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Inhibition of DT-diaphorase (NAD(P)H:Quinone Oxidoreductase, EC 1.6.99.2) by 5,6-Dimethylxanthenone-4-acetic Acid (DMXAA) and Flavone-8-acetic Acid (FAA): Implications for Bioreductive Drug Development

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ABSTRACT. The tumour blood flow inhibitors 5,6-dimethylxanthenone-4-acetic acid (DMXAA) and flavone-8-acetic acid (FAA) have been shown to potentiate the antitumour activity of several bioreductive drugs *in vivo*. Whilst the induction of hypoxia as a result of blood flow inhibition is presumed to be responsible for enhancing the activity of bioreductive drugs, no studies have examined potential interactions between DMXAA or FAA and enzymes involved in bioreductive drug activation. Both FAA and DMXAA are competitive inhibitors of the enzyme DT-diaphorase (NAD(P)H:Quinone oxidoreductase EC 1.6.99.2) with respect to NADH, with K_i values of 75 and 20 μM, respectively. Cytochromes P450 reductase and b_5 reductase activities are not significantly inhibited by FAA, whereas DMXAA partially inhibits cytochrome b_5 reductase activity. The cytotoxicity of the indoloquinone EO9 (3-hydroxymethyl-5-aziridinyl-1-methyl-2-[1*H*-indole-4,7-dione] prop-β-en-α-ol) against DLD-1 (IC_{50} = 0.32 ± 0.08 μM) was significantly reduced when combinations of EO9 and FAA (IC_{50} = 12.26 ± 5.43 μM) or DMXAA (IC_{50} > 40 μM) were used. In the case of menadione (which is detoxified by DT-diaphorase), combinations of menadione with FAA or DMXAA were more toxic (IC_{50} = 7.46 ± 2.22 and 9.46 ± 1.70 μM, respectively) than menadione alone (IC_{50} = 22.02 ± 1.59 μM). Neither DMXAA nor FAA potentiated the activity of tirapazamine *in vitro*. These results suggest that the use of DMXAA and FAA to potentiate the activity of bioreductive drugs where DT-diaphorase plays a central role in either activation or detoxification may be inappropriate. The fact that FAA in particular does not inhibit other key enzymes involved in bioreductive activation suggests that it may be useful in terms of identifying DT-diaphorase-activated prodrugs. BIOCHEM PHARMACOL **58**;2:303–310, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. DMXAA; FAA; DT-diaphorase; bioreductive drugs; tirapazamine

Manipulation of tumour blood flow with the aim of increasing tumour hypoxia is regarded as a possible therapeutic strategy for increasing the efficacy of bioreductive drugs [1]. Inhibition of tumour blood flow [2–4] is a characteristic feature of antitumour responses induced by DMXAA† and FAA, and several studies have demonstrated that the activity of bioreductive drugs (such as tirapazamine) or standard agents (such as melphalan) can be potentiated when used in combination with FAA or DMXAA [5–10]. DMXAA and tirapazamine are currently undergoing clinical evaluation and depending upon the outcome of these trials, combinations of DMXAA and tirapazamine in the clinic are becoming a real possibility. The mechanistic basis for synergistic effects between DMXAA or FAA and bioreductive drugs is presumed to be via the induction of hypoxia in tumours following blood flow inhibition, resulting in greater metabolic activation of hypoxia-selective prodrugs within the tumour. Both DMXAA and FAA, however, have complex mechanisms of action involving the production of TNF-α (tumour necrosis factor-α), interferons, nitric oxide, serotonin, etc. [11, 12]. Interactions between tirapazamine and bioactive peptides cannot be ruled out as a potential mechanism for synergy between DMXAA or FAA and tirapazamine, particularly as the activity of tirapazamine was not enhanced by vinblastine (which does not induce tumor necrosis factor-α), despite the fact that both vinblastine and FAA elicit vascular effects of a similar magnitude [13]. The mechanistic basis for synergy between DMXAA or FAA and bioreductive drugs may therefore be more complex than the induction of hypoxia following blood flow inhibition.

