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# Verapamil Enhances Doxorubicin Activity in Cultured Human Renal Carcinoma Cells

Teresa Lai, C.M.P. Collins, P. Hall, A.P. Morgan, P.J.B. Smith, B.R. Stonebridge and M.O. Symes

**Cells from 22 renal cell carcinomas (RCC) were established in culture. Sensitivity of the tumour cells to doxorubicin alone and in combination with racemic verapamil, which reverses multidrug resistance, was tested using a [$^{75}$Se]selenomethionine uptake assay to measure protein synthesis. The effect of verapamil was expressed as a potentiation index: $LD_{50}$doxorubicin/$LD_{50}$doxorubicin + verapamil. The potentiation index in 15 of these carcinomas was determined for cells within the first 14 days of culture. At 3.3 μmol/l concentration of verapamil, of the tumours sensitive to doxorubicin alone ($LD_{50}$ < 0.75 μg/ml) five of seven showed a potentiation index of > 2. For the less sensitive tumours the analogous proportion was seven of eight. Tumour cell expression of glycoprotein P-170, associated with multidrug resistance, was estimated using the monoclonal antibody C-219. Initial expression levels were unrelated to the action of verapamil. In five tumours the proportion of cells expressing P-170 declined as the period of culture increased. This was not associated with any consistent change in the $LD_{50}$ for doxorubicin or in potentiation of doxorubicin sensitivity by verapamil. Cell cloning associated with prolonged cell growth *in vitro* could mimic tumour cell cloning which accompanies the formation of metastases. Thus reduced expression of P-170 on prolonged cell growth *in vitro* may be a pointer to the efficacy of combination therapy in the treatment of patients with metastatic renal cell carcinoma.**
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## INTRODUCTION

RENAL CELL carcinomas are resistant to chemotherapy [1]. This may be due to genetically determined multidrug resistance (MDR). Evidence for this is the expression by renal carcinoma cells of mRNA encoded by the MDR I gene [2] and the consequent presence of P-170 glycoprotein in the cells [3, 4]. It has been demonstrated that MDR to vinblastine or doxorubicin may be reversed *in vitro* by culture of the tumour cells together with either verapamil [4, 5] or quinidine [2].

A limitation in some of the studies cited above was the use of a single concentration of vinblastine or doxorubicin in combination with verapamil [4, 5]. Thus a dose–response curve for the anti-tumour effect could not be determined. Furthermore, only cells freshly derived from primary renal carcinomas, which had reached their exponential growth phase, were studied [2, 4, 5] so that changes in the expression of P-170 and thus of MDR, together with its reversal on prolonged tumour growth could not be assessed. This may be of importance in patients with metastases who require drug treatment. The present communication therefore attempts to extend the above quoted studies.

## MATERIALS AND METHODS

Approximately 5 g of macroscopically viable tumour were obtained from nephrectomy specimens. The tissue was minced with scissors and then disaggregated for 45 min at 37°C in 2 mg/ml of collagenase type II (Sigma) and 0.2 mg/ml DNase (Sigma) in RPMI 1640 (Gibco). The mixed cell suspension was filtered through 100 mesh stainless steel gauze and the cell pellet obtained following centrifugation (200 *g*:5 min) was washed once in RPMI 1640 and resuspended in 4 ml of this medium. Four density gradients of nycodenz® (Nycomed UK) were prepared in 15-ml conical centrifuge tubes (Falcon). Each gradient had four layers which were, from the bottom upwards, nycodenz 27.6% w/v and nycodenz diluted 2:1, 1:1 and 1:2 with a balanced salt solution [6]. The tubes were inclined at an angle of 45° for 45 min to allow the formation of a continuous density gradient. 1 ml of the mixed cell suspension was layered on to the top of each gradient and the whole was centrifuged at 800 *g* for 45 min at room temperature. The carcinoma cells were aspirated from a distinct band which separated near the top of the gradient [7].

### *Culture of tumour cells*

Carcinoma cells obtained following separation on nycodenz were cultured in RPMI 1640 medium containing 10% (v/v) newborn calf serum, 1% (w/v) glutamine and antibiotics (pencillin 100 U/ml, streptomycin 0.1 mg/ml and amphotericin B 0.25 μg/ml) (Gibco). An initial cell concentration of $10^5$ cells/ml was used. Each carcinoma was passaged just prior to the cells becoming confluent. The cells were harvested by exposure for 5–10 min at 37°C to trypsin 0.5 mg/ml and EDTA 0.2 mg/ml in phosphate-buffered saline. Cell detachment was monitored using an inverted microscope (Wilj) and when complete the trypsin was inactivated by the addition of two volumes of medium. The cells were washed once in medium and counted using a haemocytometer.

