APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 9, 531–540 (1995)

Differential Effects of Various Trivalent and Pentavalent Organic and Inorganic Arsenic Species on Glucose Metabolism in Isolated Kidney Cells

B. Liebl,* H. Mückter, Ph.-T. Nguyen, E. Doklea, S. Islambouli, B. Fichtl and W. Forth

Walther-Straub-Institut für Pharmakologie and Toxikologie der Universität München, Nussbaumstrasse 26, D-80336 München, Germany

We have compared the acute toxicities of the trivalent arsenic species arsenite, oxophenylarsine (PhAsO), 2-chlorovinyloxoarsine (ClvinAsO), methyloxoarsine (MeAsO), and of the pentavalent arsenic species arsenate, methyl- and phenylarsonic acid in rat kidney tubules (RKT) and Madin–Darby canine kidney (MDCK) cells. In RKT, PhAsO (1 μmol l^{-1}, 60 min) almost completely (>90%) blocked gluconeogenesis without affecting cell viability as assessed by dye exclusion. In MDCK cells, PhAsO (2 μmol l^{-1}) markedly inhibited glucose uptake (60% of controls) within 30 min, while cell viability, as assessed by formazan formation, was not affected within 180 min. MeAsO and ClvinAsO were similarly effective to PhAsO in both RKT and MDCK cells. Estimated IC_{50} values for the inhibition of gluconeogenesis were 0.55 (PhAsO), 0.69 (ClvinAsO) and 0.99 μmol l^{-1} (MeAsO) and for the inhibition of glucose uptake 1.23 (PhAsO), 2.62 (ClvinAsO) and 6.99 μmol l^{-1} (MeAsO). At longer storage times, aqueous solutions of MeAsO and of ClvinAsO, but not of PhAsO, gradually lost toxic activity in RKT and MDCK cells, especially at alkaline pH. Concomitantly, a gradual decrease in content as assessed by HPLC was detected.

Roughly 10-fold higher concentrations of arsenite than of PhAsO were required for comparable effects on gluconeogenesis in RKT, whereas in MDCK cells about 100-fold higher concentrations were needed for similar inhibition of glucose uptake. Pentavalent arsenate and phenylarsonate were two orders of magnitude less effective than PhAsO in RKT, while methylarsonate had virtually no influence on gluconeogenic activity. In MDCK cells the pentavalent arsenic species showed effects only in the millimolar range.

It is concluded (1) that different mechanisms are involved in the acute toxicity of oxoarsines and inorganic arsenic and (2) that PhAsO offers advantages as a model substance for monosubstituted trivalent arsenicals, because it is more stable and more readily detectable.

Keywords: arsenicals; rat kidney tubules; MDCK cells; cytotoxicity; glucose metabolism

INTRODUCTION

From both a biological and a toxicological point of view it is important to classify arsenic compounds by their state of oxidation and to differentiate between organic and inorganic substances (Fig. 1). Among the trivalent compounds inorganic arsenite has a long history as a drug and as a poison.[1] 2-Chlorovinyloxoarsine (ClvinAsO) is an organic trivalent arsenical species, the hydrated form of which is believed to be responsible for the systemic toxicity of organochloroarsenicals such as 2-chlorovinyldichloroarsine ($ClvinAsCl_2$).[2–4] Oxophenylarsine (PhAsO) represents the prototype of a series of compounds which have been of pharmacological interest because of their antimicrobial activity.[5,6] Furthermore, PhAsO has widely been used as a biochemical tool to block func-

* Author to whom correspondence should be addressed.

Abbreviations used: $ClvinAsCl_2$, 2-chlorovinyldichloroarsine; ClvinAsO, 2-chlorovinyloxoarsine; DMEM, Dulbecco's modified Eagle's medium; ECD, electron capture detector; GLUT, glucose transporter; HBSS, Hanks' balanced salt solution; KHB, Krebs–Henseleit buffer; MDCK, Madin–Darby canine kidney; MeAsO, methyloxoarsine; MMAA, monomethylarsonic acid; PDH, pyruvate dehydrogenase; PhAsO, oxophenylarsine; RKT, rat kidney tubules; SD, standard deviation; XTT, sodium 3′-[1-(phenylaminocarbonyl)-3,4-tetrazolium]bis(4-methoxy-6-nitro)benzenesulphonic acid.

CCC 0268–2605/95/070531–10

Received 9 September 1994
Accepted 20 February 1995

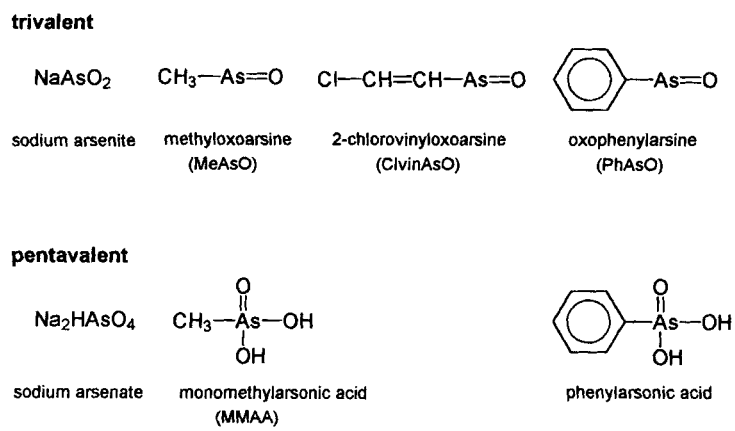

Figure 1 Chemical structures and abbreviations of the arsenic species tested.