One question that has not been addressed in studies using antivascular approaches to enhance the activity of

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† *Abbreviations:* FAA, flavone-8-acetic acid; DMXAA, 5,6 dimethylxanthenone-4-acetic acid; EO9, 3-hydroxymethyl-5-aziridinyl-1-methyl-2 [1*H*-indole-4,7-dione] prop-β-en-α-ol; DT-diaphorase, NAD(P)H:quinone oxidoreductase; AUC, area under the curve; DCPIP, 2,6-dichlorophenol-indophenol; pHMB, *p*-hydroxymercuribenzoate; and MTT, (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium; thiazolyl blue).

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bioreductive drugs is the effect that compounds such as DMXAA and FAA have on enzymes involved in bioreductive drug activation. The enzymology of tumours is one of the key factors determining the selectivity of bioreductive drugs, and several enzymes have been implicated in their mechanism of action [14]. In the case of DMXAA and FAA, their effects on bioreductive enzyme activity are not known, although flavanoids isolated from the Chinese herb *Scutellarie radix* and other plants are known to inhibit rat DT-diaphorase [15, 16]. DT-diaphorase is one of the major enzymes involved in both the activation and detoxification of quinones such as EO9 and menadione, respectively [17–19], and inhibition of this enzyme would influence the activity of quinone-based bioreductive drugs. Other enzymes such as NADPH cytochrome P450 reductase (EC 1.6.2.4) play a prominent role in the activation of compounds such as tirapazamine, although the effects of flavanoids on the activity of cytochrome P450 reductase are not known [20]. In view of the fact that interactions between flavanoids and bioreductive enzymes are poorly understood coupled with evidence that flavanoids can inhibit rat DT-diaphorase, the principal aim of this study was to determine whether DMXAA and FAA inhibit human DT-diaphorase. These studies were extended to assess whether or not other key enzymes involved in bioreductive drug metabolism are inhibited by DMXAA or FAA. Finally, *in vitro* chemosensitivity studies using combinations of DMXAA or FAA plus EO9, menadione, or tirapazamine were conducted to determine whether modulation of enzyme activity by DMXAA or FAA influences chemosensitivity.

MATERIALS AND METHODS

Materials

Purified human recombinant DT-diaphorase was obtained as described previously [21]. Clinically formulated FAA was a gift from Lipha and pure DMXAA was a gift from the Cancer Research Campaign (U.K.). Tirapazamine was a gift from Sanofi Winthrop. All compounds were dissolved in sterile deionised water and stored at −80° in light-protected vessels. NADH, NADPH, menadione, BSA, DCPIP, pHMB, and cytochrome *c* were obtained from Sigma Chemicals. EO9 was obtained from the Screening and Pharmacology Group of the EORTC (European Organisation for the Research and Treatment of Cancer). All cell culture materials were obtained from GIBCO BRL, Life Technologies.

DT-diaphorase Activity and Kinetic Analysis of Enzyme Inhibition by FAA and DMXAA

Purified DT-diaphorase enzyme activity was assayed by measuring the reduction of cytochrome *c* at 550 nm on a Beckman DU 650 spectrophotometer as described previously [21]. Each assay contained cytochrome *c* (70 μM), NADH (variable concentrations), purified DT-diaphorase (0.032 μg), and menadione (variable concentrations) in a final volume of 1 mL Tris–HCl buffer (50 mM, pH 7.4) containing 0.14% BSA. The reaction was started by the addition of NADH. Rates of reduction were calculated over the initial part of the reaction curve (30 sec), and results were expressed in terms of μmol cytochrome *c* reduced/min/mg protein using a molar extinction coefficient of 21.1 mM^{-1} cm^{-1} for reduced cytochrome *c*. Enzyme assays were carried out at room temperature and all reactions were performed in triplicate. Inhibition of purified DT-diaphorase activity was performed by the inclusion of either FAA or DMXAA (at various concentrations) in the reaction, and inhibition characteristics were determined by varying the concentration of NADH (constant menadione) or menadione (constant NADH) at several concentrations of inhibitor. K_i values were obtained by plotting 1/V against [I].

