ELSEVIER

Biochemical Pharmacology 62 (2001) 1371–1377

Biochemical Pharmacology

Genotyping of NAD(P)H:quinone oxidoreductase (NQO1) in a panel of human tumor xenografts: relationship between genotype status, NQO1 activity and the response of xenografts to Mitomycin C chemotherapy *in vivo*☆

Roger M. Phillips[a,*], Angelika M. Burger[b], Heinz-Herbert Fiebig[b], John A. Double[a]

[a]*Cancer Research Unit, University of Bradford, Bradford BD7 1DP, United Kingdom* [b]*Tumour Biology Center, University of Freiburg, Breisacher Str., 117, D-79106 Freiburg, Germany*

Received 3 January 2001; accepted 2 July 2001

Abstract

Pharmacogenetic analysis of polymorphisms in drug metabolizing enzymes is currently generating considerable interest as a means of individualizing patient therapy. Recent studies have suggested that patients that are homozygous for a polymorphic variant (a C to T transition at position 609 of the cDNA sequence) of the enzyme NAD(P)H:quinone oxidoreductase (NQO1) may be resistant to Mitomycin C (MMC). Genotyping of a panel of 54 human tumor xenografts by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP), classified tumors as wild type (40/54), heterozygotes (11/54), and homozygous mutants (3/54). Previously, 37 of these tumors had been characterized in terms of their response to MMC *in vivo*, and in this study, a further nine tumor xenografts have been characterized in terms of their response to MMC. No correlation could be found between the NQO1 polymorphic status of xenografts and their response to MMC *in vivo*. In terms of genotype/phenotype relationships, NQO1 activity in tumors genotyped as wild type, heterozygotes, and homozygous mutants were 311.1 ± 421.9 (N = 40), 76.9 ± 109.5 (N = 11), and 0.2 ± 0.17 (N = 3) nmol/min/mg, respectively. Genotyping of patients may provide a useful initial step in identifying patients who are unlikely to benefit from quinone-based chemotherapy. In the case of MMC, however, the work presented here demonstrates that genotyping of individuals with respect to NQO1 is unlikely to be beneficial in terms of predicting tumor responses to MMC.

Keywords: NQO1; Polymorphism; Mitomycin C; Xenografts; Pharmacogenetics

1. Introduction

The ability to tailor chemotherapy to individual patients has been a major objective in cancer therapy for many years, but despite extensive studies using a variety of chemosensitivity tests [1], no predictive assay is currently in widespread use today. Individualizing patient therapy remains a major issue in modern anticancer drug development, and this objective forms one of the cornerstones for the rapidly growing field of pharmacogenetics [2]. The enzyme NAD(P)H:quinone oxidoreductase (E.C. 1.6.99.2, NQO1 [also known as DT-diaphorase]) has generated considerable interest within the field of bioreductive drug development because of its ability to activate certain quinone-based compounds to cytotoxic products, and elevated levels of NQO1 have been found in several tumor types, particularly NSCLC (non-small cell lung cancer) [3,4]. In 1992, Traver *et al.* [5] identified a C to T point mutation at position 609 (C609T) in exon 6 of the NQO1 cDNA isolated from BE human colon carcinoma cells. Despite detectable levels of NQO1 mRNA, cells were devoid of NQO1 activity, and NQO1 protein was undetectable by immunoblot analysis. Subsequent studies have demonstrated that C609T mutation is a true polymorphism [6,7], suggesting that affected indi-

☆ This work was funded by the Cancer Research Campaign (program grant reference number SP 2523/0101).

* Corresponding author. Tel.: +44 1274 233226; fax: +44 1274 233234.

E-mail address: r.m.phillips@bradford.ac.uk (R.M. Phillips).

Abbreviations: NQO1, NAD(P)H,Quinone oxidoreductase; MMC, Mitomycin C; PCR-RFLP, Polymerase chain reaction restriction fragment length polymorphism; and RH1, 2,5-diaziridinyl-3-(hydroxymethyl)-6-methyl-1,4-benzoquinone.

0006-2952/01/$ – see front matter
PII: PII S0006-2952(01)00769-9

viduals may exhibit resistance to quinone-based cancer therapy. The widely used anticancer agent, Mitomycin C (MMC), is a quinone-based compound that can be reduced by NQO1 to DNA damaging species in cell free assays [8]. Resistance to MMC has been documented in cell lines that are homozygous for the C609T polymorphism [5,9]. Recent studies have indicated that patients with gastric cancer or disseminated peritoneal cancer (of gastrointestinal or other origin) with this polymorphism have reduced response to MMC at both the cellular level and in terms of overall survival [10,11]. While these reports are preliminary, they are very significant in that they represent the first direct evidence that the C609T polymorphic variant could have a major impact on the outcome of MMC chemotherapy. If substantiated, screening of patients for the NQO1 C609T polymorphism could be used to identify those patients who are unlikely to benefit from MMC therapy.

