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Research Papers

In vitro and *in vivo* studies on the action of BW502U83, an arylmethylaminopropanediol

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BW502U83, an arylmethylaminopropanediol (AMAP), showed to be partially cross-resistant in a P-glycoprotein-positive and in a P-glycoprotein-negative, doxorubicin-resistant cell line, while no cross-resistance was noticed in a cisplatin-resistant cell line. Interstrand cross-links were not observed, but BW502U83 induced extensive DNA strand breaks. In a feasibility study the effect of intra-arterially BW502U83 was tested. One patient with a hepatocellular carcinoma showed partial remission and signs of a tumor lysis syndrome, another patient with a hepatocellular carcinoma improved clinically. A patient with soft tissue sarcoma had stable disease. Transient increase in SGOT, SGPT and LDH were observed, but no systemic side effects.

Key words: Arylmethylaminopropanediol, BW502U83, DNA strand breaks, intra-arterial treatment.

Introduction

Progress in the treatment of malignancies with chemotherapeutic drugs has slowed down because of intrinsic and acquired drug resistance. Approaches aimed at solving this problem include the search for new chemotherapeutic drugs that evade drug resistance, either alone or in combination with resistance modifying agents, or the administration of high-dose chemotherapy systemically or locally.[1,2]

A new drug that could combine these options is BW502U83 (Figure 1). It belongs to a heterogenous group of compounds, mostly multicyclic and substituted with an arylmethylaminopropanediol, also called AMAPs. All AMAPs display significant activity in a broad range of murine and human tumor models.[3,4] The working mechanism has not yet been fully identified, but DNA intercalation and topoisomerase II inhibition, and membrane directed activity have been described.[5,6] *In vitro* cross-resistance with DNA intercalating agents was found, although partial activity was demonstrated in some intercalator-resistant tumor cells.[3]

Phase I studies with intravenous BW502U83 showed a very high maximum tolerated dose. This high tolerance was thought to be the result of very rapid glucuronidation in humans, presumedly taking place in the liver.[7] Dose-related toxicities consisted of transient cardiac conduction delay, occurring at a dose of 2000 mg/m^2, and of pulmonary distress leading to death in three patients at a dose of 6500–8000 mg/m^2.[8,9] Interest in this drug has waned as a result of these findings. However, the interesting properties of BW502U83 give an opportunity for an alternative pharmacological approach. When delivered locally, i.e. intra-arterially, high drug levels may be reached in the tumor with minimal systemic toxicity because of presumed hepatic detoxification. This approach may be feasible in the palliative or neo-adjuvant setting.

To elucidate the modes of action and of resistance of this drug, and the feasibility of its local administration, we studied BW502U83 *in vitro* and a feasibility study of local administration was done in patients.

Materials and methods

Chemicals and DNA

BW502U83 was a gift from Wellcome (Beckenham, UK). RPMI 1640 medium was obtained from Gibco

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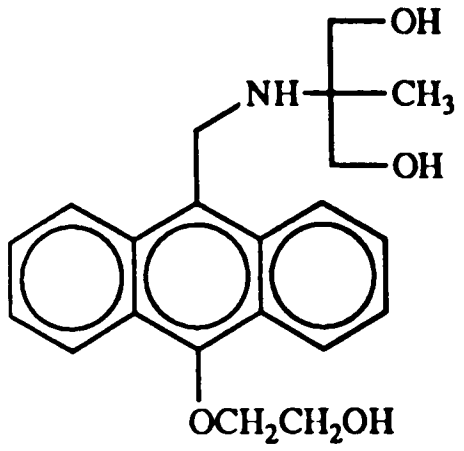

Figure 1. Structure of BW502U83.

(Paisley, UK), Dulbecco's modified Eagle medium (DME) and Ham's F12 were from Flow Laboratories (Irvine, UK). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma (St Louis, MO). Amiodarone was obtained from Sanofi (Maassluis, The Netherlands), doxorubicin from Farmitalia Carlo Erba (Milan, Italy) and mitoxantrone from Lederle (Etten-Leuer, The Netherlands). Ethidium bromide was purchased from Serva (Heidelberg, Germany) and fetal calf serum (FCS) from Sanbio (Uden, The Netherlands).