The activity of DT-diaphorase in DLD-1 cells was determined by measuring the dicumarol-sensitive reduction of DCPIP at 600 nm [22]. Briefly, DLD-1 cells in mid-exponential growth were harvested by scraping into ice-cold buffer (Tris–HCl, 25 mM, pH 7.4 and 250 mM sucrose) and sonicated on ice (3 × 30 sec bursts at 40% duty cycle/output setting 4 on a Semat 250 cell sonicator). Enzyme assay conditions were 2 mM NADH, 40 μM DCPIP, 20 μL of dicumarol (when required) in a final volume of 1 mL Tris–HCl (25 mM, pH 7.4) containing BSA (0.7 mg mL^{-1}). Results were expressed as the dicumarol-sensitive reduction of DCPIP using a molar extinction coefficient of 21 mM^{-1} cm^{-1}. Protein levels were determined using the Bradford assay [23].

Measurement of Cytochrome b_5 Reductase Activity

The activity of NADH:cytochrome b_5 reductase (EC 1.6.2.2) in DLD-1 cells was determined using the method described by Bareham *et al.* [24]. Briefly, DLD-1 cells were washed in PBS, trypsinised, and the cell suspension pelleted at 800 *g* for 10 min. Following a further wash in PBS, the cell pellet was resuspended in ice-cold nuclear buffer A (10 mM HEPES, KOH, pH 7.4, 1.5 mM $MgCl_2$, 10 mM KCl, and 0.05 mM dithiothreitol) and incubated on ice for 10 min. Cells were then sonicated as described above and left for a further 10 min on ice prior to centrifugation at 7800 *g* for 15 min at 4°. Supernatant was used either directly or stored at −80° for determination of enzyme activity. Reaction conditions were 1 mM NADH, 70 μM cytochrome *c*, 200 μM pHMB (used when required as an inhibitor of cytochrome b_5 reductase) in a final volume of 1 mL phosphate buffer (0.05 M, pH 6.8). Enzyme activity was determined by following the reduction of cytochrome *c* at 550 nm in the presence and absence of the inhibitor pHMB. Protein levels were determined using the Bradford assay and results were expressed as the pHMB-sensitive reduction of cytochrome *c*/min/mg protein.

Measurement of Cytochrome P450 Reductase Activity

Cytochrome P450 reductase was measured as described by Gibson and Skett [25]. Briefly, DLD-1 cells were harvested in 0.25 M sucrose and disrupted by sonication as described above. Following an initial centrifugation step at 12,500 *g* (Beckman Optima™ TL ultracentrifuge) for 15 min, the microsomal fraction was pelleted by centrifugation of the supernatant at 100,000 *g* for 45 min. Microsomal fractions were resuspended in 0.1 M Tris–HCl (pH 7.4) and placed on ice until use. Reaction conditions were cytochrome *c* (33.75 μM) and NADPH (200 μM) in a final volume of 1 mL Tris–HCl buffer (0.1 M, pH 7.4). Cytochrome P450 reductase activity was determined by measuring the initial rate of reduction of cytochrome *c* at 550 nm (over 30 sec), and results were expressed as nmol cytochrome *c* reduced/min/mg protein as described above. Protein levels were determined using the Bradford assay [23].