tional SH groups.[7–10] Little is known about the biochemistry of methyloxoarsine (MeAsO), the simplest trivalent mono-substituted organoarsenic species, which is difficult to synthesize in a chemically pure form.[11] Formally, MeAsO represents the reduced form of monomethylarsonic acid (MMAA), which is a major metabolite of inorganic arsenic in man.[12,13]

The toxicity of trivalent arsenicals is thought to be due to their binding to thiol groups of biologically active proteins. Acute toxicity has mainly been attributed to inhibition of metabolic enzymes, especially of the pyruvate dehydrogenase (PDH) complex (Fig. 2), leading to a serious disturbance of cellular carbohydrate/energy metbolism.[14,15] However, interaction with the cell membrane resulting in an impairment of glucose uptake might contribute as well.[16]

Arsenate, phenylarsonic acid and MMAA are the pentavalent analogues of arsenite, PhAsO and MeAsO (Fig. 1). Inorganic arsenate has been employed as a pesticide and wood preservative.[17] Phenylarsonic acid is used as a therapeutic agent in veterinary medicine.[18] MMAA is one of the main metabolites of inorganic arsenic.[19] Due to their high water solubility, these compounds are readily excreted via the kidneys.[20] Pentavalent arsenic species are generally regarded as less toxic, but partial reduction to the trivalent oxidation state might contribute to toxicity and has been demonstrated *in vitro*[21,22] and *in vivo*.[23–25]

While the individual toxicities of some of the above-mentioned inorganic and organic trivalent and pentavalent arsenic species have been investigated *in vitro* and *in vivo*, comparative studies of several compounds are scarce. We have studied the acute toxicity of various arsenic oxides in rat kidney tubules (RKT) and Madin–Darby canine kidney (MDCK) cells. The kidney represents a highly metabolizing organ which plays a key role in the elimination of arsenic.[2,26] The usefulness of RKT to detect the metabolic toxicity of arsenic species has been demonstrated previously.[15,27] MDCK cells exhibit a stereospecific uptake mechanism for glucose which is inhibited by arsenicals in a time- and concentration-dependent

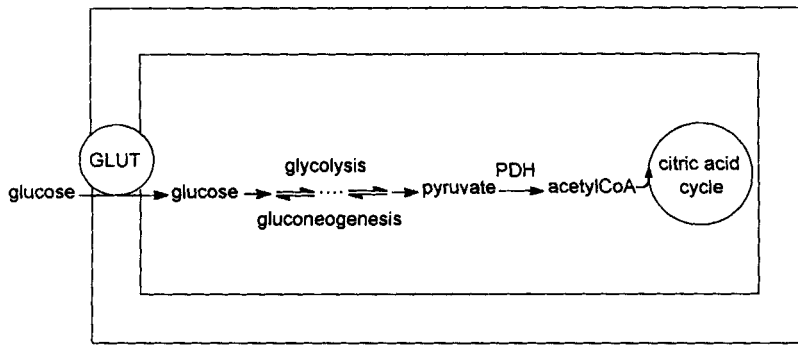

Figure 2 Cellular glucose metabolism. Trivalent arsenic species inhibit PDH, leading to a drop in acetylCoA formation and a slow-down of the citric acid cycle. Inhibition of glucose transporters (GLUT) may contribute to disturbed glucose metabolism.

manner. Studies with 2-deoxy-D-glucose indicated an interaction closely related to the process of glucose transport across the plasma membrane rather than an indirect effect due to disturbed energy metabolism.[16]

MATERIALS AND METHODS

Chemicals

Suppliers of arsenic compounds were as follows: Aldrich, Steinheim, Germany (PhAsO); Merck, Darmstadt, Germany (sodium arsenite, sodium arsenate, phenylarsonic acid); Prins-Maurits-Laboratory, TNO, Rijswijk, The Netherlands (ClvinAsO). D-[6-^{14}C]Glucose was obtained from Du Pont–NEN, Bad Homburg, Germany. All other chemicals were from various suppliers and were of the highest purity available. Silica 60 HPTLC plates were from Merck, Darmstadt, Germany.

Preparation of monomethylarsonic acid

MMAA was prepared according to the method of Favrel,[28] with modifications: 197.84 g (1 mol) As_2O_3 was added to a solution of 240 g (6 mol) NaOH in 500 ml H_2O. Methyl iodide (283.88 g in 100 ml MeOH, 2 mol) was added dropwise during 30 min. This mixture was stirred for 40 h. The resulting precipitate was dissolved in 600 ml of boiling H_2O. After cooling, 3 l ethanol (90%, v/v) was added. Crude MMAA sodium salt precipitated and the supernatant was discarded. The precipitate was dissolved in 1500 ml H_2O and 5 g $Ba(OH)_2$ was added. After standing overnight, the mixture was filtered to remove residual iodide. The filtrate was acidified (pH 2) using concentrated H_2SO_4. Following removal of $BaSO_4$, twice the volume of acetone was added. This mixture was refluxed (5 min), filtered and concentrated to 200 ml. The product partially precipitated from this solution in large crystals. The supernatant was condensed to dryness. Both fractions were recrystallized from MeOH and dried over P_2O_5.

Yield: 145.5 g (1.04 mmol), 52% of theory. Analytical data: m.p. 158 °C, lit.[11] 159.8 °C. ^{1}H NMR (DMSO-d_6): 1.88 ppm (s), 10.3 ppm (s). ^{1}H NMR (D_2O): 1.88 ppm (s). IR (KBR) 3420 (m), 2935 (s), 2801 (s), 2362 (s), 1653 (w), 1298 (w), 1260 (w), 1209 (m), 942 (s), 892 (m), 782 (s), 642 cm^{-1} (m). HPTLC (silica 60; n-BuOH acetic acid H_2O, 4:1:1): R_f 0.35 = 0.02.