For the polymorphic variant to have a direct impact on tumor response to MMC, it must be clearly demonstrated that NQO1 plays a pivotal role in MMC activation. The role of NQO1 in MMC activation is, however, controversial, and there is considerable doubt as to whether or not cellular response to MMC can be predicted on the basis of NQO1 activity [3,12]. Recent studies in this laboratory have also demonstrated that the response of a large panel of human tumor xenografts to MMC *in vivo* cannot be predicted on the basis of NQO1 enzyme activity [13]. These findings cast some doubt over whether or not analysis of the C609T NQO1 polymorphism would lead to a better prediction of MMC activity *in vivo* than does enzyme activity. It should, however, be stressed that the measurement of NQO1 activity in human tumor xenografts was performed on tumor tissues that represent a mixture of both stromal cells (predominantly of murine origin) and tumor cells. Becuase components of stromal tissue such as vascular endothelial cells have been shown the express NQO1 protein [14], it is conceivable that the NQO1 activity measurements obtained do not accurately reflect NQO1 activity in tumor cells. Genotyping of NQO1 using RFLP-PCR techniques has the potential to circumvent this problem by the use of primers specific to human NQO1. In view of this, plus the potential significance of clinical findings reported in the literature [10,11], the principle aim of this study was to examine whether or not tumor response to MMC can be predicted on the basis of NQO1 genotype status. In addition, studies using cell lines have demonstrated that NQO1 activity may be forecast on the basis of NQO1 genotype with wild-type cells having higher levels of NQO1 than do heterozygotes, whereas mutant cells are devoid of NQO1 activity [15, 16]. As NQO1 activity has been previously determined [13], a secondary aim of this study was to determine the genotype-phenotype relationship in this panel of human tumor xenografts *in vivo*.

2. Materials and methods

2.1 Tissue collection and DNA extraction

Tissues of human tumor xenograft growing subcutaneously in thymus aplastic nude mice (NMRI background) were collected from the Freiburg Tumor Bank. This bank comprises over 350 human tumor xenograft models that were established in serial passage *in vivo* [17]. A number of these xenografts have been previously characterized in terms of their response to MMC in nude mice (37 tumors [13,17]) and NQO1 activity (58 tumors [13]). In addition to the 37 tumors that have been characterized for MMC sensitivity *in vivo*, the response of a further nine tumors to MMC were included in this study (BXF 1301, CNXF 498, LXFL 1072, MAXF 401, RXF 944LX, PAXF 736, SXF 1410, UXF 1138, and XF 575). Of the 58 tumors characterized for NQO1 activity [13], nine tumors from this original panel were not available for genotyping in this study (CXF HT29X, BXF 1036, LXFA 418, CXF 609, MEXF 514, BXF 439, LEXF HL60X, MEXF 134,1 and CEXF 633). Five additional tumors (HNXF 675, MAXF 401, OVXF 1619, SXF 1186, and UXF 1138) were included in this study for evaluating genotype-phenotype relationships.

All tumor samples were initially frozen in liquid nitrogen and stored at -80^{o} for analysis. In addition, livers from non-tumor-bearing mice were also collected. Genomic DNA was extracted from tissue fragments (<10mg) using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, USA) and stored at -20^{o}.

2.2. Restriction fragment length polymorphism of Polymerase chain reaction products

Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis was conducted according to the method published by Eickelmann *et al.* [9]. Primers (synthesised by Cruachem, Glasgow, UK) for PCR amplification of NQO1 from genomic DNA were forward primer 5′ GAG ACG CTA GCT CTG AAC TGA T 3′ and reverse primer 5′ ATT TGA ATT CGG GCG TCT GCT G 3′. PCR was conducted by using the PCR core kit (Roche Diagnostics Ltd., Lewes, UK), and each reaction contained 10 μL 10X PCR buffer (without magnesium), 5 μL Magnesium Chloride (25 mM), 2 μL dNTPs (10 mM), 1 μL each of forward and reverse primers (16.3 nmol mL^{-1}), 1 μL Taq polymerase (5 units μL^{-1}), 1 μL genomic DNA (0.1 $\mu g\ mL^{-1}$) and was made up to a final volume of 100 μL using sterile, deionized water. Thermal cycling conditions following an initial denaturation step of 94^{o} for 4 min were, 94^{o} (1 min), 65^{o} (1 min), and 72^{o} (1 min). Following 35 cycles of PCR, samples were maintained at 72^{o} for 5 min. PCR product (25 μL) was then digested with *Hinf* 1 (10 U) for 2 hr at 37^{o}, and the products separated on a 2% TAE agarose gel (run at 100 V for 2 hr) and stained with ethidium bromide. Products were visualized under UV tran-

sillumination and permanent records made by using a Polaroid camera. As a positive control, primers designed to amplify murine K-ras were employed [18], the sequences of which were forward primer 5′ AAC TTG TGG TGG TTG GAG GTG 3′ and reverse primer 5′ CTC ATC CAC AAA GTG ATT CT 3′. Reaction conditions and thermal cycling parameters for amplification of K-ras were identical to that of NQO1 apart from the use of 48°C annealing temperature. Expected PCR product size was 106 bp.