### *Exposure of cells to doxorubicin*

Doxorubicin (Farmitalia) was diluted in RPMI 1640 and 10% (v/v) newborn calf serum to a concentration of 0.2, 1.0 or

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2.0 μg/ml. Racemic verapamil (Abbot) was similarly diluted to a concentration of 2.0 or 6.6 μmol/l. Equal volumes of each concentration of doxorubicin and medium alone or medium containing verapamil were mixed to give a final concentration of 0.1, 0.5 or 1 μg/ml doxorubicin ± 1.0 μmol/l or 3.3 μmol/l verapamil. For a given tumour $2 \times 10^4$ cells were exposed to increasing concentrations of doxorubicin ± verapamil for 24 h at 37°C. Similarly, aliquots of cells were exposed to each concentration of verapamil or medium alone. All drug exposures and subsequent isotope uptake assays were performed on 3–5 samples.

[$^{75}$Se] *Selenomethionine uptake assay*

Cells exposed to drug(s), or medium alone, were washed once using medium. The cell pellet was resuspended in 0.5 ml of methionine-free MEM with $9.25 \times 10^3$ Bq of [$^{75}$Se]selenomethionine [$^{75}$SeM] (CIS) in 50 μl added. After a further 48 h incubation the cells were washed (× 3) to remove unincorporated isotope and the cell pellet was counted using a Wilj 2001γ counter to determine the level of [$^{75}$SeM] incorporation and hence of protein synthesis by the cells.

The percentage inhibition of $^{75}$SeM uptake following drug exposure was determined using the formula:

$$\left[1 - \frac{\text{CPS for cells exposed drug(s)}}{\text{CPS for cells in medium alone}}\right] \times 100,$$

where CPS = counts per second.

*Statistics*

From the percentage inhibition of protein synthesis in cells exposed to 0.1, 0.5 or 1.0 μg/ml doxorubicin the $LD_{50}$ concentration of drug was determined using a computer programme based on a least squares regression analysis [8]. The $LD_{50}$ for inhibition of protein synthesis in the presence of doxorubicin + verapamil was also determined. An index for potentiation by verapamil of the anti-tumour action of doxorubicin was calculated using the formula:

$$\frac{LD_{50}\text{ doxorubicin}}{LD_{50}\text{ doxorubicin + verapamil}}.$$

Also the individual CPS for each sample of cells from a given tumour exposed to doxorubicin ± verapamil, verapamil alone or medium alone, after each culture period, were subjected to a one-way analysis of variance. The significance of each concentration of verapamil in potentiating the effect of a particular concentration of doxorubicin was then determined by Student's *t*-test, using the pooled estimate of the variance to calculate *t* for the difference between the relevant pair of observations. In order to quantify the relationship between tumour stage, sensitivity to doxorubicin and expression of P-170, an exact 2 × 2 test was used to calculate the relevant probabilites. In order to construct the 2 × 2 tables, tumour stage was classified as stages I and II (confined to the kidney) and stages III and IV (tumour spread beyond the kidney).

Sensitivity to doxorubicin was arbitrarily divided into $LD_{50}$ < 0.75 μg/ml (sensitive) and $LD_{50}$ > 0.75 μg/ml (resistant). Expression of P-170 was classified as 1 or 2 (< 50% of cells positive and 3 or 4 (> 50% cells positive).

*Determination of P-170 glycoprotein expression by tumour cells*

Tumour cells freshly obtained from the nycodenz gradient or harvested from the cell cultures were exposed to monoclonal antibody (Mab) C-219 [9] that recognises an internal epitope on mammalian P-170 molecules. The cells were stained by the immuno-alkaline phosphatase technique and counterstained with Mayers haematoxylin. Cells stained without application of the primary antibody (C-219) served as negative controls. Sections from normal kidney were used as a positive control. The number of positively stained cells in each sample was graded as: nil, 1 – < 25%, 2 – 26–50%, 3 – 51–75% and 4 – > 75%. The intensity of staining was graded nil, +, ++ or +++ according to whether the tumour cells were unstained or showed minimal, moderate or strong staining as judged by the appearance of the cell membranes and cytoplasm. In order to facilitate comparison between individual tumours or between the same tumour after increasing periods of culture, slides from several different tumours, or culture periods, were stained as a single batch.

