Transport and reduction processes controlling the toxicity of arsenate

Final report on research findings

Research Project: OTKA K 73228 (2008-2014), Principal Investigator: Zoltán Gregus

BACKGROUND. Arsenic has been known as a poison from antiquity. Its public health importance now lies in its chronic toxicity and carcinogenicity (Chan and Huff, 1997). Arsenic leads the CERCLA priority list published by the *Agency for Toxic Substances and Disease Registry* (http://www.atsdr.cdc.gov). The main source of environmental arsenic exposure is the contaminated drinking water, which endangers millions of human beings worldwide. In South-Eastern Hungary, well water often contains arsenic over the allowable level (Csanády et al., 1985; Börzsönyi et al., 1992). According to a survey, arsenic levels in drinking water and human urine were highest in Hungary (Csongrád and Békés counties) within Central Europe (Lindberg et al., 2006).

In oxygenated water arsenic is present mainly as arsenate (As^V), which is absorbed from the gut. After cellular uptake, As^V is biotransformed in sequential steps, the first being its reduction to arsenite (As^{III}). Reduction of As^V is significant for two reasons: (1) it produces As^{III} which is SH-reactive and much more toxic than As^V, and (2) it is a prerequisite for further biotransformation by methylations and reductions. The As^{III} formed is first methylated to monomethylarsenate ($MMAs^V$), then is reduced to monomethylarsenite ($MMAs^{III}$), which in turn is methylated to dimethylarsenate ($DMAs^V$), the major product of arsenic metabolism in urine. Yet another reduction may yield dimethylarsenite ($DMAs^{III}$).

Thus, the metabolic scheme is: $As^V \rightarrow As^{III} \rightarrow MMAs^V \rightarrow MMAs^{III} \rightarrow DMAs^V \rightarrow DMAs^{III}$ (Thomas et al., 2001). It is important to note that the trivalent arsenicals (e.g., As^{III}, $MMAs^{III}$ and $DMAs^{III}$), due to their SH-reactivity, are much more toxic than the pentavalent ones (e.g., As^V, $MMAs^V$ and $DMAs^V$). The trivalent arsenic metabolites play significant role in the toxic and carcinogenic effects (in skin, bladder, kidney, and lung) of inorganic arsenic; therefore it is of importance to fully understand the mechanisms of their formation and disposition in the body, as well as factors influencing these processes.

PREVIOUS STUDIES. The present plans are based on our previous findings, which are summarized as follows.

We demonstrated long ago that rats given As^V or As^{III} rapidly excrete arsenic in bile (Gyurasics et al., 1991), provided hepatic glutathione (GSH) is available (Gyurasics et al., 1991; 1992). We hypothesized that arsenic is transported in bile in trivalent form, as unstable GSH-conjugates, which hydrolyze readily. Then we confirmed by HPLC-HG-AFS analysis that rats excrete arsenic in bile only in trivalent forms, as As^{III} and $MMAs^{III}$ (Gregus et al., 2000). We verified formation of $MMAs^{III}$ in vivo for the first time, and also demonstrated that this supertoxic metabolite appears in the bile of other species as well (Csanaky and Gregus, 2002).

Because in the toxicity of As^V its cellular uptake and intracellular reduction to As^{III} is of key importance, we have studied the influencing factors and mechanisms of these processes. We have shown that foscarnet, an antiviral drug using the Na-phosphate cotransporter, diminished formation of toxic metabolites from As^V because it inhibited the hepatic uptake and renal reabsorption of As^V, thereby accelerating its urinary excretion (Csanaky and Gregus, 2001). We have also verified that reduction of As^V in vivo requires GSH (Csanaky and Gregus, 2005). On studying the mechanism of As^V reduction, we have found that hepatic mitochondria (Németi and Gregus, 2002) as well as cytosol (Németi and Gregus, 2002, 2005) can reduce As^V. Although the enzymes of mitochondrial As^V reduction were then unknown, we have

identified three cytosolic enzymes that can reduce As^V to As^{III}. These are purine nucleoside phosphorylase (PNP; Gregus and Németi, 2002), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Gregus and Németi, 2005), and glycogen phosphorylase-a (GPa, Németi and Gregus, 2007, Gregus and Németi, 2007). Using enzyme inhibitors, we presented circumstantial evidence that GAPDH contributes to in vivo As^V reduction (Németi et al., 2006), whereas we have failed to obtain such evidence for PNP (Németi et al., 2003).

