Lipid profiling in renal and hepatic tissue slices following exposure to atractyloside.

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The diterpene glycoside, atractyloside (ATR) is present in a number of plants used as popular medicines throughout Africa and along the Mediterranean. These plants cause fatal intoxication in a significant number of individuals [1-3], especially children. Clinical data from humans poisoned by these plants and experimental studies in animals treated with ATR have shown an acute cellular necrosis, largely confined to the proximal tubule of the kidney [4] and centrilobular portion of the liver [5]. The main target of ATR is thought to be the mitochondria, where it inhibits the adenine nucleotide translocase and thus cellular energy production [6]. ATR also alters catabolic and anabolic functions *in vivo* [2], and although several clinical abnormalities have been reported details of the underlying metabolic changes have not been investigated or studied *in vitro*. The present study is part of a long-term interest in using *in vitro* systems to help understand the toxic effects and alterations in intermediary metabolism caused by diterpenoids. We have examined renal and liver tissue slices exposed to ATR (for relatively short periods) and used high performance thin layer chromatography (HPTLC) with densitometry to study changes in cholesterol, total phospholipid and triacyglycerol profiles.

Precision-cut renal cortical and liver slices (200 μm thick) were made from cylindrical cores of rat kidney (8 mm diameter) and liver (10 mm diameter) tissues using a Krumdieck tissue slicer [7] containing pH 7.4, 150 mM-phosphate buffered saline at 4°C. Slices were preincubated in media to allow the tissue to stabilise and then used for investigation. Individual slices were placed in a 24-well plate (Linbro, UK) containing ATR (0.2-2.0 mM) dissolved in 1 ml serum-free, phenol-red-free Dulbecco Modified Eagles nutrient mixture: F-12 Ham. This was incubated for 3 h at 37°C on an orbital shaking platform (70 cycle/min). At the end of the incubation period, slices were removed, blotted, weighed, homogenized and extracted with 1 ml chloroform:methanol (2:1, containing 0.1% w/v butylated hydroxytoluene). The lipid profile was assessed in 5 μl of tissue extract, delivered under N_2 by a Camag Linomat IV applicator to Merck silica gel HPTLC plates (10 x 20 cm). The plates were developed with petroleum ether:diethyl ether:acetic acid, 80:20:3 by volume in a Camag horizontal developing chamber. Separated bands were stained using the manganese chloride-sulphuric acid reagent [8] heated in an oven at 110°C for 40 min. The pinkish coloured bands appeared on a whitish background and were quantified using a Desaga CD-60 densitometer with a Hg lamp, scanned at an optimized wavelength of 250 nm, and using a slit width of 0.02 mm and 3 mm in height. Commercially available cholesterol, phospholipid and triacyglycerol standards (Sigma, Poole) were processed and used to calibrate the system.

ATR caused a significant and dose-related decrease of triacylglycerol in liver slices, but not in kidney slices (Table 1). By contrast ATR increased phospholipids and cholesterol in renal, but had no effects in hepatic tissue. These data show that intermediary metabolism can be affected by ATR in both kidney and liver slices within a relatively short period.

The decrease in liver triacylglycerol by ATR suggests a depletion in one of the vital cellular energy sources, as much of the energy to be derived from lipids relates to the metabolism of the fatty acid chains of triacylglycerol. This, in addition to the usual hypoglycaemic effect of ATR [2], could account for the associated decline in liver function [5] following exposure to ATR.

Table 1. Effect of varying concentrations of ATR on the lipid profile in rat kidney and liver slices incubated for 3 hours.

Tissue	ATR (mM)	PLs	Chol (ng/mg tissue protein)	TAG
Kidney	0.0	972.4 ± 47.8	164.7 ± 8.5	25.0 ± 0.3
	0.2	1267.8 ± 41.7#	215.9 ± 8.9*	21.5 ± 3.8
	0.5	1264.4 ± 8.1**	207.2 ± 11.7*	28.9 ± 0.8
	1.0	1198.3 ± 36.7	208.3 ± 2.3*	24.6 ± 1.2
	2.0	1206.7 ± 56.6	242.6 ± 3.6 #	24.0 ± 1.8
Liver	0.0	708.8 ± 37.5	75.1 ± 9.4	203.2 ± 13.5
	0.2	750.2 ± 0.9	84.3 ± 9.5	184.1 ± 13.6
	0.5	692.1 ± 28.4	62.7 ± 5.6	136.1 ± 21.1*
	1.0	665.2 ± 34.7	79.9 ± 17.1	132.7 ± 12.2*
	2.0	600.1 ± 3.2	64.0 ± 6.8	124.6 ± 3.9#

Each values represents mean ± S.E.M. for 3 separate experiments.
* $P < 0.05$; ** $P < 0.01$; # $P < 0.001$ compared to the respective control tissue.
PLs = phospholipids; Chol = cholesterol; TAG = triacylglycerol.

There were no changes in triacylglycerols in the kidney slices under these conditions. This suggests that there are fundamental differences in the way the kidney and liver respond to ATR. This is supported by the observation that kidney phospholipid and cholesterol were significantly increased by ATR in a dose-related manner, but liver was not affected (Table 1). The increased total phospholipids and cholesterol may be explained by the crucial roles these molecules play in the maintenance of membrane integrity. A significant alteration of these components may likely affect the membrane dynamism, permeability and functions. It is therefore possible that part of the selective toxicity of ATR resulting from the altered membrane permeability which only affects proximal tubular cells, but not the glomerular [9] may be due to the increased in phospholipids and cholesterol.

In conclusion, our findings show that different ATR-related metabolic alterations occur in the rat liver and kidney slices. This suggests different effects on each of these tissues. These data also raise the possibility that the mechanistic basis of lipid metabolic changes in these tissues following ATR exposure may be relevant to target selective toxicity.

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