Cell Culture and Chemosensitivity Studies

DLD-1 human colon carcinoma and H460 human non-small cell lung carcinoma cells were routinely maintained as monolayer cultures in RPMI 1640 culture medium supplemented with foetal calf serum (10%), sodium pyruvate (2 mM), penicillin/streptomycin (50 IU mL^{-1}/50 μg mL^{-1}) and L-glutamine (2 mM). Chemosensitivity was assessed using the MTT assay [26] and all assays were performed under aerobic conditions. Briefly, 1–2 × 10^3 cells were plated into each well of a 96-well culture plate and incubated overnight at 37° in an atmosphere containing 5% CO_2. Culture medium was completely removed from each well and replaced with medium containing a range of drug concentrations. For experiments using tirapazamine alone, cells were exposed to a range of drug concentrations for 3 hr. In the case of EO9 or menadione alone, the duration of drug exposure was 1 hr, after which the cells were washed twice with Hanks' balanced salt solution prior to the addition of 200 μL fresh RPMI 1640 medium to each well of the plate. In the case of FAA or DMXAA alone, the duration of drug exposure was 3 hr. Following a four-day incubation at 37°, cell survival was determined using the MTT assay [26]. For combinations of FAA or DMXAA with either menadione or EO9, cells were initially set up as described above and a non-toxic (>95% cell survival) concentration of FAA or DMXAA was selected. Cells were then initially exposed to FAA (2 mM) or DMXAA (2 mM) for a period of 2 hr, following which the medium was removed and replaced with medium containing the inhibitor (FAA or DMXAA at a constant concentration of 2 mM) and either menadione or EO9 (at a range of drug concentrations). Following a further 7-hr incubation at 37°, cells were washed twice with Hanks' balanced salt solution prior to the addition of growth medium. For combination chemosensitivity studies using tirapazamine, cells were exposed to tirapazamine (range of drug concentrations) plus FAA or DMXAA (at a constant concentration of 2 mM) for 3 hr. Chemosensitivity was assessed four days later using the MTT assay as above.

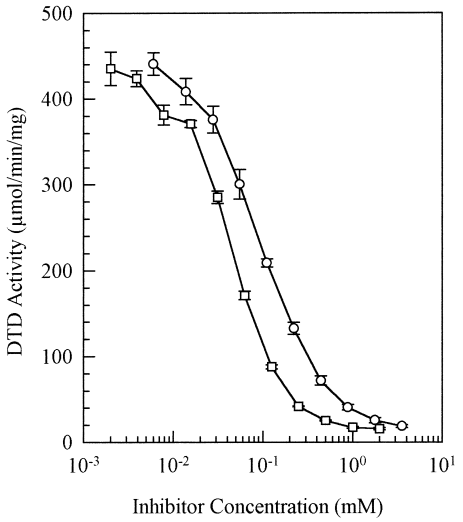

FIG. 1. Inhibition of purified human DT-diaphorase by FAA (○) and DMXAA (□). Each value represents the mean of three independent experiments ± standard deviations.

RESULTS

Inhibition of Purified DT-diaphorase by FAA and DMXAA

Purified human DT-diaphorase had a specific activity in the absence of inhibitors of 435.3 ± 19.5 μmol/min/mg. Both FAA and DMXAA inhibited purified DT-diaphorase with IC_{50} (dose required to reduce enzyme activity to 50% of control values) values of 103.3 and 62.5 μM, respectively (Fig. 1). K_i values as determined by Dixon plots of 1/V against [I] (data not shown) were 20 and 75 μM for DMXAA and FAA, respectively. Kinetic analysis of the inhibition of DT-diaphorase by FAA and DMXAA demonstrated that both compounds were competitive inhibitors with respect to NADH but non-competitive inhibitors with respect to menadione (Fig. 2).

Inhibition of DT-diaphorase, Cytochrome b_5 Reductase, and Cytochrome P450 Reductase in DLD-1 Human Colon Carcinoma Cells

The specific activities of DT-diaphorase, cytochrome b_5 reductase, and cytochrome P450 reductase in the absence of any inhibitors were 566.9 ± 85.2, 35.1 ± 1.7, and 41.5 ± 7.5 nmol/min/mg, respectively (mean ± standard deviation of three independent experiments). The results presented in Fig. 3 demonstrate that both FAA and DMXAA inhibited DT-diaphorase activity in DLD-1 cells, with IC_{50} values of 110.9 and 49.6 μM, respectively. FAA did not significantly inhibit (6.51% inhibition) the activity of cytochrome b_5 reductase at the highest dose tested (3.55 mM). The activity of cytochrome P450 reductase was partially inhibited by FAA at concentrations above 1.77