## RESULTS

*Tumours studied*

The stage and histology of the tumours is shown in Table 1. There was no relation between stage and sensitivity to doxorubicin alone, $P = 29.3\%$ (exact 2 × 2 test) (Table 2) or expression of P-170 (Table 3) by the tumour cells when first tested, $P = 27.9\%$.

*Characterisation of tumour cells*

Cytospin preparations of cells obtained from nycodenz gradients and from the cell cultures were stained with May–Grunwald Giemsa stain, and they showed a homogeneous population of carcinoma cells.

Cultures from tumours K39, K42, K44, K45, K50, K52,

*Table 1. Characteristics of the renal cell carcinoma studied*

| Tumour no. | Stage | Histology |
|---|---|---|
| K34 | III | Clear cell carcinoma |
| K37 | II | Granular cell carcinoma |
| K38 | II | Clear cell carcinoma |
| K39 | III | Clear cell carcinoma |
| K40 | II | Clear cell carcinoma |
| K42 | III | Granular cell carcinoma |
| K44 | I | Clear cell carcinoma |
| K45 | III | Clear granular cell carcinoma |
| K48 | III | Clear cell carcinoma |
| K49 | II | Clear cell carcinoma |
| K50 | II | Granular cell carcinoma |
| K51 | III | Mixed cell carcinoma |
| K52 | III | Clear cell carcinoma |
| K56 | III | Mixed cell carcinoma |
| K57 | III | Granular cell carcinoma |
| K59 | III | Granular cell carcinoma |
| K60 | II | Papillary carcinoma |
| K62 | III | Clear cell carcinoma |
| K63 | II | Papillary carcinoma |
| K67 | III | Clear cell carcinoma |
| K69 | III | Clear cell and spindle cell carcinoma |
| K70 | III | Clear cell carcinoma |

Stage I, No invasion; stage II, invasion of parenchyma; stage, III, penetration through the capsule or invasion of the inferior vena cava; stage IV, distant metastases.

*Table 2. The $LD_{50}$ concentration (μg/ml) of doxorubicin for inhibition of protein synthesis by renal cell carcinoma cells in tissue culture. Inhibition was measured by reduction in the uptake of [$^{75}$Se] selenomethionine*

| Tumour no. | Day of culture | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | 6 | 11 | 13 | 19 | 26 | 32 | 37 | 43 | 46 | 52 | 63 | 75 | 81 | 95 | 103 | 136 | 174 | 187 |
| K34 | | | | | | | | | | 0.26 | 0.36 | 0.72 | | | | | | | |
| K37 | 0.47 | | 1.40 | | | | | | | | | | | | | | | | |
| K38 | | | 0.76 | | 1.68 | | | | | | | 29.46 | | | | | | | |
| K39 | | 1.19 | | | | | | | 3.41 | | | | | | | | | | |
| K40 | | | | | 0.31 | | | | 13.63 | | | | | | 0.50 | | | | |
| K42 | | | | 0.90 | 1.44 | | 1.15 | | | | | | 1.15 | 1.96 | | 3.23 | | 1.31 | 2.25 |
| K44 | | | | | | 0.80 | 4.04 | | | | | | 8.25 | | | | | | |
| K45 | 0.12 | | | | | | | | | | | | | | | | | | |
| K48 | | | 0.23 | | | | | | | | | | | | | | | | |
| K49 | | | 0.30 | | | | | | | | | | | | | | | | |
| K50 | | 4.04 | | | | | | | | | | | | | | | | | |
| K51 | | | | 0.91 | | | | | | | | | | | | | | | |
| K52 | | | | 0.45 | | | | | | | | | | | | | | | |
| K56 | | | | | | 0.35 | | | | | | | | | 0.20 | | 0.46 | | |
| K57 | | | 0.25 | | | | | | | | | | | | 0.45 | | 0.41 | | |
| K59 | | 0.18 | | | | | | | | | | | | | | | | | |
| K60 | | 2.73 | | | | | | | | | | | | | | | | | |
| K62 | 10.1 | | | | | | | | | | | | | | | | | | |
| K63 | 1.38 | | 1.23 | | | | | | | | | | | | | | | | |
| K67 | | | | | 0.78 | | | | | | | | | | | | | | |
| K69 | | | | | | | | 1.05 | | | | | | | | 1.02 | | | |
| K70 | | | | | 0.63 | | | | | | | | | | | 0.74 | | | |

K56, K59, K60, K61, K62 and K70 were allowed to become confluent. At this point the cells showed lack of contact inhibition and formed multiple layers.

