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Original Paper

Carbonyl Reductase and NADPH Cytochrome P450 Reductase Activities in Human Tumoral Versus Normal Tissues

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The use of bioreductive agents in enzyme-directed bioreductive therapy has been proposed to take advantage not only of hypoxia in tumours, but also of the presence of reductases that metabolise such compounds. In this study, we studied the activities of NADPH cytochrome P450 reductase (P450R) and carbonyl reductase (CR) in 17 human lung tumours and 18 human breast tumours, together with the corresponding normal tissues. For lung cancer but not for breast cancer there was a significant difference in the CR activity between normal and tumour tissue. CR activity was increased with respect to the normal tissue between 2-fold and 40-fold indicating heterogeneity in tumour samples. No relationship was found between CR activity and the histological type, tumoral grade or TNM stage of the tumours. Although some variation in P450R activity in tumoral versus normal tissues was found in the majority of the samples studied, no significant differences could be demonstrated. © **1999 Elsevier Science Ltd. All rights reserved.**

Key words: carbonyl reductase, NAPDH P450 reductase, lung tumour, breast tumour, bioreductive agents

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INTRODUCTION

SEVERAL ATTEMPTS have been made to take advantage of the presence of hypoxic cells in solid tumours for therapeutic purposes. A strategy was proposed, which consisted of developing compounds that are activated by reductive mechanisms, and therefore, are favoured in an environment of low oxygen concentration; these are the so-called bioreductive agents [1]. Mitomycin C, considered to be the bioreductive agent prototype, and a series of analogues of mitomycin C, as well as the nitroimidazoles, were the first compounds of this type to be developed. At present, some of these compounds, such as the indoloquinone E09 and the N-oxide SR 4233 or tirapazamine, are in advanced phases of clinical evaluation [2–4].

In the first preclinical and clinical trials, it was clear that the effectiveness of these compounds depended not only on the oxygen level but also on the presence of reductases which metabolise the compounds. Up to now, cytochrome P450 (several isoenzymes), NADPH cytochrome P450 reductase (P450R), NADH cytochrome b5 reductase, xanthine oxidase/dehydrogenase, aldehyde oxidase, carbonyl reductase (CR) and DT diaphorase have been identified, although other enzymes may also be implicated. Another variable to be considered is the enzyme activity in non-target tissues, where the metabolism of the bioreductive agent could produce undesirable effects. Therefore, some authors proposed the use of bioreductive agents, taking advantage of the differences that might exist in the levels of expression of the reductases involved in their bioactivation in different tissues and human tumours [5]. In order to develop this strategy termed 'enzyme-directed bioreductive therapy', it is necessary to determine the activities of the enzymes in normal as well as in tumoral tissue.

CR (EC 1.1.1.184) is a cytosolic monomeric, NADPH-dependent oxidoreductase with broad specificity for carbonyl compounds. It differs from aldehyde reductase (EC 1.1.1.2) and aldose reductase (EC 1.1.1.21) in its ability to reduce aromatic ketones and in its sensitivity to specific inhibitors [6]. Like DT diaphorase, CR catalyses the 2 electron (e^-) reduction of quinones. It has been isolated from a number of human tissues: liver, brain, testis and others [6–9]. The enzyme has been implicated in the metabolism of a variety of endogenous and xenobiotic carbonyl compounds: prostaglandins [10],

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anthracycline antibiotics, such as daunorubicin [11] and quinones derived from polycyclic aromatic hydrocarbons [12]. It seems to be the predominant reductase for the anticancer drug daunorubicin in human liver cytosol [13].

P450R (EC 1.6.2.3) is a microsomal enzyme found in a great number of human tissues, especially in lung and pancreas, which shows a coinciding distribution pattern with the principal sites of tumour formation. Studies using liver microsomes and tumour cells have shown that both cytochrome P450 and P450R contribute to the reductive activation of tirapazamine [14, 15]. Moreover, tirapazamine is reduced by purified rat liver cytochrome P450 reductase [16, 17].

