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ORGAN TOXICITY AND MECHANISMS

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The toxic mechanism and metabolic effects of atractyloside in precision-cut pig kidney and liver slices

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Abstract The toxic and cellular metabolic effects of atractyloside, a diterpenoid glycoside, which causes fatal renal and hepatic necrosis in vivo in animals and humans, have been investigated in tissue slices prepared from male domestic pig kidney and liver. Precision-cut slices (200 μm thick) were incubated with atractyloside at concentrations of 200 μM, 500 μM, 1.0 mM and 2.0 mM for 3 h at 37 °C and changes in lipid profile and pyruvate-stimulated gluconeogenesis investigated. Lipid peroxidative changes, reduced glutathione (GSH) and ATP content, the release of lactate dehydrogenase (LDH), alkaline phosphatase (ALP), alanine and aspartate aminotransferase (ALT/AST) were also assessed. After 3 h of incubation, atractyloside caused a significant ($P < 0.01$) and concentration-dependent leakage of LDH and ALP from kidney slices. Only LDH leakage was significantly elevated in liver slices while ALT and AST leakage showed marginal increase. Atractyloside at concentrations of ≥200 μM caused a significant increase in lipid peroxidation, but only in liver slices. However, atractyloside at concentrations of ≥200 μM caused a marked depletion of GSH and ATP content in both kidney and liver slices. There was a marked decrease in total and individual phospholipid in kidney but not in liver slices. However, cholesterol and triacylglycerol levels were not affected by atractyloside in both kidney and liver slices. Renal and hepatic pyruvate-stimulated gluconeogenesis were significantly ($P < 0.05$) inhibited at atractyloside concentrations of ≥500 μM. Accumulation of organic anion *p*-aminohippuric acid (PAH) was also inhibited in renal cortical slices at atractyloside concentrations of ≥500 μM. These results suggest that the observable in vivo effect of atractyloside can be reproduced in slices and that basic mechanistic differences exist in the mode of toxicity in liver and kidney tissues. The data also raise the possibility that the mechanistic basis of metabolic alterations in these tissues following treatment with atractyloside may be relevant to target selective toxicity.

Key words Atractyloside · Pig kidney and liver slices · Metabolic alterations · Energy status · Selective toxicity

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Introduction

The diterpene glycoside, atractyloside, is present in a number of plants used as ethnomedicines throughout Africa, the Mediterranean areas and the Far Eastern countries (Debetto 1978; Obatomi and Bach 1998). These plants are known to cause acute fatal renal and liver damage in humans and domestic animals foraging on atractyloside-containing plants (Stuart et al. 1981; Martin et al. 1986). Pure atractyloside also produces a similar lesion in animals (Carpenedo et al. 1974; Hatch et al. 1982). Histologically, there was usually marked centrilobular degeneration of the liver (Caravaca-Magarinos et al. 1985; Georgiou et al. 1988; Hedili et al. 1989) and proximal tubular necrosis of the kidney (Bhoola 1983; Koechel and Krejci 1993). The effect of atractyloside seen in vivo is often explained partly by the fact that it is a powerful inhibitor of oxidative phosphorylation in isolated mitochondria from a variety of animals (Luciani et al. 1978). Atractyloside was also found to alter catabolic and anabolic functions in vivo (Georgiou et al. 1988), and its consumption in dried roasted beans (*Coffea arabica*) has been linked to pancreatic cancer (Pegel 1981).

Despite significant human and animal exposure and many reported deaths (Bhoola 1983; Hutchings and Terblanche 1989), there is no established mechanism of

injury and thus there has been no rational approach to limit or prevent atractyloside toxicity. It is also not clear whether atractyloside metabolism is the same in man and laboratory animals although both species have been reported to exhibit common target toxicity in vivo and the pig has been shown to be specifically sensitive to this compound (Guercio et al. 1971; Stuart et al. 1981). Swine accidentally grazed on plants containing atractyloside provided similar effects to humans poisoned with remedies containing atractyloside (Stuart et al. 1981). Thus the porcine kidney could provide a good model for the study of human toxicity. In addition, porcine tissues are readily available from the slaughterhouse and their usage may lead to a reduced consumption of laboratory animals for toxicity studies. As part of a study to evaluate the probable mechanisms of atractyloside toxicity in different animal species, we compared the toxic and cellular metabolic aspects of kidney and liver using 'precision-cut' tissue slices. This could help to provide a greater insight into the potential to modulate atractyloside toxic effects in man.