2.3. Assessment of in vivo activity of MMC in human tumor xenograft models and NQO1 enzyme activity

The response of 37 human tumor xenografts following the intraperitoneal administration of MMC *in vivo* has been described in detail elsewhere [13,17] and indicated as such in Table 1. The response of nine tumors that have not previously been characterized for MMC sensitivity were also included in this study. These tumors were BXF 1301, CNXF 498, LXFL 1072, MAXF 401, RXF 944LX, SXF 1410, XF 575, PAXF 736, and UXF 1138. Chemotherapy and assessment protocols were identical to those described previously [13,17]. Briefly, all tumors were implanted subcutaneously into both flanks of outbred, athymic nude mice of NMRI genetic background, and treatment was started when the tumors reached a median size of 6 mm in diameter (Day 0). MMC was administered intravenously at the maximum tolerated dose of 2 mg Kg^{-1} on Days 1 and 15. Antitumor effects were determined by two dimensional caliper measurements, and measurements were normalized relative to tumor volume on Day 0. Experiments were terminated when tumors reached a size of approximately 1.5 cm in diameter. Activity was expressed in terms of percentage of the optimal T/C (i.e. relative tumor volume of treated tumors divided by the relative tumor volume of control tumors × 100 at the time of maximum drug effect). All animal experiments were performed in accordance with German Animal License Regulations (Tierschutzgestz), which are identical to United Kingdom Co-ordinating Committee on Cancer Research Guidelines for the Welfare of Animals in Experimental Neoplasia [19]. NQO1 activity in five additional tumors (HNXF 675, MAXF 401, OVXF 1353, SXF 1186, and UXF 1138) was determined in this study according to procedures published elsewhere [13].

3. Results

The results of PCR-RFLP analysis of three xenografts representing the three possible genotypes of NQO1 is presented in Fig. 1. PCR amplification of NQO1 produced products of 240 bp (Fig. 1, lanes 2–4) that, on digestion with *Hinf* I, generated clear products at 218 bp for wild type; 218, 165, and 53 for heterozygotes; and 165 and 53 for homozygous mutants (Fig. 1, lanes 5–7). The 22 bp fragment produced following *Hinf* I digestion of all three PCR products was not visible because of poor detection on ethidium bromide-stained agarose gels, but its presence was

Table 1
Relationship between response of tumors to MMC *in vivo* and NQO1 polymorphic status

Tumor	Tumor response (% T/C)*	NQO1 status
BXF 1258	41.6[a]	wt
BXF 1299	88.6[a]	wt
BXF 1301	24.0	wt
CNXF 498	60.8	wt
CXF 1103	57.8[b]	het
CXF 158	67.5[b]	wt
CXF 280	5.2[b]	wt
CXF HCT116	40.0[a]	wt
GXF 209	6.3[b]	wt
GXF 214	59.4[b]	wt
GXF 251	31.1[b]	wt
GXF 97	2.6[b]	wt
HNXF 536	74.4[a]	wt
LXFA 289	8.3[b]	wt
LXFA 526	76.9[b]	wt
LXFE 211	8.3[b]	wt
LXFE 397	67.8[b]	wt
LXFE 409	3.6[b]	wt
LXFE 839	6.4[a]	wt
LXFL 1072	96.0	wt
LXFL 529	5.2[b]	wt
LXFS 538	1.5[b]	wt
LXFS 650a	57.2[b]	wt
LYXF 1189	63.2[a]	wt
MAXF 1162	70.90[b]	mut
MAXF 401	2.6	het
MAXF 449	8.1[b]	mut
MAXF 857	79.2[b]	wt
MEXF 535	85.3[b]	mut
MEXF 989	21.7[b]	wt
OVXF 1023	7.9[b]	wt
OVXF 1353	100[b]	wt
OVXF 899	29.8[b]	wt
PAXF 546	49.1[a]	wt
PAXF 736	70.2	wt
PRXF 1369	69.2[a]	wt
PRXF DU145	79.6[a]	wt
PRXF PC3MX	100[a]	wt
RXF 1220	86.4[b]	wt
RXF 423	74.2[b]	het
RXF 486	88.0[b]	het
RXF 944LX	42.2	wt
SXF 1410	89.0	wt
TXF 881	31.4[a]	het
UXF 1138	2.8	het
XF 575	42.7	wt

