PI3K inhibition using a novel inhibitor deregulates choline kinase resulting in PC depletion detected by MRS

N. M. Al-Saffar[1], L. E. Jackson[1], F. Raynaud[2], A. Ramírez de Molina[3], J. C. Lacal[3], P. Workman[2], and M. O. Leach[1]

[1]CR-UK Clinical MR Research Group, The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust, Sutton, Surrey, United Kingdom, [2]CR-UK Centre for Cancer Therapeutics, The Institute of Cancer Research, Sutton, Surrey, United Kingdom, [3]Department of Molecular and Cellular Biology of Cancer, Instituto de Investigaciones Biomédicas, Madrid, Spain

Introduction

The phosphoinositide 3-kinase (PI3K) pathway plays an important role as a regulator of mammalian cell proliferation and survival. This pathway is deregulated in a variety of cancers, providing convincing evidence for the potential benefit from developing PI3K inhibitors for the treatment of cancer (1). The rational development of new molecular cancer therapeutics requires the discovery and validation of biomarkers of drug action (2). The development of surgically non-invasive endpoints is particularly important because it avoids the need for tumor biopsy. The aims of this work are: first, to use MRS to identify biomarkers for PI3K pathway inhibition. Second, to investigate mechanisms underlying the detected changes. Using MRS we have monitored response to PI103, a novel class 1A PI3K inhibitor that was developed in a collaboration between The Institute of Cancer Research, Ludwig Institute for Cancer Research, Cancer Research UK London Centre, and the Yamanouchi Pharmaceutical Company (3). PI103 inhibits the proliferation and invasion of human cancer cells both *in vitro* and *in vivo* (3). In the human prostate cell line PC3, a dose- and time-dependent drop in PC, PE and NTP levels was observed by ^{31}P MRS. The drop in PC correlated with choline kinase (ChoK) depletion, indicting that ChoK deregulation following PI3K inhibition is the mechanism underlying PC drop detected by MRS.

Materials & Methods

The human prostate carcinoma cell line PC3 which has an activated PI3K-Akt pathway via loss of PTEN was used. A dose response experiment with PI103 at 1x, 2.5x, 5x and 10x IC_{50} (IC_{50} = 100 nM) for 24h, and a time course treatment at 5x IC_{50} for 2, 4, 8, 12, 24 and 48h were performed. PI3K pathway inhibition was verified using Western blotting for P-Akt (Ser^{473}). Adherent Control and PI103-treated cells ($5x10^7$) were extracted using the DPE method and the aqueous fractions were analyzed by ^{31}P MRS at room temperature on a 500 MHz Bruker spectrometer using a 30° flip angle and a 1s relaxation delay. Metabolite levels were corrected for saturation and cell number. Levels of ChoK were measured by Western blotting using a monoclonal antibody against human ChoK (4) and were quantified by densitometry. Results represent (average ± SD, n ≥ 3).