RESULTS OF THE PRESENT PROJECT. Our research was carried out on two specific topics**: 1.** We studied the reduction of arsenate to arsenite with three objectives: (a) to identify enzymes that can mediate the reduction of arsenate to arsenite, (b) to clarify the mechanism whereby these enzymes can promote reduction of arsenate to arsenite, and (c) to clarify the mechanism whereby mitochondria can reduce arsenate to arsenite **2.** We studied the reduction of the dimethylarsinic acid ($DMAs^V$) to dimethylarsinous acid ($DMAs^{III}$) in rats in vivo and in rat liver cytosol in vitro with the ultimate goal to identify the enzyme(s) that are involved in this reduction. In these studies, arsenicals were analyzed with HPLC - hydride generation - atomic fluorescence spectrometry (HPLC-HG-AFS).

<u>Ad 1.a.:</u> Apparently all phosphorolytic-arsenolytic enzymes can mediate the reduction of arsenate to arsenite.

Since three cytosolic phosphorolytic-arsenolytic enzymes, i.e., mammalian purine nucleoside phosphorylase (PNP), glycogen phosphorylase-a (GPa) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) could mediate reduction of arsenate (As^V) to the more toxic arsenite (As^{III}) in a thiol-dependent manner; we tested whether other phosphorolytic-arsenolytic enzymes can also facilitate As^V reduction.

Ornithine carbamoyl transferase (OCT) catalyzes phosphorolytic or arsenolytic citrulline cleavage within hepatic mitochondria. We found that glutathione-supplemented permeabilized or solubilized rat liver mitochondria could indeed reduce As^V (Németi and Gregus, *Toxicol. Appl. Pharmacol*. 239: 154-161, 2009). Citrulline (substrate for OCT-catalyzed arsenolysis) increased As^V reduction. The citrulline-stimulated As^V reduction was abolished by ornithine (OCT substrate inhibiting citrulline cleavage), phosphate (OCT substrate competing with As^V), and the OCT inhibitor norvaline or PALO, indicating that As^V reduction is coupled to OCT-catalyzed arsenolysis of citrulline. Corroborating this conclusion, purified bacterial OCT mediated As^V reduction in presence of citrulline and glutathione with similar responsiveness to these agents. In contrast, As^{III} formation by intact mitochondria was unaffected by PALO and slightly stimulated by citrulline, ornithine, and norvaline, suggesting minimal role for OCT in As^V reduction in intact mitochondria. Collectively, mitochondrial and bacterial OCT can promote glutathione-dependent As^V reduction with coupled arsenolysis of citrulline, supporting the hypothesis that As^V reduction is mediated by phosphorolytic-arsenolytic enzymes (Németi and Gregus, *Toxicol. Appl. Pharmacol*. 239: 154-161, 2009). Nevertheless, because citrulline cleavage is disfavored physiologically, OCT may have little role in As^V reduction in vivo.

In a subsequent work, we tested further enzymes, namely the bacterial **phosphotransacetylase (PTA)** and the **bacterial PNP**, for As^V reduction. PTA, which arsenolytically cleaves acetyl-CoA producing acetyl-arsenate, was compared to **GAPDH**, which can also form acetyl-arsenate by arsenolysis of its non-physiological substrate, acetyl-phosphate. As these enzymes also mediated As^V reduction in presence of their phosphorolytic-arsenolytic substrates and a thiol compound, we could assert that facilitation

of thiol-dependent As^V reduction is a general property of enzymes that catalyze phosphorolytic-arsenolytic reactions (Németi and Gregus, *Toxicol. Sci.* 110: 270-281, 2009).