* Superscript text [a] and [b] denote data previously published by Phillips et al. [13] and Fiebig et al. [17], respectively BXF: Bladder, CNXF Central Nervous System, CXF: Colorectal, GXF: Gastric, HNXF: Head and Neck, LXFA: Lung (Adenocarcinoma) LXFE: Lung (Epidermoid), LXFL: Lung (Large cell), LXFS: Lung (SCLC), LYXF: Lymphoma, MAXF: Mammary, MEXF: Melanoma, OVXF: Ovarian, PAXF: Pancreatic, PRXF: Prostate, RXF: Renal, SXF: Sarcoma, TXF: Testis, UXF: Uterine, XF: mixed histology. ND: not determined. Wt, het, and mut denote tumors classified as wild type, heterozygotes, and homozygous mutants with respect to NQO1 C609T, respectively.

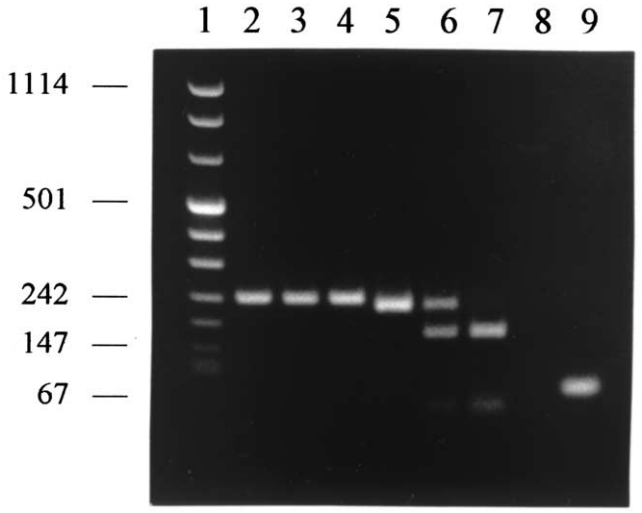

Fig. 1. PCR-RFLP analysis of NQO1. Lane 1 represents molecular weight markers (pUCBM21 cut with *Hpa* II, *Dra* I, and *Hind* III, Roche Diagnostics Ltd, Lewes, UK). Lanes 2–4 represent undigested PCR products of genomic DNA derived from CNXF 498, RXF 486, and MAXF 449 tumors, respectively. Lanes 5 to 7 represent *Hinf* I digest of PCR products derived from CNXF 498, RXF 486, and MAXF 449 tumors, respectively. Lane 8 represents mouse genomic DNA (isolated from mouse liver) amplified by using NQO1 primers, and lane 9 represents mouse genomic DNA amplified with K-ras primers.

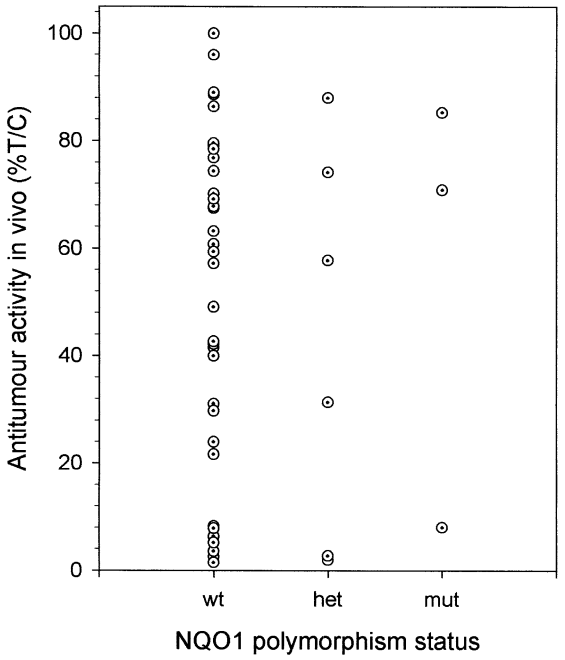

Fig. 2. The relationship between NQO1 genotype and the response of a panel of human tumor xenografts to MMC.

confirmed by capillary electrophoresis (data not shown). No PCR products at 240 bp were produced following the amplification of murine genomic DNA (Fig. 1, lane 8) demonstrating that the primers used to amplify NQO1 are specific for human NQO1. Possible errors in genotyping arising from host cell infiltration into tumor tissue are, therefore, unlikely. As a positive control to check the integrity of murine genomic DNA, PCR amplification of K-ras produced the expected product at 106 bp (Fig. 1, lane 9).

