31P and 1H MRS studies of choline kinase inhibitor MN58b in a colon carcinoma model

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INTRODUCTION: Choline kinase (ChoK) is a cytosolic enzyme that catalyses the phosphorylation of choline to form phosphocholine (PC), which is involved in cell membrane synthesis. Elevated levels of PC and ChoK found in tumours are associated with cell proliferation and malignant transformation. Inhibition of ChoK with MN58b, a novel anticancer drug and putative competitive inhibitor, demonstrated an antiproliferative effect in human tumour xenografts (1). The aims of this work were: a) to confirm the mechanism of action of MN58b; and b) to develop a robust and non-invasive surrogate marker for tumour response following MN58b treatment.

EXPERIMENTAL METHODS:

Animal Model: Human colon (HT29) xenografts were grown subcutaneously in MF1 nude mice. Once a tumour size of ~500mg was established, mice were randomly divided into 2 groups. 1 group was treated with MN58b (4mg/kg i.p. once a day for 5 days) and 1 group was treated with saline alone. *In vivo* ^{31}P and ^{1}H MRS of the tumours was carried out on day 1 (before treatment) and on day 6.

In vivo ^{31}P MRS: ISIS localised ^{31}P MR spectra were obtained at 37^{o}C on a Varian 4.7T spectrometer with a 12mm 2-turn surface coil. Spectra were quantified using VARPRO.

In vivo ^{1}H MRS: PRESS localised ^{1}H MR spectra with water suppression was used to detect choline which was then quantified (2).

In vitro ^{1}H and ^{31}P MRS: After the final *in vivo* MRS studies, tumours were freeze-clamped and extracted either for *in vitro* ^{31}P MRS studies or for ChoK activity and western blots for ChoK expression.

RESULTS: A significant growth delay was observed in the MN58b-treated HT29 xenografts when compared with controls. *In vivo* ^{31}P and ^{1}H MRS of the HT29 xenografts showed a decrease in the phosphomonoester/total phosphorus signals (PME/TotP) ($p<0.05$) ratio and total choline concentration ($p<0.01$) after 5 days of MN58b treatment (Table 1). No significant changes were observed in the control group. *In vitro* ^{31}P MRS of extracts from HT29-treated tumours showed significant decreases in PC ($p<0.03$) when compared with controls (Table 2). No changes in other phospholipid metabolites (phosphoethanolamine (PE), glycerophosphocholine (GPC) and glycerophosphoethanolamine(GPE)) were observed. No significant changes in ChoK activity or expression were found in extracts from MN58b-treated tumours when compared with control. This is consistent with MN58b being a competitive inhibitor of ChoK.

DISCUSSION: Treatment with MN58b resulted in tumour growth delay and altered phospholipid metabolism *in vivo*. These MRS changes suggest inhibition of ChoK and are consistent with the mechanism of action of MN58b. The decrease of total choline, PC and PME may have potential as surrogate non-invasive pharmacodynamic markers for determining tumour response following treatment with MN58b or other ChoK inhibitors.

Table 1. *In vivo* ^{31}P and ^{1}H MRS of HT29 tumors pre- and post- MN58b treatment.

Metabolite ratio	Pre-MN58b	Post-MN58b
	^{31}P-MRS (N = 7)	
PME/TotP	0.21± 0.02	0.17 ± 0.02*
β-NTP/TotP	0.18 ± 0.01	0.20 ± 0.01
Pi/TotP	0.08 ± 0.01	0.09± 0.02
	^{1}H-MRS (N =5)	
Total Choline (mM)	10.11 ± 0.88	7.61 ± 0.60*

Table 2. *In vitro* ^{31}P MRS of vehicle and MN58b-treated HT29 tumor extracts.

Metabolites (μmol/g w.wt)	Vehicle (N = 10)	MN58b (N = 9)
PE	1.40 ± 0.13	1.55 ± 0.12
PC	2.02 ± 0.25	1.37 ± 0.08*
GPE	0.99 ± 0.06	0.91 ± 0.07
GPC	1.77 ± 0.18	1.60 ± 0.18

* Statistically significant from control, $p < 0.05$. Data expressed as Mean ± S.E.M.

1. R Hernandez-Alcoceba, et al. Cancer Res 59: 3112-3118 (1999). 2. B Madhu et al. ISMRM 11: 1287 (2003).

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