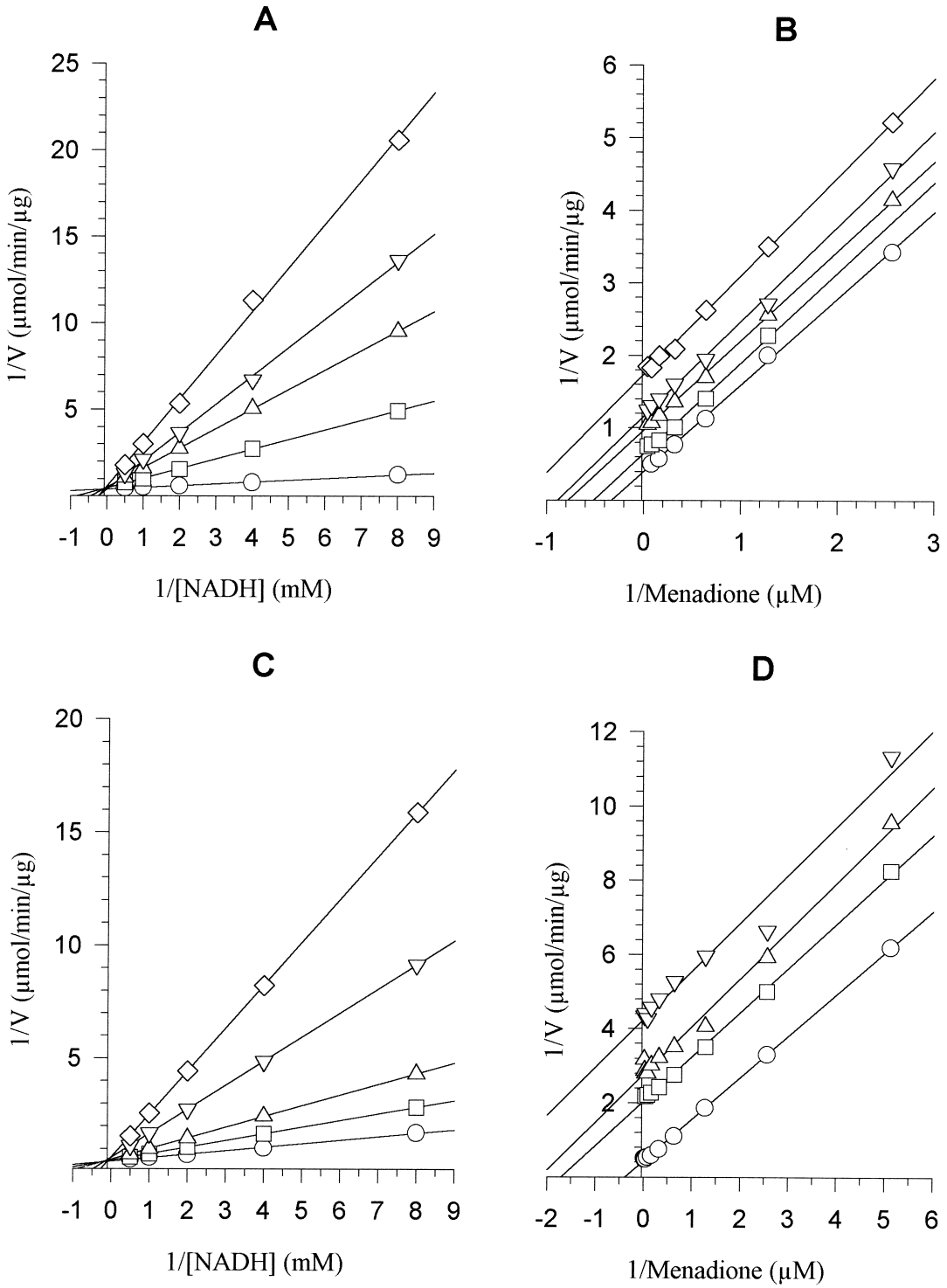

FIG. 2. Kinetic analysis of the inhibition of DT-diaphorase by FAA (Panels A and B) and DMXAA (Panels C and D) using variable NADH (A and C) and variable menadione (B and D) concentrations. The concentrations of FAA (panels A and B) used were control (○), 50 μM (□), 100 μM (△), 150 μM (▽), and 200 μM (◇). In panel C (variable NADH), the concentrations of DMXAA used were control (○), 10 μM (□), 20 μM (△), 40 μM (▽), and 60 μM (◇). In panel D (variable menadione), the concentrations of DMXAA used were control (○), 100 μM (□), 150 μM (△), and 200 μM (▽). Each data point represents the mean of three independent experiments (standard deviations not shown in the interests of clarity).

mM (<10% inhibition). In the case of DMXAA, partial inhibition (approx. 25% inhibition) of cytochrome b_5 reductase occurred, although inhibition plateaud between 250 μM and 2 mM. The activity of cytochrome P450 reductase was not significantly inhibited by DMXAA (8.9% inhibition at 2 mM).