A total of 54 human tumor xenografts have been genotyped with respect to NQO1. The incidence of the three NQO1 variants and their relationship to the response of 46 of these tumors to MMC *in vivo* is presented in Table 1 and Fig. 2. The remaining eight tumors (BXF 1352, CXF 883, CXF DLD1LX, HNXF 675, OVXF 1619, RXF488, SXF 1186, and SXF 627) were genotyped for NQO1, but no data is available with regards to their sensitivity to MMC *in vivo.* The genotype status of these tumors is reported in Table 2. Of the 54 tumors genotyped, 40 (74.1%) were wild type, 11 (20.3%) were heterozygous, and 3 (5.6%) were homozygous mutant with respect to NQO1. The response of tumors to MMC *in vivo* and the relationship between NQO1 genotype status and antitumor activity is presented in Fig. 2 and Table 1. Antitumor data exists for 46 tumors (37 tumors previously characterized, and 9 tumors where response to MMC has been determined in this study). Mean tumor responses ± SD (expressed as % T/C) were 48.4 ± 32.6 (N = 37), 42.7 ± 36.4 (N = 6), And 54.7 ± 41.0 (N = 3) for wild type, heterozygotes, and mutants, respectively. Differences between the three groups did not reach significance ($P > 0.05$ as determined by the Mann–Whitney U test). Considerable heterogeneity in tumor response to MMC existed within each group, particularly within tumors classified as wild type (Fig. 2). In this case, tumor response to MMC ranged from resistance (T/C = 100%) to complete response (T/C = 1.5%). No correlation exists between NQO1 genotype and tumor response to MMC (Fig. 2).

In terms of genotype-phenotype relationships, NQO1 activity and genotype status for 54 human tumor xenografts is presented in Table 2 and Fig. 3. NQO1 activity (expressed as mean ± SD) in wild-type tumors was generally higher than in heterozygotes [311.1 ± 421.9 (N = 40) And 76.9 ± 109.5 (N = 11) nmol/min/mg, respectively], although the difference between the groups was not significant ($P > 0.05$ as determined by the Mann–Whitney U test). A broad spectrum of NQO1 activity was observed in wild-type tumors ranging from 1843.8 to 1.1 nmol/min/mg. Tumors classified as mutant with respect to NQO1 were devoid of enzyme activity (0.2 ± 0.17 nmol/min/mg, N = 3).

4. Discussion

The results of this study clearly demonstrate that genotyping of tumors with respect to the NQO1 C609T polymorphic variant does not correlate with tumor response to MMC *in vivo.* This finding is consistent with our previous study, which demonstrated a lack of correlation between NQO1 activity and tumor response to MMC *in vivo* [13], and both studies together strongly argue against NQO1 playing a major role in MMC activation. This point is

Table 2
Relationship between DT-diaphorase activity and NQO1 polymorphic status

Tumor	DT-diaphorase (nmol/min/ms)	NQO1 status
BXF 1258	11.9	wt
BXF 1299	362.5	wt
BXF 1301	6.0	wt
BXF 1352	169.2	wt
CNXF 498	9.7	wt
CXF 1103	61.8	het
CXF 158	120.3	wt
CXF 280	238.9	wt
CXF 883	93.3	wt
CXF HCT116	155.0	wt
CXF DLD1LX	55.3	het
GXF 209	660.5	wt
GXF 214	510.6	wt
GXF 251	980.5	wt
GXF 97	582.8	wt
HNXF 536	159.3	wt
HNXF 675	4.90*	het
LXFA 289	554.4	wt
LXFA 526	20.5	wt
LXFE 211	64.6	wt
LXFE 397	15.2	wt
LXFE 409	1.1	wt
LXFE 839	564.4	wt
LXFL 1072	800.3	wt
LXFL 529	322.2	wt
LXFS 538	125.4	wt
LXFS 650a	1.9	wt
LYXF 1189	1.7	wt
MAXF 1162	0.4	mut
MAXF 401	71.6*	het
MAXF 449	<0.1	mut
MAXF 857	1.4	wt
MEXF 535	<0.1	mut
MEXF 989	229.4	wt
OVXF 1023	4.3	wt
OVXF 1619	352.4*	het
OVXF 1353	58.3	wt
OVXF 899	1375.4	wt
PAXF 546	300.9	wt
PAXF 736	415.6	wt
PRXF 1369	16.2	wt
PRXF DU145	1075.1	wt
PRXF PC3MX	144.4	wt
RXF 1220	799.2	wt
RXF 423	23.0	het
RXF 486	6.1	het
RXF 488	120.5	wt
RXF 944LX	1.8	wt
SXF 1410	25.4	wt
SXF 1186	35.0*	het
SXF 627	3.6	het
TXF 881	15.9	het
UXF 1138	216.8*	het
XF 575	1843.8	wt