Materials and methods

Chemicals and reagents

All chemicals and reagents were of analytical grade and obtained from Sigma Chemical Company (Poole, Dorset, UK) unless otherwise stated.

Slice preparation and incubation

Adult male domestic pigs, moved straight from farms to a local abattoir for slaughter, were used as sources of fresh tissue. One kidney (usually the right) and a lobe of liver were removed within 5 min of slaughter and the tissues transported to the laboratory in ice-cold rinse medium (Krebs-HEPES buffer, pH 7.4) gassed with 95% O_2/5% CO_2. Tissues were cored for slicing within 30 min of harvesting. Different areas of the liver lobes and renal cortex were cored with a motor-driven stainless steel corer (Alabama Research and Development Corp., Munford, Ala., USA) of 8 mm in diameter. The cores were placed in fresh ice-cold Krebs-HEPES buffer (gassed with 95% O_2/5% CO_2) and transferred one at a time to a Krumdieck tissue slicer (Krumdieck et al. 1980). Tissue slices (200 $\pm$ 15 μM in diameter) were prepared as previously described (Obatomi et al. 1997). Slices were collected and placed one slice per well of 24-well culture plates (LINBRO, Flow laboratories Ltd, Herts, England) for preincubation in 1 ml serum-free, phenol red-free Dulbecco's modified Eagle's medium and Ham's nutrient mix F12 (1:1) on an orbital shaker (Bellco, Biotechnology, Vineland, N.J., USA) in an humidified incubator at 37 °C for 1 h to allow slices to stabilize (Obatomi et al. 1997).

In vitro exposure of slices to atractyloside

After 1 h of preincubation, the medium was replaced with freshly prepared medium containing atractyloside to obtain final concentrations of 200 μM, 500 μM, 1.0 mM and 2.0 mM. Control slices were incubated in medium only. The slices were incubated for 3 h at 37 °C on a rocker platform rotated at approximately 3 rpm. The 3 h incubation period was established in a previous study (Obatomi et al. 1998) as the minimum time required to observe significant biochemical and morphological changes following exposure to atractyloside.

Slice viability assessment

Fresh and preincubated slices from each preparation were usually assessed for viability after a short incubation with 200 μg/ml ethidium bromide and 5 μg/ml fluorescein diacetate in phosphate-buffered saline (Edindin 1970). Slices were observed under a Leitz Dialux 20 (Leica UK Ltd., Milton Keynes, UK) microscope (390–490 nm excitation and 510–515 nm emission). Viable cells fluoresced bright green whereas non-viable cells fluoresced red. This test showed that slices had high viability on slicing and throughout the period of incubation.

Assay of enzyme leakage

Injury was assessed by measuring leakage of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) from renal cortical slices or LDH, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) from liver slices into the incubation medium. The activities of ALP (EC 3.1.3.1) and LDH (EC 1.1.1.27) were assayed by the methods described by Obatomi and Plummer (1995) while ALT (EC 2.6.1.2) and AST (EC 2.6.1.1) were determined by commercial kits purchased from Sigma Chemical Co. (Sigma catalogue no 505). Enzyme release was initially calculated as nmoles/mg protein and expressed as a percentage of total enzyme. Slices were solubilized in 1% (w/v) Triton X-100 to obtain total enzyme activity.