Of the 14 tumours examined, 11 (K42, K44, K45, K50, K52, K59, K60, K62, K63, K69 and K70) expressed cytokeratins on a proportion of the cells. Expression was detected by reactivity with Mab MNF 116 [10] (Dako) that is specific for an epitope on a wide range of cytokeratins including numbers 10, 17 and 18. Cells from tumours K56, K57 and K67 did not express cytokeratins. In four tumours, K42, K50, K52 and K63 expression of cytokeratins decreased as the period of culture increased. Of these in 3 (K42, K50 and K63) and also in K56, a proportion of the cells were aneuploid as determined by FACS analysis of nuclear DNA content.

*Table 3. Expression of P-170 by renal carcinoma cells from the primary neoplasm or its first passage* in vitro

| Tumour | Passage no. | |
|---|---|---|
| | 0 | 1 |
| K44 | | $2^{+++}$ |
| K45 | | $2^{++}$ |
| K48 | | $3^{++}$ |
| K49 | $3^{+++}$ | $4^{+++}$ |
| K50 | Nil | $1^{++}$ |
| K51 | $4^{+++}$ | |
| K52 | $2^{+++}$ | |
| K56 | | $2^{++}$ |
| K60 | | $3^{++}$ |
| K62 | $4^{++}$ | $3^{++}$ |
| K63 | $1^{++}$ | |
| K67 | | $3^{++}$ |
| K69 | $4^{++}$ | |
| K70 | $4^{++}$ | |

Expression was graded: nil, 0% cells +ve; 1, < 25% +ve; 2, 25–50% +ve; 3, 51–75% +ve; 4, > 75% +ve. + Staining minimal; ++ moderate; +++ marked.

*Drug sensitivity*

17 tumours were examined for sensitivity to doxorubicin alone during their first passage, 15 within the first 14 days of culture. Taking an $LD_{50}$ of > 0.75 μg/ml on first testing within 14 days as a threshold to indicate relative drug insensitivity, eight tumours (K38, K39, K42, K50, K51, K60, K62 and K63) fall into this category (Table 2). In all of these tumours except K63 there was a corresponding initial potentiation index ≥ 2 due to 3.3 μmol/l verapamil (Fig. 1). Seven tumours had an initial $LD_{50}$ of < 0.75 μg/ml (K37, K45, K48, K49, K52, K57 and K59). Five of these (K48, K49, K52, K57 and K59) also showed a potentiation index for doxorubicin sensitivity of > 2 in the presence of 3.3 μmol/l verapamil (Fig. 1).

In three tumours K38, K39 and K44 there was a rise in the $LD_{50}$ concentration of doxorubicin alone as the period of cell culture increased (Table 2). Of these two, K38 and K44, showed a progressive rise in the potentiation index due to 3.3 μmol/l verapamil (Fig. 1). In general, the tumour cells displayed increasing sensitivity to increasing concentrations of doxorubicin alone (Figs 2, 3). Furthermore, 3.3 μmol/l verapamil was more effective than 1.0 μmol/l in potentiating the action of a given concentration of doxorubicin (Figs 2, 3). Verapamil alone at either 1.0 μmol/l or 3.3 μmol/l concentrations had no effect on protein synthesis.