Cell lines

COLO 320 is a cell line derived from a human adenocarcinoma of the colon.[10] It has a doubling time of 23.8 h. In COLO 320, the presence of P-glycoprotein was demonstrated with the monoclonal antibodies C219 and JSB-1.[11] GLC4 is a human small cell lung carcinoma cell line derived from a pleural effusion and kept in continuous culture. GLC4-Adr is its doxorubicin resistant subline. GLC4 and GLC4-Adr have doubling times of 16.5 and 21.8 h, respectively. GLC4-Adr has an atypical multidrug resistance phenotype and does not show overexpression of MDR*1* mRNA or P-glycoprotein,[12,13] but has an energy-dependent efflux pump, different from P-glycoprotein, and overexpression of a 110 kDa, mainly cytoplasmic, localized protein.[14–16] Moreover, in this cell line the so-called multidrug resistance associated protein (MRP) is overexpressed, which is a member of the energy-dependent superfamily of transport systems, to which also the MDR*1* gene belongs.[17,18] GLC-4-CDDP is a subline of GLC4 with an acquired resistance for cisplatin, its doubling time is 28 h, it has an increased glutathione, decreased DNA platination and increased repair of platinum adducts.[19,20]

The topoisomerase I activity in these four cell lines is equal.[21] The topoisomerase II activity of GLC4-Adr is 3-fold reduced compared with GLC4 and in GLC4-CDDP slightly enhanced compared with GLC4. The topoisomerase II activity in COLO 320 is comparable with that of GLC4-Adr.[13,21] All cell lines were cultured in RPMI 1640 medium and 10% FCS in a humidified atmosphere with 10% CO_2 at 37°C.

Drug sensitivity assay

The microtiter tetrazolium assay (MTA) is dependent of the cellular reduction of MTT by the mitochondrial dehydrogenase of viable cells to a blue formazan product which can be measured spectrophotometrically.[22,23]A total volume of 0.1 ml was used per microculture well (96-well microtiter well plates; Nunc, Gibco, Paisley, UK). Cells (6250/well for COLO 320, 5000/well for GLC4, 12 500/well for GLC4-Adr and 15 000/well for GLC4-CDDP) in logarithmic growth phase were incubated in culture medium with doxorubicin, mitoxantrone and BW502U83 or with the combination of BW502U83 and the P-glycoprotein efflux blocker amiodarone (1 h). Experiments were performed with either continuous or 1 h incubation. In the case of 1 h incubation, after 1 h the cells were washed three times by removal of medium after centrifugation (10 min, 180 *g*) followed by addition of fresh medium. Cell survival was estimated on day 4. Experiments were performed two to four times in quadruplicate.

Fluorometric analysis of DNA unwinding (FADU) for DNA strand breakage detection

This assay is based on detection of drug-induced DNA strand breaks by alkaline unwinding and determination of ethidium bromide fluorescence on a Kontron spectrofluorometer (excitation wavelength at 525 nm and emission wavelength at 580 nm) as described.[24,25] Cells (respectively GLC4, GLC4-Adr and COLO 320) were incubated with increasing concentrations of BW502U83 for 1 h. The concentrations used were based on the previous results of the MTAs for each cell line. In order to stop the 1 h incubation, the cells were washed three times with ice-cold phosphate buffered saline (PBS, 0.14 M NaCl, 2.7 mM KCl, 6.4 mM NaH_2PO_4 and 1.5 mM KH_2PO_4 pH 7.4). Results were expressed as D (percentage double-stranded DNA) = $(F-F_{min})$/

$(F_{max}-F_{min}) \times 100$, where F is the fluorescence of the sample, F_{min} is the background fluorescence determined in samples that were sonicated at the beginning of the unwinding period in order to induce maximal unwinding and F_{max} is the fluorescence of samples kept at pH 11.0, which is below the pH needed to induce unwinding of DNA. All experiments were performed at least three times in duplicate.