Measurement of malondialdehyde and glutathione contents of slices

The extent of lipid peroxidation in slices was estimated by measuring the concentration of malondialdehyde (MDA) using the thiobarbituric acid (TBA) assay (Burge and Aust 1978). After incubation, the slices were removed, weighed and homogenized in 3 ml of the TBA reagent [0.026 M thiobarbituric acid, 0.92 M trichloroacetic acid (TCA) and 0.25 M HCl]. The homogenate was heated to boiling for 15 min, cooled, and centrifuged. The MDA concentration of the sample was determined spectrophotometrically at 535 nm utilizing the molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} (Obatomi and Bach 1996a). The slice content of reduced (GSH) and oxidized (GSSG) glutathione was determined in slices homogenized in 5% TCA using a modified method of Hissin and Hilf (1976). Samples were centrifuged for 10 min at 3000 rpm and treated as previously reported (Obatomi et al. 1998). Fluorescence was measured (LS-5L luminescence spectrometer, Perkin-Elmer) at excitation of 350 nm and emission of 420 nm. Both GSH and GSSG were expressed as ng/mg of slice protein, based on a standard curve.

ATP content of slices

Adenosine triphosphate (ATP) content was determined with a luciferin-luciferase assay (Deluca and McElroy 1978) using a Luminometer 1251 (LKB Wallac, Finland). Following incubation, slices were placed in 5% TCA, sonicated and centrifuged for 10 min. The supernatant was used for ATP assay while the pellet was used for protein analysis and normalization.

Determination of glucose production

After incubation with atractyloside for 3 h, slices were incubated for a further 60 min following the addition of 10 mM pyruvate (Roobol and Alleyne 1974). At the end of the incubation period, an aliquot of the incubation medium and slices were assessed for glucose by the glucose oxidase and peroxidase assay (Plummer 1987). Glucose formation was expressed as mg glucose/g tissue wet weight.

Lipid analysis

The kidney and liver slices were subjected to detailed lipid analysis. These included the quantization of the individual phospholipid,

total cholesterol and cholesterol ester. Extraction of tissue was performed as previously described (Thanh et al. 1997). Briefly, slices were homogenized with an Ultra Turrax T8 homogenizer and extracted in 1 ml of the solvent mixture chloroform methanol (2:1, v/v) with 0.01% w/v butylated hydroxytoluene, and 5 µl of tissue extract was delivered under nitrogen by a Linomat IV Applicator to silica gel high-performance thin layer chromagraphy (HPTLC) plates (Merck; 10 × 20 cm). The plates were developed in a Camag horizontal developing chamber. Detection by staining was carried out using manganese chloride-sulphuric acid reagent (1 g of $MnCl_2$: 150 ml methanol: 10 ml conc H_2SO_4: 150 ml deionized water). This was heated at 110 °C for 60 min. The pinkish coloured bands on a whitish background were quantified using a Desaga CD60 densitometer with a Hg lamp, scanned at 250 nm (optimized wavelength), slit width 0.02 mm and height 3 mm. Commercially available cholesterol, phospholipid and triacylglycerol standards were processed and used to calibrate the system.

Uptake of *p*-aminohippurate (PAH)

PAH uptake was determined in renal slices using a modified method of Smith et al. (1944). After 3 h incubation with atractyloside, slices were further incubated in medium containing 0.074 mM PAH (Fujimoto and Fujita 1982) at 37 °C for 60 min. At the end of the incubation, slices were removed, blotted, weighed, homogenized (10 ml/100 mg tissue) in 0.184 M TCA acid and centrifuged. A 1 ml aliquot of the incubation medium was treated similarly. Uptake of PAH was expressed as the slice to medium (S/M) ratio.

Total protein measurement

Slices were dissolved in 1.0 ml of 0.5 M NaOH for 24 h and protein content was assessed by the Coomassie Brilliant G method (Read and Northcote 1981) using bovine serum albumin as a standard. Protein concentrations were used to normalize data obtained in all other assays except gluconeogenesis.

Statistical analysis

All data are expressed as means ± SE. Statistical evaluation of the results was carried out by analysis of variance and Student's *t*-test. Differences between the means of experimental and control groups were considered significant at $P < 0.05$.

Results

Enzyme leakage from slices

The leakage of ALP and LDH from rat kidney slices and also LDH, ALT and AST from liver slices exposed to atractyloside are presented in Fig. 1. There were significant increases ($P < 0.01$) in both ALP and LDH leakage from the kidney slices (Fig. 1a) at atractyloside concentrations of ≥0.5 mM. Similarly in the liver slices, LDH leakage was significantly elevated at atractyloside concentrations of ≥0.5 mM (Fig. 1b). There were, however, significantly fewer increases in ALT and AST leakage from the liver slices at all atractyloside exposure levels.