Glutathione synthetase (GS) is another phosphorolytic/arsenolytic enzyme. GS can catalyze the arsenolysis of GSH (γ-Glu-Cys-Gly) yielding two arsenylated products, i.e. γ-Glu-Cys-arsenate and ADP-arsenate. Thus, GS may also promote the reduction of As^V by GSH. This hypothesis was tested with human recombinant GS, a Mg^{2+} dependent enzyme. GS markedly increased As^{III} formation when incubated with As^V, GSH, Mg^{2+} and ADP, but not when GSH, Mg^{2+} or ADP was separately omitted. Phosphate, a substrate competitive with As^V in the arsenolysis of GSH, as well as the products of GSH arsenolysis or their analogs, e.g. glycine and γ-Glu-aminobutyrate, decreased As^V reduction. Replacement of ADP with ATP or an analog that cannot be phosphorylated or arsenylated abolished As^V reduction, indicating that GS-supported As^V reduction requires formation of ADP-arsenate. In the presence of ADP, however, ATP (but not its metabolically inert analog) tripled As^V reduction because ATP permits GS to remove the arsenolysis inhibitory glycine and γ-Glu-Cys by converting them into GSH. GS failed to promote As^V reduction when GSH was replaced with ophthalmic acid, a GSH analog substrate of GS containing no SH group (although ophthalmic acid did undergo GS-catalyzed arsenolysis), indicating that the SH group of GSH is important for As^V reduction. Our findings support the conclusion that GS promotes reduction of As^V by catalyzing the arsenolysis of GSH, thus producing ADP-arsenate, which upon being released from the enzyme is readily reduced by GSH to As^{III}. Thus GS is yet another enzyme that may contribute to reduction of As^V into the more toxic As^{III} (Németi et al., *Biochimie* 94: 1327-1333, 2012).

<u>Ad 1.b.:</u> Arsenolytic enzymes promote the reduction of As^V by forming arsenylated metabolites which are more reducible to As^{III} by thiols than inorganic As^V.

Because with all phosphorolytic enzymes tested arsenolysis is obligatory for As^V reduction, we analyzed the **relationship between arsenolysis and As^V reduction** in presence of various thiol compounds, using PNP. While no thiol influenced the rate of PNP-catalyzed arsenolysis, all enhanced the PNP-mediated As^V reduction, albeit differentially (Németi and Gregus, *Toxicol. Sci.* 110: 270-281, 2009). Furthermore, the relative capacity of thiols to support As^V reduction mediated by PNP, GPa, PTA, and GAPDH apparently depended on the type of arsenylated metabolites (i.e. arsenate ester or anhydride) produced by these enzymes. Importantly, As^V reduction by both acetyl-arsenate-producing enzymes (i.e. PTA and GAPDH) exhibited striking similarities in responsiveness to various thiols, thus highlighting the role of arsenylated metabolite formation. This observation, together with the finding that PNP-mediated As^V reduction lags behind the PNP-catalyzed arsenolysis, endorsed the following hypothesis: arsenolytic enzymes promote reduction of As^V by forming arsenylated metabolites which are more reducible to As^{III} by thiols than inorganic As^V (Németi and Gregus, *Toxicol. Sci.* 110: 270-281, 2009).

The above-mentioned hypothesis was evaluated subsequently, using a multidisciplinary approach. It is known that PNP cleaves inosine with As^V into hypoxanthine and ribose-1-arsenate. In presence of inosine, As^V and DTT, PNP mediates As^{III} formation. In this study, we incubated PNP first with inosine and As^V, allowing the arsenolytic reaction to proceed, then blocked this reaction with the PNP inhibitor BCX-1777, added DTT and continued the incubation. Despite inhibition of PNP, large amount of As^{III} was formed in these incubations, indicating that **PNP does not reduce As^V directly but forms a product (i.e. ribose-1-arsenate) that is reduced to As^{III} by DTT** (Gregus et al., *Toxicol. Sci.* 110: 282-292, 2009). Similar studies with the other arsenolytic enzymes (GPa, GAPDH, and PTA)

