BIOCHEMICAL PHARMACOLOGY 71 (2006) 772–778

available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.elsevier.com/locate/biochempharm

Methionine dependence of tumours: A biochemical strategy for optimizing paclitaxel chemosensitivity in vitro

Valerie Pavillard [a], *Anna Nicolaou* [b], *John A. Double* [a], *Roger M. Phillips* [a,*]

[a] *Institute of Cancer Therapeutics, University of Bradford, Bradford BD7 1DP, UK*
[b] *School of Pharmacy, University of Bradford, Bradford BD7 1DP, UK*

ARTICLE INFO

Article history:
Received 7 October 2005
Accepted 9 December 2005

Keywords:
Methionine-dependence
Paclitaxel
Chemosensitivity
Vinblastine
Homocysteine

ABSTRACT

Methionine dependence is a unique feature of cancer cells characterized by growth and cell cycle arrest (typically in S and G2/M) under conditions of methionine depletion. Following replenishment of media with methionine, the cell cycle blockade is reversible and during this recovery period, cells may become more susceptible to the action of cell cycle specific drugs. The response of a panel of methionine dependent (HTC, Phi-1, PC3 and 3T3) cells to vinblastine and paclitaxel was compared to methionine independent Hs-27 cells under conditions of methionine depletion (M^-H^+; methionine depleted media supplemented with homocysteine) and starvation (M^-H^-; media without methionine or homocysteine). All cell lines were significantly more resistant to both agents under M^-H^+ and M^-H^- conditions compared to controls under normal culture conditions [M^+H^-]; however, the magnitude of resistance was reduced in the methionine independent Hs-27 cells. During recovery from methionine depletion and starvation, the response of the methionine dependent cells to vinblastine and paclitaxel was significantly enhanced compared to controls. Although the activity of vinblastine on the Hs-27 cell line was comparable to controls, these methionine independent cells became significantly more resistant to paclitaxel during recovery studies (IC50 = 2.13 ± 0.5 μM) compared to control cultures (IC50 = 0.13 ± 0.15 μM). Whilst the mechanism responsible for this remains uncertain, the increased activity of paclitaxel against methionine dependent cells in conjunction with the decreased activity against Hs-27 cells suggests that methionine depletion strategies may enhance the therapeutic index of paclitaxel.

1. Introduction

A characteristic feature of many solid tumours is their requirement for exogenous methionine in order to proliferate whereas normal cells are generally methionine independent [1,2]. This unique feature is not specific to the tissue of origin and is characterized in vitro by the inability of cells to grow in methionine-deprived medium supplemented with homocysteine, which is the metabolic precursor for de novo methionine synthesis [2,3]. Whilst the biochemical basis for methionine dependence is still not well understood, methionine depletion is known to have marked effects upon cell cycle kinetics producing cell arrest in the S and G2 phases of the cell cycle in vitro [4–6]. Similar cell cycle changes have been reported in xenografts models and in cancer patient tumours treated with methionine-free diets [7–12].

As methionine depletion affects both the growth and the cell cycle of tumour cells, this treatment has been considered for selectively targeting methionine dependent cells. Two

* *Corresponding author*. Tel.: +44 1274 233226; fax: +44 1274 233234.
E-mail address: r.m.phillips@bradford.ac.uk (R.M. Phillips).
0006-2952/$ – see front matter
doi:10.1016/j.bcp.2005.12.014

main strategies have been evaluated. First, depletion of methionine through the use of enzymatic treatments (such as methioninase) has demonstrated tumour regression following prolonged methionine restriction [13]. Prolonged methionine depletion is not however suitable for clinical use because of potential toxicity and quality of life issues and the subsequent interruption of treatment results in re-growth of the tumour [14]. An alternative approach is to use methionine restriction strategies in combination with chemotherapy [15] where enhanced activity of cytotoxic agents could be achieved through exploitation of the changes in cell cycle kinetics that occur during methionine depletion and recovery from methionine depletion conditions. In the case of DNA intercalating agents (such as doxorubicin) that exert their greatest cytotoxic effects in S and G2, enhanced sensitivity of methionine dependent cells in vitro has been reported [11,16]. During recovery from methionine depletion, cells become synchronized in M phase where they become more sensitive to anti-mitotic agents such as vincristine [11]. Paclitaxel belongs to the anti-mitotic class of agents but has a different mechanism of action compared to the vinca alkaloids in that it promotes tubulin polymerization [17]. As paclitaxel is now widely used to treat a range of malignancies, the principal objective of this study is to determine whether or not the activity of paclitaxel can be modulated by methionine depletion studies in a panel of cell lines where methionine dependent growth characteristics and cell cycle analysis has been previously characterized [6]. Furthermore, analysis of methionine depletion and its effect on the response of methionine independent cells was also determined with a view to identifying whether or not potentiation of cytotoxicity is restricted to the methionine dependent phenotype. The vinca alkaloid, vinblastine was also included in this study as a positive control compound in order to determine whether or not differences in the mechanism of action between paclitaxel and vinca alkaloids influence the efficacy of methionine depletion strategies.