Lipid peroxidation in slices

The formation of MDA was used as a measure of lipid peroxidation in both liver and kidney slices. MDA formation in control slices remained stable over the period of incubation, while there was a significant increase ($P < 0.05$) in liver slices at atractyloside concentration of ≥0.2 mM (Fig. 2). No significant change, however, was observed in kidney slices at any atractyloside concentration (Fig. 2). There was no detectable amount of MDA in the incubation media of both control and treated slices.

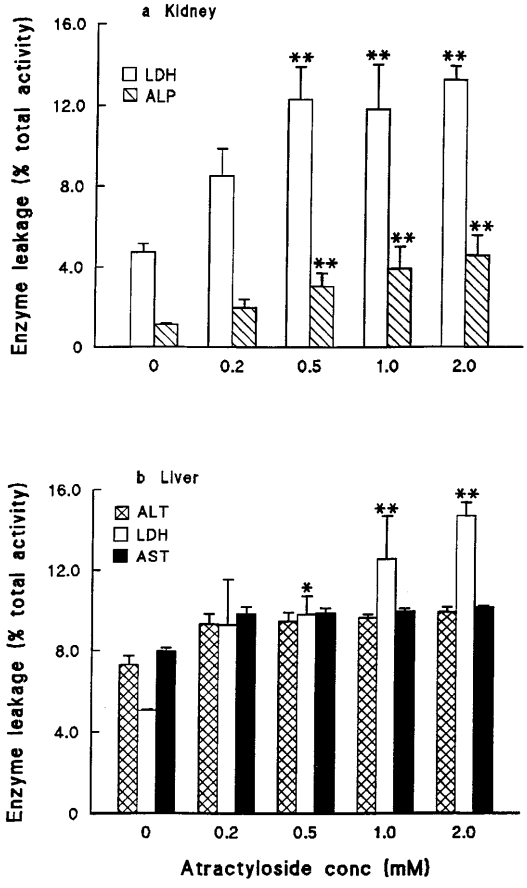

Fig. 1a, b Enzyme leakage from kidney (ALP, LDH) and liver (AST, ALT, LDH) slices exposed to atractyloside. The data represent percentage of total enzyme in slices and are presented as mean ± SE ($n = 3$ of 4 slices). $^*P < 0.05$, $^{**}P < 0.01$, compared to control groups. (*ALP* Alkaline phosphatase, *LDH* lactate dehydrogenase, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase)

Reduced and oxidized glutathione levels in slices

Atractyloside induced a dose-dependent depletion of intracellular GSH in kidney and liver slices (Fig. 3a). Enhanced atractyloside toxicity was also shown by significantly higher increases in GSSG in liver slices at atractyloside concentrations of ≥0.5 mM. In contrast, there was no significant change in GSSG levels in kidney slices at any atractyloside exposure levels (Fig. 3b). The GSH/GSSG ratio (obtained by calculation) in the liver slices showed remarkably low values, while in kidney slices this ratio remained at a comparable level with the

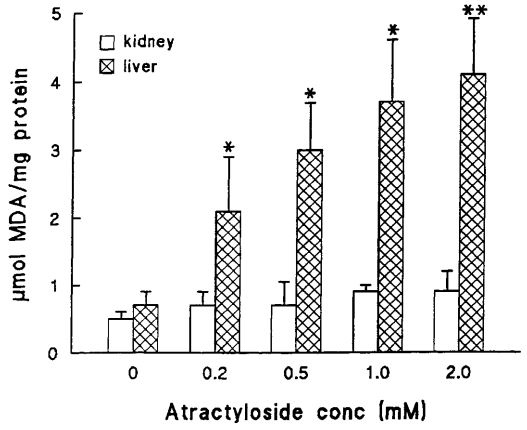

Fig. 2 Effects of atractyloside on malondialdehyde (MDA) production in kidney and liver slices. MDA production was measured by the thiobarbituric acid assay. Results represent mean values ± SE of 3 experiments performed in quadruplicate. Statistically significant differences between treated and control groups are indicated as $*P < 0.05$, $**P < 0.01$

