



## Contribution of glutathione and MRP-mediated efflux to intracellular oxaliplatin accumulation\*

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### Key words

oxaliplatin – efflux –  
MRP1 – MRP2 –  
resistance – glutathione

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### Introduction

Thirty years of experience using cisplatin in the treatment of cancer have resulted in a growing understanding of its mode of action and led to the successful development of second and third generation analogues. In contrast to the first clinically available compounds cisplatin and carboplatin which exhibit primary resistance against colorectal cancer, oxaliplatin is also effective in this tumor entity. However, some mechanisms associated with acquired resistance against oxaliplatin resemble those reported for cisplatin, including lower intracellular platinum accumulation [1]. Detoxification of platinum complexes by intracellular formation of platinum-glutathione (GSH) adducts and their subsequent efflux via the ABC transporters MRP1 and MRP2 contribute to a decreased accumulation and have been repeatedly suggested as resistance mechanism for cisplatin [2] but have also been the subject of controversy [3]. The present study focuses on oxaliplatin and its detoxification via GSH and MRP-mediated efflux. Electrospray ionization mass spectrometry (ESI-MS) was applied to detect oxaliplatin-GSH adducts formed after incubation of oxaliplatin with GSH. Gü83 (Figure 1), a 4-aminobenzoic acid derivative recently shown to inhibit MRP1 ( $IC_{50} = 1.2 \mu M$ ) and MRP2 ( $IC_{50} = 21.5 \mu M$ ) [4], was used to investigate the contribution of the transporters to oxaliplatin efflux in oxaliplatin-sensitive and oxaliplatin-resistant ileum carcinoma cells.

### Material and methods

#### Identification of platinum-GSH adducts

Oxaliplatin was incubated with GSH in a ratio of 1 : 10 (55  $\mu M$  oxaliplatin and 550  $\mu M$

GSH) in aqueous solution. A solution containing methanol (60%) and formic acid (1%) was added immediately or after a 12 h incubation time at 37 °C. Subsequently, electrospray ionization-mass spectrometry measurements were performed using an ESI-Q-qTOF (QSTAR XL; Applied Biosystems, Darmstadt, Germany) equipped with a nanospray ion source.

#### Cell culture

The human ileocecal colorectal adenocarcinoma cell line HCT8 and its oxaliplatin-resistant variant HCT8ox were kindly provided by Dr. R.A. Hilger, University of Essen, Germany. Cells were cultivated in RPMI-1640™ medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin (37 °C, 5% CO<sub>2</sub>).

#### Cellular platinum accumulation

To investigate the cellular platinum accumulation, cells were allowed to attach in 6-well plates for 12 – 14 hours and incubated with oxaliplatin (100  $\mu M$ ) and in some experiments additionally with Gü83 (100  $\mu M$ ). At certain time points, cells were harvested and platinum was quantified using flameless atomic absorption spectrometry (SpectrAA™ Zeeman 220; Varian, Darmstadt, Germany). The platinum concentration was related to the protein content determined by the bicinchonic acid (BCA) assay (Pierce, Rockford, IL, USA).

#### Statistical analysis

Statistical analysis was performed using Mann-Whitney test. P values of < 0.05 were considered statistically significant.

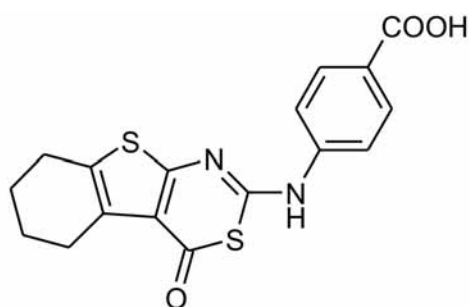


Figure 1. Chemical structure of Gü83.

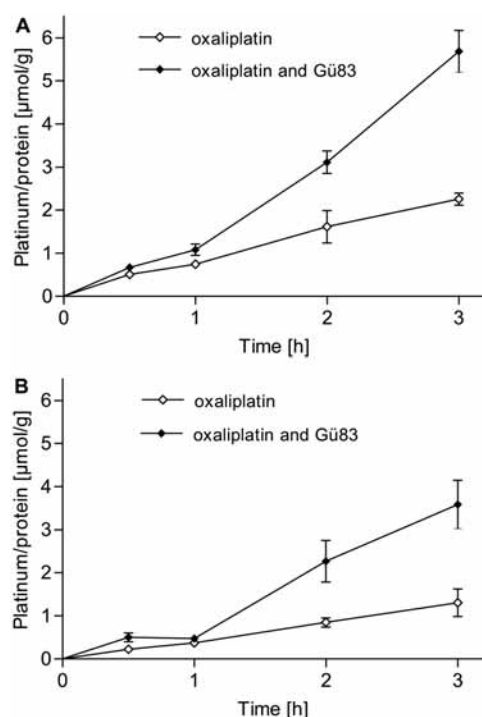


Figure 2. Cellular platinum accumulation after 100  $\mu\text{M}$  oxaliplatin alone and in the presence of 100  $\mu\text{M}$  Gü83 in A HCT8 and B HCT8ox cells (mean  $\pm$  SEM,  $n = 3$  in triplicate).

## Results and discussion

Following incubation of oxaliplatin with glutathione a 1 : 1 oxaliplatin-glutathione adduct ( $[\text{M}+\text{H}]^+ = 614.28$ ) was identified using ESI-MS. Most of this compound was bound to oxalic acid forming the molecule  $[\text{M}+\text{H}]^+ = 704.29$ . Fragmentation of the product resulted in elimination of oxalic acid and appearance of  $[\text{M}+\text{H}]^+ = 614.28$ . Formation of the adduct took place rapidly as it was found immediately after incubation of oxaliplatin with GSH. Since it was also detected after 12 h of incubation it can be considered as stable over this time period. The mass signal was identified in a cell-free environment but its presence is indicative of a potential intra-

cellularly formed adduct representing a possible substrate for MRP efflux pumps.

To investigate whether oxaliplatin or its intracellularly formed adducts are actually pumped out of the cell via MRP1 or MRP2, oxaliplatin accumulation was determined in HCT8 and HCT8ox cells after treatment with oxaliplatin alone and in the presence of the MRP inhibitor Gü83. After incubation with oxaliplatin, a statistically significant lower platinum accumulation was found in the resistant variant at all time points (0.5, 1, 2 and 3 h) suggesting that influx and/or efflux were altered (Figure 2). Co-incubation of the cells with the MRP inhibitor Gü83 (100  $\mu\text{M}$ ) led to an increase in the cellular platinum accumulation in the sensitive and resistant cells. After 2 h the platinum accumulation expressed as platinum per gram protein ( $\mu\text{mol/g}$ ) increased by 1.5-fold in the sensitive ( $p = 0.003$ ,  $n = 3$  in triplicate) and by twofold in the resistant cells ( $p = 0.003$ ,  $n = 3$  in triplicate) (Figure 2). These findings indicate that there is an efflux via MRP1 and/or MRP2 starting rapidly after oxaliplatin exposure.

## Conclusions

The observed increase in platinum accumulation in the presence of the MRP inhibitor Gü83, seen with oxaliplatin-sensitive and -resistant cells, suggests that oxaliplatin and/or an oxaliplatin adduct are substrates of MRP1 and/or MRP2. Since the effect was comparable in sensitive and resistant cells no conclusions about the contribution of MRP mediated efflux to oxaliplatin resistance can be drawn so far. A 1 : 1 oxaliplatin-GSH adduct was identified in a cell-free environment representing a candidate MRP substrate. However, further experiments are required to confirm this finding in a cellular system.

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