2. Materials and methods

2.1. Cell lines and culture conditions

Five different cell lines were used in this study. These were HTC, Phi-1 (rat hepatocarcinoma cells), PC3 (hormone-dependent human prostate cancer), 3T3 (murine fibroblast cells) and Hs-27 (human new-born fibroblast cell line), all of which were obtained from the European collection of cell cultures. HTC, Phi-1 (previously reported as being partially methionine dependent [18]), PC3 and 3T3 cells exhibited methinone dependent growth characteristics whereas Hs-27 cells are methionine independent, details of which have been published elsewhere [6]. Culture conditions for all cell lines were identical to those described previously [6], brief details of which are described below. Methionine-free media was purchased from ICN (Basingstoke, UK) and supplemented with 10% 1 kDa cut-off dialyzed foetal calf serum (First Link, Birmingham, UK), 1 mM sodium pyruvate, 50 IU/ml penicillin, 50 μg/ml streptomycin, non-essential amino acid solution, 2 μM glutamine, 50 μg/ml insulin (used only for Phi-1 and HTC cells), all purchased from Sigma (Dorset, UK), and 24 μM HEPES (for PC3 cells only, InVitrogen, Paisley, UK). The following notations were used to describe the various culture conditions employed: M^+H^- denotes standard or control media conditions where media was supplemented with 100 μM of L-methionine; M^-H^+ denotes methionine depletion conditions where media was supplemented with 100 μM of L-homocysteine thiolactone; M^-H^- denotes methionine starvation conditions where no methionine or L-homocysteine thiolactone supplementation occurred. All cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2.

2.2. Chemosensitivity studies

Paclitaxel and vinblastine were obtained from Sigma (Dorset, UK). Both drugs were reconstituted in DMSO. Stock solutions were batched out into aliquots and stored at −20 °C until required. Experimental conditions and drug exposure parameters used for the various conditions of methionine depletion, starvation and recovery are specified below. It is important to state however that during conditions of methionine depletion and starvation, methionine dependent cells remain viable but do not proliferate and therefore cultures do not reach confluence during this initial phase of the study design. The methionine independent Hs-27 cells do continue to proliferate under methionine depletion conditions but their doubling time is long (85 h) and therefore cultures do not reach confluence during this initial phase of the study design. Once methionine is added after methionine depletion, cells begin to proliferate with doubling times of 24–85 h depending on the cell line in question. Chemosensitivity is assessed 5–6 days after recovery from methionine depletion commenced and all cells were in the exponential phase of the growth curve at the time chemosensitivity was assessed. Full details of all growth curves under the conditions used in this study are presented elsewhere [6].

2.3. Drug treatment combined with M^+H^- medium

Cells were plated in 96-well plates in M^+H^- medium (between 500 and 2000 cells per well according to cell line). After 24–48 h of incubation at 37 °C to obtain cells in exponential growth, the cells were treated with either paclitaxel or vinblastine at concentrations ranging from 100 to 0.01 μM for 1 h at 37 °C. A 1 h drug exposure period was employed as this time period is pharmacologically relevant in view of the fact that the major drug exposure in vivo occurs within the first hour after drug exposure [19].

2.4. Drug treatment combined with M^-H^+ and M^-H^- medium

Cells were plated in 96-well plates as described earlier in M^+H^- medium. In exponential growth, the medium was changed for either M^-H^+ or M^-H^- medium and cells were maintained under these conditions for 5 days at 37 °C. After this period of time, cells were treated with either vinblastine or paclitaxel for 1 h at 37 °C in M^-H^+ or M^-H^- media.

2.5. Drug treatment applied after M^-H^+ and M^-H^- treatment (recovery)

Cells were plated in 96-well plates as described earlier in M^+H^- medium. In exponential growth the medium was replaced by either M^-H^+ or M^-H^- medium and cells were maintained for 5 days at 37 °C. The medium was removed and replaced by fresh M^+H^- medium and the cells were maintained at 37 °C for 5 or 24 h before 1 h of drug treatment.

