

# Imaging of changes in p-Glycoprotein activity *in vivo* with $^{68}\text{Ga}$ -Schiff base derivatives

M. Fellner<sup>1</sup>, W. Dillenburger<sup>2</sup>, H-G. Buchholz<sup>3</sup>, M. Schreckenberger<sup>3</sup>, F. Renz<sup>4</sup>, F. Rösch<sup>1</sup>, O. Thews<sup>2</sup>

<sup>1</sup>Nuclear Institute, Johannes Gutenberg University of Mainz, D-55128 Mainz; <sup>2</sup>Institute of Physiology and Pathophysiology, University Medicine Mainz, D-55128 Mainz; <sup>3</sup>Department of Nuclear Medicine, University Hospital, D-55101 Mainz; <sup>4</sup>Institute of Inorganic Chemistry, Leibniz University of Hannover, D-30167 Hannover

## Objectives

P-glycoprotein (pGP) is an active drug transporter of the ABC family pumping a wide number of xenobiotics out of the cell (under ATP consumption). Since many tumours overexpress this transporter and several chemotherapeutics are substrates of the pGP, the cytotoxicity of these drugs is markedly reduced leading to a multidrug resistant phenotype of tumours. Recent studies showed that the metabolic microenvironment of tumour affects the functional pGP-activity. Especially an extracellular acidosis (pH 6.6) leads to more than doubling of the transport rate resulting in a reduced cytotoxicity of chemotherapeutics *in vitro* and *in vivo* [1]. In this mechanism, MAP kinases (p38 and ERK1/2) play an important role in the signal pathway.

With Gallium-68 Schiff base complexes [2], in particular a novel derivative  $^{68}\text{Ga}$ -MFL6.MZ, it became possible to visualize the functional activity of the pGP *in vivo* [3]. This compound allows the analysis of alterations of the tumour microenvironment (acidosis) as well as interrupting the signal pathway (inhibition of p38 and ERK1/2) on the pGP-transport activity non-invasively.

## Methods

The  $^{68}\text{Ge}/^{68}\text{Ga}$  generator provides the positron emitter Gallium-68 ( $T_{1/2} = 68$  min) as a relatively inexpensive source of a PET nuclide. Using a recently published purification method [4] the ligand MFL6.MZ was labelled in a fast and easy process, directly ready for injection. Tumours were induced by subcutaneous injection of R3327-AT1 cells (which express pGP functionally) into the hind foot dorsum of male Copenhagen rats. Tumours were used when they had reached a volume of 1-2 mL. Acidification of the tumour was achieved by injection of small amounts (20  $\mu\text{L}$ ) of lactic acid (0.222 mM) directly into the tumour. The same amount of sodium lactate was injected into the contralateral tumour and served as control. The MAP kinases were inhibited by intratumoural injection of SB203580 (p38) and U0126 (ERK1/2).

## Results

Acidifying the tumour led to a pronounced local reduction of the tracer accumulation in the tumour (Fig. 1-3). Since the tracer is a substrate of pGP, a reduced tissue concentration indicates a higher pGP transport activity. On average, the tracer concentration of acidified tumours was only 80% of controls.

In contrast, MAP kinase inhibitors lead to a reduced pGP transport rate which should result in a higher tracer accumulation in the tissue. Inhibition of p38 led to almost a doubling of the tracer activity as compared to the contralateral control tumour, whereas with the ERK1/2 inhibitor the concentration increased by 30%.

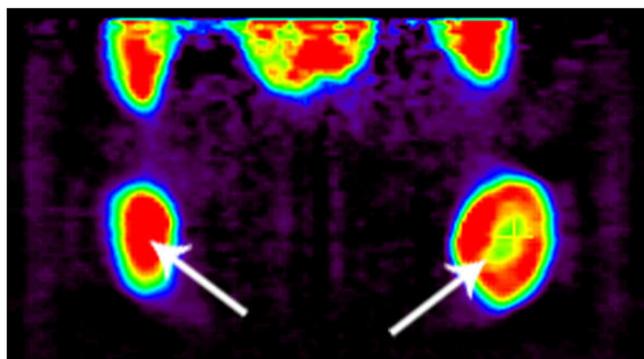


Fig. 1: coronal slice (acid injection in right tumour)

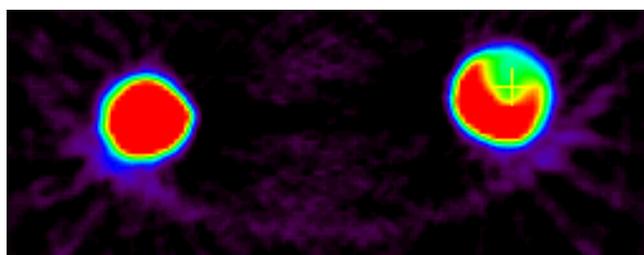


Fig. 2: transversal slice

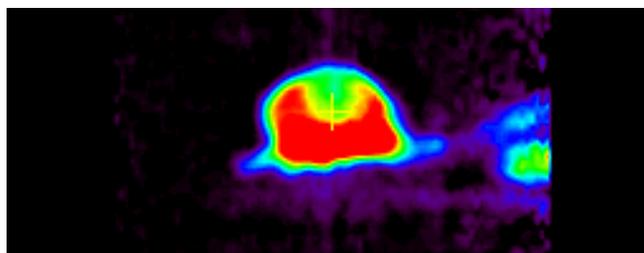


Fig. 3: sagittal slice

## Conclusions

The newly developed Schiff base derivative MFL6.MZ labelled with the positron emitter  $^{68}\text{Ga}$  allows a non-invasive monitoring of the functional pGP-activity in tumours. The results obtained with this new technique confirm previous *in vitro* studies that the transport rate in acidic tumour is markedly increased and that the MAP kinases p38 and ERK1/2 play a central role in the signalling pathway. The new tracer will be helpful in the development of new pGP-inhibitors in order to overcome multidrug resistance. In addition, this tracer will allow identifying patients overexpressing pGP, eventually needing a more aggressive treatment regime or other therapy modalities (e.g. radiotherapy).

## References

- [1] Sauvart et al, Int J Cancer 123: 2532-2542
- [2] Sharma et al, J Nucl Med 46: 354-364
- [3] Fellner et al, in preparation
- [4] Zhernosekov et al, J Nucl Med 48: 1741-1748