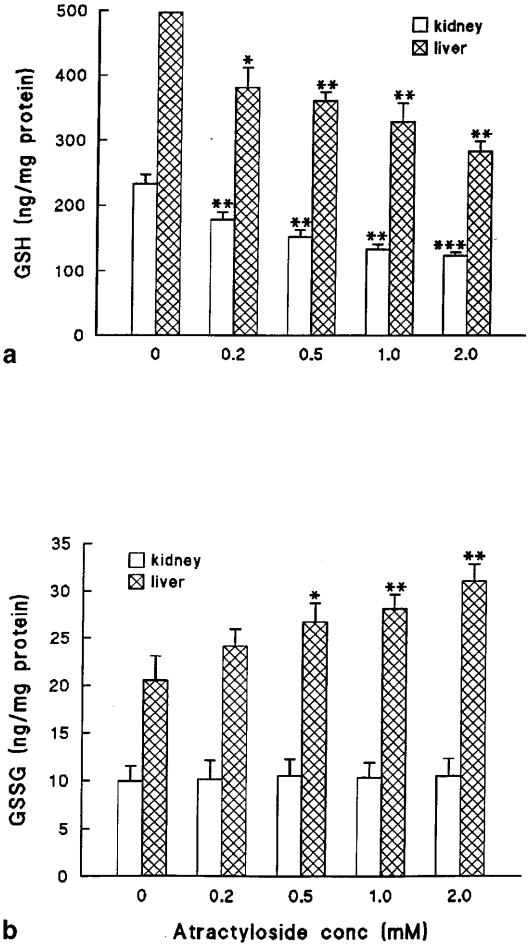

Fig. 3a, b Effects of different atractyloside concentrations on (**a**) reduced (GSH) and (**b**) oxidized (GSSG) glutathione levels in kidney and liver slices. Results represent mean values ± SE of 3 experiments performed in quadruplicate. Significantly different from corresponding control: $*P < 0.05$, $**P < 0.01$

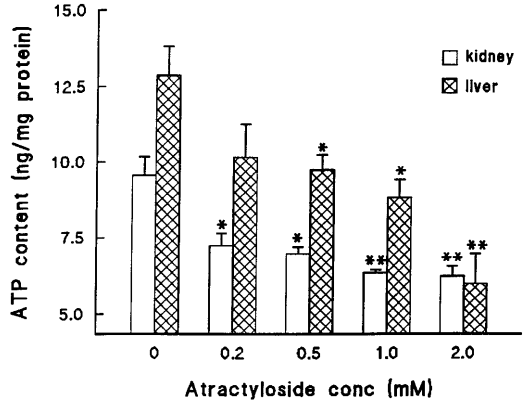

Fig. 4 Effects of atractyloside on adenosine triphosphate (ATP) production in kidney and liver slices. Results represent mean values ± SE of 3 experiments performed in quadruplicate. Significantly different from corresponding control: $*P < 0.05$, $**P < 0.01$

control. There were no detectable amounts of GSH and GSSG in the incubation media of both control and treated slices.

Energy status of slices

Intracellular ATP levels in control slices remained stable over the 3 h incubation period. There was, however, a marked depletion of ATP in both kidney and liver slices following exposure to atractyloside (Fig. 4).

Gluconeogenic capacity and lipid changes in slices

Atractyloside markedly inhibited gluconeogenesis in a concentration-dependent manner (Fig. 5). The concentration response curve observed for gluconeogenesis in kidney slices was very similar to that observed for liver slices (Fig. 5). Endogenous gluconeogenesis (in the absence of exogenous pyruvate) was also inhibited to the same extent by atractyloside, an indication that inhibition of pyruvate uptake is not the cause of atractyloside-induced decrease of gluconeogenesis. Both total and individual phospholipid in the kidney slices were significantly reduced by atractyloside in a concentration-dependent manner (Table 1). In the liver slices, there was a slight but non-significant increase in total and individual phospholipid following exposure to atractyloside (Table 2). There were no changes in triacylglycerol and cholesterol or cholesterol ester in both kidney and liver slices (data not shown). Cholesterol ester was, however, not detected in the kidney slices.