yielded similar results. Various thiols that differentially supported As^V reduction when present during PNP-catalyzed arsenolysis (DTT ~ DMPS > mercaptoethanol > DMSA > GSH) similarly supported As^V reduction when added only after a transient PNP-catalyzed arsenolysis, which preformed ribose-1-arsenate. Experiments with progressively delayed addition of DTT after BCX-1777 indicated that ribose-1-arsenate is short-lived with a half-life of 4 min. In conclusion, phosphorolytic enzymes, such as PNP, GAPDH, GPa, and PTA, promote thiol-dependent As^V reduction because they convert As^V into arsenylated products that are reducible by thiols more readily than As^V in its inorganic form. In support of this view, reactivity studies using conceptual density functional theory reactivity descriptors (local softness, nucleofugality) indicated that reduction by thiols of the arsenylated metabolites was favored over As^V (Gregus et al., *Toxicol. Sci.* 110: 282-292, 2009).

<u>Ad 1.c.:</u> Mitochondria reduce arsenate to arsenite by forming ADP-arsenate which is more reducible to As^{III} by glutathione than inorganic As^V.

The recognition that phosphorolytic enzymes promote reduction of As^V because they convert As^V into arsenylated products (i.e., arsenate esters or arsenate anhydrides) in which the pentavalent arsenic is more reducible by thiols, such as glutathione (GSH), than the pentavalent arsenic in inorganic As^V induced us to return to our early observation on reduction of As^V by isolated mitochondria with the aim of clarifying the underlying mechanism. Several years earlier we demonstrated that rat liver mitochondria can rapidly reduce As^V in a process requiring intact oxidative phosphorylation and intramitochondrial GSH (Németi and Gregus, Mitochondria work as reactors in reducing arsenate to arsenite. *Toxicol. Appl. Pharmacol.* 182: 208-218, 2002). Thus, these organelles might reduce As^V because mitochondrial ATP synthase, using As^V instead of phosphate, arsenylates ADP to ADP-arsenate, which in turn is readily reduced by GSH.

To test this hypothesis we first examined whether the RNA-cleaving enzyme polynucleotide phosphorylase (PNPase), which can split poly-adenylate (poly-A) by arsenolysis into units of AMP-arsenate (a homologue of ADP-arsenate), could also promote reduction of As^V to As^{III} in presence of thiols (Németi et al., *Toxicol. Sci.* 117: 270-281, 2010). Indeed, bacterial PNPase markedly facilitated formation of As^{III} when incubated with poly-A, As^V and GSH. PNPase-mediated As^V reduction depended on arsenolysis of poly-A and presence of a thiol. PNPase can also form AMP-arsenate from ADP and As^V (termed arsenolysis of ADP). In presence of GSH, this reaction also facilitated As^V reduction in proportion to AMP-arsenate production. While various thiols did not influence the arsenolytic yield of AMP-arsenate, they differentially promoted the PNPase-mediated reduction of As^V, with GSH being the most effective. Circumstantial evidence indicated that AMP-arsenate formed by PNPase is more reducible to As^{III} by GSH than inorganic As^V (Németi et al., *Toxicol. Sci.* 117: 270-281, 2010).

Then we demonstrated that As^V reduction by isolated mitochondria was markedly inhibited by an ADP analogue that enters mitochondria but is not phosphorylated or arsenylated (Németi et al., *Toxicol. Sci.* 117: 270-281, 2010). Furthermore, inhibitors of the export of ATP or ADP-arsenate from the mitochondria diminished the increment in As^V reduction caused by adding GSH externally to these organelles whose intramitochodrial GSH had been depleted. Thus, while PNPase promotes reduction of As^V by incorporating it into AMP-arsenate, the mitochondrial ATP synthase facilitates As^V reduction by forming ADP-arsenate; then GSH can easily reduce these arsenylated nucleotides to As^{III} (Németi et al., *Toxicol. Sci.* 117: 270-281, 2010).

