In vivo pH measurements by 31P MRS in subcutaneous melanoma tumors in SCID mice: effect of proton pump inhibitors

R. Canese[1], F. Podo[1], A. De Milito[2], E. Iessi[2], M. Spada[1], A. Cesolini[1], and S. Fais[2]

[1]Cell Biology and Neurosciences, Istituto Superiore di Sanità, Rome, Italy, [2]Drug Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy

Introduction Tumour microenvironment may play a key role in tumour malignancy. In particular, acidity has been shown to have a role in resistance to chemotherapy, proliferation and tumour progression[1]. It has been recently shown that proton pump inhibitors (PPI), currently used in the antiacid treatment of peptic diseases, can inhibit the acidification of tumour microenvironment and increase tumour cells sensitivity to cytotoxic drugs [2]. Magnetic resonance spectroscopy (MRS) and spectroscopic imaging (MRSI) offer the most powerful approaches today available to non-invasively measure extracellular pH (pHe) and intracellular/extracellular pH gradient (ΔpH) in intact cancer cells and tissues [3,4].

Aim Aim of this work was to evaluate by in vivo ^{31}P MRS pHe and intracellular pH (pHi) in human melanoma xenografts implanted in SCID mice and their alterations following treatment with PPIs (omeprazole, esomeprazole).

Methods The pHe value was measured from the chemical shift difference between the exogenous cell-impermeant ^{31}P reporter 3-aminopropyl phosphonate (3-APP) resonance and that of α-ATP at -7.57 ppm. pHi was measured from the chemical shift difference between Pi and α-ATP. In vivo solid tumours were obtained by s.c. injection of 5 x 10^6 human melanoma cells in the dorsum of female SCID/SCID mice aged 4-5 weeks. Once the tumour became larger than 600 µl PPI was orally administered by gavage. The 3-APP probe was administered i.p. at the dose of 128 mg/kg immediately prior to MRI/MRS analyses. Animals underwent MRI/MRS analysis at different times, ranging from 2 to 48 h, after PPI administration (2.5 or 62.5 mk/kg by gavage).

A Varian Inova 200/183 MRI/MRS system for small animals operating at 4.7 T was used for MRI/MRS analysis. A 1cm two-turn or a 2cm three-turn ^{31}P surface coil were used in combination with a volume (6 cm diameter) ^{1}H coil for shimming and positioning of the VOI for localised MR spectroscopy. T1-weighted GE multislice contiguous images (TR/TE=123/4.3 ms, α= 20°, thickness=2mm, 8 averages, FOV= 3x3 cm^2) were acquired to localise the tumour. ^{1}H localised spectra were used to optimise the shim values and therefore to increase the signal resolution within the tumour (^{1}H PRESS, TR/TE=2000/23ms). ^{31}P localised spectra were acquired from the tumour with ISIS (TR/TM= 2000/80 ms, 2048 averages, VOI ranging from 300 to 400 µl). In the animals who underwent repeated MRS analyses, in order to reduce the duration of each MRS examination and therefore the time of anaesthesia, a ^{1}H-decoupled pulse-acquire sequence was used (TR= 3000 ms, α=25°, garp, 256 averages).

Results The S/N ratio and the resolution of both in vivo global and localised spectra were sufficient to determine pHe and pHi values in melanoma tumor xenografts. A pHe shift towards alcaline values was measured in vivo in the tumours treated with PPI 3-5 h before MRS examinations, as shown in Table 1. No effect was instead observed in tumours treated with the same PPI doses 48 h after treatment. One way ANOVA and post-hoc analysis (Bonferroni) showed that the differences between groups were statistically significant (P=0.016). The intracellular/extracellular Δph gradient progressively decreases as shown in Table 1 (P<0.001).

Discussion and conclusions Tumour pHe was clearly shifted towards alkaline values for each of the doses used in this pilot work. The effect is maximum at 3-4 h and no more visible at ≥ 48 h after treatment. The possibility to modulate the effectiveness of chemotherapy by manipulating tumour pH has been explored by several groups [5-7]. We will therefore continue our study by investigating on the correlations of tumour pH (pHe, pHi and ΔpH) with the effects of PPI (administered alone or in combination with cytotoxic drugs) on tumour growth and progression.

These exploratory investigations showed that in vivo ^{31}P MRS methods, coupled with the use of an appropriate cell-impermeant pH reporter such as 3-APP, allow simultaneous monitoring of both pHe and pHi in melanoma xenografts implanted in SCID mice. In vivo administration of PPI was capable to change tumour pHe and pHi values in a dose and timing dependent manner.

Table 1. pHe, pHi and ΔpH in tumours measured by ^{31}P in vivo MRS 3-5 h with and without PPI treatment.

	Controls (n=10)	**PPI treated 2.5 mg/kg (n=5)**	**PPI treated 62.5 mg/kg (n=4)**
pHe	6.42 ± 0.20	6.67 ± 0.20	6.78 ± 0.23*
pHi	7.10 ± 0.23	6.91 ± 0.21	6.80 ± 0.30
ΔpH	0.68 ± 0.11	0.24 ± 0.28*	0.03 ± 0.31**

*p=0.05 ** p<0.001 vs controls

References

[1] De Milito et al. 2005, *Future Oncol* 1:779-786

[2] Luciani F et al. 2004, *J Natl Cancer Inst* 96:1702-1713

[3] Gillies et al.2004, *IEEE Eng Med Biol* 23: 57-64

[4] Raghunand N et al. 2006, *Methods Mol Med*.124: 347-64

[5] Raghunand N et al. 1999, *Br J Cancer*.80: 1005-15

[6] Mc Sheehy PMJ et al. 2005 Cancer Chemother Pharmacol 55: 117-128

[7] Matsubara T et al 2006 Anticancer Res 2006, 26: 187-194