Organic anion uptake

Uptake of PAH, which increased linearly in control slices with period of incubation, was significantly

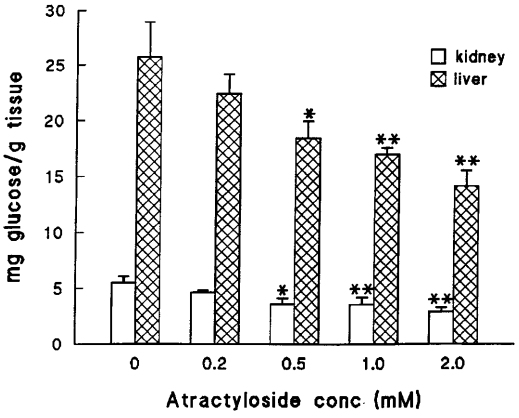

Fig. 5 Effects of different atractyloside concentrations on pyruvate-stimulated gluconeogenesis in kidney and liver slices. Pyruvate-stimulated gluconeogenesis was measured as glucose production after a subsequent 1 h incubation in pyruvate containing atractyloside-free medium. Data represent mean values ± SE of 3 experiments performed in quadruplicate. Glucose production after incubation with atractyloside was significantly different compared to corresponding control values: $*P < 0.05$, $**P < 0.01$

inhibited in kidney slices at atractyloside concentrations of ≥0.5 mM (Fig. 6).

Discussion

In the present study, tissue slices from liver and kidney of male domestic pigs were used as an in vitro model to provide a better understanding of the toxic mechanism and cellular metabolic alterations induced by atractyloside. A dose-dependent study was conducted to establish the minimum concentration of this diterpenoid glycoside required to bring about toxic changes in pig liver and kidney tissues over a relatively short period (3 h) of exposure. Major alterations in biochemistry and morphology of these tissues are known to occur within this period of exposure to atractyloside both in vivo (Carpenedo et al. 1974; Koechel and Krejci 1993) and in vitro (Obatomi et al. 1998). We have also shown previously that slices prepared from rat and pig striated muscle and peritoneum tissues were not sensitive to atractyloside toxicity (Obatomi et al. 1998). Similarly, swine exposed to atractyloside (Stuart et al. 1981) were more sensitive than calves (Martin et al. 1986) exposed to same level of the toxicant.

Under our experimental conditions, the kidney and liver slices showed increased leakage of LDH in response to atractyloside, which demonstrates comparable cytotoxicity in the two organs. Futhermore, ALP which is a regioselective marker of proximal brush border (Guder and Ross 1984) was also significantly elevated in the kidney suggesting that the proximal tubular cells may be the major site of atractyloside-induced toxicity. It is of interest to observe that the levels of both ALT and AST in liver slices were not remarkably affected at any atractyloside exposure. This observation was in marked contrast to the reported elevation of ALT and AST in animals and human sera, following exposure to atractyloside (Bhoola 1983; Georgiou et al. 1988).

The mechanism by which atractyloside induces nephrotoxicity and hepatotoxicity remains to be determined. Studies have shown that the allyl group of atractyloside is capable of being biotransformed in the liver (Obermann et al. 1973), suggesting that it can undergo redox cycling resulting in the production of oxygen free radicals. The significant increase of MDA formation leading to GSH depletion and elevation in GSSG levels in the liver slices at all atractyloside exposures suggests that oxidative process may be the toxic mechanism in this organ. It is not clear if the kidney is able to bioactivate atractyloside to a reactive metabolite. The lack of any substantial increase in lipid peroxidation in kidney slices together with the unchanged levels of GSSG and GSH/GSSG status following atractyloside exposure rules out the possibility of oxidative stress as the mechanism of toxicity in this organ. Further studies are needed both in vivo and in vitro to determine the ultimate toxicant species produced in the liver or kidney following exposure to atractyloside.