Ethidium bromide fluorescence cross-link assay

Renaturation of DNA after heat denaturation and rapid cooling is related to the extent of DNA cross-linking. Ethidium bromide can stain double-stranded renaturated DNA selectively, which can be measured by fluorescence spectrophotometry.[26] For this experiment COLO 320 cells were incubated for 1, 2, 3 and 4 h with different concentrations of, respectively, BW502U83 and cisplatin, of which the formation of DNA interstrand cross-links with this assay was previously demonstrated.[19,26] To stop the incubation period, cells were washed three times with ice-cold PBS and subsequently lysed overnight. Measurements were performed as described.[26] Results were expressed as percentage fluorescence, which represents the amount of DNA interstrand cross-links. The experiments were performed three times in duplicate with 1 h incubation and once with incubation up to 4 h.

Clinical feasibility study

Patients with histologically confined malignancies not curable with standard therapy were included in the study. All patients gave informed consent. An angiogram was performed to identify feeding arteries and, subsequently, the catheter was left in place for the local infusion. A starting dose of BW502U83 of 3 g/m^2 was chosen for the first two patients based on previous phase 1 studies.[8] It was infused over 24 h in 500 ml 5% glucose. Patients were retreated after at least 4 weeks. The dose was escalated within the patient when no toxicity occurred. Toxicity was evaluated according to standard WHO criteria.[27] Efficacy of the treatment was evaluated using standard WHO criteria including radiological techniques and, in addition, follow-up of serological tumor markers. Patients went off study in case no more clinical improvement was reached after every second course. The study was approved by the local medical ethical committee.

Results

The results of the cell survival studies are shown for the four cell lines in Table 1. There is limited cross-resistance for BW502U83 in the doxorubicin-resistant GLC4-Adr compared with GLC4. Whereas this cell line is 3- and 1.8-fold cross-resistant for BW502U83 after, respectively, 1 h and continuous incubation, the factor cross-resistance is 91 for 1 h doxorubicin and 6 for 1 h mitoxantrone. In GLC4-CDDP no obvious difference was found in the cytotoxicity of doxorubicin, mitoxantrone or BW502U83 compared with GLC4. In COLO 320 co-incubation with 10 μM amiodarone for 1 h did not change the ID_{50} of BW502U83 (results not shown). Previously, we have demonstrated that in COLO 320 the ID_{50} of 1 h doxorubicin decreased 1.6-fold with 10 μM amiodarone and 4.2-fold with 50 μM amiodarone.[11] In the same cell line, the ID_{50} of 1 h mitoxantrone decreased 2.0- and 2.3-fold, respectively, with 10 and 50 μM amiodarone.[28] Figure 2 shows the results of the percentage of double-stranded DNA after 1 h incubation of GLC4, GLC4-Adr and COLO 320 with different concentrations of BW502U83. At ID_{50} concentrations of BW502U83 for each cell line, respectively, about 60% in GLC4, 45% in GLC4-Adr and 20% in COLO 320 of DNA breaks were demonstrated. In the ethidium bromide cross-link assay no DNA interstrand cross-links were observed in COLO 320 after 1–4 h incubation with BW502U83 at 80 and 320 μM, whereas a rise in interstrand cross-link formation after increasing concentrations or longer exposure to cisplatin was found. After 1 h incubation the untreated cells showed 22% fluorescence; the cells incubated with 75 and 100 μM cisplatin demonstrated 30.0 and 30.7% fluorescence, respectively, and after 4 h demonstrated 35.0 and 43.7%, respectively.

Clinical feasibility study

The patients received a total of eight courses.