The work above was published in *Toxicological Sciences* and was selected by the Editor-in-Chief as **"Toxicological Highlight"** in that specific issue of the journal. The issue

also published a short communication in order to introduce our work (Thomas, D.J.: Arsenolysis and thiol-dependent arsenate reduction. *Toxicol. Sci.* 117: 249-252, 2010).

Our studies on the role of PNPase in thiol-mediated As^{V} reduction also lead to a spin-off discovery on the catalytic mechanism of PNPase. This enzyme also catalyzes the apparent arsenolysis of ADP to AMP-arsenate and inorganic phosphate, with the former hydrolyzing rapidly into AMP and As^{V}. However, in presence of glutathione, AMP-arsenate may also undergo reductive decomposition, yielding AMP and As^{III}. Further experimentation allowed us to determine the mechanisms of **PNPase-catalyzed arsenolysis of ADP**. For this purpose, we characterized ADP arsenolysis mediated by PNPase with respect to its time course in presence of increasing concentrations of ADP with or without polyadenylate (poly-A) supplementation. These studies revealed that increasing supply of ADP enhanced the consumption of ADP but inhibited the production of both AMP and As^{III}. Formation of these products was amplified by adding trace amount of poly-A. Furthermore, AMP and As^{III} production accelerated with time, whereas ADP consumption slowed. These observations collectively suggest that PNPase does not catalyze the arsenolysis of ADP directly but in two separate steps: the enzyme first converts ADP into poly-A then it cleaves the newly synthesized poly-A by arsenolysis. It is inferred that one active site of PNPase can catalyze only one of these reactions at a time and that high ADP concentrations favor poly-A synthesis, thereby inhibiting the arsenolysis (Németi and Gregus, *Biochimie* 93: 624-627, 2011).

<u>Ad 2.a.:</u> Rats reduce dimethylarsinic acid ($DMAs^{V}$) to dimethylarsinous acid ($DMAs^{III}$) in a glutathione dependent manner – a novel method to follow $DMAs^{V}$ reduction in rats in vivo.

$DMAs^{V}$ is the major urinary metabolite of inorganic arsenic in humans and most experimental animals. Reduction of $DMAs^{V}$ to $DMAs^{III}$ is a toxification reaction because the trivalent As-containing $DMAs^{III}$ is 100 to 10 000 times more cytotoxic than the pentavalent As-containing $DMAs^{V}$.

We studied the reduction of $DMAs^{V}$ to $DMAs^{III}$ in vivo in anesthetized rats, using a novel method to measure $DMAs^{V}$ reduction in rats. This was based on the unique property of rat hemoglobin: $DMAs^{III}$ is known to bind very strongly to rat hemoglobin (by reacting covalently with a reactive Cys in hemoglobin) and thus it accumulates in red blood cells. These experiments were performed in anesthetized rats which were injected i.v. with $DMAs^{V}$. Reduction of $DMAs^{V}$ to $DMAs^{III}$ was followed by measuring erythrocyte-bound DMAs in serially collected blood samples. We have shown that reduction of $DMAs^{V}$ in vivo is also GSH dependent, as depletion of hepatic GSH in rats by buthionine sulfoximine or phorone markedly diminished DMAs accumulation in the red blood cells (assumed to be $DMAs^{III}$) and diminished the disappearance of red blood cell-unbound DMAs, which is purportedly largely $DMAs^{V}$ (Németi and Gregus, *Chem. Res. Toxicol.* 26: 432-443, 2013).

<u>Ad 2.b.:</u> Rat liver cytosol reduces dimethylarsinic acid ($DMAs^{V}$) to dimethylarsinous acid ($DMAs^{III}$) in a glutathione dependent manner – characterization of the enzymatic reaction.

