The Pharmacodynamic effects of the novel anticancer drug 17-AAG on HT29 xenografts in mice monitored by Magnetic Resonance Spectroscopy

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ABSTRACT:- We monitored the action of 17-AAG, a new anti-cancer drug that inhibits HSP90, in HT29 xenografts. *In vivo* ^{31}P MRS showed reduction in the β-NTP/TotP ratio and elevation in the PME/PDE, PME/TotP and PME/β-NTP ratios post-treatment. No significant changes were observed in the control group. *In vitro* ^{1}H and ^{31}P MRS of the extracts from 17-AAG-treated tumours showed an increase in PC, PE and valine levels when compared with controls. Western blots showed induction of HSP70 and reduced RAF1 in the treatment group, confirming the expected drug action. Inhibition of HSP90 resulted in altered tumour bioenergetics and phospholipid membrane metabolism.

INTRODUCTION:- 17-AAG is an inhibitor of heat shock protein 90 (HSP90), a chaperone which ensures the correct folding of several proteins involved in tumour growth regulation (RAF1, CDK2, ERB2 and mutant P53). Inhibition of HSP90 results in degradation of oncogenic client proteins by proteosomal degradation. We have used *in vivo* ^{31}P MRS: a) to study the efficacy of 17-AAG on HT29 xenografts, b) to examine the MR changes that are associated with specific therapeutic targets (HSP90 inhibition in this case) and c) to determine if ^{31}P MRS can provide a useful non-invasive surrogate marker of tumour response in clinical trials.

EXPERIMENTAL METHODS:-

Animal Model: Human colon (HT29) xenografts were grown subcutaneously in MF1 nude mice. Tumour volume was calculated by measuring length, width and depth using calipers and the formula: $l*w*d*(\pi/6)$. Once a tumour size of ~500mg (484±36 mg) was established, mice were randomly divided into 2 groups. 14 mice were treated with 17-AAG (80mg/kg i.p. once a day for 4 days) and 5 controls with vehicle alone.

In vivo ^{31}P MRS: ^{31}P MRS of the tumours was carried out on day 1 (before treatment) and day 5. ISIS localised ^{31}P MR spectra were obtained at 37 °C on a Varian 4.7T spectrometer with a 12mm 2-turn surface coil. Spectra were quantified using VARPRO.

In vitro ^{1}H and ^{31}P MRS: After the final ^{31}P MRS study, tumours were freeze-clamped. ^{1}H and ^{31}P MRS and Western blots for RAF1 and HSP70 were carried out to complement the *in vivo* MR data. ^{1}H and ^{31}P MRS of the extracts were performed on a 500 MHz Bruker system.

RESULTS:- 17-AAG treatment for 4 days caused tumours to decrease in size by 4%, while control tumours increased by 16%, demonstrating a clear anti-tumour effect. Western blots showed induction of HSP70 (which indicated an inhibition of HSP90) and reduced RAF1 in the 17-AAG treated group, confirming the expected action of the drug.

In vivo ^{31}P MRS of the treatment group showed a significant reduction in the β-nucleoside triphosphate/total phosphorus signal (β-NTP/TotP) ratio. Significant increases in the phospho-monoester/phospho-diester (PME/PDE), PME/TotP and PME/β-NTP ratios were also observed post-treatment (Table 1). No significant changes were found in the vehicle group.

Table 1: *In vivo* ^{31}P MRS of tumours pre and post 17-AAG

(n = 14)	Pre 17-AAG	Post 17-AAG	$p <$
PME/Total P	0.15 ± 0.01	0.18 ± 0.01	0.05*
β-NTP/Total P	0.15 ± 0.01	0.13 ± 0.01	0.03*
PME/β-NTP	1.04 ± 0.05	1.52 ± 0.17	0.03*
PME/PDE	1.35 ± 0.12	2.16 ± 0.34	0.02*
pHi	7.03 ± 0.03	7.11 ± 0.02	0.10

Data are expressed as Mean ± S.E.M. * statistically different.

In vitro ^{31}P MRS of the extracts showed an elevated level of phosphoethanolamine (PE) and phosphocholine (PC) in the 17-AAG treated group when compared with vehicle controls (Figure Ia, Table 2).

Table 2: *In vitro* ^{31}P MRS of tumour extracts

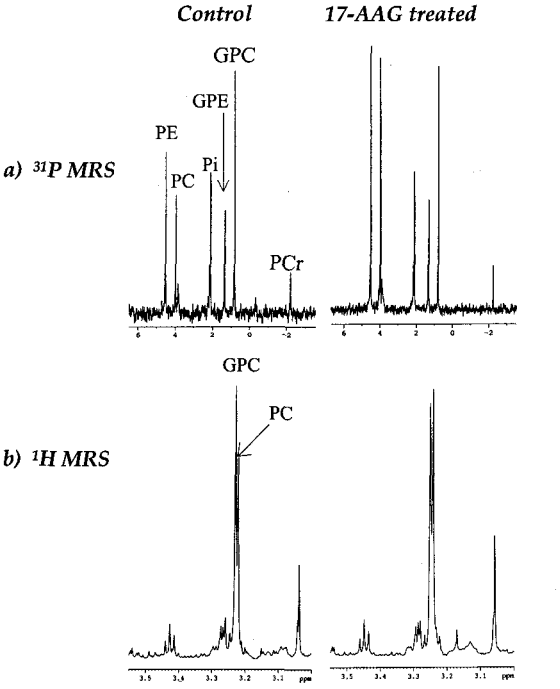
Metabolite (μmol/g w.wt)	17-AAG (n = 11)	Vehicle (n = 9)	$p <$
PE	0.70 ± 0.05	0.51 ± 0.05	0.02*
PC	0.59 ± 0.04	0.45 ± 0.06	0.08
GPE	0.42 ± 0.04	0.40 ± 0.03	NS
GPC	0.80 ± 0.10	0.79 ± 0.07	NS

^{1}H MRS of the tumour extracts from the 17-AAG-treated mice showed a significant increase in the phosphocholine/glycero-phosphocholine (PC/GPC) ratio and the levels of PC and valine when compared with vehicle-controls (Figure 1b, Table 3).

Table 3: *In vitro* ^{1}H MRS of tumour extracts

Metabolite (μmol/g w.wt)	17-AAG (n = 11)	Vehicle (n = 9)	$p <$
PC	1.66 ± 0.18	1.23 ± 0.06	0.04*
GPC	1.49 ± 0.21	1.55 ± 0.10	NS
PC/GPC	1.15 ± 0.07	0.81 ± 0.02	0.001*
Valine	0.24 ± 0.03	0.14 ± 0.01	0.003*

Figure 1: *In vitro* ^{31}P and ^{1}H MRS of the tumour extracts



Keys: PE (phosphoethanolamine), PC (phosphocholine), Pi (inorganic phosphate), GPE (glycero-phosphoethanolamine), GPC (glycero-phosphocholine), PCr (phosphocreatine)

DISCUSSION:- This study showed that 17-AAG inhibited HSP90 and altered the tumor bioenergetics and phospholipid membrane metabolism, as well as decreasing tumour growth rate. Monitoring the pharmacodynamic effects of 17-AAG by MRS may provide a non-invasive surrogate marker of tumour response in clinical trials.

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