2.6. Post drug exposure procedures and chemosensitivity determination

Following drug exposure, monolayers were washed twice with Hanks Balanced Salt Solution prior to the addition of 200 μl of M^+H^- media to each well. Following a 5 day incubation period at 37 °C, chemosensitivity was determined using the MTT assay (which is an established endpoint for assessing cell viability [20]), details of which are described elsewhere [21]. Briefly, the medium of each plate was completely removed and replaced by fresh medium containing 0.6 mg/ml of MTT. Following 4 h incubation at 37 °C, the medium was completely removed and replaced by 150 μl of DMSO to dissolve the formazan crystals. The absorbance of the resulting solution was measured at 550 nm using a Labsystems Multiskan® PLUS plate reader. Cell viability was determined as the ratio of absorbances in treated wells to control wells expressed as a percentage. IC50 values were obtained from plots of percentage cell viability against drug concentration. Each experiment was performed in triplicate for each condition. In order to compare the response of methionine dependent and independent lines under the various culture conditions specified, results were expressed as the ratio of IC50 for methionine dependent lines to the IC50 value for HS-27 cells. Values >1 indicate that methionine dependent lines are more resistant to drug treatment than the methionine independent Hs-27 cell line.

2.7. Statistical analysis

All statistical analysis was performed with the SPSS 12.0 Software. Independent-samples Student's *t*-test was used to compare IC50 values generated as a result of the various methionine depletion strategies outlined above.

3. Results

3.1. Chemosensitivity of cells to paclitaxel under conditions of methionine depletion, starvation and during recovery

The influence of methionine depletion and starvation strategies on the response of cells to paclitaxel is presented in Fig. 1. Under standard M^+H^- cell culture conditions, a broad spectrum of sensitivity exists with IC50 values ranging from 0.05 ± 0.06 to 48.7 ± 16.5 μM. Under conditions of M^-H^+ methionine depletion and M^-H^- starvation, all methionine dependent cell lines were significantly less responsive to paclitaxel with IC50 values of >100 μM. In the case of the methionine independent Hs-27 cell line, IC50 values increased from 0.13 ± 0.15 μM in methionine supplemented M^+H^- medium to 10.9 ± 7.1 μM under conditions of M^-H^+ methionine depletion and 51.9 ± 46.3 μM under conditions of M^-H^- methionine starvation. During the initial phase (5 h) of recovery from M^-H^+ methionine depletion, all methionine dependent lines (with the exception of Phi-1) were more responsive to paclitaxel than control M^+H^- conditions and in HTC and Phi-1 cells, the response of cells was further potentiated after a 24-h recovery period (Fig. 1). In contrast, the methionine independent Hs-27 cell line was less sensitive than control cells (IC50 = 0.13 ± 0.15 μM) following both a 5 (IC50 = 0.99 ± 0.40 μM) and 24 h (IC50 = 2.13 ± 0.5 μM) recovery period from methionine depletion. During recovery from methionine starvation (M^-H^-), a similar pattern of chemosensitivity profiles exists in HTC, Phi-1 and 3T3 cells as described above although Phi-1 and 3T3 cells are particularly sensitive to paclitaxel following a 5 h recovery period (IC50 values of 0.017 ± 0.018 and 0.089 ± 0.014 μM, respectively) compared to control values (IC50 values of 15.4 ± 4.15 and 24.4 ± 7.30 μM, respectively). In contrast, the activity of paclitaxel against PC3 cells was not potentiated during recovery from M^-H^- conditions (Fig. 1).

3.2. Chemosensitivity of cells to vinblastine under conditions of methionine depletion and during recovery

The influence of methionine depletion on the response of cells to vinblastine is presented in Fig. 2. Under standard M^+H^-, cell culture conditions IC50 values ranged from 7.22 ± 8.39 to 0.16 ± 0.13 μM. During recovery from M^-H^+ conditions, the activity of vinblastine was potentiated after 24 h of recovery for HTC and Phi-1 cells whereas for 3T3 and PC3 cells, the activity of vinblastine was potentiated after 5 h of recovery only (Fig. 2) although this potentiation did not reach statistical significance (except for PC3 cells treated after 5 h of recovery). In all cases however, the magnitude of potentiation was less than that observed with paclitaxel. In the case of Hs-27 cells, IC50 values during recovery (0.13 ± 0.13 and 0.08 ± 0.06 μM for 5 and 24 h recovery, respectively) were similar to IC50 values in controls (0.16 ± 0.13 μM). During recovery from M^-H^- conditions, only minor potentiation of vinblastine activity against HTC, Phi-1 and PC3 cells was observed. In the case of 3T3 cells however, significant decreases in IC50 values were observed following both 5 (IC50 = 0.038 ± 0.016 μM) and 24 h (0.14 ± 0.076 μM) recovery compared to controls (IC50 = 0.54 ± 0.12 μM). In contrast, the activity of vinblastine against Hs-27 cells decreased following a 24 h recovery period.