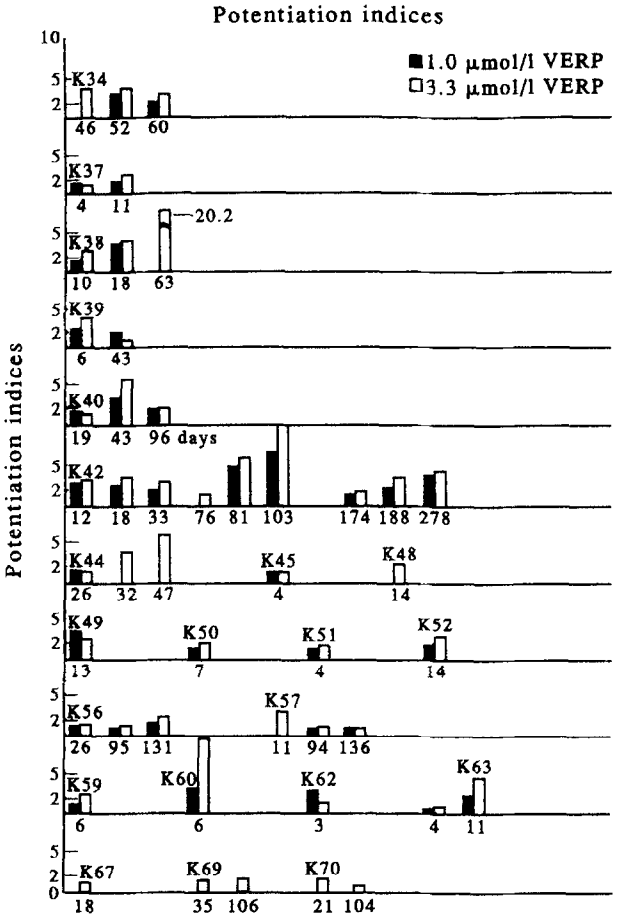


**Fig. 1. The effect of verapamil in potentiating the action of doxorubicin in inhibiting protein synthesis by renal carcinoma cells *in vitro*. The figure beneath each column(s) indicates the period of culture (days).**

Following a dose of 60 mg/m$^2$ intravenously, the concentration time product for doxorubicin in man is 3.84 μg/h/ml [11]. Therefore, for a 24-h exposure period, concentrations of 0.16 μg/ml or less are clinically relevant. It is thus clear that the $LD_{50}$ concentrations recorded in the present studies are above those obtainable in man. However, it is of interest to note that in 10 of the 22 tumours studied, 3.3 μmol/l verapamil significantly potentiated the action of 0.1 μg/ml doxorubicin when first tested.

*Expression of P-170*

When examined either as cells obtained direct from the nycodenz gradient (passage 0) or in primary culture (passage 1) the tumours could be classified according to the percentage of cells (< 50% or > 50%) expressing P-170. On this basis there were six tumours (K44, K45, K50, K52, K56 and K63) in which a minority of cells expressed P-170 and eight (K48, K49, K51, K60, K62, K67, K69 and K70) wherein P-170 was present in the majority of cells (Table 3).

*Correlation of drug sensitivity to expression of P-170*

In nine of the 14 tumours the $LD_{50}$ for doxorubicin was determined within 14 days of culture. There was no relation between sensitivity to doxorubicin and the proportion of cells expressing P-170 in a particular tumour (Tables 2, 3), $P = 47.6\%$.

In seven tumours, expression of P-170 was examined after progressively increasing periods of culture. The level declined in five (K42, K45, K56, K57 and K69) (Table 4).

## DISCUSSION

The uptake of [$^{75}$Se]selenomethionine by tumour cells is a measure of protein synthesis [12]. The dependence of isotope uptake on the number of tumour cells and the concentration of isotope has been reported previously [13]. In the present study the suitability of this assay is confirmed by the dose–responses obtained. Thus inhibition of isotope uptake by a given number of carcinoma cells was related to the concentration of doxorubicin to which they were exposed. Also, the action of verapamil in increasing the effectiveness of doxorubicin was concentration dependent. We found no relationship between sensitivity to doxorubicin alone and the ability of verapamil to potentiate this in contrast to the results of Mickisch *et al.* [4]. This may reflect the use of the microculture tetrazolium assay in their work and $^{75}$SeM uptake in ours. Furthermore, in some tumours, e.g. K42, the $LD_{50}$ for doxorubicin and the potentiation index due to verapamil showed no consistent relationship to the period of culture.

Initial sensitivity to doxorubicin alone was not related to the degree of expression of P-170. This is in contrast to the findings of Mickisch *et al.*[4]. However, in their study 16 of 35 tumours