Preparation of methyloxoarsine

MeAsO was synthesized from di-iodomethylarsine which was prepared according to the method of Samaan,[11] with modifications. Briefly, 2.0 g (14.3 mmol) MMAA was dissolved in 50 ml glacial acetic acid. HI solution (24.4 ml of 56%; 110 mmol) was added. The resulting dark-violet mixture was refluxed for 15 min and was then allowed to stand for 4 h at 4 °C. The crude product was collected and crystallized from glacial acetic acid.

Yield: 3.48 g (10.1 mmol), 71% of theory. Analytical data: m.p. 30 °C, lit.[11] 30 °C. ^{1}H NMR ($CDCl_3$): 3.10 ppm. UV (cyclohexane): λ_{max} 209.8, 230.0, 274.0 nm.

MeAsO was prepared following the method of Baeyer:[29] 3.44 g (10 mmol) di-iodomethylarsine was dissolved in dried benzene. After addition of 50 g $NaHCO_3$ the yellow colour slowly disappeared. The benzene fraction was dried with K_2CO_3. The supernatant was concentrated to yield a colourless solid residue of MeAsO.

Yield: 1.00 g (9.4 mmol), 94% of theory. Analytical data: m.p. 95 °C, lit.[29] 95 °C. ^{1}H NMR (D_2O): 1.20 ppm. ^{13}C NMR (D_2O): 26.24 ppm.

Analysis of oxoarsines

Stock solutions of MeAsO, ClvinAsO and PhAsO were analysed by chromatographic separation (precolumn, ChromHypersil ODS 5μ, Shandon, Astmoor, UK; separation column μBondapak C18, Waters, Eschborn, Germany; pump, model 480, Gynkotek, Germering, Germany; eluent, 5 mmol l^{-1} tetrabutylammonium acetate/5% MeOH, pH 6; flux: 1.0 ml min^{-1}) and electrochemical detection (EP 30 carbon cell, oxidation potential 0.8 V, 10 nA, Biometra, Göttingen, Germany), or UV detection at 218 nm (model 1706, BioRad, Munich, Germany). Quantitation was achieved by comparing peak heights.

Stability of oxoarsines

In the course of the functional studies performed with RKT and MDCK cells we noticed that aqueous solutions of ClvinAsO and MeAsO, but

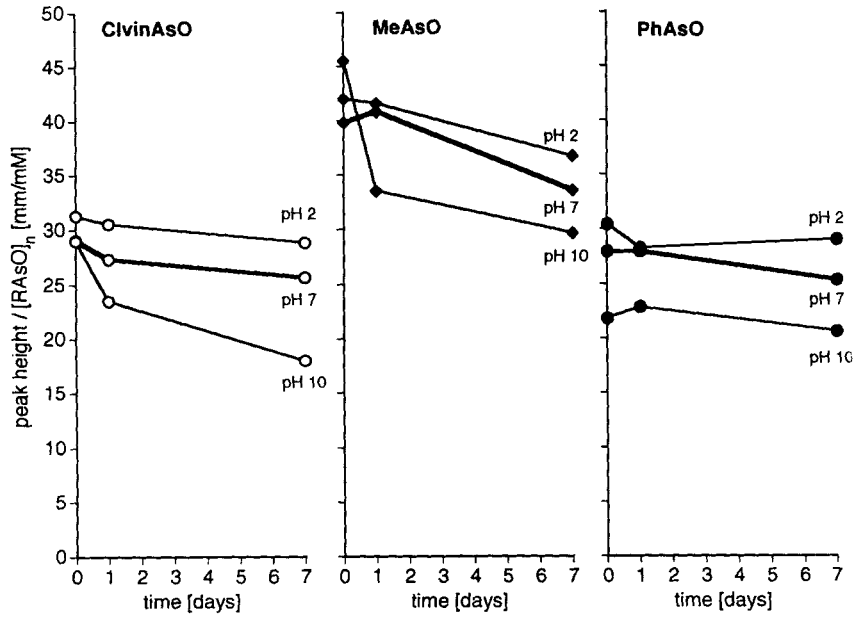

Figure 3 Change of ECD signal for the oxidation As(III)→As(V) depending on the storage time and the pH of aqueous stock solutions (5 mmol l^{-1}) of MeAsO, ClvinAsO and PhAsO.

not of PhAsO, gradually lost their toxic activity within days to weeks, emphasizing the need for freshly prepared solutions. Since this loss of effectiveness might be due to a decrease in content, we investigated the stability of stock solutions (5 mmol l^{-1}) at various pH values (pH 2, pH 7, pH 10) and for various storage times at room temperature. With PhAsO, quantitation in the micromolar range was possible by electron capture detection (ECD) as well as by UV absorption, whereas with MeAsO and ClvinAsO sufficient sensitivity could only be achieved by ECD under oxidizing conditions (potential + 0.8 V, current 10 nA). With PhAsO, virtually no changes of concentration were observed at all three pH values (Fig. 3). However, with MeAsO and ClvinAsO, especially at pH 10, a distinct decrease of content became evident within several days of observation (Fig. 3).

Cells

RKT were prepared from starved (48 h, tap-water *ad libitum*) male Sprague–Dawley rats (200–280 g; Interfauna, Tuttlingen, Germany) according to Guder *et al.*[30] with modifications described previously.[27] Briefly, kidneys were excised, perfused with glucose-free Krebs–Henseleit buffer (KHB)[31] and dissociated mechanically and enzymically (collagenase 10 mg g^{-1} wet weight). The resulting cell suspension was sedimented (1 min) to get rid of larger cell aggregates. The supernatant was centrifuged (1 min; 50 g). The pellet was washed with ice-cold KHB (10 ml g^{-1} kidney wet weight) and centrifuged again. The final pellet was resuspended in KHB (protein concentration *ca* 10 mg ml^{-1}).