Patient 1. A 20 year old man with an α-1-fetoprotein producing hepatocellular carcinoma had previously been treated with chemotherapy containing bleomycin, etoposide and cisplatin without a tumor response. This patient received two courses of BW502U83, 3 g/m^2 each, at 4 week intervals. Before each course the patient underwent ateriography with placement of a catheter in the hepatic artery. Neither subjective toxicity nor hematological

Table 1. ID_{50} ± SD (μM) after 1 h or continuous incubation (n = 2–4 in quadruplicate)

Cell lines	Cisplatin (continuous)	Doxorubicin (1 h)	Mitoxantrone (1 h)	BW502U83 (1 h)	BW502U83 (continuous)
GLC4	1.4 ± 0.3	0.33 ± 0.07	0.25 ± 0.09	29.2 ± 5.1	3.3 ± 0.5
GLC4-Adr	1.4 ± 0.3	30.1 ± 8.0	1.53 ± 0.19	89.5 ± 7.9	5.8 ± 0.2
GLC4-CDDP	10.3 ± 3	0.19 ± 0.04	0.31 ± 0.13	33.7 ± 4.5	3.2 ± 0.9
COLO 320	4.2 ± 0.2	1.69 ± 0.37	0.37 ± 0.02	42.5 ± 14.3	6.0 ± 0.9

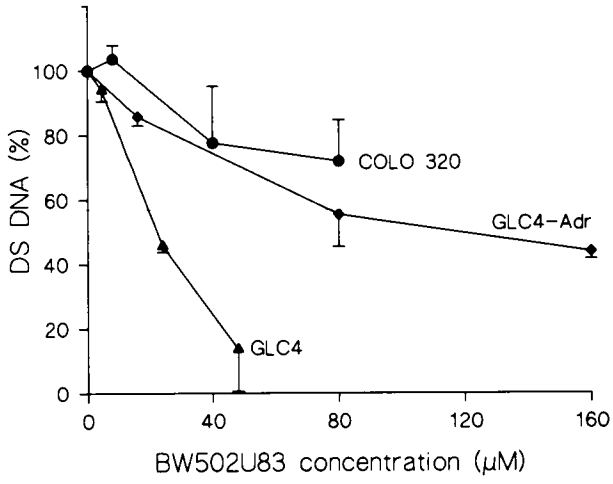

Figure 2. Percentage double-stranded DNA ± SD after 1 h incubation with BW502U83 (n = 3 in duplicate).

toxicity of the drug was observed. However, a marked reversible increase in lactic dehydrogenase and also a rise, although less pronounced, in other liver enzymes was measured (Table 2). The subsequent rapid increase in the blood levels of potassium and uric acid were compatible with the presence of a tumor lysis syndrome. Probably secondary to this event, a reversible partial renal insufficiency developed after the first course. In the second course, renal insufficiency could be prevented by prehydration and allopurinol. Again a transient, but less pronounced, increase in lactic dehydrogenase (to a maximum of 4257 U/l on the second day after administration of BW502U83) and a transient doubling in values of SGOT and SGPT were observed. Other toxicity was not noticed in this patient. Physical examination revealed about 50% reduction in liver size after both courses. This partial remission was confirmed by arteriography, which also excluded an occlusion of the hepatic artery as a cause of the remission. The clinical and radiographic remission was also reflected in a decrease in α-1- fetoprotein (from 580 000 μg/l on day 0 to a minimum of 165 000 μg/l on day 12 after the first course; from 290 000 μg/l at the start of the second course to 170 000 μg/l 1 week later). Some weeks after the second course the condition of the patient deteriorated, he developed ascites and died from a hepatorenal syndrome.

Patient 2. A 29 year old man with a hepatocellular carcinoma without the production of α-1-fetoprotein. He underwent a laparotomy for the evaluation of operability which appeared technically impossible. An intra-arterial catheter with a subcutaneous access port was left behind in the hepatic artery. He was in pain and bedridden most of the day before the start of treatment. He received four courses of 3, 5, 7.5 and 7.5 g/m^2, respectively. Computed tomography showed stable disease, but the clinical condition of the patient improved significantly. After the first course a very slight, 1 day long, increase

Table 2. Biochemical profile of patient 1 following the first intrahepatic arterial infusion of 3 g/m^2 BW502U83