3.3. Relationship between the response of methionine dependent and independent cells to paclitaxel and vinblastine under normal culture conditions (M^+H^-), recovery from methionine depletion (M^-H^+) and recovery from methionine starvation (M^-H^-)

The results are expressed in terms of the ratio of IC50 values for the methionine dependant cell lines to the IC50 value for Hs-27 cells under the various experimental conditions employed (values >1 indicate resistance of methionine dependent cells compared to Hs-27 cells), details of which

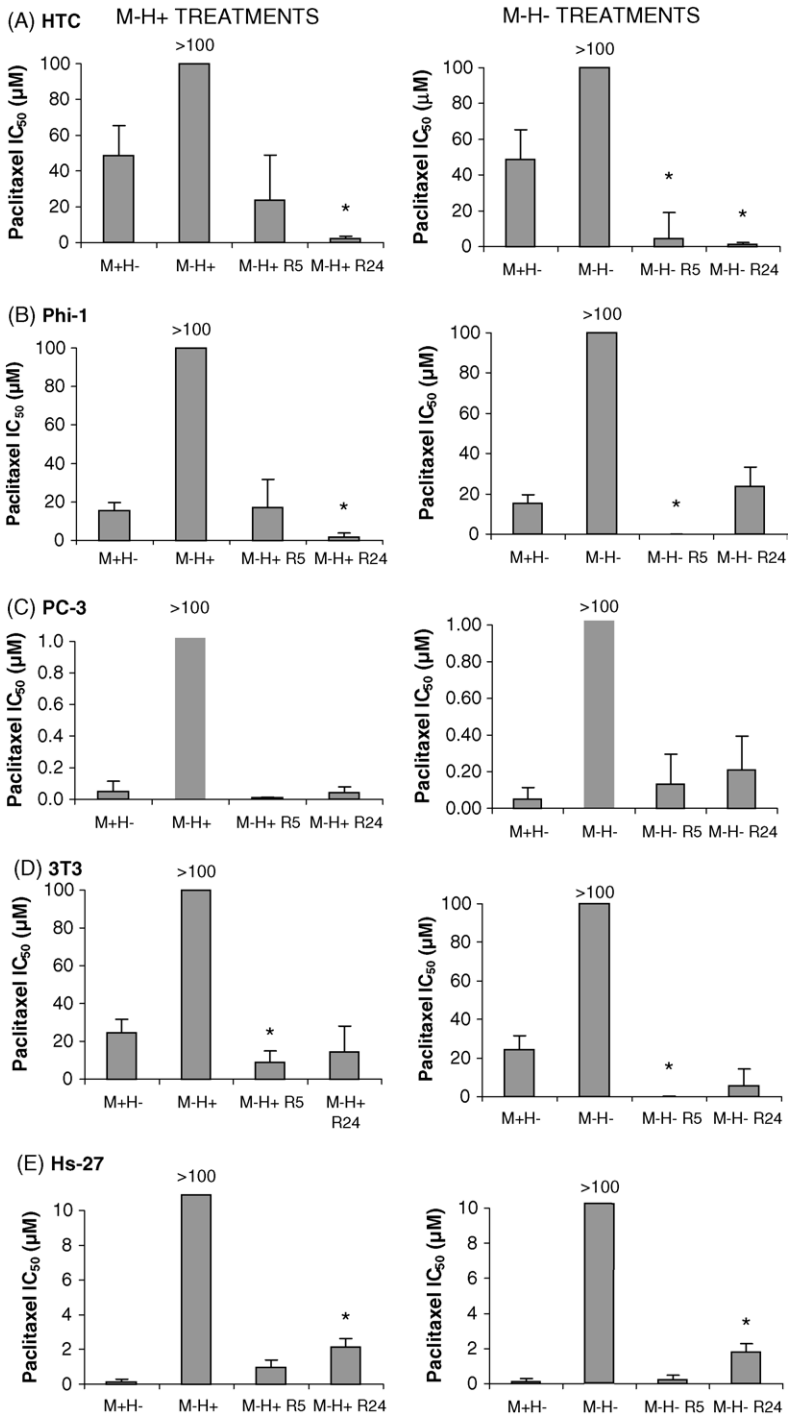

Fig. 1 – The response of a panel of cell lines exposed to paclitaxel under standard (M^+H^-) media conditions, during methionine depletion (M^-H^+) or starvation (M^-H^-) and during recovery from M^-H^+ and M^-H^- treatment (R5 and R24 denote drug treatment after 5 or 24 h recovery). (*) statistical significance ($p < 0.05$) and all statistical analyses were made by comparing treated cells to control M^+H^- cultures.