MDCK cells were obtained from the American Type Culture Collection, Rockville, USA. Cells were grown in 50-ml flasks or in 96-well tissue culture plates in a moist atmosphere at 37 °C and 5% CO_2 with Dulbecco's modified Eagle's medium (DMEM/F12; Gibco, Eggenstein, Germany) containing 3.7 g l^{-1} D-glucose, 10% (v/v) fetal calf serum, 50 U ml^{-1} of penicillin and 50 µg ml^{-1} streptomycin. Experiments were performed on day 3 or 4 with confluent cultures. The medium was changed 12–15 h prior to experiments.

Gluconeogenesis studies

For each test, 8.8 ml ice-cold glucose-free KHB, 0.1 ml sodium pyruvate (1 mol l^{-1}) and 0.1 ml arsenical solution or buffer (control) were pipetted into a 250-ml Cautex flask. Ice-cold RKT suspension was added (1 ml; final protein content *ca* 1 mg ml^{-1}) and gluconeogenesis was started by placing the flasks in a water-bath shaker at 37 °C. Before closing the flasks, the mixtures were aer-

ated with carbogen (95% O_2, 5% CO_2) for 30 s, then 1-ml samples for glucose determination were withdrawn every 10 min during the following 60 min. The remainder was aerated each time before closing the flasks. At the beginning and at the end of incubation, cell viability was assessed by Trypan Blue exclusion.

Glucose determination

The 1-ml aliquots drawn for glucose determination were acidified with 0.1 ml ice-cold $HClO_4$ (3.3 mol l^{-1}) to stop glucose formation. The acid aliquots were neutralized with 0.2 ml $KHCO_3$ (2.2 mol l^{-1}) and centrifuged. The clear supernatant was assayed for glucose using the hexokinase/glucose-6-phosphate dehydrogenase reaction.[32]

Glucose uptake studies

Before each experiment cells were freed from medium, washed and pre-incubated (37 °C) with (Ca^{2+}-, Mg^{2+}-free) Hanks' balanced salt solution (HBSS)[33] (10 ml/flask) for 30 min. Incubation with arsenical solutions in HBSS or buffer alone (control) was performed at 37 °C for 30 min. After removal of the incubation mixture, uptake studies were performed as described previously.[16] Briefly, cells were incubated with 10 μmol l^{-1} D-[6-^{14}C]-glucose in HBSS (3 ml per flask, 37 °C) for 10 min. Uptake was terminated by removing the supernatant, adding ice-cold HBSS (5 ml/flask) and placing the flasks on ice. Monolayers were rinsed twice with ice-cold HBSS (5 ml/flask) to remove excess radioactivity and solubilized in 4 ml 0.5 mol l^{-1} NaOH (12 h, 37 °C). Aliquots of 1 ml were added to 5 ml Omniszintisol® for radioactivity determination in a 1215 RackBeta scintillation counter (Pharmacia–LKB, Freiburg, Germany). The remainder was used for protein determination.

XTT-based viability assay

Cells, grown in a 96-well tissue culture plate, were washed (200 μl HBSS/well) and incubated at 37 °C in the absence or presence of arsenic species in culture medium without Phenol Red. After removal of the test mixture, cells were washed again (400 μl HBSS/well) and incubated with a mixture of XTT (sodium 3'-[1-(phenylaminocarbonyl)-3,4-tetrazolium]bis(4-methoxy-6-nitro)benzenesulphonic acid) labelling reagent and electron-coupling reagent as recommended by the supplier (cell proliferation kit II, Boehringer–Mannheim, Germany). The assay is based on the cleavage of the yellow tetrazolium salt XTT to form an orange formazan dye by mitochondrial dehydrogenase activity in living cells. Formazan formation was quantified spectrophotometrically at 450 nm (reference wavelength 690 nm) using a microtiter plate reader (Multiscan MCC/340, Merlin, Bornheim-Hersel, Germany).

Protein measurement

Cellular protein of RKT was measured by the biuret method.[34] Coomassie Blue dye binding as described by Read and Northcote[35] was used for MDCK cells. In both cases bovine serum albumin served as standard.

Calculations

Glucose concentrations and ^{14}C activities were related to protein contents of the tested cells. Glucose formation was calculated as the slope of linear regression curves fitted to concentrations versus time. Individual rates were expressed as a percentage of the corresponding control. Concentration-effect curves were calculated by fitting a sigmoid function to effects (relative rates of glucose formation, relative glucose uptake) measured at various arsenic concentrations.[27] IC_{50} values were obtained as half-maximum-effect concentrations from the fitted curves. Data processing was performed on an Apple Macintosh II and an IBM-compatible personal computer using MS-Excel® (Microsoft Corporation, Redmond, USA), proFit® (QuantumSoft, Zürich, Switzerland) and SigmaPlot® software (Jandel Scientific, Corte Madera, USA).