DT-diaphorase enzyme activity measurements taken from Phillips et al. [13] with the exception of those marked * where DT-diaphorase activity was determined in this study according to previously published methodology [13].

strikingly illustrated in the case of MAXF 449, which despite being homozygous for the polymorphism and devoid

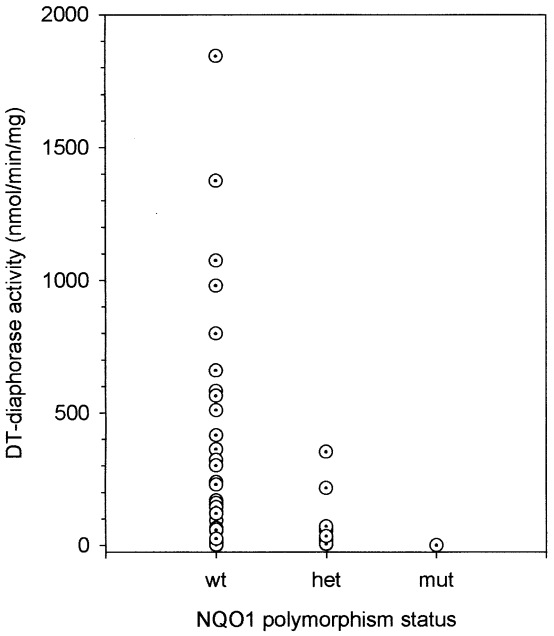

Fig. 3. The relationship between NQO1 genotype status and NQO1 activity in a panel of human tumor xenografts.

of NQO1 activity, is responsive to MMC (T/C = 8.1%). Furthermore, tumors that are wild type with respect to NQO1 and have high NQO1 activity, such as PRXF DU145 and LXFL 1072, are relatively resistant to MMC, with T/C values of 79.6% and 96.0%, respectively (Tables 1 and 2). The reasons why MAXF 449 responds to MMC *in vivo* are not clear, although other enzymes have been implicated in MMC activation [12]. Physiological parameters, such as hypoxia, have a significant influence on MMC activation with high hypoxia selectivity ratios reported, particularly for NQO1 deficient tumors [20,21]. In addition the final outcome of chemotherapy *in vivo* is likely to be determined by several parameters involved not only in bioreductive drug activation, but also by drug delivery to tumors and the inherent ability of tumor cells to repair DNA damage. Further studies are required to determine why tumors such as MAXF 449 and LXFL 1072 respond quite differently to MMC, but it is clear from this and previous studies in this laboratory [13] that accurate predictions of tumor response to MMC *in vivo* on the basis of the activity or genotype of NQO1 are unlikely.

In terms of genotype-phenotype relationships (Table 2), the results of this study are consistent with previous reports in that cells or tissues genotyped as wild type tend to have higher levels of NQO1 activity than do heterozygotes, whereas mutant cells are devoid of NQO1 activity [15,16]. Lack of NQO1 activity in xenografts genotyped as mutant (Table 2, Fig. 3) are entirely consistent with studies using cell lines and human tissues [5,6,15,16]. Previous studies have reported that a significant difference exists between wild type and heterozygotes in terms of either NQO1 activity [16] or NQO1 protein levels as determined by immu-

noblotting [15]. These results suggest that genotype status may provide a reliable initial estimate of NQO1 activity, although some overlap in values between cells genotyped as wild type and heterozygotes was observed [16]. In this study, however, the differences in NQO1 activity between tumors classified as wild type [311.1 ± 421.9 (N = 40) nmol/min/mg] and heterozygotes and [76.9 ± 109.5 (N = 11) nmol/min/mg] were not significant, with considerable heterogeneity observed in NQO1 activity within both groups. Heterogeneity was particularly marked in the wild-type group (Table 2, Fig. 3), with several tumors having very low or barely detectable levels of NQO1 activity. These included RXF 944LX, LXFE 409, MAXF 857, LYXF 1189, OVXF 1023, and LXFS 650a, all of which have NQO1 activities of less than 5.0 nmol/min/mg (Table 2). While the results of this study are in general agreement with previously published data (particularly with regards to cells genotyped as mutants), characterization of tumors with respect to wild type or heterogeneous NQO1 does not necessarily provide an indication of NQO1 activity in tumor tissue. In a clinical setting, genotyping of patients would certainly be of use for identifying patients who are unlikely to respond (i.e. those who are devoid of NQO1 activity as a result of the C609T polymorphism) to quinone-based therapeutics where NQO1 plays a prominent role in drug activation.