Some previous studies have shown abnormally high DT diaphorase levels (both activity or mRNA) in human tumours [18–22]. This enzyme has been implicated in the metabolism of E09 and mitomycin C amongst others. To advance the knowledge of human tumour enzyme profiling, we studied differences in expression or activity of CR and P450R in human tumoral samples versus normal adjacent tissues.

MATERIALS AND METHODS

Tissue specimens

Biopsy specimens of lung solid tumours were obtained from 11 patients at the Hospital of Navarra and from 6 patients at the University Clinic, both in Pamplona, Spain. Tissue specimens of breast solid tumours were obtained from 18 patients at the University Clinic. Primary solid tumours were obtained together with macroscopically normal tissue from the same subject. Tissues were frozen in liquid nitrogen immediately after each operation. They were maintained at $-80\,^{\circ}C$ in the pathology laboratory until they could be transported in liquid nitrogen to the research laboratory, where they were stored at $-135\,^{\circ}C$ until processed. Tumour and normal tissues were frozen and stored separately.

Patient characteristics

The following data were obtained from the medical records after the tissues had been analysed for reductase levels: age, sex, ethnic origin, current pathologies and medication, tobacco use, tumour histology and grade, presence of metastasis and the tumour staging according to the TNM classification status [23].

The mean age of the patients with lung tumours was 67 years. All the patients were smokers or ex-smokers except for the 2 women in the study (patients 7 and 10), who had no recorded tobacco use. In general, they were receiving concomitant medication for non-malignant pathologies such as cardiac and/or respiratory pathologies. The mean age of the patients with breast tumours was 51 years. All were females undergoing radical or conservative surgery. Histological characteristics are included in Table 1.

Tissue processing

Batches of three or four paired tissues were processed for enzyme assays at the same time. A piece of liver from a rat, which had been previously frozen and stored in the same way as the human tissues, was processed with each batch. The frozen tissues were cut into small pieces and washed in ice-cold sucrose (0.25 M) so as to eliminate the contaminating haemoglobin. Then, the samples were dried and pulverised with liquid nitrogen in a porcelain mortar. The powder was weighed and resuspended using a hand-held glass homogeniser, in 3 volumes of ice-cold homogenising buffer (KPO_4/KCl 10 mM/0.1 M pH 7.8, ethylene diamine tetraacetic acid (EDTA) 0.1 mM, phenazine methosulfate (PMSF) 2 mM and trypsin inhibitor 80 mg/l). Finally, the samples were homogenised using an Ultra Turrax-T25 homogeniser (Janke & Kunkel, Germany, IKA-labortechnik). The temperature was maintained at $4\,^{\circ}C$ throughout the whole time by working on ice. The homogenate was then centrifuged at $4\,^{\circ}C$, at 15000 ***g***, for 20 min. The supernatants were separated, aliquoted and stored at $-80\,^{\circ}C$ until used for enzyme activity measurements. Reproducibility was tested with the rat liver samples. Inter- and intra-assay variation was approximately 10%. The protein concentration of the supernatants was determined by the bicinchoninic acid assay [24].

Enzyme assays

CR activity was determined spectrophotometrically by following the reduction of cytochrome *c* at 550 nm, using a

Table 1. *Histological characteristics of lung and breast tumours*

Patient	Histology	Grade	Stage†	Receptors‡
Lung tumours				
1	AD	G2	I	NA
2	EC	G2	II	NA
3	AD	G1	I	NA
4	AD[1]	G2	I	NA
5	EC	G3	IIIB	NA
6	EC	G1	I	NA
7	AD	G1	I	NA
8	EC	G1	IIIA	NA
9	AD[2]	?	I	NA
10	C	G2	I	NA
11	EC	G3	I	NA
12	EC	G2	IIIA	NA
13	MC	G3	I	NA
14	EC	G2	IIIA	NA
15	EC	G3	IIIA	NA
16	EC	G2	IIIA	NA
17	EC or LCC	G2	IIIB	NA
Breast tumours				
18	DIC*	G3	IIA	RE(+)RP(+)
19	DC	G1	IIA	RE(−)RP(+)
20	LIC	G3	IIIB	RE(−)RP(−)
21	DIC	G3	I	RE(−)RP(+)
22	DIC	G3	IIB	RE(+)RP(+)
23	DIC*	G2	IIA	RE(−)RP(−)
24	CA	G1	I	RE(+)RP(+)
25	MC*	G2	IIIA	RE(−)RP(+)
26	DIC*	G3	IIA	RE(+)RP(+)
27	DIC*	G3	IIB	RE(−)RP(−)
28	DIC	G3	IIA	RE(+)RP(−)
29	DIC	G2	IV	?

