport using sandwich cultured primary human hepatocytes support a role for human MRP2 in the biliary excretion of arsenic (Roggenbeck et al., 2015). Inter-individual differences existed in the biliary excretion of arsenic between sandwich cultured primary human hepatocyte preparations, with only five of fourteen preparations having detectable arsenic biliary excretion. For these five preparations, BSO treatment resulted in the complete loss of biliary excretion, this GSH-dependence is consistent with an MRP2-dependent process (Roggenbeck et al., 2015). Consistent with this is the ability of human MRP2-enriched membrane vesicles to transport $As(GS)_3$ (Carew and Leslie, 2010) and $MMA(GS)_2$ (Roggenbeck, Kaur, and Leslie, unpublished data).

The purpose of arsenic biliary excretion is unclear, considering that arsenic is predominantly eliminated in urine. The majority of $As(GS)_3$ and $MMA(GS)_2$ exported across the apical/canalicular surface of the hepatocyte by human MRP2 or rat Mrp2 does not undergo elimination in the feces. These As-GSH conjugates are unstable (Kala et al., 2000; Raab et al., 2004; Yehiayan et al., 2009), especially in bile which has an alkaline pH, resulting in the opportunity for free As^{III} and MMA^{III} to be reabsorbed across the gastrointestinal tract and enter the portal circulation to be carried back to the liver (thus undergoing enterohepatic cycling) (Dietrich et al., 2001; Leslie, 2012; Suzuki et al., 2001). In WT-Wistar and -Sprague Dawley rats a substantial portion of arsenic (~27% of the dose) is excreted into bile (Bu et al., 2011; Suzuki et al., 2001). In Mrp2-deficient rats, arsenic accumulates more in liver, kidney, red blood cells, and plasma, suggesting that enterohepatic cycling is protective, especially to the liver (Bu et al., 2011; Suzuki et al., 2001). Interestingly, evidence suggests that Mrp2-dependent enterohepatic cycling of arsenic is required for the formation of certain thioarsenicals (Bu et al., 2011). It has been proposed that this cycling permits the thiolation of arsenic by the gastrointestinal microbiota (Bu et al., 2011).

In contrast with $As(GS)_3$ and $MMA(GS)_2$, which are unstable at alkaline biliary pH, formation and MRP2-mediated biliary excretion of $[(GS)_2AsSe]^-$ is thought to play a more important role in elimination. $[(GS)_2AsSe]^-$ is more stable at alkaline pH and is thought to remain intact for fecal elimination (Carew and Leslie, 2010; Gailer et al., 2002; Leslie, 2012; Levander and Baumann, 1966). Recently, rat bile isolated from WT-Wistar rats treated with equimolar As^{III} and Se^{IV} was found to contain >90% of arsenic and selenium as $[(GS)_2AsSe]^-$ (George et al., 2016), suggesting that this conjugate is important. What dictates the formation and biliary excretion of selenium-containing versus non-

Table 3 – Summary of human transport pathways for different chemical species of arsenic in the hepatocyte. [a]

Arsenic species	Uptake (basolateral/ sinusoidal)	Efflux (apical/ canalicular)	Efflux (basolateral/ sinusoidal)
As^{III}	AQP9	[b]/[e]	AQP9
As^{V}	[b]	[b]/[e]	[b]/[e]
MMA^{III}	[b]	[b]/[e]	AQP9
MMA^{V}	[b]	[b]/[e]	AQP9
DMA^{III}	[b]	[b]/[e]	[b]/[e]
DMA^{V}	[b]	[b]/[e]	AQP9 MRP4
[c]$As(GS)_3$	[b]/[e]	MRP2	[b]
[c]$MMA(GS)_2$	[b]/[e]	MRP2	MRP4
[d]$[(GS)_2AsSe]^-$	[b]/[e]	MRP2	[b]/[e]

[a] Only human transporters that have been demonstrated to directly transport arsenic when overexpressed in a cell system are included.
[b] Not known.
[c] Chemically unstable and never isolated from blood.
[d] Thought to undergo biliary excretion and be eliminated in feces (Carew and Leslie, 2010; Gailer et al., 2002; Levander and Baumann, 1966).
[e] Potentially irrelevant.

selenium-containing arsenic-GSH conjugates is currently unknown, and worthy of investigation.