In conclusion, the results of this study demonstrate that genotyping of tumors with respect to the NQO1 C609T polymorphism is unlikely to be of benefit in terms of predicting tumor response to MMC. These results do not support the clinical findings of Fleming *et al.* [10] and Yano *et al.* [11], where the NQO1 polymorphic variant was associated with poor response to MMC at both the cellular level and in terms of survival times. It should, however, be stressed that the *in vivo* endpoint used in this study represents maximal tumor response and may not be directly comparable with the final outcome of chemotherapy in terms of patient survival times. Further studies are required to validate the findings of Yano *et al.* [11]. Nevertheless, the results of this study in conjunction with the controversy in the literature surrounding NQO1 and its role in determining cell and tumor response to MMC [8,12,13,22–25] suggest that tailoring MMC therapy to patients on the basis of NQO1 genotype status is unlikely to be clinically useful. In the case of novel compounds entering clinical evaluation [e.g. RH1 (2;5-diaziridinyl-3-(hydroxymethyl)-6-methyl-1; 4-benzoquinone) and 17-allylamino 17-demthoxygldanomycin] [26,27], where there is evidence for NQO1 involvement in their mechanism of action, genotyping of tumors may prove valuable in terms of identifying patients who are unlikely to benefit from such therapy. For novel compounds in preclinical development, it is essential to demonstrate that NQO1 plays a central role in determining tumor response *in vivo* if genotyping or enzyme profiling is to be of any value. In this respect, the use of xenografts that have been characterized in terms of both NQO1 activity and genotype status should provide a useful experimental tool for establishing proof of principle *in vivo* for NQO1 activated prodrugs.

References

[1] Phillips RM, Bibby MC, Double JA. A critical appraisal of the predictive value of *in vitro* chemosensitivity assays. J Natl Cancer Inst 1990;82:1457–68.

[2] Roses AD. Pharmacogenetics and the practice of medicine. Nature 2000;405:857–65.

[3] Workman P. Enzyme directed bioreductive drug development revisited: a commentary on recent progress and future prospects with emphasis on quinone anticancer agents and quinone metabolizing enzymes, particularly DT-diaphorase. Oncol Res 1994;6:6–8.

[4] Malkinson AM, Siegel D, Forrest GL, Gazdar AF, Oie HK, Chan DC, Bunn PA, Mabry M, Dykes DJ, Harrison SD, Ross D. Elevated DT-diaphorase activity and messenger RNA content in human non-small cell lung carcinoma: relationship to the response of lung tumor xenografts to mitomycin C. Cancer Res 1992;52:4752–7.

[5] Traver RD, Horikoshi T, Danenberg KD, Stadlbauer THW, Danenberg PV, Ross D, Gibson NW. NAD(P)H:Quinone oxidoreductase gene expression in human colon carcinoma cells: characterisation of a mutation which modulates DT-diaphorase activity and mitomycin sensitivity. Cancer Res 1992;52**:**797–802,

[6] Traver RD, Siegel D, Beall HD, Phillips RM, Gibson NW, Franklin WA, Ross D. Characterisation of a polymorphism in NAD(P)H: quinone oxidoreductase (DT-diaphorase). Br J Cancer 1997;**75:**69–75.

[7] Gaedigk A, Tyndale RF, Jurima-Romet M, Sellers EM, Grar DM, Leeder JS. NAD(P)H:quinone oxidoreductase: polymorphism and allele frequency in Caucasian, Chinese and Canadian native Indian and Inuit populations. Pharmacogenetics 1998;8:305–13.

[8] Siegel D, Gibson NW, Preusch PC, Ross D. Metabolism of MMC by DT-diaphorase: role in MMC induced DNA damage and cytotoxicity in human colon carcinoma cells. Cancer Res 1990;50:7483–89.

[9] Eickelmann P, Schulz WA, Rohde D, Schmitz-Dräger B, Sies H. Loss of heterozygosity at the NAD(P)H:Quinone oxidoreductase locus associated with increased resistance against MMC in a human bladder carcinoma cell line. Biol Chem Hoppe Seyler 1994;375: 439–45.

[10] Fleming RA, Drees J, Loggie BW, Russell GB, Geisinger KR, Morris RT, Sachs D, McQuellon RP. Clinical significance of NAD-(P)H:quinone oxidoreductase 1 C609T polymorphism in patients with disseminated peritoneal cancer receiving intraperitoneal MMC. Proc Am Assoc Cancer Res 2000;41:721.

[11] Yano M, Akiyama Y, Shiozaki H, Inoue M, Doki Y, Fujiwara Y, Ross D, Sadler A, Monden M. Genetic polymorphism of NAD(P)H: Quinone oxidoreductase and MMC sensitivity in gastric cancer. Proc Am Assoc Cancer Res 2000;41:721.