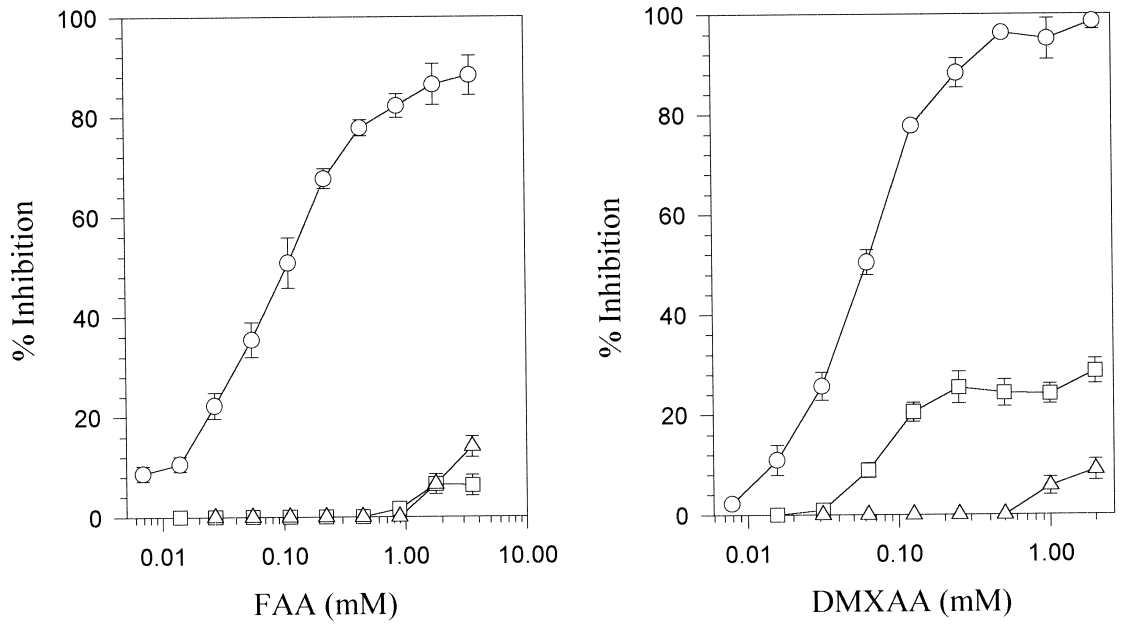

FIG. 3. Inhibition of DT-diaphorase (○), cytochrome b_5 reductase (□), and cytochrome P450 reductase (△) activity in DLD-1 human colon carcinoma cells. Each point represents the mean of three independent experiments ± standard deviations.

Response of DLD-1 Cells to EO9 and Menadione in the Presence or Absence of FAA or DMXAA

The response of DLD-1 cells to menadione and EO9 alone and in the presence of either FAA or DMXAA is presented in Table 1. In the case of EO9, combination with either FAA or DMXAA resulted in a significant decrease in chemosensitivity (Table 1). In the case of menadione, however, combination with FAA or DMXAA resulted in an increase in the antiproliferative activity of menadione (Table 1).

Response of DLD-1 and H460 Cells to Tirapazamine in the Presence or Absence of DMXAA or FAA

The antiproliferative effect of tirapazamine against DLD-1 cells or the DT-diaphorase-rich H460 (1,139 ± 120 nmol/min/mg [21]) cell line was not significantly altered by combination with FAA or DMXAA (Table 1).