are presented in Table 1. With regards to paclitaxel, HTC, 3T3 and Phi-1 cells were significantly more resistant than Hs-27 cells with IC50 ratios of 369, 187 and 118, respectively, under standard methionine (M^+H^-) rich media conditions. In contrast, PC3 cells were more responsive than normal Hs-27 cells (IC50 ratio of 0.38) under similar conditions. Under M^+H^- conditions, chemosensitivity ratios were 369 and 118 for HTC and Phi-1 cells, respectively, but following a 24 h recovery from M^-H^+ conditions, the response of HTC and Phi-1 cells were similar to the response of Hs-27 cells (IC50 ratios decreased from 369 and 118 to 0.98 and 0.76 for HTC and Phi-1 cells, respectively, Table 1). With regards to recovery from M^-H^- conditions, the only cell line not to show any major reductions in chemosensitivity ratios to

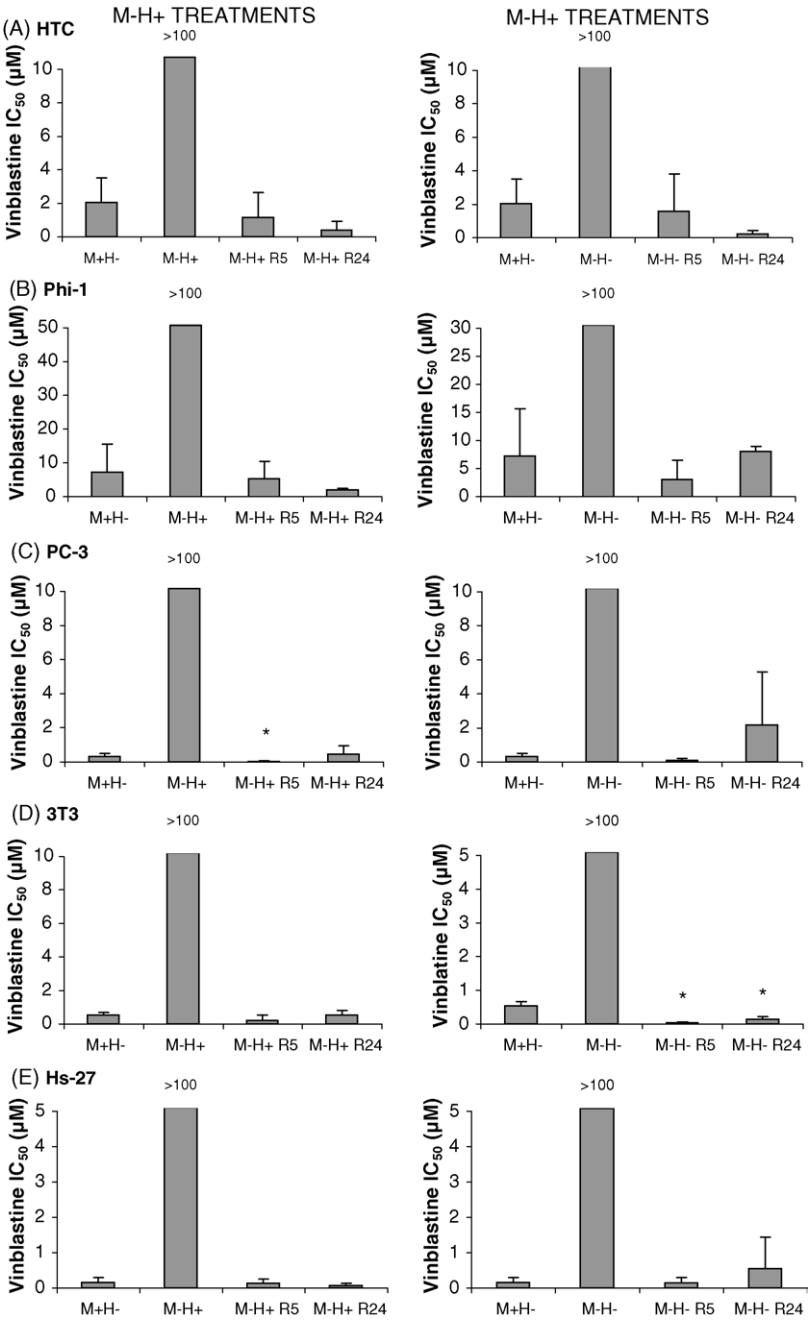

Fig. 2 – The response of a panel of cell lines exposed to vinblastine under standard (M^+H^-) media conditions, during methionine depletion (M^-H^+) or starvation (M^-H^-) and during recovery from M^-H^+ and M^-H^- treatment (R5 and R24 denote drug treatment after 5 or 24 h recovery). (*) statistical significance ($p < 0.05$) and all statistical analyses were made by comparing treated cells to control M^+H^- cultures.

paclitaxel was PC3. Particularly marked reductions in chemosensitivity ratios were observed in the case of Phi-1 and 3T3 cells (following a 5 h recovery time) and HTC cells (following a 24 h recovery period). In contrast to studies on paclitaxel, the effect of methionine depletion or starvation and recovery from either treatment condition on cytotoxicity ratios for vinblastine was comparatively modest (Table 1). Whilst all cell lines showed some reduction in chemosensitivity ratios at either 5 or 24 h recovery compared to standard M^+H^- conditions, the most significant reductions were observed in HTC cells (following a 24 h recovery from M^-H^- conditions) and 3T3 cells (following 5 and 24 h recovery from M^-H^- conditions).