RESULTS

Rat kidney tubules

Gluconeogenic activity of isolated RKT was investigated using pyruvate as substrate. In the absence of substrate no glucose formation could be measured during 60 min of observation (data not shown), while in the presence of pyruvate (10 mmol l^{-1}) glucose levels rose steadily at an average rate of 9.74 ± 0.90 nmol (mg

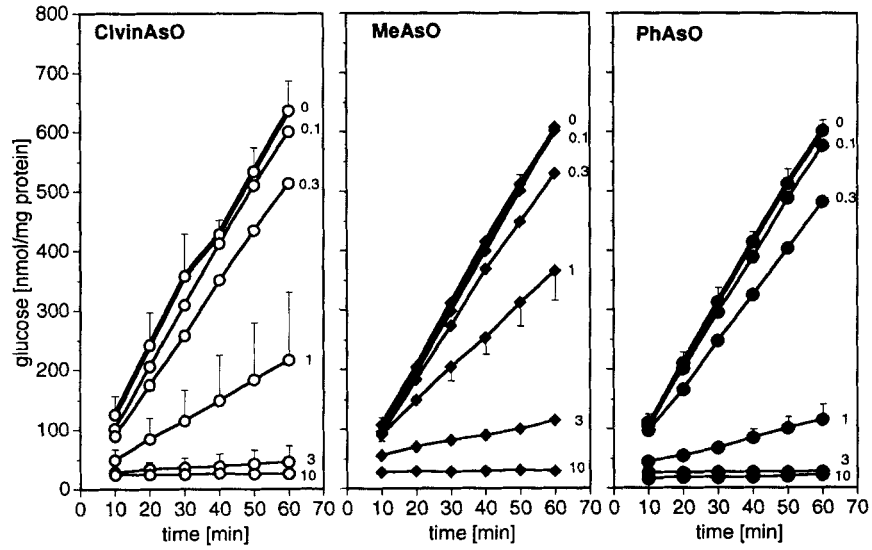

Figure 4 Inhibition of gluconeogenesis in RKT by MeAsO, ClvinAsO and PhAsO. Glucose formation was determined at various times after addition of pyruvate (10 mmol l^{-1}) as substrate. Concentrations of R—As=O are indicated in μmol l^{-1} at the respective curve (0, control).

protein)$^{-1}$ min^{-1} over 60 min. RKT suspensions could be kept on ice for several hours without considerable loss of gluconeogenic activity. When the rate of glucose formation during the first half-hour was compared with the second half-hour, a slight decrease from 10.08 ± 1.39 to 9.29 ± 0.78 nmol (mg protein)$^{-1}$ min^{-1} ($n = 61$) was observed. This was paralleled by a slight loss of cell viability as assessed by dye exclusion (Trypan Blue) from >90% at the beginning to >80% after 60 min. Viability was not affected by the tested oxoarsines up to 2 μmol l^{-1} (60 min).

Arsenic species inhibited glucose formation in a concentration-dependent manner (Fig. 4). A comparison of concentration-effect curves, generated as described above, revealed that PhAsO,

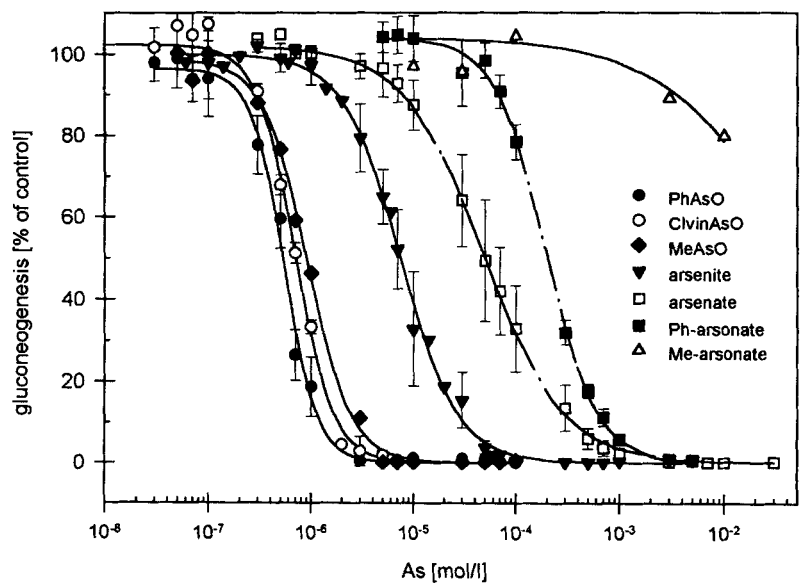

Figure 5 Effect of various arsenic species on gluconeogenesis when incubated with RKT for 60 min at 37 °C. Data points represent mean rates of glucose formation from pyruvate relative to control cells ($n = 2$–7). Error bars give the standard deviation (SD). Curves are drawn from parameters obtained by fitting a sigmoid function to the data points.

Table 1 Inhibitory effect of various arsenicals on gluconeogenesis in RKT and on glucose uptake in MDCK cells

Substance	IC_{50}[a] (μmol l^{-1}) for inhibition of: Gluconeogenesis in RKT	Glucose uptake in MDCK cells
PhAsO	0.55	1.23
ClvinAsO	0.69	2.62
MeAsO	0.99	6.99
Arsenite	7.48	114
Arsenate	48.3	985
Phenylarsonate	195	>1000
Methylarsonate	>10 000	>10 000

[a] Calculated from the fitted sigmoid curves shown in Figs 5 and 6.

ClvinAsO and MeAsO were similarly effective (Fig. 5; Table 1); 1 μmol l^{-1} PhAsO completely (>90%) blocked gluconeogenesis from pyruvate. The trivalent organoarsenic species were roughly one order of magnitude more effective than inorganic arsenite and about two orders of magnitude more effective than the pentavalent derivatives arsenate or phenylarsonate, while methylarsonate had virtually no effect (Fig. 5; Table 1). While, with oxoarsines, cell viability was not affected at concentrations where gluconeogenesis was markedly inhibited, the high concentrations of pentavalent arsenic species required for notable gluconeogenic inhibition at the same time caused a significant decrease of cell viability as assessed by dye exclusion (data not shown).