Patient 1	Day 0	Day 1	Day 2	Day 7	Day 28
Sodium (132–144 mmol/l)	137	132	132	125	129
Potassium (3.6–4.8 mmol/l)	4.9	5.0	5.2	6.0	5.9
Urea nitrogen (3.3–6.7 mmol/l)	4.2	4.5	6.2	24.5	7.2
Creatinine (62–106 μmol/l)	73	52	77	262	70
Uric acid (0.1–0.45 mmol/l)	0.52		0.64	1.0	0.36
Alkaline phosphatase (13–120 U/l)	2015	2260	3363	2995	2452
Lactic dehydrogenase (114–235 U/l)	1215	8310	15723	2325	1628
SGOT (0–40 U/l)	150	704	1382	120	213
SGPT (0–30 U/l)	105	231	514	153	95
Bilirubin total (3–25 μmol/l)	35	39	39	70	109
Bilirubin direct (0–5 μmol/l)	20	34	27	51	90

in SGOT and SGPT, but not in lactic dehydrogenase, was noticed. After the second course a transient 3-fold increase in SGOT, SGPT and lactic dehydrogenase was observed, whilst after the third course only an isolated transient doubling of SGOT and SGPT was measured. After the fourth course a transient maximal 10-fold rise in SGOT and SGPT, and a 2- to 3-fold rise in alkaline phosphatase, γ-glutamyl transferase and lactic dehydrogenase on the second day after the administration of BW502U83 were measured. Other side effects were not observed.

Patient 3. A 25 year old woman with an alveolar soft part tissue sarcoma localized in the pelvis and metastases in the lung. She was pretreated with chemotherapeutic regimens containing cisplatin, doxorubicin, methotrexate and ifosfamide without signs of regression. Her major clinical problem was pain due to a local invasion of the pelvic muscles. Arteriography revealed two feeding arteries, the last lumbar artery and the left internal iliac artery. In the first course both arteries were cannulated and in both arteries 2.5 g/m^2 BW502U83, thus a total dose of 5 g/m^2, was infused over 24 h. The catheter position was confirmed afterwards by radiography. Six weeks later the second course of 7.5 g/m^2 was given in the internal iliac artery only. There was no clinical toxicity except for a slight transient erythematous rash of the left leg. Apart from a transient doubling of the previously normal lactic dehydrogenase, no hematological, renal or hepatic toxicity was observed. After both courses she had stable disease.

None of the patients had alterations on electrocardiography, during or after treatment. Pulmonary dysfunction was also not noticed.

Discussion

Cell lines with doxorubicin resistance (GLC4-Adr and COLO 320) are partially cross-resistant to BW502U83, which seems not to be influenced by the presence of P-glycoprotein as there was limited cross-resistance for the MDR-related drug doxorubicin in COLO 320 and there was no increase in cytotoxicity by adding amiodarone, an agent effective in the circumvention of P-glycoprotein-mediated drug resistance.[11] The lowered activity of topoisomerase II in GLC4-Adr might have played a role in the decreased cytotoxicity of BW502U83 in this cell line. Cross-resistance is, however, low (3.0-fold) in comparison with that for doxorubicin (91-fold). Cross-resistance in a cisplatin-resistant cell line was not noticed. In the tested cell lines the patterns of cytotoxicity of mitoxantrone and BW502U83 were similar. Previously, Carter and Bair also observed a pattern of AMAP action in MCF-7 cells resembling not only that of mitoxantrone, but also that of doxorubicin.[6] The working mechanism of the drug seems to be related to interaction with DNA. There is an extensive DNA damaging effect as shown by DNA strand breaks, which is probably not the direct cause of cell death as there is no relation between the amount of DNA strand breaks and cytotoxicity in our cell lines. Bellamy *et al.* also found no such correlation with an alkaline elution assay.[5] DNA interstrand cross-links could not be demonstrated. Although the exact mechanism of action of BW502U83 is not fully elucidated, the presence of extensive DNA strand breaks, its activity in cisplatin-resistant tumor cells, and the very low level of cross-resistance in P-glycoprotein-positive and -negative MDR cell lines suggest that this mechanism differs from the mechanism of most clinically used chemotherapeutic drugs.