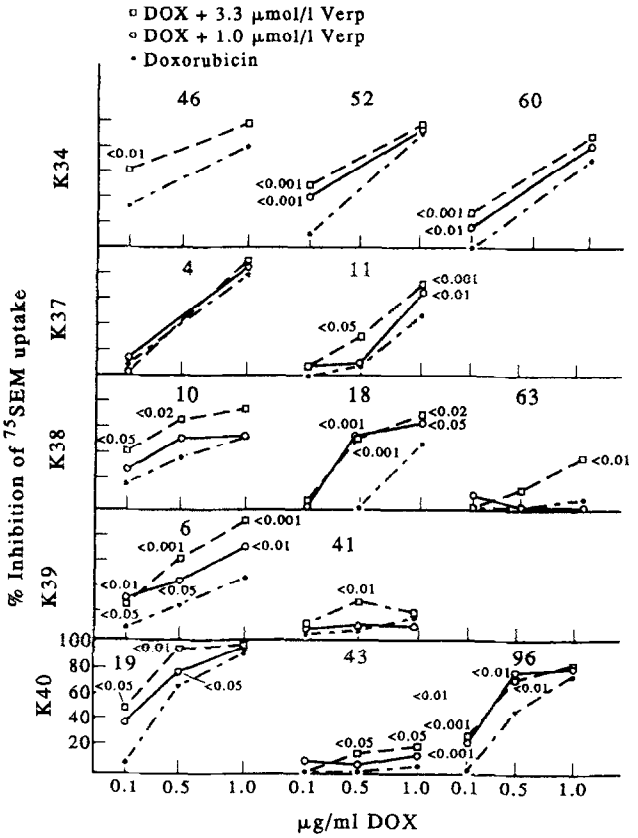


**Fig. 2. The effect of doxorubicin with or without the addition of racemic verapamil on [$^{75}$Se]selenomethionine uptake by renal carcinoma cells. Cells were cultured with drug(s) for 24 h and then with isotope for 48 h. Each point represents the mean of three to five determinations for drug exposed cells, expressed as the percentage inhibition of isotope uptake relative to the mean of three to five determinations for cells cultured in medium alone. The figure above each set of graphs is the time in days for which the cells were cultured prior to testing for drug sensitivity.**