MDCK cells

In MDCK cells arsenicals inhibited glucose uptake (Fig. 6). Again, PhAsO, ClvinAsO and MeAsO were almost identically effective and were the most potent inhibitors. Compared with organic oxoarsines, inorganic arsenite was roughly two orders of magnitude less effective. The pentavalent arsenic species showed only slight effects at concentrations in the millimolar range (Fig. 6; Table 1). Like glucose uptake, cell viability as assessed by formazan formation was affected by the mono-substituted trivalent organoarsenic species in a similar manner (Fig. 7). Concentrations up to 2 μmol l^{-1} showed no significant effects within 180 min of incubation. At 5 μmol l^{-1} a loss of viability first became evident after 90–120 min, whereas glucose uptake was half-maximally inhibited by 1–7 μmol l^{-1} of PhAsO, ClvinAsO and MeAsO after 30 min (Fig. 6; Table 1). Accordingly, higher concentrations (≥10 μmol l^{-1}) leading to maximal inhibition of glucose uptake within 30 min caused a comparable inhibition of viability 30–150 min later. In contrast to the trivalent organoarsenic species,

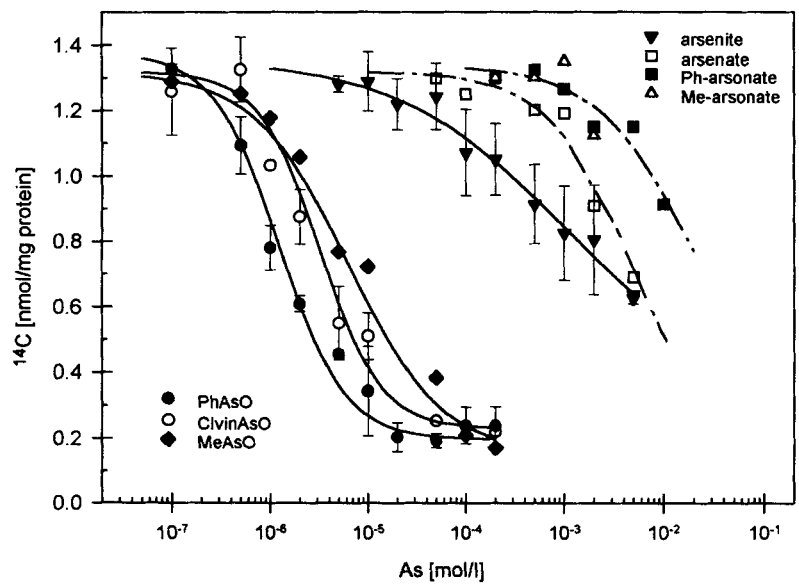

Figure 6 Effect of various arsenic species on glucose uptake in MDCK cells. Cells were incubated (37 °C) in the absence (controls) and in the presence of arsenic species for 30 min and then with D-[6-^{14}C]-glucose (10 μmol l^{-1}) for 10 min. Data points represent cellular tracer accumulation (means ± SD for $n \geq 3$) (controls: 1.36 ± 0.8 nmol ^{14}C (mg protein)$^{-1}$. Curves are drawn from parameters obtained by fitting a sigmoid function to the data points.

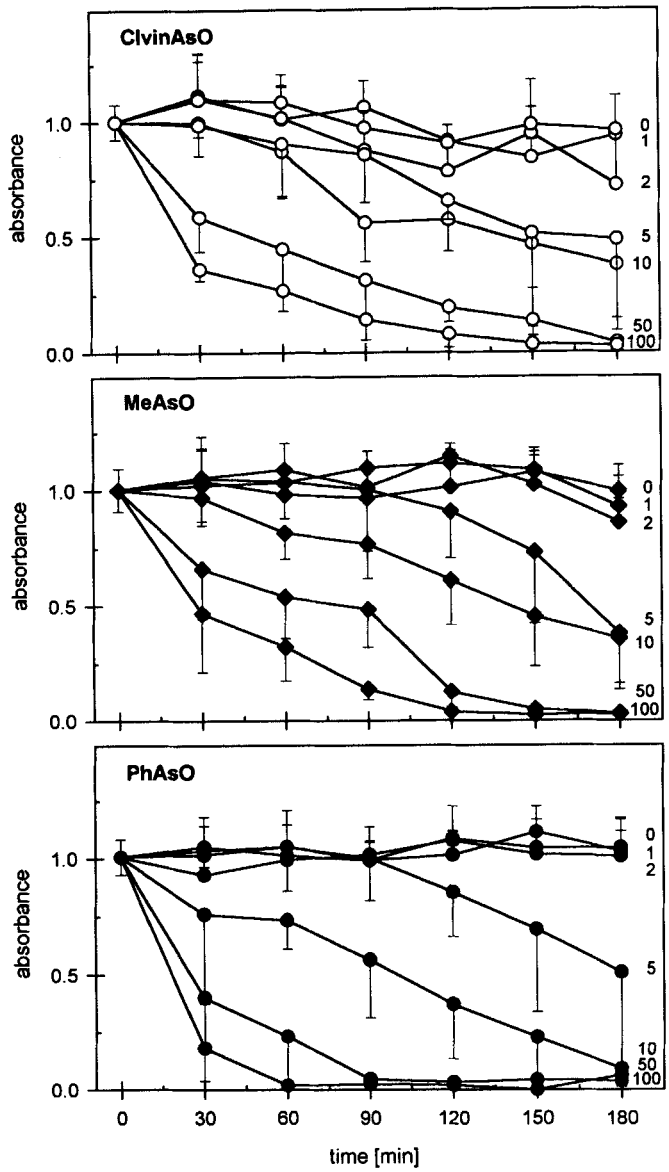

Figure 7 Influence of MeAsO, ClvinAsO and PhAsO on the viability of MDCK cells. Following incubation of cells without or with the respective arsenic species for various times, formazan dye formed from XTT by mitochondrial dehydrogenase activity in living cells was detected spectrophotometrically. Concentrations of R—As=O are indicated in μmol l^{-1} at the respective curve (0, control). Means ± SD; $n = 3$–5.

even 1 mmol l^{-1} of arsenite and the pentavalent arsenic species showed no measurable effects on cell viability within 180 min (Fig. 8).