2.2.3. *Arsenic export across the basolateral surface of the hepatocyte*

Given the extensive hepatic metabolism and urinary elimination of arsenic, it is important to understand how arsenic and its metabolites are exported across the basolateral surface of hepatocytes into blood. As described in Section 1.5.1.1.3 we have shown that human MRP4, which is localized to the basolateral surface of hepatocytes, is a high affinity transporter of DMA^V and $MMA(GS)_2$ (Banerjee et al., 2014) (Tables 1, 3, and Fig. 3). The transport of DMA^V, the main urinary metabolite of arsenic found in human urine, by MRP4 suggests that this protein could play a pivotal role in the elimination of methylated arsenic species. Our study of the hepatobiliary transport of arsenic using sandwich cultured primary human hepatocytes revealed that basolateral transport of arsenic was temperature- and partially GSH-dependent and inhibited by MK-571, a commonly used MRP inhibitor (Roggenbeck et al., 2015). MRP4 levels were increased in a dose dependent manner by low (μmol/L) levels of As^{III}. Treatment of sandwich cultured primary human hepatocytes with the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) activator, oltipraz, increased MRP4 levels and basolateral efflux of arsenic. In contrast, oltipraz increased MRP2 levels without increasing biliary excretion. These results suggest arsenic basolateral transport prevails over biliary excretion (consistent with the predominant urinary elimination in vivo) and is mediated at least in part by MRPs, most likely including MRP4 (Roggenbeck et al., 2015).

Presumably transport proteins and channels in addition to MRP4 contribute to the basolateral efflux of arsenic from hepatocytes. Speciation analysis of culture media from sandwich cultured primary human hepatocytes treated with As^{III} revealed the presence of iAs (predominantly), MMA, and DMA (Roggenbeck et al., 2015). As^{III}, As^V, and $As(GS)_3$ are not transported by MRP4 (Banerjee et al., 2014). It is worth noting that MRP6 is expressed at the basolateral membrane of hepatocytes and its role in arsenic transport is unknown (Madon et al., 2000). MRP6 might contribute to iAs efflux through the export of $As(GS)_3$ and/or other arsenic metabolites.

In addition to the MRPs, several of the transport proteins discussed in Section 2.2.1 as potential uptake pathways for arsenic might contribute to efflux as well. The evidence for this is strongest for AQP9 (Mukhopadhyay et al., 2014). Under conditions where the intracellular concentration of As^{III} and neutral methylated species exceeds that outside the cell, AQP9 and other bidirectional channels and transporters could allow the permeation of arsenic across the basolateral surface of hepatocytes into blood.

Once arsenic is exported across the basolateral surface of the hepatocyte it enters the sinusoidal blood and is carried to the hepatic central vein. From the central vein, blood then flows into the hepatic veins followed by the inferior vena cava. At this point, iAs and metabolites formed in the liver and intestine are circulated in the blood to different organs, most importantly for elimination, the kidney.

2.3. *Arsenic renal transport and metabolism*

While arsenic is predominantly eliminated in urine very little is known about the specific pathways that allow this to occur. At the kidney, arsenic (as for all solutes) has numerous pathways it can potentially take prior to urinary elimination. Glomerular filtration (a passive process not involving transporters) can remove a fraction of any free solute (i.e., non-protein bound) from the blood. After glomerular filtration, different chemical species of arsenic might undergo tubular reabsorption or be directly eliminated in the urine from the filtrate. Arsenic species might also be taken up from blood across the basolateral surface of the proximal tubules. Once inside the proximal tubule epithelium, arsenic can be metabolized and then secreted across the apical surface for elimination. While all free chemical species of arsenic (methylated, glutathionylated, thiolated, and inorganic) will undergo glomerular filtration, the extent of the other processes (tubular reabsorption, uptake across the basolateral surface of the proximal tubule cells, and secretion into urine) will be dictated by the different chemical species of arsenic and involve different transport pathways. The following sections review what is known about the renal elimination of arsenic from in vivo studies, and what transporters are predicted to be involved based on expression studies and in vitro transport experiments.