AD, adenocarcinoma; AD[1], possible mixed oat cell/epidermoid carcinoma; AD[2], recurrence; C, carcinoid; EC, epidermoid carcinoma; MC, mucoepidermoid carcinoma; LCC, large cell carcinoma; DIC, ductal infiltrating carcinoma; LIC, lobular infiltrating carcinoma; DC, *in situ* ductal carcinoma; CA, colloid adenocarcinoma; MC, mixed ductal and lobular infiltrating carcinoma; NA, not applicable; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated; ?, not registered. *Although this was the most prevailing histological type in this sample, they presented areas or focus where other histological types were represented. †Stage according to AJCC. ‡Presence (+) or absence (−) of the oestrogen (RE) or progesterone (RP) receptors in the tumour.

modification of the method reported elsewhere [12]. Reaction mixtures consisted of 0.1 M KPO_4 (pH6), 30 μM cytochrome *c* as the electron acceptor, 0.2 mM menadione as the substrate and 0.2 mM NADPH as the cofactor. Controls without sample, NADPH or substrate were routinely included. The reaction was initiated with the addition of 10 μl of the tissue sample. Reactions were conducted at 37 °C in a total volume of 1 ml, in the presence and absence of the specific inhibitor rutin (20 μM). CR activity was expressed as the fraction of activity measured which was inhibited by rutin. P450R activity was determined spectrophotometrically by following the reduction of cytochrome *c* at 550 nm, using a modification of the Ernster method [25], as reported in detail elsewhere [26]. Both activities were expressed as nmol cytochrome *c* (ε 21.1×10^3 M/cm) reduced/min/mg protein.

Statistical analysis

The statistical analyses were performed using the SPSS program. Normality was assessed by the Shapiro–Wilks test. When the samples were not normally distributed, the Mann–Whitney *U* rank sum test was used for assessing differences between samples. When the samples showed a normal distribution, Student's *t*-test was used for the same purpose.

RESULTS

CR activity

Lung. Final CR activities found in paired lung tumour and normal tissues are shown in Figure 1a. The mean activity in normal tissue was 15.2 ± 2.8 nmol/min/mg protein, with the highest value being 47.9 ± 0.9 nmol/min/mg protein (found in patient 1) and the lowest value being 2.1 ± 0.4 nmol/min/mg protein (found in patient 9). There was more heterogeneity in the tumour samples. The mean was equal to 50.0 ± 14.0 nmol/min/mg protein, the range being between 10.1 ± 0.7 nmol/min/mg protein (patient 13) and 248.1 ± 3.1 nmol/min/mg protein (patient 8). In 15 of the patients studied (88%), CR activity was elevated in tumour tissue with respect to normal tissue and the activity had only decreased in 2 of the patients (patients 5 and 13). The Mann–Whitney *U* rank sum test revealed very significant differences between tumour and normal tissue (two-tailed probability = 0.0006).

The lung tumours analysed were non-small cell lung cancers (NSCLC): most of them epidermoid carcinomas or adenocarcinomas, grade 1, 2 or 3 (Table 1). No relationship has been found between CR activity and histological type (data not shown). With respect to grade, six of the 12 tumours which were well differentiated (G1) or moderately differentiated (G2) had high CR levels (> 50 nmol/min/mg protein). The four poorly differentiated (G3) tumours (patients 13, 5, 11 and 15) showed no increase in CR activity compared with normal tissue.