DISCUSSION

Among the procedures used to assess metabolic toxicity of arsenicals *in vitro*, gluconeogenesis in RKT has been shown to be a very sensitive parameter[15,27] because it is tightly linked to many aspects of cellular energy metabolism, and formation of glucose is easily quantitated. However, laboratory animals are required and biological variation is considerable. RKT cannot be kept fully functional under conventional tissue-culture conditions for more than one day.[27] Since these limitations do not exist with permanent cell lines, they might represent an alternative to primary

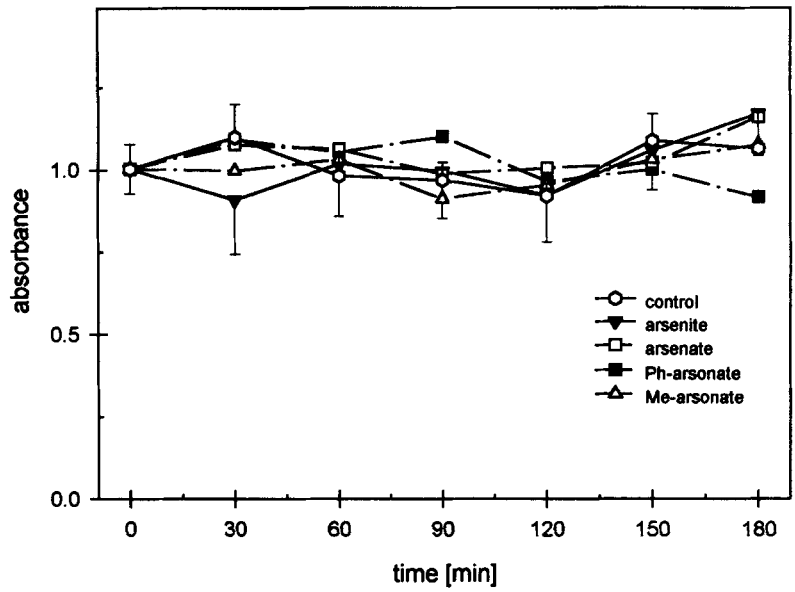

Figure 8 Effect of 1 mmol l^{-1} of arsenite, arsenate, methylarsonate or phenylarsonate on the viability of MDCK cells. Same protocol as indicated in Fig. 7. Means ± SD; $n = 2–4$.

cultures such as RKT. However, not a single report about such cells capable of effective gluconeogenesis was found in the literature.

Although PhAsO has been widely used as a biochemical tool to block internalization processes, presumably by an interaction with functional sulphydryl groups located in the plasma membrane,[7-10] little attention has been paid so far to the possible role of membrane interactions in arsenic toxicity. There is evidence for sulphydryl groups of functional importance for membrane transport mechanisms such as the uptake of glucose.[38-40] Our results show that glucose uptake is inhibited by trivalent arsenic species in MDCK cells. Previous studies indicated that this inhibition is due to an interaction with glucose transporters rather than an indirect consequence of disturbed energy metabolism.[16] Glucose uptake was very sensitive to trivalent organoarsenic species, the IC_{50} being comparable with the value found for inhibition of gluconeogenesis in RKT. On the other hand, this parameter was much less sensitive to arsenite. While in other *in vitro* models (e.g. RKT) and *in vivo ca* 10-fold higher concentrations of this toxicant than of trivalent organoarsenic species were required for comparable effects, in MDCK cells this factor amounted to about 100. The high efficacy of organoarsenic species as compared with the other tested compounds can be explained by the combination of trivalent arsenic, responsible for reactivity towards functional (e.g. sulphyrdyl) groups, with an organic moiety which may improve accessibility of the affected structures.

Animal studies have shown that trivalent organic arsenic species are more toxic to mammals than inorganic arsenic,[4,14] while pentavalent arsenic species are generally regarded as less toxic.[1,36,37] We have found the same graduation of toxicities in our *in vitro* experiments, in which trivalent organoarsenic species proved to be more potent inhibitors of gluconeogenesis in RKT and glucose uptake in MDCK cells, respectively, than arsenite, which in turn was more toxic than the pentavalent arsenic species investigated. Interestingly, all three trivalent mono-substituted organoarsenic species tested (MeAsO, ClvinAsO, PhAsO) exerted similar effects in both test systems. This finding is consistent with *in vivo* data obtained with rabbits in which PhAsO and $ClvinAsCl_2$ showed similar LD_{50} values.[4,5]

Our data further indicate that the use of the trivalent mono-substituted arsenic species requires close monitoring of the identity of the compounds. Spontaneous oxidation and/or polymerization have been reported,[3] but have not been investigated in biological media so far. Our findings suggest that the substituent is critical for the stability of the compound.

We conclude (1) that different mechanisms are involved in the acute toxicity of oxoarsines and inorganic arsenic and (2) that PhAsO offers

several advantages as a model substance for mono-substituted trivalent arsenic species, because it is more stable and more readily detectable.

Acknowledgement The skilful technical assistance of Ms E. Mattausch is gratefully acknowledged.