4. Discussion

The results of this study demonstrate that methionine depletion, starvation and recovery from both treatments have a significant effect on the response of methionine dependent

Table 1 – Relationship between the response of methionine dependent and methionine independent cells under standard cell culture conditions (M^+H^-) and during recovery from methionine depletion (M^-H^+) and starvation (M^-H^-)

Drug cell line	Ratio (M^+H^-)	Ratio R5 (M^-H^+)	Ratio R24 (M^-H^+)	Ratio R5 (M^-H^-)	Ratio R24 (M^-H^-)
Paclitaxel					
HTC	369 ± 82	23.9 ± 1.4*	0.98 ± 0.3*	18.5 ± 6.2*	0.62 ± 0.14*
Phi-1	118 ± 28	17.2 ± 4.6*	0.76 ± 0.5*	0.073 ± 0.05*	13.2 ± 1.9*
3T3	187 ± 39	8.9 ± 2.1*	6.76 ± 0.6*	0.38 ± 0.01*	3.05 ± 0.23*
PC3	0.38 ± 0.10	0.009 ± 0.003*	0.019 ± 0.04	0.56 ± 0.19	0.11 ± 0.08
Vinblastine					
HTC	12.8 ± 1.28	8.92 ± 1.08	5.0 ± 0.60	11.3 ± 2.90	0.40 ± 0.38**
Phi-1	45.1 ± 3.8	41.1 ± 10.9	25.7 ± 5.8	22.1 ± 5.2	14.9 ± 1.70
3T3	3.4 ± 0.8	1.61 ± 0.10	6.5 ± 0.40	0.28 ± 0.10**	0.26 ± 0.14***
PC3	1.9 ± 1.20	0.15 ± 0.08**	5.8 ± 1.76	0.78 ± 0.16	4.0 ± 0.89

Results are expressed in terms of the ratio (±S.D., $n = 3$) of IC50 values for individual methionine dependent cells relative to the methionine independent Hs-27 cell line under the different culture conditions used. R5 and R24 denote recovery from depletion or starvation for 5 and 24 h, respectively. Statistical analysis was conducted by comparing all individual values to M^+H^- results.

* p values of <0.01.

** p values of <0.05.

*** p values of <0.001.

cell lines to vinblastine and paclitaxel. Under conditions of methionine starvation (M^-H^-) and depletion (M^-H^+), all methionine dependent cell lines were considerably more resistant to both vinblastine and paclitaxel compared to sensitivity under M^+H^- conditions (Figs. 1 and 2). These results are consistent with the fact that these cells are blocked in various positions of the cell cycle (S and G2/M for HTC; PC3 and 3T3 cells; G1 for Phi-1 cells) during the period of drug exposure [6]. During recovery from M^-H^+ and M^-H^- conditions, the cytotoxic effects of both agents increased (Figs. 1 and 2). These results are consistent with previous studies using the closely related vinca alkaloid vincristine in that reversal of the cell cycle blockade following the addition of methionine allows cells to enter mitosis where they become more sensitive to anti-mitotic agents [11,15].