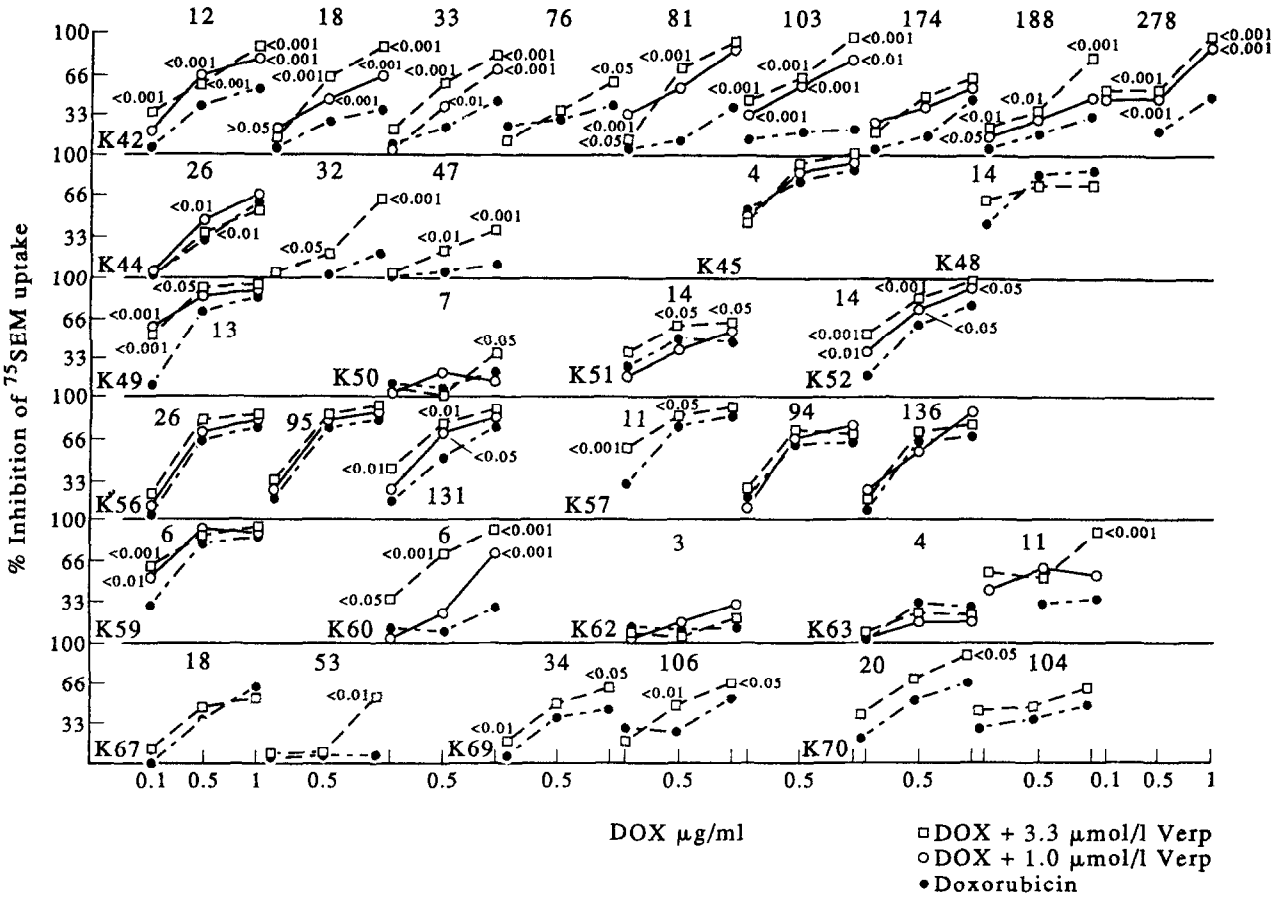


**Fig. 3. The effect of doxorubicin ± verapamil on protein synthesis by renal carcinoma cells.**

failed to express P-170 whereas we found initial expression in a proportion of the cells from all the tumours examined, the level of expression being classified according to the number of cells reacting with Mab C-219.

Decline in the proportion of tumour cells expressing P-170 with increasing culture periods may be a reflection of decreasing cell differentiation associated with loss of tumour heterogeneity resulting from cell cloning. Expression of P-170 is directly related to the degree of RCC differentiation [2, 3]. The decline in the level of P-170 expression was not associated with any consistent change in the $LD_{50}$ for doxorubicin alone or in potentiation of doxorubicin sensitivity by verapamil, but further data are required to verify this point.

*Table 4. Changes in expression of P-170 by renal carcinoma cells with increasing periods of growth in vitro*

| Passage no. | Tumour | | | | | | |
|---|---|---|---|---|---|---|---|
| | K42 | K45 | K56 | K57 | K63 | K69 | K70 |
| 0 | | | | | $1^{++}$ | $4^{++}$ | $4^{++}$ |
| 1 | | $2^{++}$ | $2^{++}$ | | | | |
| 2 | | | | | | $1^{++}$ | $4^{++}$ |
| 3 | $4^{++}$ | Nil | | | | | |
| 4 | | | | | $1^{++}$ | | |
| 5 | | | | | Nil | | |
| 6 | | | | | | Nil | |
| 7 | | | $3^{++}$ | $3^{++}$ | | | $4^{++}$ |
| 9 | $1^{+}$ | | Nil | Nil | | | |
| 11 | $1^{++}$ | | Nil | Nil | | | |
| 28 | Nil | | | | | | |

Potentiation of drug action by verapamil in our experiments was very much less than that previously reported by others for multi-passaged tumour cell lines [14, 15]. However, these lines were selected by growth in medium containing increasing concentrations of doxorubicin, a procedure which substantially enhanced P-170 expression by the tumour cells and high concentrations of verapamil, 10–80 μmol/l, were necessary to reverse drug resistance. By contrast, in the present experiments, an increasing culture period in drug-free medium was associated with reduced expression of P-170. The relative level of change in expression of P-170, associated with differing conditions of culture may be such that an effect upon drug sensitivity is noted only with continued growth in drug-containing medium.

It is possible that there is an analogy between *in vitro* RCC cloning due to culture and *in vivo* cloning associated with the formation of metastases [16]. If this is so, it is predicted that the cells in a metastasis will show diminished expression of P-170. Thus, a given concentration of verapamil might be more effective in increasing the action of doxorubicin on metastatic tumour cells compared to those in the primary neoplasm.

If reduced expression of P-170 by metastatic cells is associated with their increased sensitivity to verapamil, the use of this drug to potentiate the action of appropriate cytotoxic agents in the treatment of metastases is indicated.