REFERENCES

1. K. A. Winship, *Adv. Drug React. Acute Poisoning Rev.* **3**, 129 (1984).
2. T. H. Snider, R. L. Wientjes, R. L. Joiner and G. L. Fisher, *Fund. Appl. Toxicol.* **14**, 262 (1990).
3. R. A. Waters and J. H. Williams, *J. Chem. Soc.* 18 (1950).
4. R. H. Inns, J. E. Bright and T. C. Marss, *Toxicology* **51**, 213 (1988).
5. H. Eagle, R. B. Hogan, G. O. Doak and H. G. Steinman, *J. Pharmacol. Exp. Ther.* **80**, 142 (1944).
6. H. Eagle and G. O. Doak, *Pharmacol. Rev.* **3**, 107 (1951).
7. J. E. Lamb, F. Ray, J. H. Ward, J. P. Kushner and J. Kaplan, *J. Biol. Chem.* **258**, 8751 (1983).
8. C. Hertel, S. J. Coulter and J. P. Perkins, *J. Biol. Chem.* **260**, 12547 (1985).
9. A. E. Gibson, R. J. Noel, J. T. Herlihy and W. F. Ward, *Am. J. Physiol.* **257**, C182 (1989).
10. A. G. Douen and M. N. Jones, *Biofactors* **2**, 153 (1990).
11. S. Samaan, in: *Houben-Weyl: Methoden der Organischen Chemie*, 4th edn, Müller, E. (ed.) Georg Thieme Verlag, Stuttgart, 1978, Vol. XIII/8, pp. 179–181.
12. E. A. Crecelius, *Environ. Health Persp.* **19**, 147 (1977).
13. J. P. Buchet, R. Lauwerys and H. Roels, *Int. Arch. Occup. Environ. Health* **48**, 71 (1981).
14. R. A. Peters, *Bull. Johns Hopkins Hosp.* **97**, 1 (1955).
15. L. Szinicz and W. Forth, *Toxicology* **61**, 444 (1988).
16. B. Liebl, H. Mückter, E. Doklea, B. Fichtl and W. Forth, *Analyst (London)* **117**, 681 (1992).
17. N. Ishinishi, K. Tsuchiya, M. Vather and B. A. Fowler, in: *Handbook on the Toxicology of Metals*, 2nd edn, Friberg, L., Nordberg, G. F. and Vouk, V. B. (eds), Elsevier, Amsterdam, Vol. 2, pp. 43–83.
18. A. E. Ledet and W. B. Buck, in: *Toxicity of Heavy Metals in the Environment*, Oehme, F. W. (ed.), Marcel Dekker, New York, 1978, Vol. 2, pp. 375–392.
19. H. V. Aposhian, in *Reviews in Biochemical Toxicology*, Hodgson, E., Bend, J. R. and Philpot, R. M. (eds), Elsevier, Amsterdam, 1989, Vol. 10, pp. 265–289.
20. C. Voegtlin and T. W. Thompson, *J. Pharmacol. Exp. Ther.* **20**, 85 (1923).
21. J. P. Buchet and R. R. Lauwerys, *Arch. Toxicol.* **57**, 125 (1985).
22. A. B. Fischer, J. P. Buchet and R. R. Lauwerys, *Arch. Toxicol.* **57**, 168 (1985).
23. T. B. B. Crawford and G. A. Levvy, *Biochem. J.* **41**, 333 (1947).
24. M. Vahter and E. Marafante, *Arch. Toxicol.* **57**, 119 (1985).
25. M. Vahter and E. Marafante, *Biol. Trace Element Res.* **21**, 233 (1989).
26. G. K. H. Tam, S. M. Charbonneau, F. Bryce, C. Pomroy and E. Sandi, *Toxicol. Appl. Pharmacol.* **50**, 319 (1979).
27. H. Mückter, S. Islambouli, E. Doklea, C. Hopfer, L. Szincz, B. Fichtl and W. Forth, *Toxicol. Appl. Pharmacol.* **121**, 118 (1993).
28. G. Favrel, *Chem. Zbl.* **84**, 576 (1913).
29. A. Baeyer, *Ann. Chem. Pharm.* **107**, 257 (1858).
30. W. Guder, W. Wiesner, B. Stukowski and O. Wieland, *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 1319 (1971).
31. H. A. Krebs and K. Henseleit, *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33 (1932).
32. A. Kunst, B. Draeger and J. Ziegenhorn, in: *Methods of Enzymatic Analysis*, 3rd edn., Bergmeyer, H. U., Bergmeyer, J. and Grassl, M. (eds). Verlag Chemie, Weinheim, 1988, Vol. 6, pp. 163–172.
33. J. H. Hanks and R. E. Wallace, *Proc. Soc. Exp. Biol. Med.* **71**, 196 (1949).
34. G. Beisenherz, H. J. Boltze, T. Bücher, R. Czok, K. H. Garbade, E. Meyer-Arendt and G. Pfleiderer, *Z. Naturforsch.* **8B**, 555 (1953).
35. S. M. Read and D. H. Northcote, *Anal. Biochem.* **116**, 53 (1981).
36. B. L. Vallee, D. D. Ulmer and W. E. C. Wacker, *Arch. Ind. Health* **21**, 132 (1960).
37. C. D. Klaassen, in: *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th edn, Gilman, A. G., Rall, T. W., Nies, A. S. and Taylor, P. (eds), Pergamon, New York, 1990, pp. 1592–1614.
38. M. Mueckler, C. Caruso, S. A. Baldwin, M. Panico, I. Blench, H. R. Morris, W. J. Allard, G. E. Lienhard and H. F. Lodish, *Science* **229**, 941 (1985).
39. J. M. May, *J. Biol. Chem.* **260**, 462 (1985).
40. A. G. Lowe and A. R. Walmsley, *Biochim. Biophys. Acta* **857**, 146 (1986).