2.3.1. *Glomerular filtration and tubular reabsorption of arsenic*

In terms of renal elimination, As^V is the best characterized arsenic species. Using a dog model system, Ginsburg and Lotspeich (1963) demonstrated that after glomerular filtration As^V undergoes net tubular reabsorption, and that reabsorption is inversely correlated to plasma P_i levels (Ginsburg and Lotspeich, 1963). The reduction of As^V reabsorption by P_i could indicate that Na^+/P_i transporters (discussed in Section 1.4.1) are involved in the reabsorption of As^V. Indeed, Na^+/P_i-IIa, Na^+/P_i-IIc, and P_iT-2 are appropriately localized at the apical surface of the renal proximal tubule epithelial cells for reabsorption (Forster et al.,

2013) (Fig. 4 and Table 4). However, as discussed in Section 1.4.1 the affinity of these transporters for As^V is >10-fold lower than for As^V with K_i values of 1.1–3.8 mmol/L in heterologous expression systems (Villa-Bellosta and Sorribas, 2008, 2010) and 1.1 mmol/L in renal brush border membranes isolated from rats (Hoffmann et al., 1976). Furthermore, the concentration of P_i in the glomerular filtrate is ~6 orders of magnitude greater than As^V (~1 mmol/L P_i vs nmol/L As^V), thus these transporters are unlikely to be involved in As^V reuptake under physiological conditions (Villa-Bellosta and Sorribas, 2008, 2010). Thus, more work is required to understand the mechanism(s) of tubular reabsorption of As^V.

Studies of As^V renal handling in dog revealed that ~14% of intravenously administered As^V is reduced to As^{III}, and As^{III} undergoes net tubular secretion (Ginsburg and Lotspeich, 1963). Data suggested that the reduction of As^V to As^{III} occurred within the tubular epithelium (Ginsburg and Lotspeich, 1963). Further study showed that when As^{III} is the infused species it undergoes net tubular reabsorption from the glomerular filtrate (Ginsburg, 1965). AQP7 and GLUT5 are two candidate As^{III} transport pathways at the apical surface of the renal proximal tubule cell that could allow the tubular reabsorption of As^{III}. As described in Section 1.4.2, hAQP7 is capable of allowing the permeation of As^{III} while mAQP7 allows the permeation of As^{III} and MMA^{III} (Liu et al., 2006b; McDermott et al., 2010). The evidence for the involvement of GLUT5 is weaker (knockdown of GLUT5 reduced As^{III} accumulation in Caco-2 cells) (Calatayud et al., 2012a) (Section 1.4.3), and the arsenic species potentially transported by GLUT5 (other than As^{III}) are unknown.

There are no other uptake transport pathways described in Section 1/Table 1 or related transporters (other AQPs, GLUTs, or OATPs) present at the apical surface of the renal tubule epithelium (Fig. 4 and Table 4). To our knowledge there are no reports regarding the tubular reabsorption of methylated, glutathionylated, or thiolated arsenic species, thus, it is not known if these species of arsenic are reabsorbed or undergo elimination in the glomerular filtrate. Further work is required to determine if other transporters present at the apical surface of renal proximal tubule cells are capable of taking up iAs and metabolites.

2.3.2. *Uptake of inorganic and methylated arsenic species into renal proximal tubule cells from blood*

During glomerular filtration not all arsenic will be filtered in a single pass through the kidney. Thus, arsenic continues in the peritubular circulation, and if transport proteins with specificity for the arsenic chemical form are available, arsenic will be taken up by the tubular epithelium. The focus of this review will be on the proximal tubule as this is the major site of xenobiotic secretion into urine (Morrissey et al., 2013).

The only candidate transporter with any in vitro evidence for arsenic uptake expressed at the basolateral surface of the proximal tubule is GLUT1. As described in Section 1.4.3 rGLUT1 has been shown to increase the cellular accumulation of As^{III} and MMA^{III} and hGLUT1 at least MMA^{III} (Liu et al., 2004a). GLUT2,