The clinical part of this study, with intra-arterially administered BW502U83, showed unexpected results. The drug was tolerated in very high dosages without serious systemic toxicity. Although transient rises in liver enzymes were observed in both patients with a hepatocellular carcinoma, the rise in lactic dehydrogenase in all three patients is probably not a sign of toxicity, but suggests an effect on the tumor. In patient 1 this effect seems to have been very pronounced. In this patient a tumor lysis syndrome very likely took place after the first course because of the transient renal failure combined with a rise in uric acid, occurring after the impressive rise in serum lactic dehydrogenase, and the fact that after the second course, which was administered after prehydration and allopurinol, no repeat of deterioration of renal function was observed. Toxicity, such as QT interval prolongation on ECG and pulmonary distress, which has been described after high-dose intravenously administered BW502U83, was not observed.[9] Thus, the concept of local treatment with a drug which is rapidly detoxified avoiding systemic toxicity seems feasible, although this patient population is too small to draw conclusions about efficacy. More patients would need to be treated in this way in order to define the optimal dose and treatment schedule. It would also be necessary to investigate whether the response in the first patient is specific for an α-1-fetoprotein producing hepatocellular carcinoma.[29]

Conclusion

BW502U83 has limited cross-resistance in cell lines with various types of drug resistance. It produces extensive DNA breaks, but this does not explain its mode of action. Intra-arterial treatment of tumors with a high dosage of this drug without life-threatening toxicity is possible.