[12] Cummings J, Spanswick VJ, Tomaz M, Smyth JF. Enzymology of MMC metabolic activation in tumor tissue. Implications for enzyme directed bioreductive drug development. Biochem Pharmacol 1998; 56:405–14.

[13] Phillips RM, Loadman PM, Jarret CM, Burger AM, Swaine DJ, Fiebig HH. Predicting tumor responses to mitomycin C on the basis of DT-diaphorase activity or drug metabolism by tumor homogenates: implications for enzyme directed bioreductive drug development. Cancer Res 2000;60:6384–90.

[14] Siegel D, Franklin WA, Ross D. Immunohistochemical detection of NAD(P)H:Quinone oxidoreductase in human lung and lung tumors. Clin Cancer Res 1998;4:2065–70.

[15] Siegel D, McGuinness SM, Winski SL, Ross D. Genotype-phenotype relationships in studies of a polymorphism in NAD(P)H:quinone oxidoreductase 1. Pharmacogenetics 1999;9:113–21.

[16] Misra V, Grondin A, Klamut HJ, Rauth AM. Assessment of the relationship between genotypic status of a DT-diaphorase point mutation and enzymatic activity. Br J Cancer 2000;83:998–1002.
[17] Fiebig HH, Berger DP, Dengler WA, Wallbrecher E, Winterhalter BR. Combined *in vivo*/*in vivo* test procedure with human tumour xenografts. In: Fiebig HH, Berger DP, editors. Immunodeficient mice in oncology. Karger: Basel, 1992. p. 321–51.
[18] Au Zaidi NH, Pretlow TP, Oriordan MA, Dumenco LL, Allay E, Gerson SL. Transgenic expression of human MGMT protects against azoxymethane induced aberrant crypt foci and G to A mutations in the K-ras oncogene of mouse colon. Carcinogenesis 1995;16:451–56.
[19] Workman P, Twentyman P, Balkwill F, Balmain A, Chaplin D, Double JA, Embleton J, Newell D, Raymond R, Stables J, Stevens T, Wallace J. United Kingdom Co-ordinating committee on Cancer Research (UKCCCR) guidelines for the welfare of animals in experimental neoplasia. 2nd ed. Br J Cancer 1998;77:1–10.
[20] Plumb J, Workman P. Unusually marked hypoxic sensitisation to indoloquinone EO9 and Mitomycin C in a human colon carcinoma cell line which lacks DT-diaphorase. Int J Cancer 1994;56:134–39.
[21] Belcourt MF, Hodnick WF, Rockwell S, Sartorelli AC. Differential toxicity of mitomycin C and porfiromycin to aerobic and hypoxic Chinese hamster ovary cells over expressing human NADPH:cytochrome c (P450) reductase. Proc Natl Acad Sci USA 1996;93:456–60.
[22] Sharp SY, Kelland LR, Valenti MR, Brunton LA, Hobbs S, Workman P. Establishment of an isogenic human colon tumour model for NQO1 gene expression: application to investigate the role of DT-diaphorase in bioreductive drug activation in vitro and *in vivo*. Mol Pharmacol 2000;58:1146–55.
[23] Gustafson DL, Beall HD, Bolton EM, Ross D, Waldren CA. Expression of human NAD(P)H:Quinone oxidoreductase (DT-diaphorase) in Chinese hamster ovary cells: effect on the toxicity of antitumour quinones. Mol Pharmacol 1996;50:728–35.
[24] Robertson N, Stratford IJ, Houlbrook S, Carmichael J, Adams GE. The sensitivity of human tumour cells to quinone bioreductive drugs: what role for DT-diaphorase? Biochem Pharmacol 1992;44:409–12.
[25] Fitzsimmons SA, Workman P, Grever M, Paull K, Camalier R, Lewis AD. Reductase enzyme expression across the National Cancer Institute tumor cell line panel: correlation with sensitivity to mitomycin C and EO9. J Natl Cancer Inst 1996;88:259–69.
[26] Winski SL, Hargreaves RH, Butler J, Ross D. A new screening system for NAD(P)H: quinone oxidoreductase (NQO1) directed antitumour quinones: identification of a new aziridinylbenzoquinone, RH1, as a NQO1 directed antitumor agent. Clin Cancer Res 1998;4:3083–88.
[27] Kelland LR, Sharp SY, Rogers PM, Myers TG, Workman P. DT-diaphorase expression and tumor cell sensitivity to 17-allylamino 17-demethoxygeldanomycin, an inhibitor of heat shock protein 90. J Natl Cancer Inst 1999;91:1940–49.