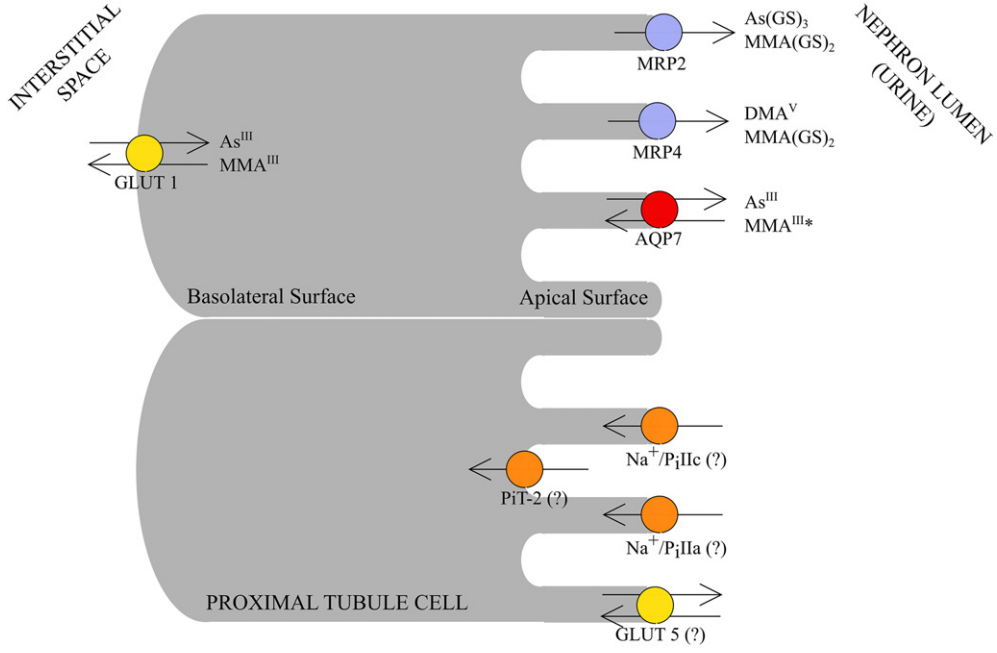

Fig. 4 – Potential arsenic transport pathways in the mammalian proximal tubular epithelium. Arrows denote the direction of transport, into or out of the renal proximal tubule epithelium across apical or basolateral surfaces. Transporters with arrows in both directions are theoretically capable of moving arsenic species into or out of the cell, down the concentration gradient. While all potential arsenic transporters are discussed in the text, only transporters with experimental evidence for their involvement in arsenic transport are included in this figure. Transporters with some experimental evidence, but that require further validation for their role in arsenic transport, are indicated with "(?)". The chemical species of arsenic transported are only indicated for pathways with previously published direct transport data. *MMA^{III} is transported by mAQP7, but not hAQP7 (McDermott et al., 2010).

Table 4 – Summary of human transport pathways for different chemical species of arsenic in the renal proximal tubule epithelium. [a]

Arsenic species	Tubular reabsorption (uptake across apical surface)	Uptake (across basolateral surface)	Efflux (across apical surface)
As^{III}	AQP7	GLUT1	AQP7
As^{V}	Na^+/P_i transporters/[b]	[b]	[b]
MMA^{III}	[b]	GLUT1	[b]
MMA^{V}	[b]	[b]	[b]
DMA^{III}	[b]	[b]	[b]
DMA^{V}	[b]	[b]	MRP4
[c]$As(GS)_3$	[b]/[e]	[b]/[e]	MRP2
[c]$MMA(GS)_2$	[b]/[e]	[b]/[e]	MRP2
[d]$[(GS)_2AsSe]^-$	[b]/[e]	[b]/[e]	[b]/[e]

[a] Only human transporters that have been demonstrated to directly transport arsenic when overexpressed in a cell system are included.
[b] Not known.
[c] Chemically unstable and never isolated from blood.
[d] Predominant elimination pathway thought to be biliary/fecal (Carew and Leslie, 2010; Gailer et al., 2002; Levander and Baumann, 1966).
[e] Potentially irrelevant.

GLUT9, and OATP4C1 (*SLCO4C1*) are also localized to the basolateral membrane of proximal tubule cells (Douard and Ferraris, 2008; Kimura et al., 2014; Morrissey et al., 2013), and their relatedness to other transporters capable of taking up arsenic make them worthy of further investigation.