1. Harris DT. Hormonal therapy and chemotherapy of renal cell carcinoma. *Semin Oncol* 1983, **10**, 422–430.
2. Kanamaru H, Kakeni Y, Yoshida O, Nakanishi S, Pastan I, Gottesman MM. MDRI RNA levels in human renal carcinomas: correlation with grade and prediction of reversal of doxorubicin resistance by quinidine in tumour explants. *J Natl Cancer Inst* 1989, **81**, 844–849.
3. Van Kalken CK, Van der Valk P, Hadisaputro MMN, *et al.* Differentiation dependant expression of P-glycoprotein in the normal and neoplastic human kidney. *Ann Oncol* 1991, 2, 55–62.
4. Mickisch GH, Roehrich K, Koessig J, Forster S, Tschada RK, Alken PM. Mechanisms and modulation of multidrug resistance in primary human renal cell carcinoma *J Urol* 1990, **144**, 755–759.
5. Mickisch GH, Koessig J, Keilhaver G, Schlick E, Tschada RK, Alken PM. Effects of calcium antagonists in multidrug resistant primary human renal cell carcinomas. *Cancer Res* 1990, **50**, 3670–3674.
6. Umpleby HC, Fermor B, Symes MO, Williamson RCN. Viability of exfoliated colorectal carcinoma cells. *Br J Surg* 1984, **71**, 659–663.
7. Ford TC,Lai T, Symes MO. Morphological and functional characteristics of mouse mammary carcinoma cells separated on Nycodenz columns. *Br J Exp Path* 1987, **68**, 453–460.
8. Lai T, Stonebridge BR, Black J, Symes MO. Inhibition of protein synthesis, pulmonary localisation and pulmonary tumour formation by drug treated tumour cells as a means of predicting their chemosensitivity. *Clin Exp Metastasis* 1989, 7, 427–436.
9. Kartner N, Everden-Porelle D, Bradley G. Detection of *P*-glycoprotein in multidrug resistant cell lines by monoclonal antibodies. *Nature* 1985, **316**, 820–823.
10. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalog of human cytokeratins: Patterns of expression in normal epithelia, tumours and cultured cells. *Cell* 1982, **31**, 11–24.
11. Alberts DS, Chen HSG. Tabular Summary of pharmokinetic parameters relevant to *in vitro* drug assay. In *Cloning of Human Tumor Stem Cells*. New York, AR Liss, 1980, 351–359.
12. Liebold W, Bridge S. $^{75}$Se Release—A short and long term assay for cellular cytotoxicity. *Z Immun Forsch* 1979, **155**, 287–311.
13. Ferro MA, Heinemann D, Smith PJB, Symes MO. Effect of stilboestrol and testosterone on incorporation of $^{75}$Selenomethionine by prostatic carcinoma cells. *Br J Urol* 1988, **62**, 166–172.
14. Schuurhuis GJ, Broxterman HJ, Van der Hoeven JM, *et al.* Potentiation of doxorubicin cytotoxicity by the calcium antagonist nepridil in anthracycline-resistant and sensitive cells. A comparison with verapamil. *Cancer Chemother Pharmacol* 1987, **20**, 285–290.
15. Keizer HG, Joenje H. Increased cytosolic pH in multidrug resistant human lung tumour cells: Effect of verapamil. *J Natl Cancer Inst* 1989, **81**, 706–709.
16. Alam SM, Whitford P, Cushley W, George WD, Campbell AM. Aneuploid subpopulations in tumour invaded lymph nodes from breast cancer patients. *Eur J Cancer* 1992, **28A**, 357–362.

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# Coamplification of the *hst* and *bcl*-1 Oncogenes in Advanced Squamous Cell Carcinomas of the Head and Neck

**Peter Volling, Markus Jungehülsing, Manfred Jücker, Hartmut Stützer, Volker Diehl and Hans Tesch**

**Squamous cell carcinomas of the head and neck from 40 untreated patients were analysed for rearranged or amplified proto-oncogenes by Southern blot hybridisation. The *bcl*-1 and the *hst* genes were coamplified 8–32-fold in 5 patients (12.5%). Only males with stage III and IV disease showed coamplification of these oncogenes. Northern blot analysis of the positive samples did not show expression of *bcl*-1 or *hst* genes. In contrast, a third oncogene located on chromosome 11 (Ha-*ras*-1) was not amplified in these tumours. Disease development was observed in all cases over a minimum period of 3 years. Survival of the patients with coamplification of *hst*/*bcl*-1 seemed to be shorter than of those with stage III and IV disease without amplification. This difference was not significant probably due to the small number of investigated patients.**
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## INTRODUCTION

LIMITED INFORMATION is available on the involvement and role of oncogene amplification in the development and prognosis of head and neck squamous cell carcinomas (SCC). In contrast to other human tumours, statistically significant correlations between amplified oncogenes and clinicopathological parameters of head and neck cancer are not known [1, 2].

Recently some authors have described an uncommonly high rate of gene amplification of two oncogenes, *bcl*-1 and *int*-2, which have been shown to share the same gene locus [3, 4]. *Bcl*-1 has been recognised as the breakpoint of a chromosome translocation in various B-cell malignancies. The breakpoint has been cloned and the region with a putative oncogene has been named bcl-1 (B-cell lymphoma/leukaemia-1) [5]. The *int*-2 gene is known as a member of the family of fibroblast growth factor-related genes. These genes are suggested as important factors in cell growth modulation, because they are active in the early embryogenesis but not in normal adult tissues [6]. Furthermore, a third oncogene, *hst*, is located at the same locus. It shows coamplification with the *int*-2 oncogene in some human cancers [7].