Results & Discussion

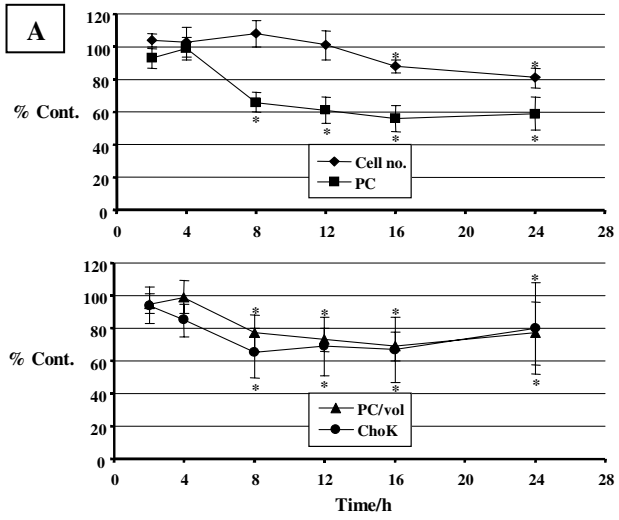
The number of PI103-treated PC3 cells was significantly reduced to 80% relative to controls at concentrations of 5xIC_{50} and 10xIC_{50} (500 & 1000nM, 24h). At 5xIC_{50}, a decrease to 88%, 81% and 55% in treated cells compared to controls was observed at 16, 24 and 48h respectively, consistent with decreased proliferation (Fig. 1A). PI103 caused a decrease in P-Akt, providing molecular evidence for PI3K inhibition. A minimum concentration of 2.5xIC_{50} of PI103 was required to deplete P-Akt levels at 24h, while P-Akt was depleted as early as 2h following treatment with PI103 (5xIC50). PI103 also depleted levels of P-p70S6K, P-S6, P-GSK-3 and CyclinD1. Cell cycle distribution by flow cytometry was performed to further characterize the effects of PI103 on PC3 cells. As indicated by cyclinD1 depletion, a 24h exposure at 2.5xIC_{50} caused an increase in G1 cellular population and a decrease in S phase. At 5xIC_{50}, a G1 arrest was detected from 12h post treatment. MRS was used to identify metabolic changes following PI3K inhibition by PI103. ^{31}P MRS showed that PI103 treatment for 24h led to a significant concentration-dependent decrease in PC levels with a decrease to 68±11%, P=0.01 at 2.5xIC_{50}. At 5xIC50, PI103 also caused a time-dependent drop in PC, PE and NTP levels which started as early as 8h (PC, 80±12%, P=0.01), (PE, 62±21%, P=0.02), (NTP, 76±5%, P=0.002) and was down to 59±10%, 60±23%, and 72±8% (P<0.0001) respectively at 24h relative to controls (Fig. 1A&B). PI3K is well known to control cell size. This is particularly important for MRS mesurements of cellular metabolite levels as cells will adjust the rate of metabolic processess, for example membrane phospholipid metabolism, in response to changes in cellular size. Hence we carried out cell volume measurements using flow cytometry on samples from the experiments analysed by MRS in order to find out whether our MRS detected changes are caused by PI103 affecting cell size. PI103 caused a 10 and 20% decrease in cell volume at 8h and up to 24h respectively compared to controls. Controlling for cell size (Fig 1A) reduces the drop in PC, but a significant drop in PC is still seen, that is independent of changes in cell size, reflecting the direct affects of PI3K inhibition. To further investigate mechanisms underlying MRS detected PC depletion, we have performed Western blotting on samples from the same experiments, using antibody against human ChoK, the enzyme responsible for the generation of PC from its precursor choline. Our results (Fig. 1A) showed that PI103 causes a decrease in ChoK expression which is time dependent and correlates very well with the drop in PC levels (Fig. 1A), indicating that ChoK inhibition is indeed one of the mechanisms causing the MRS detected drop in PC levels following PI3K inhibition.

Conclusions

Using ^{31}P MRS, we have detected a decrease in PC, PE and NTP levels following inhibition of PI3K pathway by the novel inhibitor PI103 in the human prostate cell line PC3. This decrease is both dose- and time-dependent and correlates with the decrease in P-Akt. PI103 also depleted levels of ChoK, the enzyme responsible for PC synthesis, indicating that ChoK is likely to be the mechanism underlying the drop in PC following inhibition of PI3K pathway. Monitoring the pharmacodynamic effects by MRS may provide a non-invasive biomarker(s) for PI3K inhibition and potentially of tumor response in solid tumors in clinical trials.

Acknowledgments This work is supported by Cancer Research UK (grant no. C1060/A5117) and the AICR (grant no. 03-304).

References (1) Vivanco I. et al. Nature Reviews-Cancer (2002) *2*: 489.
(2) Workman P. EJC (2002) *38*: 2189.
(3) Workman P. Biochemical Society Transactions (2004) *32*: 393.
(4) Gallego-Ortega D. Int J Oncol (2006) 29(2): 335.



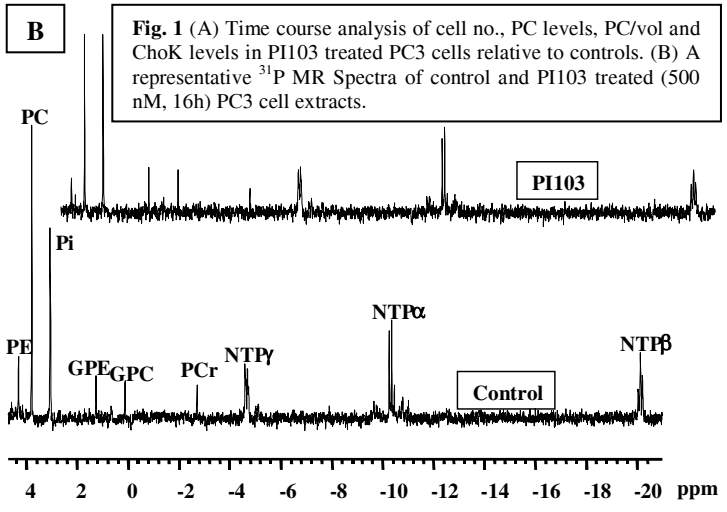


Fig. 1 (A) Time course analysis of cell no., PC levels, PC/vol and ChoK levels in PI103 treated PC3 cells relative to controls. (B) A representative ^{31}P MR Spectra of control and PI103 treated (500 nM, 16h) PC3 cell extracts.