While putative pathways exist for the proximal tubule cell uptake of As^{III} and MMA^{III} from blood, Na^+-dependent P_i transporters are not present at the basolateral surface of the proximal tubule, raising the possibility that As^{V} enters the lumen of the tubule solely through glomerular filtration. $As(GS)_3$ and $MMA(GS)_2$ are chemically unstable (Section 1.3 and reviewed in Leslie (2012)) and have never been isolated from blood, thus it is possible that they are handled by the kidney only as the dissociated As^{III} and MMA^{III}. Rabbit renal cortical slices incubated with As^{III}, As^{III} + GSH, or $As(GS)_3$ had no difference in the cellular accumulation of arsenic, potentially suggesting that the kidney is capable of taking up both $As(GS)_3$ and As^{III} (Burton et al., 1995). Alternatively, $As(GS)_3$ was enzymatically degraded by γ-glutamyl transpeptidase in the renal cortical slices (Kala et al., 2004), resulting in the release of As^{III} for accumulation. Lastly, a transport pathway for the uptake of DMA^{V} into the renal proximal tubule has not yet been identified. It is possible that DMA^{V} formed outside of the renal proximal tubule epithelium is eliminated solely through glomerular filtration, however, this won't be understood until the renal handling of this important urinary metabolite is investigated further.

2.3.3. *Efflux of inorganic and methylated arsenic species across the apical surface of renal proximal tubule cells*

Once arsenic has entered the proximal tubule epithelium (either through reabsorption from the glomerular filtrate (Section 2.3.1) or from the blood (Section 2.3.2)) it can be metabolized (or not) and effluxed across the apical surface (into urine). Efflux across the basolateral surface into the interstitial space for passage into blood can also occur. iAs is extensively methylated by kidney tissue (Healy et al., 1998; Lerman and Clarkson, 1983), therefore, iAs/MMA entering the renal tubule cells has the potential to undergo initial/further methylation. The kidney is also rich in GSH (Meister et al., 1979), which would allow the renal formation of As-GSH conjugates.

As described in Section 2, arsenic in human urine is found predominantly as iAs (10%–30%), monomethylated arsenic (10%–20%), and dimethylated arsenic (60%–80%). Evidence from mice deficient in γ-glutamyl transpeptidase suggest that $As(GS)_3$ and $MMA(GS)_2$ are the major forms of iAs and MMA, respectively, transported into urine along with DMA^{V} (Section 1.3) (Kala et al., 2004). Urinary elimination of arsenic in mice was predominantly GSH-dependent suggesting the MRPs as likely candidates for $As(GS)_3$ and $MMA(GS)_2$ transport (Kala et al., 2004). The work of Kala et al. (2000, 2004) combined with the apical localization of MRP2 and MRP4 in renal proximal tubule cells, and our in vitro transport work showing that $As(GS)_3$ and $MMA(GS)_2$ are transported by human MRP2 (Carew and Leslie, 2010; Roggenbeck, Kaur, and Leslie, unpublished data), and that $MMA(GS)_2$ and DMA^{V} are transported with high affinity by human MRP4 (Banerjee et al., 2014), make these two ABC transporters strong candidates for involvement in the urinary elimination of arsenic (Fig. 4). In conflict with a role for Mrp2 in urinary elimination, Mrp2-deficient TR^- rats treated with As^{III} (1 mg/kg, subcutaneous) had an increased urinary level of total arsenic (Kala et al., 2004). This is likely due to the loss of biliary excretion and enterohepatic cycling (Section 2.2.2) of $As(GS)_3$ and $MMA(GS)_2$, and might suggest a more prominent role for MRP4, and possibly other unknown arsenic renal transporters, in the urinary elimination of arsenic.

AQP7 and GLUT5 are also present at the apical surface of renal proximal tubule cells (described in Section 2.3.1) and could be responsible for the movement of select arsenic species from the proximal tubule into urine. As described in Section 2.3.1 there are no other known candidate arsenic transporters present in the brush border (apical surface) of the renal proximal tubule cell.

3. Future perspectives and conclusions

3.1. *Studies of arsenic cellular transport using in vitro systems*

As described in Sections 1.4 and 1.5, multiple arsenic cellular uptake and export pathways have been reported (e.g., AQPs as channels for neutral arsenic species, Na^+/P_i transporters for As^{V} uptake, and MRPs for GSH conjugate and DMA^{V} export). Characterization has been completed for individual transporters overexpressed in different cell systems (including yeast, *Xenopus laevis* oocytes, and mammalian cell lines) or through knockdown of endogenous levels in mammalian cell lines. These experimental systems have allowed the identification of transporters capable of mediating the cellular uptake and efflux of certain arsenic species. These studies are extremely valuable because they are the starting points for further work to determine the importance of these transport pathways in arsenic absorption, distribution, and elimination. Once important players are

identified, work can begin to understand how genetic variation in transport pathways might contribute to arsenic-induced carcinogenesis and other diseases.