Our studies on rat liver cytosol indicated that cytosolic enzyme(s) can catalyze reduction of $DMAs^{V}$ to $DMAs^{III}$ in a GSH dependent manner. The enzyme(s) involved are sensitive to thiol reagents, disulfide compounds, and several other inhibitors, including aurothioglucose and trivalent antimony, which are inhibitors of thioredoxin reductase (TRR). However,

purified TRR when supplemented with NADPH and GSH failed to catalyze reduction of $DMAs^V$. Other investigators have demonstrated that GSH-transferase-omega (GSTO1) can reduce $DMAs^V$; however, our experiments, using centrifugal ultrafiltration of cytosol, did not support the role of GSTO1 in the reduction of $DMAs^V$ by rat liver cytosol (Németi and Gregus, *Chem. Res. Toxicol.* 26: 432-443, 2013).

In the last part of this grant period we focused on further characterization of the reduction of $DMAs^V$ to $DMAs^{III}$ with the aim of identification of the enzyme(s) involved These studies have yielded preliminary results which were demonstrated on the 53rd Annual Meeting of the Society of Toxicology but not yet published. For example, in order to further analyze the role of TRR, we tested the effects of an Nrf2 activator (an oleanolic acid derivative called CDDO) in rats on $DMAs^V$ reduction. As expected, CDDO increased cytosolic TRR activity in the liver markedly, yet $DMAs^{III}$ formation was only 30% higher in the cytosol of CDDO-treated rats as compared to controls. However, when the incubations were supplemented with NADPH or the disulfide-reducing TCEP, the difference disappeared. Treatment of rats with CDDO did not alter the time course of the accumulation of $DMAs^{III}$ in the blood of rats. In conclusion, the contribution of TRR to $DMAs^V$ reduction appears indirect. The activity of TRR may be indispensable for the reduction of $DMAs^V$; however, at the quantity normally present in rat liver, TRR does not limit the rate of $DMAs^V$ conversion to the highly toxic $DMAs^{III}$.

Because our studies on reduction of $DMAs^V$ to $DMAs^{III}$ in rat liver cytosols yielded mixed findings, some compatible with involvement of GSTO1 others not (Németi and Gregus, *Chem. Res. Toxicol.* 26: 432-443, 2013), we further investigated the role of this enzyme. For this purpose, we developed an improved GSTO1 assay that allows quantification of GSTO1 activity in presence of GSH as a supporting substrate and which permits comparison of the cytosolic GSTO1 activity with the $DMAs^V$ reducing activity under identical circumstances in response to various conditions. Some of our findings indicate similar responsiveness of the GSTO1 activity to that of the $DMAs^V$ reducing activity, whereas others attest divergent characteristics: 1. Potent GSTO1 inhibitors inhibited both GSTO1 activity and $DMAs^V$ reduction by rat liver cytosol in the same concentration range. 2. Aurothioglucose and trivalent antimony weakly inhibited GSTO1 activity, but strongly inhibited $DMAs^V$ reduction by rat liver cytosol. 3. The $DMAs^V$ reducing activity of some cytosols were low, but were normalized by NADPH. In contrast, the GSTO1 activity of all cytosols was similar and was not affected by NADPH addition.

In other studies we analyze the role of glutathione (GSH) in the activity of the $DMAs^V$ reducing cytosolic enzyme in rat liver cytosol. These investigations seem to indicate that the cytosolic $DMAs^V$ reducing activity is best supported by GSH much less or not by other SH compounds. S-Alkyl-GSH compounds impair the $DMAs^V$ reducing activity in rat liver cytosol, suggesting that the $DMAs^V$reducing enzyme has a GSH binding site. This feature may facilitate isolation of this hitherto unknown enzyme and its positive identification.

We hope to continue this research and complete it in the framework of a grant application of Balázs Németi submitted to OTKA earlier this year.