References

1. Ling V. P-glycoprotein and resistance to anticancer drugs. *Cancer* 1992; **69**: 2603–9.
2. Frei E, III, Canellos GT. Dose: a critical factor in cancer chemotherapy. *Am J Med* 1980; **69**: 585–94.
3. Knick VC, Tuttle RL, Bair KW, *et al.* Murine and human tumour stem cell activity of three candidate arylmethylaminopropanediols. *Proc Am Ass Cancer Res* 1986; **27**: 1685.
4. Adams DJ, Watkins PJ, Knick VC, *et al.* Evaluation of arylaminopropanediols by a novel *in vitro* pharmacodynamic assay: correlation with antitumour activity *in vivo*. *Cancer Res* 1990; **50**: 3663–9.
5. Bellamy W, Dorr R, Bair K, Alberts D. Cytotoxicity and mechanism of action of 3 arylmethylaminopropanediols (AMAPS). *Proc Am Ass Cancer Res* 1989; **30**: 2236.
6. Carter CA, Bair KW. Effects of isomeric 2-(arylmethylamino)-1,3-propanediols (AMAPS) and clinically established agents on macromolecular synthesis in P388 and MCF-7 cells. *Invest New Drugs* 1991; **9**: 125–36.
7. Lam K, Alberts D, Peng YM, *et al.* Phase I and pharmacokinetic study of BW502U83 (an arylmethylaminopropanediol) in cancer patients. *Proc Am Ass Clin Oncol* 1990; **9**: 264.
8. Von Hoff DD, Kuhn JG, Havlin KA, *et al.* Phase I and clinical pharmacology trial of 502U83 using a monthly single dose schedule. *Cancer Res* 1990; **50**: 7496–500.
9. Schilsky RL, Ratain MJ, Janisch L, *et al.* Phase I clinical and pharmacology study of 502U83 given as a 24-h continuous intravenous infusion. *Cancer Chemother Pharmacol* 1993; **31**: 283–8.
10. Quinn LA, Moore GE, Morgan RT, *et al.* Cell lines from human colon carcinoma with unusual cell products, double minutes, and homogeneously staining regions. *Cancer Res* 1979; **39**: 4914–24.
11. Van der Graaf WTA, De Vries EGE, Uges DRA, *et al. In vitro* and *in vivo* modulation of multi-drug resistance with amiodarone. *Int J Cancer* 1991; **48**: 616–22.
12. Zijlstra JG, De Vries EGE, Mulder NH. Multifactorial drug resistance in an adriamycin-resistant human small cell lung carcinoma cell line. *Cancer Res* 1987; **47**: 1780–4.
13. De Jong S, Zijlstra JG, De Vries EGE, *et al.* Reduced DNA topoisomerase II activity and drug-induced DNA cleavage activity in extracts from an adriamycin resistant human small cell lung carcinoma cell line. *Cancer Res* 1990; **50**: 304–9.
14. Versantvoort CHM, Broxterman HJ, Pinedo HM, *et al.* Energy dependent processes involved in reduced drug accumulation in multidrug-resistant human lung cancer cell lines without P-glycoprotein expression. *Cancer Res* 1992; **52**: 17–23.
15. De Jong S, Kooistra AJ, De Vries EGE, *et al.* Topoisomerase II as a target of VM-26 and m-AMSA in atypical multidrug resistant human small cell lung carcinoma cells. *Cancer Res* 1993; **53**: 1064–71.
16. Scheper RJ, Broxterman HJ, Scheffer GL, *et al.* Overexpression of M_r 110,000 vesicular protein in non-P-glycoprotein-mediated multidrug resistance. *Cancer Res* 1993; **53**: 1475–9.
17. Cole SPC, Bhardwaj G, Gerlach JH, *et al.* Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science* 1992; **258**: 1650–4.
18. Zaman GJR, Versantvoort CHM, Smit JJM, *et al.* Analysis of the expression of MRP, the gene for a new putative transmembrane drug transporter, in human multidrug resistant lung cancer cell lines. *Cancer Res* 1993; **53**: 1747–50.
19. Hospers GAP, Mulder NH, De Jong B, *et al.* Characterization of a human small cell lung carcinoma cell line with acquired resistance to *cis*-diamminedichloroplatinum *in vitro*. *Cancer Res* 1988; **48**: 6803–7.
20. Meijer C, Mulder NH, Hospers GAP, *et al.* The role of glutathione in resistance to cisplatin in a human small cell lung cancer cell line. *Br J Cancer* 1990; **62**: 72–7.
21. Van der Graaf WTA, De Vries EGE, Mulder NH. Modulation of mitoxantrone cytotoxicity with amiodarone, cyclosporin A, and PSC 833 in a sensitive human small cell lung carcinoma cell line, a cisplatin resistant subline, and in P-glycoprotein positive and negative MDR cell lines. In: *Proc Int Symp Resistance against Anticancer Drugs: Molecular Mechanisms and Clinical Opportunities*. Toronto: General Motors Cancer Research Foundation, 1993: A26.
22. Carmichael J, Degraff WG, Gazdar AF, *et al.* Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res* 1987; **47**: 936–42.
23. De Vries EGE, Meijer C, Timmer-Bosscha H, *et al.* Resistance mechanisms in three human small cell lung carcinoma cell lines established from one patient during clinical follow-up. *Cancer Res* 1989; **49**: 4175–8.
24. Birnboim HC, Jevcak JJ. Fluorometric method for rapid detection of DNA strand breaks in human white blood cells produced by low doses of radiation. *Cancer Res* 1981; **37**: 1889–92.
25. Meijer C, Mulder NH, Timmer-Bosscha H, *et al.* Role of free radicals in adriamycin-resistant human small cell lung cancer cell line. *Cancer Res* 1987; **47**: 4613–17.
26. De Jong S, Zijlstra JG, Timmer-Bosscha H, *et al.* Detection of DNA cross-links in tumour cells with the ethidium bromide fluorescence assay. *Int J Cancer* 1986; **37**: 557–61.
27. *WHO handbook for reporting results of cancer treatment (WHO Offset Publication 48)*. The Hague: Nijhoff 1979.
28. Van der Graaf WTA, Mulder NH, Timmer-Bosscha H, *et al.* Different effects of amiodarone, cyclosporin A, and PSC833 on mitoxantrone sensitivity in human tumour cell lines with various resistance mechanisms. *Proc Am Ass Cancer Res* 1993; **34**: 1899.
29. Yamasita Y, Takahashi M, Koga Y, *et al.* Prognostic factors in the treatment of hepatocellular carcinoma with transcatheter arterial embolization and arterial infusion. *Cancer* 1991; **67**: 385–91.

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