Breast. CR activity was studied in 12 paired tumour and normal breast tissues (Figure 1b). The patients have been ordered according to the criteria mentioned previously, except patient 19, who showed a very high activity level in normal tissue. Excluding this patient, the mean activity in normal tissue was 21.3 ± 4.8 nmol/min/mg protein (range 4.6 ± 0.0 and 59.1 ± 3.9 nmol/min/mg protein). CR activity was elevated (> 60 nmol/min/mg protein) in seven tumour samples (58%), six of which showed an increase in activity with respect to the normal tissue. The Mann–Whitney *U* test applied to the 12 pairs of samples studied revealed no significant differences between normal and tumoral tissues ($P = 0.15$). No relationship could be found between CR activity and the histological type, grade, TNM stage or even with the presence of progesterone or oestrogen receptors (data not shown).

P450R activity

P450R activity was studied in all of the samples. The mean activity in lung normal tissue was 6.1 ± 0.5 nmol/min/mg protein and in breast normal tissue was 5.7 ± 0.7 nmol/min/mg protein. Student's *t*-test revealed significant differences between normal lung and breast at the level of 5% ($t = 2.14$, $P = 0.036$). The mean activity in tumours was 6.8 ± 0.6 nmol/min/mg protein in lung tumours and 4.6 ± 0.6 nmol/min/mg protein in breast tumours (Table 2). In many cases the ratio between tumoral and normal tissues was increased (T/N was between 1.1 and 1.8 in eight lung tumours and between 1.1 and 6.8 in 10 breast tumours) but in other cases this ratio was below 1. However, the statistical analysis revealed no significant differences of activity between both tissues (lung: $U = 122$, $P = 0.45$; breast: $t = 0.81$, $P = 0.42$).

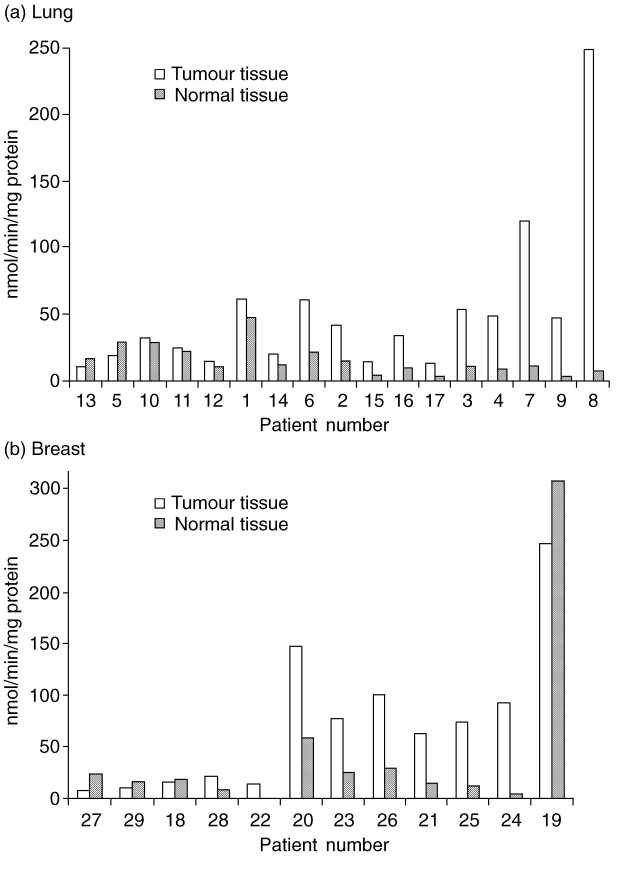

Figure 1. Carbonyl reductase activity measured as described in the text from patient paired (a) lung normal and tumour tissue; (b) breast normal and tumour tissue. Patients have been ordered, from left to right, according to the quotient resulting from the division of the enzyme activity found in tumour tissue, by the enzyme activity found in normal tissue. Reduction rates are the mean of three experiments, expressed in nmol cytochrome *c* reduced/min/mg protein. Standard errors of the mean (SEM) were below 5%.