TABLE 1. Response of DLD-1 and H460 cells to EO9, menadione, or tirapazamine in the presence and absence of DMXAA or FAA

Drugs ± inhibitor	DLD-1 (IC_{50} values)	H460 (IC_{50} values)
EO9	0.32 ± 0.08 μM	—
EO9 + FAA	12.26 ± 5.43 μM	—
EO9 + DMXAA	>40 μM	—
Menadione	22.02 ± 1.59 μM	—
Menadione + FAA	7.46 ± 2.22 μM	—
Menadione + DMXAA	9.46 ± 1.70 μM	—
Tirapazamine	0.74 ± 0.25 mM	0.92 ± 0.32mM
Tirapazamine + FAA	1.01 ± 0.34 mM	0.62 ± 0.27mM
Tirapazamine + DMXAA	0.78 ± 0.19 mM	0.59 ± 0.25mM

Each value presented represents the mean of three independent experiments ± standard deviations.

DISCUSSION

The results of this study demonstrate that both DMXAA and FAA are competitive inhibitors of human DT-diaphorase with respect to NADH. Whilst K_i values are relatively high (20–75 μM) compared with dicumarol (0.5–10 nM, [27]) and other flavanoids (3377−20 nM, [16]), high levels of both DMXAA and FAA are achievable *in vivo*. In terms of therapeutically achievable levels of FAA, peak plasma and plasma AUC levels of FAA following i.p. administration of FAA (200 mg Kg^{-1}) to non-tumour-bearing NMRI mice were 1.65 mM and 5.27 mM · hr, respectively [28]. In tumour-bearing animals (MAC 15A s.c.), plasma AUC levels for FAA of 13.88 mM · hr and tumour AUC values of equivalent to 4.57 mM · hr have been reported [28]. In the case of DMXAA, peak plasma and AUC values were 600 μM and 2.4 mM · hr respectively, following i.v. administration of DMXAA (99 μmol Kg^{-1}) in male BDF_1 mice [29]. These results suggest that therapeutically achievable levels of both FAA and DMXAA would be sufficient to significantly inhibit DT-diaphorase activity *in vivo* despite the fact that they are not potent inhibitors of DT-diaphorase. With regards to the effects of DMXAA and FAA on other enzymes involved in bioreductive activation, FAA has little effect upon the activity of cytochromes P450 reductase and b_5 reductase in DLD-1 cells. DMXAA, on the other hand, partially inhibits cytochrome b_5 reductase (Fig. 3). The inhibitory effects of DMXAA and FAA on two other bioreductive enzymes, xanthine oxidase and dehydrogenase, could not be determined in DLD-1 cells, as

enzyme activity was below the level of detection (data not shown). It is therefore unlikely that FAA or DMXAA will have a significant effect upon the activity of these enzymes *in vivo*.

With regard to the use of DMXAA or FAA to potentiate the activity of bioreductive drugs, the inhibition of DT-diaphorase could have significant effects upon the outcome of chemotherapy. This is certainly the case for menadione and EO9, where combination chemotherapy with DMXAA or FAA *in vitro* has a marked effect upon quinone-induced toxicity. The activity of menadione is potentiated by both DMXAA and FAA whereas the activity of EO9 is reduced by both flavanoids, which is consistent with DT-diaphorase's proposed role in the detoxification of menadione and the activation of EO9 [17–19]. These results suggest that the use of DMXAA or FAA to enhance the activity of bioreductive drugs, which are good substrates for DT-diaphorase, may be inappropriate. It should be stressed that these conclusions are based upon studies conducted under aerobic conditions using compounds which are good substrates for DT-diaphorase and cell lines which have high levels of DT-diaphorase activity. There are at least two possible scenarios where the activity of quinone-based compounds could be potentiated *in vivo* by DMXAA or FAA. In the first case, high hypoxic cytotoxicity ratios (HCR) have been reported for compounds which are good substrates for DT-diaphorase (e.g. EO9) in cell lines which have low DT-diaphorase activity [30]. Induction of tumour hypoxia via DMXAA or FAA could therefore potentiate the activity of compounds such as EO9, but only in DT-diaphorase-deficient tumours. In the second scenario, high HCR values have been reported for compounds which are poor substrates for DT-diaphorase (e.g. EO8 [31]), and in these cases synergistic effects might be expected following DMXAA or FAA administration. Future studies designed to potentiate the activity of quinone-based compounds need to be carefully designed to accommodate the points raised above. In the case of tirapazamine, no significant differences exist between the response of cells to tirapazamine alone compared with tirapazamine plus DMXAA or FAA (Table 1). DT-diaphorase has been implicated in the detoxification of tirapazamine by virtue of its ability to convert tirapazamine into its inactive 2- and 4-electron reduction products [32–34]. Subsequent studies have, however, suggested that DT-diaphorase plays a limited role in the mechanism of action of tirapazamine [20], and the results of this study are consistent with these findings. Both DMXAA and FAA have a minor effect upon cytochrome P450 reductase, which plays a prominent role in the metabolism of tirapazamine. The mechanistic basis for synergy between DMXAA or FAA and tirapazamine *in vivo* is therefore likely to be the result of either the induction of tumour hypoxia or entrapment of the drug as a consequence of vascular collapse. As described previously, other factors such as the interaction of tirapazamine with bioactive peptides released as a result of DMXAA or FAA treatment cannot be entirely ruled out as a mechanistic explanation for synergy between tirapazamine and DMXAA/FAA *in vivo* [9, 13].