CHANGES IN PARTICIPANTS. There were three changes in the research group that carried out the project: 1. *Iván Csanaky* did not return from his sabbatical at the University of Kansas Medical Center, Kansas City. His departure unfavorably affected our progress. This necessitated extension of the grant period. 2. *Balázs Németi* spent 10 months during the grant period in Karolinska Institute, Stockholm, and worked on topics unrelated to our OTKA-supported research. 3. From 2013 September, *Miklós Poór*, a PhD student, joined our group.

RESEARCH ARTICLES

1. Németi, B., Gregus, Z.: Mechanism of thiol-supported arsenate reduction mediated by phosphorolytic-arsenolytic enzymes. I. The role of arsenolysis. *Toxicol. Sci.* 110: 270-281, 2009. (IF=4.814)
2. Gregus, Z., Roos, G., Geerlings, P., Németi, B.: Mechanism of thiol-supported arsenate reduction mediated by phosphorolytic-arsenolytic enzymes. II. Enzymatic formation of arsenylated products susceptible for reduction to arsenite by thiols. *Toxicol. Sci.* 110: 282-292, 2009. (IF=4.814)
3. Németi, B., Gregus, Z.: Glutathione-supported arsenate reduction coupled to arsenolysis catalyzed by ornithine carbamoyl transferase. *Toxicol. Appl. Pharmacol*. 239: 154-161, 2009. (IF=3.359)
4. Németi, B., Regonesi, M.E., Tortora, P., Gregus, Z.: Polynucleotide phosphorylase and mitochondrial ATP synthase mediate reduction of arsenate to the more toxic arsenite by forming arsenylated analogues of ADP and ATP. *Toxicol. Sci.* 117: 270-281, 2010. (IF=5.093)
5. Styblo, M., Gregus, Z., Devesa, V., Velez, D.: Preface: 2nd International Congress, As 2008: arsenic from nature to humans (Valencia, Spain, May 21-23). *Environ. Res.* 110: 411-412, 2010. (IF=3.500)
6. Németi, B., Regonesi, M.E., Tortora, P., Gregus, Z.: The mechanism of the polynucleotide phosphorylase-catalyzed arsenolysis of ADP. *Biochimie* 93: 624-627, 2011. (IF=3.022)
7. Németi, B., Anderson, M.E., Gregus, Z.: Glutathione synthetase promotes the reduction of arsenate via arsenolysis of glutathione. *Biochimie* 94: 1327-1333, 2012. (IF=3.142)
8. Németi, B., Gregus, Z.: Reduction of dimethylarsinic acid to the highly toxic dimethylarsinous acid by rats and rat liver cytosol. *Chem. Res. Toxicol.* 26: 432-443, 2013. (IF=3.667)

Remark: Articles referenced under Background and Previous Studies are not listed here. These can be found in PubMed data base.

BOOK CHAPTERS

1. Gregus, Z.: Általános méregtan. *In*: Gyires Klára és Fürst Zsuzsanna (szerk.): *Farmakológia és farmakoterápia*, Medicina, Budapest, 987-1003, 2007. *In*: Gyires Klára és Fürst Zsuzsanna (szerk.): *A farmakológia alapjai*, Medicina, Budapest, 997-1013, 2011.
2. Gregus, Z.: Fémek okozta mérgezések. *In*: Gyires Klára és Fürst Zsuzsanna (szerk.): *Farmakológia és farmakoterápia*, Medicina, Budapest, 1004-1020, 2007. *In*: Gyires Klára és Fürst Zsuzsanna (szerk.): *A farmakológia alapjai*, Medicina, Budapest, 1014-1030, 2011.
3. Gregus, Z.: Mechanisms of toxicity. *In:* Klaassen, C.D. (ed.): *Casarett and Doull's Toxicology. The Basic Science of Poisons*. Eighth Edition. McGraw-Hill, Inc., New York, NY. pp. 49-122, 2013.

ORAL PRESENTATIONS AND POSTERS

From the topic of the OTKA-supported research, 5 presentations were given on scietific conferences in Hungary and 7 presentations were given on the annual meetings of the Society of Toxicology in the USA during the grant period.