Table 2. NADPH cytochrome P450 reductase activity

Patient No.	Tumour*	Normal	T/N
Lung			
1	5.4 ± 0.2	13.1 ± 0.5	0.4
2	6.9 ± 1.0	6.3 ± 0.4	1.1
3	5.9 ± 0.0	4.9 ± 0.2	1.2
4	7.9 ± 1.3	7.8 ± 0.5	1
5	7.5 ± 0.0	5.3 ± 0.0	1.4
6	4.8 ± 0.2	6.2 ± 0.3	0.8
7	7.6 ± 0.2	5.2 ± 0.1	1.4
8	10.7 ± 1.1	5.9†	1.8
9	7.2 ± 0.4	10.3 ± 0.1	0.7
10	4.7 ± 0.3	4.1 ± 0.0	1.2
11	5.4 ± 0.3	5.4 ± 0.3	1
12	4.4 ± 0.1	5.6 ± 0.0	0.8
13	3.5 ± 0.2	10.4 ± 0.5	0.3
14	9.9 ± 0.4	6.6 ± 0.3	1.5
15	2.7 ± 0.0	4.1 ± 0.0	0.6
16	3.7 ± 0.0	10.5 ± 0.2	0.3
17	5.2 ± 0.1	4.8 ± 0.2	1.1
Breast			
18	7.0 ± 0.4	7.7 ± 0.5	0.9
19	8.1 ± 0.4	4.2 ± 0.7	1.9
20	4.9 ± 0.2	4.3 ± 0.0	1.1
21	16.0 ± 1.1	7.5 ± 0.5	2.1
22	7.5 ± 0.5	1.1 ± 0.2	6.8
23	6.0 ± 1.1	6.1 ± 0.4	1
24	2.3 ± 0.0	3.0 ± 0.1	0.7
25	4.5 ± 0.0	3.7 ± 0.0	1.2
26	3.9 ± 0.2	4.0 ± 0.3	1
27	6.2 ± 0.2	2.0 ± 0.1	3.1
28	4.2 ± 0.1	3.5 ± 0.8	1.2
29	4.1 ± 0.2	6.4 ± 0.6	0.6
30	4.6 ± 0.0	10.0 ± 0.5	0.5
31	7.1†	5.5 ± 0.2	1.3
32	5.4 ± 0.4	3.0 ± 0.2	1.8
33	4.1 ± 0.0	4.1 ± 1.0	1
34	4.8 ± 0.3	2.8 ± 0.2	1.7
35	1.9 ± 0.8	10.0 ± 0.5	0.2

*Reduction rates are mean ± standard error of the mean (SEM) of three experiments, expressed in nmol cytochrome *c* reduced/min/mg protein. †Only one experiment could be carried out.

DISCUSSION

There have been few studies on CR activity in human tumours. The results obtained in this study show that the activity of such an enzyme is clearly augmented in some lung and breast tumours. Nevertheless, the behaviour is totally heterogeneous: while in some patients the enzyme activity was comparable to that of normal tissue, in others there was an increase of two to 40 times the normal value. Other authors have found a mean value of CR activity in human tumours which was superior to that of normal tissue; the increase was approximately double in the case of breast tumours and six times greater in the case of lung tumours [19]. When considering the mean value of activity in tumour and normal tissue, we found an increase of approximately three times, in both lung and breast tissues.

CR metabolises a variety of antitumour drugs containing carbonyl groups, such as the anthracycline antibiotics daunorubicin and doxorubicin. CR reduces the methyl ketone in the carbon side-chain of daunorubicin to its corresponding alcohol, which has been demonstrated to be less toxic than daunorubicin [27, 28]. The high CR activity level found in some tumour cells could contribute to tumoral resistance to some drugs metabolised in such a way.

Some authors have found that, unlike in rodent tissues, the major quinone reductase in centrifuged homogenates of human liver and placenta is a CR rather than DT diaphorase [12]. This enzyme, acting as a quinone reductase, may protect humans against quinone-mediated carcinogenic, mutagenic and toxic effects, in a concerted action with superoxide dismutase, similar to that proposed for DT diaphorase [29]. In relation to that, it has been demonstrated that CR mRNA is induced in tumoral cell lines by compounds that induce enzymes involved in xenobiotic detoxification [30], which implies that CR is also important in cell protection. In the samples in which a higher level of CR activity was found, a higher DT diaphorase activity (data not shown) was also found. This would support the idea suggested by these authors: a similar induction mechanism in which the locus Ah would be implicated.