With regards to the methionine independent cell line Hs-27, resistance to both paclitaxel and vinblastine during methionine depletion and starvation was also observed although the magnitude of this effect is greatly reduced compared to methionine dependent lines. The effects of methionine depletion and starvation on cell cycle kinetic parameters demonstrated a slight but statistically significant accumulation of cells in G1 compared to controls [6]. During recovery of cells from M^-H^+ and M^-H^- conditions, only minor differences in IC50 exist in the response of cells to vinblastine were observed compared to controls (Fig. 2). In the case of paclitaxel however, a different pattern of chemosensitivity was observed particularly with regards to the response of methionine independent and dependent cells during recovery from methionine depletion and starvation. In contrast to vinblastine, Hs-27 cells were significantly more resistant to the effects of paclitaxel after both 5 (IC50 = 0.99 ± 0.40 μM) and 24 h (IC50 = 2.13 ± 0.50 μM) recovery from M^-H^+ conditions compared to controls (IC50 = 0.16 ± 0.13 μM, Fig. 1). The mechanistic basis for these observations is not clear. It is conceivable that because Hs-27 cells continue to grow during M^-H^+ conditions that the cell population would be approaching plateau phase at the time of drug treatment and therefore be less sensitive to paclitaxel. This is however unlikely as a similar effect would have been obtained with vinblastine. Recent studies have suggested that paclitaxel induces apoptosis in G1 and S phases of the cell cycle whereas both apoptosis and necrosis occur in G2/M [22]. During M^-H^+ and M^-H^- conditions, a small but significant increase in G1 and reduction in S phase was observed in Hs-27 cells whereas cells in G2 remained at a similar level to controls [6]. It is conceivable therefore that the extent of cell death caused by necrosis would decrease and this could explain the increase in resistance observed. Furthermore, methionine restriction is known to have multiple biochemical effects including changes to cellular glutathione, *S*-adenosyl homocysteine and *S*-adenosyl methionine levels that could have broad ranging effects on both drug sensitivity and cell cycle kinetics [23,24]. It has been recently suggested that reactive oxygen and nitrogen species are involved in paclitaxel cytotoxicity and the tumour antioxidant capacity is directly linked to resistance to paclitaxel [25]. Our previous studies have shown that methionine depletion reduces the levels of glutathione in vitro [6]; this effect may lead to a reduction in the antioxidant capacity of methionine dependent cells under methionine depletion and may render these cells more susceptible to paclitaxel. Further studies are required to dissect the mechanism responsible for the chemosensitivity effects reported in this study.

The increase in the response of methionine dependent cells coupled with the decreased sensitivity of the methionine independent Hs-27 cell line to paclitaxel during recovery from methionine depletion has significant implications in that the potential therapeutic index of paclitaxel could be enhanced by methionine restriction strategies. HTC cells for example are 369 times more resistant than cells to paclitaxel under standard methionine rich control conditions (M^+H^-) whereas 24 h after the initiation of recovery from methionine depleted (M^-H^+) conditions, IC50 values for HTC and Hs-27 cells are comparable (Table 1). Similar results have been obtained for Phi-1 and PC3 cells (Table 1). Clearly Hs-27 cells cannot be regarded as being representative of all normal cells but in the context of their methionine independent phenotype (which is a characteristic feature of normal cells in general), similar effects may occur in other

normal cell types. To our knowledge, increased resistance of methionine independent cells during recovery from methionine depletion has not been reported previously and further studies in the in vivo setting are warranted. Both dietary methionine starvation in vivo [8,26] and PEGylated recombinant methioninase [27] have been used in the in vivo setting to lower methionine levels and alternate cycles of methionine restriction and chemotherapy have been suggested as a suitable schedule for improving the efficacy of anticancer drugs [28]. Further studies in the in vivo setting are required to determine whether or not methionine restriction strategies in combination with paclitaxel chemotherapy can improve the therapeutic index of paclitaxel.

Acknowledgement

This study was supported by Cancer Research UK (program grant number C459/A2579).

