Dichloroacetate treatment resulted in altered phospholipid metabolism and compromised tumour bioenergetics in human colon carcinoma xenografts

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INTRODUCTION: Dichloroacetate (DCA) is a pyruvate dehydrogenase kinase (PDK) inhibitor and is found to be an anti-cancer agent [1]. PDK is a negative regulator of pyruvate dehydrogenase (PDH). PDH is the key mitochondrial enzyme that determines whether pyruvate formed during glycolysis from glucose will be metabolised to lactate or oxidised in the TCA cycle. Treatment with DCA decreases proliferation and growth in cancer cells and solid tumours (1).

AIM: To develop a non-invasive and robust pharmacodynamic biomarker for tumour response following PDK inhibition.

METHODS: HT29 human colon carcinoma xenografts were grown subcutaneously in NCr nude mice. Once a tumour size of ~500mm^3 was established, mice were treated with oral DCA (200 mg/kg) or with vehicle (saline) alone for 4 days. *In vivo* localised PRESS ^{1}H-MRS (TE = 136ms and TR = 4s) and ISIS ^{31}P-MRS (TR = 2s) of the tumours were carried out on day 0 (before treatment) and day 4 on a 7T Bruker MR system. After the *in vivo* study, tumours were freeze-clamped and neutralised extracts made. *In vitro* ^{1}H- and ^{31}P-MRS were performed on the tumour extracts on a Bruker 500MHz MR system. Two-tailed paired and unpaired t-tests were used to analyse *in vivo* and *in vitro* MR measurements, respectively.

RESULTS AND DISCUSSION: Significant tumour growth inhibition ($p<0.001$) was observed in HT29 xenografts after 4 days of DCA treatment when compared with vehicle-treated controls. *In vivo* ^{1}H- and ^{31}P-MRS spectra of an HT29 tumour before and after DCA treatment are shown in Fig. 1. *In vivo,* significant decreases in the ratios of phosphomonoesters/total phosphorus signal (P=0.01) and total choline/water signal (P=0.05) were found in DCA-treated HT29 xenografts. The *in vivo* results were confirmed by significantly lower phosphocholine (PC) (P=0.02), glycerophosphocholine (P=0.01) and glycerophosphoethanolamine (P=0.05) levels, as found by ^{31}P-MRS of tumour extracts from DCA-treated animals when compared with controls (Table 1). Increases in leucine (P=0.04), iso-leucine (P=0.02), valine (P=0.04) and succinate levels (P=0.03) and lower NTP (P=0.003), glucose (P=0.03) and creatine (P=0.05) levels were also found in DCA-treated HT29 tumour extracts when compared with controls (Table 1).

DISCUSSION: The drop in PC and other choline-related metabolites following DCA treatment is probably due to tumour stasis caused by the PDK inhibition, since DCA upregulates the mitochondrial membrane potential, induces apoptosis, decreases proliferation and inhibits tumour growth (1). These and the non-phospholipid changes including lower NTP are consistent with previous findings where similar metabolic profiles were associated with antiangiogenic effects induced by LAQ824 (2).

CONCLUSIONS: Inhibition of PDK by DCA resulted in altered phospholipid metabolism and compromised tumour bioenergetics. PC and phosphomonoesters decreases and metabolic changes associated with compromised tumour bioenergetics may have potential as surrogate non-invasive markers for determining tumour response following treatment with DCA or other PDK inhibitors.

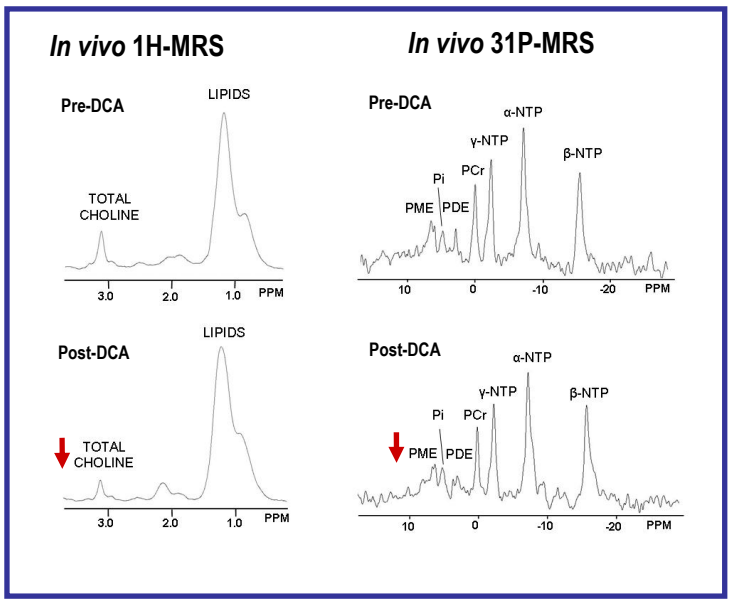

Table 1: *In vitro* 1H- and 31P-MRS of vehicle- and DCA-treated HT29 tumor extracts

Metabolites (µmol/g w.wt)	Vehicle (n = 5)	DCA (n = 6)	*P* =
PC	1.88 ± 0.16	1.27 ± 0.15	0.02
GPE	1.04 ± 0.14	0.68 ± 0.09	0.05
GPC	2.40 ± 0.39	1.16 ± 0.19	0.01
NTP	0.45 ± 0.04	0.22 ± 0.03	0.002
Leucine	0.24 ± 0.07	0.42 ± 0.04	0.04
Iso-Leucine	0.08 ± 0.02	0.24 ± 0.05	0.02
Valine	0.23 ± 0.04	0.38 ± 0.04	0.04
Creatine	3.12 ± 0.39	2.11 ± 0.25	0.05
Glucose	0.73 ± 0.11	0.32 ± 0.10	0.03

Data are expressed as Mean ± SEM. Two-tailed unpaired t-test was used to compare changes between the vehicle- and DCA-treated groups.

Fig. 1: *In vivo* ^{1}H- and ^{31}P-MRS spectra of an HT29 tumour before and after DCA treatment (200mg/kg p.o. for 4 days). Peak assignments: phosphomonoesters (PME), phosphodiesters (PDE), inorganic phosphate (Pi), phosphocreatine (PCr), nucleoside triphosphates (α-, β-, γ-NTP).

1) Bonnet et al., Cancer Cell 11, 37-51 (2007). 2) Chung et al., Neoplasia 10: 303-313 (2008).

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