With regard to the P450R, the activity found in both the tumours and the normal tissues was extremely low. In a study in which this enzyme and other microsomal enzymes from normal lung tissue adjacent to tumour tissue were measured, other authors obtained values of P450R activity of 33.3 ± 7.6 nmol/min/mg microsomal protein [31]. The mean level of activity for this enzyme which we found in normal tissue was lower. The differences could be due to the fact that our measurements were carried out in a subcellular fraction which was less purified than the microsomal fraction obtained by McManus and colleagues [31]. In any case, the values found in the tumours were of the same order of magnitude as those of normal tissue in most of the samples analysed; in only three was there an increase of more than 2-fold in tumour versus normal tissue.

According to these results, CR would be a better target than P450R for designing bioreductive compounds. However, it must be borne in mind that the activities were measured under non-physiological conditions using an artificial substrate. Although it is true that, *in vitro*, absolute values and the T/N ratio are much smaller for P450R than for CR, *in vivo*, where many prodrugs may partition into the lipophilic environment of the endoplasmic reticulum, P450R may, in fact, be more active than CR.

The heterogeneity in reductase activity in normal and tumour tissues indicates that enzyme-directed bioreductive therapy does not seem applicable in the clinic at present.

1. Martin JM, Giaccia AJ. The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. *Cancer Res* 1998, **58**, 1408–1416.
2. Miller VA, Ng KK, Grant SC, *et al*. Phase II study of the combination of the novel bioreductive agent, tirapazamine, with cisplatin in patients with advanced non-small-cell lung cancer. *Ann Oncol* 1997, **8**, 1269–1271.
3. Pavlidis N, Hanauke AR, Gamucci T, *et al*. A randomized phase II study with two schedules of the novel indoloquinone E09 in non-small-cell lung cancer: a study of the EORTC Early Clinical Studies Group (ECSG). *Ann Oncol* 1996, **7**, 529–531.
4. Bedikian AY, Legha SS, Eton O, *et al*. Phase II trial of tirapazamine combined with cisplatin in chemotherapy of advanced malignant melanoma. *Ann Oncol* 1997, **8**, 363–367.
5. Workman P, Stratford IJ. The experimental development of bioreductive drugs and their role in cancer therapy. *Cancer Metastasis Rev* 1993, **12**, 73–82.