REFERENCES

[1] Mecham JO, Rowitch D, Wallace CD, Stern PH, Hoffman RM. The metabolic defect of methionine dependence occurs frequently in human tumor cell lines. Biochem Biophys Res Commun 1983;117(2):429–34.
[2] Hoffman RM. Altered methionine metabolism, DNA methylation and oncogene expression in carcinogenesis. A review and synthesis. Biochim Biophys Acta 1984;738(1–2):49–87.
[3] Hoffman RM, Erbe RW. High in vivo rates of methionine biosynthesis in transformed human and malignant rat cells auxotrophic for methionine. Proc Natl Acad Sci USA 1976;73(5):1523–7.
[4] Hoffman RM, Jacobsen SJ. Reversible growth arrest in simian virus 40-transformed human fibroblasts. Proc Natl Acad Sci USA 1980;77(12):7306–10.
[5] Lu S, Epner DE. Molecular mechanisms of cell cycle block by methionine restriction in human prostate cancer cells. Nutr Cancer 2000;38(1):123–30.
[6] Pavillard V, Drbal AA, Swaine DJ, Phillips RM, Double JA, Nicolaou A. Analysis of cell-cycle kinetics and sulfur amino acid metabolism in methionine-dependent tumor cell lines; the effect of homocysteine supplementation. Biochem Pharmacol 2004;67(8):1587–99.
[7] Poirson-Bichat F, Lopez R, Bras Goncalves RA, Miccoli L, Bourgeois Y, Demerseman P, et al. Methionine deprivation and methionine analogs inhibit cell proliferation and growth of human xenografted gliomas. Life Sci 1997;60(12):919–31.
[8] Guo HY, Herrera H, Groce A, Hoffman RM. Expression of the biochemical defect of methionine dependence in fresh patient tumors in primary histoculture. Cancer Res 1993;53(11):2479–83.
[9] Guo H, Lishko VK, Herrera H, Groce A, Kubota T, Hoffman RM. Therapeutic tumor-specific cell cycle block induced by methionine starvation in vivo. Cancer Res 1993;53(23):5676–9.
[10] Hoshiya Y, Kubota T, Matsuzaki SW, Kitajima M, Hoffman RM. Methionine starvation modulates the efficacy of cisplatin on human breast cancer in nude mice. Anticancer Res 1996;16(6B):3515–7.
[11] Stern PH, Hoffman RM. Enhanced in vitro selective toxicity of chemotherapeutic agents for human cancer cells based on a metabolic defect. J Natl Cancer Inst 1986;76(4):629–39.
[12] Goseki N, Yamazaki S, Shimojyu K, Kando F, Maruyama M, Endo M, et al. Synergistic effect of methionine-depleting total parenteral nutrition with 5-fluorouracil on human gastric cancer: a randomized, prospective clinical trial. Jpn J Cancer Res 1995;86:484–9.
[13] Tan Y, Xu M, Guo H, Sun X, Kubota T, Hoffman RM. Anticancer efficacy of methioninase in vivo. Anticancer Res 1996;16(6C):3931–6.
[14] Kokkinakis DM, Hoffman RM, Frenkel EP, Wick JB, Han QH, Xu M, et al. Synergy between methionine stress and chemotherapy in the treatment of brain tumour xenografts in athymic mice. Cancer Res 2001;61:4017–23.
[15] Cellarier E, Durando X, Vasson MP, Farges MC, Demiden A, Maurizis JC, et al. Methionine dependency and cancer treatment. Cancer Treat Rev 2003;29(6):489–99.
[16] Nagahama T, Goseki N, Endo M. Doxorubicin and vincristine with methionine depletion contributed to survival in the Yoshida sarcoma bearing rats. Anticancer Res 1998;18(1A):25–31.
[17] Mekhail TM, Markman M. Paclitaxel in cancer therapy. Expert Opin Pharmacother 2002;3(6):755–66.
[18] Kenyon SH, Waterfield CJ, Timbrell JA, Nicolaou A. Methionine synthase activity and sulphur amino acid levels in the rat liver tumour cells HTC and Phi-1. Biochem Pharmacol 2002;63(3):381–91.
[19] Alberts D, Chen H-S, Salmon S. In vitro drug assay: pharmacologic considerations. In: Salmon SE, editor. Cloning of human tumour stem cells. New York: Alan R Liss Inc.; 1980. p. 197–207.
[20] Twentyman P, Luscombe M. A study of some variables in a tetrazolium dye (MTT) based assay for cell growth and chemosensitivity. Br J Cancer 1987;56:279–85.
[21] Phillips RM, Hulbert PB, Bibby MC, Sleigh NR, Double JA. In vitro activity of the novel indoloquinone EO-9 and the influence of pH on cytotoxicity. Br J Cancer 1992;65(3):359–64.
[22] Liao PC, Lieu CH. Cell cycle specific induction of apoptosis and necrosis by paclitaxel in the leukemic U937 cells. Life Sci 2005;76(14):1623–39.
[23] Poot M, Teubert H, Rabinovitch PS, Kavanagh TJ. De novo synthesis of glutathione is required for both entry into and progression through the cell cycle. J Cell Physiol 1995;163(3):555–60.
[24] Mizunuma M, Miyamura K, Hirata D, Yokoyama H, Miyakawa T. Involvement of S-adenosylmethionine in G1 cell-cycle regulation in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 2004;101(16):6086–91.
[25] Ramanathan B, Jan K-Y, Chen C-H, Hour T-C, Yu H-J, Pu Y-S. Resistance to paclitaxel is proportional to cellular total antioxidant capacity. Cancer Res 2005;65:8455–60.
[26] Hoshiya Y, Guo H, Kubota T, Inada T, Asanuma F, Yamada Y, et al. Human tumors are methionine dependent in vivo. Anticancer Res 1995;15(3):717–8.
[27] Yang Z, Wang J, Lu Q, Xu J, Kobayashi Y, Takakura T, et al. PEGylation confers greatly extended half-life and attenuated immunogenicity to recombinant methioninase in primates. Cancer Res 2004;64(18):6673–8.
[28] Epner DE, Morrow S, Wilcox M, Houghton JL. Nutrient intake and nutritional indexes in adults with metastatic cancer on a phase I clinical trial of dietary methionine restriction. Nutr Cancer 2002;42(2):158–66.