From a drug development viewpoint, DMXAA and FAA may have potential applications as an experimental tool for evaluating DT-diaphorase-activated prodrugs. The use of the DT-diaphorase inhibitor dicumarol has featured prominently in several studies designed to determine the role played by DT-diaphorase in bioreductive activation [35]. Dicumarol, however, is not a specific inhibitor of DT-diaphorase, and the fact that other bioreductive enzymes (e.g. cytochrome b_5 reductase) are inhibited by dicumarol casts doubt upon the validity of data obtained for DT-diaphorase-activated prodrugs using this inhibitor [35]. As described previously, FAA does not significantly inhibit cytochrome P450 reductase and cytochrome b_5 reductase, whereas partial inhibition of cytochrome b_5 reductase occurs with DMXAA. Whether FAA is a specific inhibitor of DT-diaphorase is, however, questionable, particularly as other NADH-dependent enzymes may be inhibited by FAA and evidence from the literature demonstrates that flavanoids can influence the activity of a variety of enzymes and molecules involved in signal transduction [36, 37]. Nevertheless, both FAA and DMXAA are capable of distinguishing between compounds which are detoxified by (e.g. menadione) or activated by (e.g. EO9) DT-diaphorase (Table 1). In addition, compounds such as tirapazamine, where DT-diaphorase plays a limited role in the mechanism of action, could also be identified in that FAA and DMXAA have little or no effect upon the activity of tirapazamine *in vitro*. These results suggest that FAA in particular may be a useful experimental tool in the search for DT-diaphorase-activated prodrugs, although further studies to address the question of whether FAA is a specific inhibitor of DT-diaphorase are required.

In conclusion, this study has demonstrated that both FAA and DMXAA inhibit human DT-diaphorase, one of the major enzymes involved in the activation and detoxification of quinone-based drugs. These findings have specific implications for the use of DMXAA and FAA as antivascular agents to potentiate the activity of bioreductive drugs, particularly for those drugs where DT-diaphorase plays a prominent role in either detoxification or activation. It should be stressed that DT-diaphorase can catalyse the reduction of a broad range of substrates (not just quinones [14, 27]), and future studies using DMXAA or FAA in combination with bioreductive drugs should be carefully designed based upon whether DT-diaphorase plays a role in the drugs' mechanism of action. As a general point, a variety of antivascular agents (such as tubulin binders, nitric oxide synthase inhibitors, etc.) are currently being evaluated with a view to potentiating the activity of therapeutic agents such as bioreductive drugs [13, 38]. The effect that these blood flow inhibitors have on tumour enzymology is generally overlooked and the results of this study suggest that this question needs to be addressed prior to *in vivo* studies being conducted. Finally, the fact that FAA does not significantly inhibit cytochromes P450 and

b_5 reductase suggests that FAA may be a useful experimental tool for identifying DT-diaphorase-activated prodrugs. Further studies are, however, required to determine if FAA in particular is a specific inhibitor of DT-diaphorase.

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