6. Wermuth B. Purification and properties of an NADPH-dependent carbonyl reductase from human brain. Relationship to prostaglandin 9-ketoreductase and xenobiotic ketone reductase. *J Biol Chem* 1981, **256**, 1206–1213.
7. Nakayama T, Hara A, Yashiro K, Sawada H. Reductases from carbonyl compounds in human liver. *Biochem Pharmacol* 1985, **34**, 107–117.
8. Inazu N, Ruepp B, Wirth H, Wermuth B. Carbonyl reductase from human testis: purification and comparison with carbonyl reductase from human brain and rat testis. *Biochem Biophys Acta* 1992, **116**, 50–56.
9. Wirth H, Wermuth B. Immunohistochemical localization of carbonyl reductase in human tissues. *J Histochem Cytochem* 1992, **42**, 1857–1863.
10. Lee SC, Levine L. Prostaglandin metabolism I. Cytoplasmic reduced nicotinamide adenine dinucleotide phosphate-dependent and microsomal reduced nicotinamide adenine dinucleotide-dependent prostaglandin E9-ketoreductase activities in monkey and pigeon tissues. *J Biol Chem* 1974, **249**, 1369–1375.
11. Ahmed NK, Felsted RL, Bachur NR. Heterogeneity of anthracycline antibiotic carbonyl reductases in mammalian livers. *Biochem Pharmacol* 1978, **27**, 2713–2719.
12. Wermuth B, Platts KL, Seidel A, Oesch F. Carbonyl reductase provides the enzymatic basis of quinone detoxification in man. *Biochem Pharmacol* 1986, **35**, 1277–1282.
13. Ohara H, Miyabe Y, Deyashiki Y, Matshura K, Hara A. Reduction of drug ketones by dihydrodiol dehydrogenases, carbonyl reductase and aldehyde reductase of human liver. *J Biochem* 1995, **50**, 221–227.
14. Lloyd RV, Duling DR, Rumyantseva GV, Mason RO, Bridson PK. Microsomal reduction of 3-amino-1,2,4-benzotriazine 1,4-dioxide to a free radical. *Molec Pharmacol* 1991, **40**, 440–445.
15. Wang J, Biederman KA, Wolff CR, Brown JM. Metabolism of the bioreductive cytotoxin SR4233 by tumour cells: enzymatic studies. *Br J Cancer* 1993, **67**, 321–325.
16. Walton MI, Wolff CR, Workman P. The role of cytochrome P450 and cytochrome P450 reductase in the reductive bioactivation of the novel benzotriazine di-N-oxide hypoxic cytotoxin 3-amino-1,2,4-benzotriazine-1,4-dioxide (SR4233, WIN59075) by mouse liver. *Biochem Pharmacol* 1992, **44**, 251–259.
17. Cahill A, White INH. Reductase metabolism of 3-amino-1,2,4-benzotriazine-1,4-dioxide (SR4233) and the induction of unscheduled DNA synthesis in rat and human derived cell lines. *Carcinogenesis* 1990, **11**, 1407–1411.
18. Schor NA, Cornelisse CJ. Biochemical and quantitative histochemical study of reduced pyridine nucleotide dehydrogenation by human colon carcinomas. *Cancer Res* 1983, **43**, 4850–4855.
19. Schlager JJ, Powis G. Cytosolic NAD(P)H: (quinone acceptor) oxidoreductase in human normal and tumor tissue: effects of cigarette smoking and alcohol. *Int J Cancer* 1990, **45**, 403–409.
20. Cresteil T, Jaiswal AK. High levels of expression of the NAD(P)H: quinone oxidoreductase (NQO1) gene in tumor cells compared to normal cells of the same origin. *Biochem Pharmacol* 1991, **42**, 1021–1027.
21. Smitskamp-Wilms E, Giaccone G, Pinedo HM, Van der Laan BFAM, Peters GJ. DT-diaphorase activity in normal and neoplastic human tissues; an indicator for sensitivity to bioreductive agents? *Br J Cancer* 1995, **72**, 917–921.
22. Marin A, Lopez de Cerain A, Hamilton E, *et al.* DT-diaphorase and cytochrome b5 reductase in human lung and breast tumours. *Br J Cancer* 1997, **76**, 923–929.
23. American Joint Committee on Cancer (AJCC). *Manual for Staging of Cancer*, 4th edn. Beahrs OH, Henson DE, Hutter RVP, Kennedy BJ, eds. Philadelphia, J.B. Lippincott, 1992.
24. Smith PK, Krohn IR, Hermanson GT, *et al.* Measurement of protein using bicinchoninic acid. *Anal Biochem* 1985, **150**, 76–85.
25. Ernster L. DT-diaphorase. *Methods Enzymol* 1967, **10**, 309–317.
26. Riley RJ, Workman P. DT-diaphorase and cancer chemotherapy. *Biochem Pharmacol* 1992, **43**, 1657–1659.
27. Kuffel MJ, Reid JM, Ames MM. Anthracyclines and their C-13 alcohol metabolites: growth inhibition and DNA damage following incubation with human tumor cells in culture. *Cancer Chemother Pharmacol* 1992, **30**, 51–57.
28. Schott B, Robert J. Comparative activity of anthracycline 13-dihydrometabolites against rat glioblastoma cells in culture. *Biochem Pharmacol* 1989, **38**, 4069–4074.
29. Jarabak J, Harvey RG. Studies on three reductases which have polycyclic aromatic hydrocarbon quinones as substrates. *Arch Biochem Biophys* 1993, **303**(2), 394–401.
30. Forrest GL, Akman S, Krutzik S, *et al.* Induction of a human carbonyl reductase gene located on chromosome 21. *Biochem Biophys Acta* 1990, **1048**, 149–155.
31. McManus ME, Boobis AR, Pacifici GM, *et al.* Xenobiotic metabolism in the human lung. *Life Sci* 1980, **26**, 481–487.

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