Experimental results from different labs have not always provided consistent evidence for each transport pathway (e.g., in *Xenopus laevis* hAQP3 and hAQP10 were excluded as As^{III} cellular permeation pathways (Liu et al., 2004b), while studies in mammalian cell lines have shown these two channels are capable of increasing As^{III} cellular accumulation (Calatayud et al., 2012a; Lee et al., 2006) (discussed in Section 1.4.2)). The reason for these differences are currently unknown, but could be related to the different expression systems. Understanding the differences is important for not only confirming the identification of specific membrane proteins that are arsenic transporters, but also could provide clues for understanding how these pathways function.

Research efforts utilizing heterologous expression systems should also focus more on understanding the physiological relevance of arsenic transport by a specific transport pathway. For example, the characterization of As^{V} transport by Na^+/P_i transporters has been done very thoroughly, and conclusions drawn about the importance of these transporters have been made while considering the influence of the high physiological concentrations of competing P_i (Villa-Bellosta and Sorribas, 2008, 2010). For other transporters it is critical to do the same. When possible, kinetic parameters should be determined to evaluate if blood and tissue arsenic levels are relevant to K_m values. Inhibitory constants should also be determined in the presence of physiological substrates for the transport pathway of interest. If arsenic is not transported in the presence of relevant concentrations of physiological substrates, then research efforts should be re-prioritized to other pathways.

Lastly, while cell models have allowed the identification of transport pathways for iAs and most of its methylated metabolites, more research is needed to understand how other metabolites pass across cell membranes (e.g., thiolated and thiomethylated arsenic species). The rate of cellular accumulation of thiomethylated and other arsenic species has been shown to be directly proportional to the level of toxicity, suggesting that uptake pathways could be critical (Naranmandura et al., 2011).

3.2. Transport of arsenic in a physiological context

While the study of individual transport pathways has been valuable, more research is needed to understand how arsenic transporters work in a physiological context to allow the passage of arsenic across epithelial layers for absorption, distribution, and ultimately elimination. When the tissue distribution, cellular localization (apical vs basolateral), and substrate specificity of known arsenic transporters are considered it is evident that unidentified arsenic transport pathways are possibly involved (Figs. 2-4 and Tables 2–4). For example, at the basolateral surface of the hepatocyte MRP4 potentially exports $MMA(GS)_2$ and DMA^V into blood, while AQP9 allows the permeation of free neutrally charged arsenic species (e.g., As^{III} and MMA^{III}). However, it is likely that trivalent arsenic species are not freely available for export from cells, but thiol bound (either present in cells as GSH conjugates or protein thiols). Therefore, how $As(GS)_3$ is exported across the basolateral surface of the hepatocyte is important to understand. While the knowledge regarding the handling of arsenic by the enterocyte (Section 2.1) and hepatocyte (Section 2.2) are incomplete, the mechanisms of arsenic renal handling (Section 2.3) are understudied and poorly understood. Due to the importance of the kidney in eliminating 60–80% of arsenic, it is critical that renal handling of arsenic is investigated further.

In addition to the identification and characterization of arsenic transporters in tissues important for arsenic absorption, distribution, metabolism, and elimination more work is needed to understand the involvement of transporters in additional tissues with barrier functions (e.g., placenta, blood testes barrier, blood brain barrier). Furthermore, transport pathways in tissues that are targets of arsenic-induced carcinogenesis (lung, bladder, and skin) could function to protect tissue from arsenic accumulation or be harmful by allowing its entry. Genetic variability in arsenic transport pathways in any of the above tissues could be critical for predicting an individual's susceptibility to tumors and deserve further attention.

3.3. Conclusions

Cellular transport pathways potentially have an important influence on regulating the cellular/tissue levels, epithelial passage, and ultimately elimination of arsenic. Thus, transport pathways are likely important regulators of the overall body burden of arsenic. In turn, arsenic body burden will influence arsenic-induced disease susceptibility. Genetic differences in transport pathways could contribute to inter-individual susceptibility to arsenic-induced disease and are highly deserving of more research. Ultimately, understanding arsenic transport pathways will contribute to the development of strategies to prevent and treat arsenic-induced toxicity and disease.

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