There were differences in the phospholipid profiles in kidney and liver slices following atractyloside exposure. While there was a marked decrease of the individual phospholipid components in the kidney, there was hardly any change at all in the liver. In contrast, both renal and hepatic gluconeogenesis were similarly inhibited following exposure to atractyloside. The later mirrored the known in vivo effect of atractyloside (Roeder

Table 1 Effect of atractyloside on phospholipid profile in pig kidney slices. (*SPM* Sphingomyelin, *PC* phosphatidyl choline, *PS* phosphatidyl serine, *PI* phosphatidyl inositol, *CAR* cardiolipin, *PE* phosphatidyl ethanolamine)

Atractyloside conc (μM)	Phospholipid (μg/mg protein)					
	SPM	PC	PS	PI	CAR	PE
0	52.6 ± 6.13	157.6 ± 12.0	18.1 ± 4.23	28.2 ± 3.77	38.8 ± 4.58	99.3 ± 10.3
200	29.9 ± 4.29**	135.4 ± 18.0	19.4 ± 7.13	22.2 ± 1.39*	29.6 ± 1.44*	71.2 ± 14.4*
500	23.2 ± 11.9*	113.1 ± 3.25**	13.0 ± 4.91	19.9 ± 4.04*	26.4 ± 4.05**	65.3 ± 9.57**
1000	27.5 ± 13.0*	109.1 ± 12.9**	14.4 ± 4.20	16.7 ± 3.88*	24.6 ± 0.3**	63.6 ± 10.6**
2000	30.6 ± 4.22**	109.0 ± 5.3**	19.2 ± 6.20	14.2 ± 1.81**	20.2 ± 3.55**	58.9 ± 17.2**

Each value represents means ± SE for 3 separate experiments
$*P < 0.05$, $**P < 0.01$ compared to the respective control tissue

Table 2 Effect of atractyloside on phospholipid profile in pig liver slices. For explanation of phospholipid abbreviations see the heading to Table 1

Atractyloside conc (μM)	Phospholipid (μg/mg tissue protein)					
	SPM	PC	PS	PI	CAR	PE
0	7.23 ± 1.61	167.6 ± 7.08	9.65 ± 1.44	19.2 ± 2.26	10.7 ± 1.71	47.0 ± 3.26
200	6.43 ± 1.83	183 ± 12.70	9.9 ± 1.46	19.3 ± 1.84	12.0 ± 0.94	51.0 ± 8.14
500	4.33 ± 2.22	162.6 ± 10.8	9.3 ± 1.21	18.2 ± 0.50	9.05 ± 1.43	39.2 ± 11.1
1000	6.43 ± 0.90	187.2 ± 6.91	11.7 ± 4.12	23.0 ± 1.18	11.3 ± 1.44	53.3 ± 5.07
2000	5.52 ± 1.21	188.0 ± 33.1	13.3 ± 3.48	20.7 ± 3.36	11.8 ± 2.47	56.5 ± 10.1

Each value represents means ± SE for 3 separate experiments

et al. 1994) and indicates that atractyloside interferes with renal and hepatic intermediary metabolism. Abnormalities in renal lipid metabolism have been previously identified as an important factor in the pathogenesis of chronic progressive renal injury (Kasiske et al. 1989). The effect of atractyloside on the lipid profile obtained in this study is similar to an observed effect in animals treated with ochratoxin A (Rahimtula et al. 1989). We have also shown previously (Thanh et al. 1997) that atractyloside-induced toxicity is accompanied by alterations in lipid metabolism in rat kidney and liver slices. Since the major lipid constituent of cell membrane structure is phospholipid, a decrease could lead to malfunction of the cell membrane, which may result in lipid peroxidation or enhanced leakage of intracellular materials such as cytosolic enzymes (e.g. LDH). However, it is of interest to observe a decrease in phospholipid components in the kidney without lipid peroxidation. Thus, the abnormal renal lipid changes in the kidney may be due to the observed nephrotoxic effect of atractyloside exhibited by the leakage of enzymes and alterations in the toxicant frame of other parameters.

The kidney (especially the proximal tubule) and the liver rely most exclusively on oxidative phosphorylation for ATP synthesis, which is inhibited by atractyloside

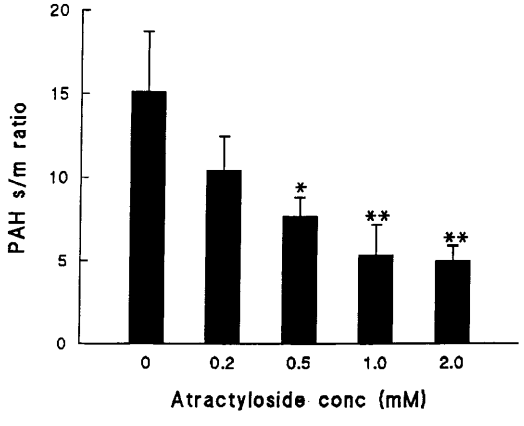

Fig. 6 Effects of atractyloside on uptake of *p*-aminohippurate (PAH) in kidney slices. PAH uptake was expressed as the slice-to-medium (S/M) ratio, where S represents the concentration of PAH/g tissue in the slice, and M represents PAH/ml in medium. The data represent mean values ± SE of 3 experiments performed in quadruplicate. Significantly different from corresponding control: $*P < 0.05$, $**P < 0.01$

(Allman et al. 1967; Obatomi and Bach 1996b). This inhibitory effect of atractyloside on cellular energy was also observed in the present study. Both the kidney and liver ATP levels were depleted to the same extent. The changes in ATP levels following exposure to atractyloside closely resembled the pattern of changes obtained for the inhibition of gluconeogenesis, depletion of GSH and LDH leakage. A decline in ATP levels can be critical for all cellular oxidative process; the decline observed in this study may therefore be a common event in the cause of atractyloside toxicity, a situation that usually precedes the irreversible stages of cell injury.

Functional assessments of a variety of organic ions is a key function of the kidney. This process could easily be altered by a toxicant which competes for such transport processes. The targeting of atractyloside for the proximal tubular cells (Luciani et al. 1978; Koechel and Krejci 1993) suggests a localized transport process, which facilitates the entry of this compound selectively into these cells. The presence of a sulphated glycoside with an aglycone moiety suggests that atractyloside may undergo anion transport. Our data showed that atractyloside inhibits the anion (PAH) uptake in a dose and time dependent manner. This invariably suggests that atractyloside may be exerting its toxic effect by interfering with this transport process in the kidney.

We have shown that in vitro exposure of kidney and liver slices to atractyloside resulted in toxicity to both organs. Evidence of toxicity was shown at the atractyloside concentration of ≥200 μM. The sensitivity of pig tissues to atractyloside as observed in this study is greater than the previously reported effect of atractyloside in rat (Thanh et al. 1997) or dog (Koechel and Krejci 1993) tissues. This study has also shown that cytotoxicity occurs subsequent to renal depletion of GSH, inhibition of PAH uptake and depletion of ATP with no alteration in lipid peroxidation. In the liver, cytotoxicity occurs concomitantly with depletion of GSH, ATP and increased lipid peroxidation. Atractyloside toxicity is also accompanied by alterations of membrane integrity and permeability leading to leakage of enzymes (especially LDH) in both kidney and liver tissues.

Although atractyloside affects gluconeogenesis similarly in both tissues, only kidney phospholipid components were significantly altered in the presence of atractyloside. Thus, it is concluded that atractyloside

exhibits separate mechanisms of toxicity in the liver and kidney. In addition, as no information is available on the biokinetics and accumulation of atractyloside in tissues, direct exposure of kidney and liver tissues to atractyloside in vitro has helped in obtaining information on the minimum concentration of this compound that may be likely to produce or elicit toxicity in vivo. Use of the tissue slice system has proved to be useful for studying and establishing relative toxicity and metabolic changes caused by atractyloside in kidney and liver of the pig under identical culture conditions. Due to the physiological similarities of the tissues of pig and man, the model and data obtained could be more relevant to man than data obtained using other animals.

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