Biochemical Pharmacology, Vol. 31, No. 15, pp. 2439–2445, 1982.
Printed in Great Britain.

0006-2952/82/152439-07 $03.00/0
Pergamon Press Ltd.

COMMENTARY

HYPOXIC CELL RADIOSENSITIZERS AS POTENTIAL ADJUVANTS TO CONVENTIONAL CHEMOTHERAPY FOR THE TREATMENT OF CANCER

BARBARA C. MILLAR
Radiobiology Unit, Institute of Cancer Research, Sutton, Surrey, U.K.

Before discussing the possibility that hypoxic cell radiosensitizers may be useful as adjuvants to chemotherapy in the treatment of human tumours it is worth considering the availability of oxygen in tumour compared with normal tissues and the rationale for the development of radiosensitizers. In both normal and tumour tissue oxygen tension is dependent on the supply through the vascular system and local removal by metabolism in the tissues. Unlike normal tissue, the vascular supply of tumours undergoes continuous change. As the tumour increases in size the vascular network proportionally decreases. Vessels become larger relative to tumour mass but shorter. There is a decrease in the exchange surface area of the capillaries accompanied by an increase in the intercapillary distance. Thus, as tumours grow the oxygen supply per unit mass decreases and this leads to areas of hypoxia and necrosis at sites distant from the capillaries. Hypoxic cells are much more resistant to the lethal effects of ionising radiation than well oxygenated cells. If such cells are present in human tumours the possibility that they may form foci for regrowth after fractionated radiotherapy could lead to local recurrence. The development of radiosensitizers was based on radiation chemical considerations that oxygen mimetic compounds with a high electron affinity should selectively fix radiation damage in hypoxic cells whilst having no effect in aerobic conditions [1]. Nitroheterocyclic compounds such as nitrofurans and nitroimidazoles have received particular attention because several members of these groups were already in use clinically for the treatment of anaerobic infections. The rationale for their introduction into clinical radiotherapy was that they should be metabolised slowly and thereby have ample time to reach target cells in tumour tissue, that they should diffuse passively via cell–cell contact and that by virtue of their inability to sensitize aerobic cells should not increase normal tissue toxicity.

It is now apparent from biochemical and biological evidence that although radiosensitizers are metabolised slowly, certain products produced in hypoxia are toxic to both hypoxic and aerobic cells. In 1974 Sutherland [2] showed that metronidazole was toxic to hypoxic cells in multicellular spheroids and proposed that some product(s) produced by events in hypoxia diffused into aerobic cells and caused cell killing. Similar toxicity towards hypoxic cells *in vitro* was later demonstrated using misonidazole [3].

The observation that radiosensitizers selectively kill hypoxic cells led to a reappraisal of other agents used in conventional chemotherapy. For a number of compounds such as adriamycin [4] and 5-fluorouracil [5] their efficacy against hypoxic cells is less than against aerobic cells *in vitro*. Additionally, in large tumours, drugs which rely on the vascular supply to gain access to cells may not reach sites distant to the capillary network. Thus the possibility arose that radiosensitizers may provide a useful addition to the chemotherapist's armoury. The implication was that the addition of a radiosensitizer to a drug cocktail may enhance the antitumour effect by removing a subpopulation of hypoxic and therefore potentially drug-resistant cells.

Experiments *in vitro* have since shown an added bonus from using radiosensitizers in combination with other cytotoxic agents. If cells are pretreated with radiosensitizers in hypoxia and subsequently challenged with a second chemotherapeutic agent in aerobic conditions, the toxicity of the second agent is enhanced more than would be predicted on the basis of the simple additivity of the two separate toxicities. Furthermore, both *in vitro* and *in vivo* the use of radiosensitizers at non-toxic concentrations has been shown to enhance the toxicity of other agents [6–11]. This phenomenon has been called 'potentiation' and if applicable in human tumours may greatly broaden the usefulness of radiosensitizers in the treatment of human cancer. In man any increased effects against tumour tissue should not be paralleled by similar increased adverse effects to normal tissue. Nitroheterocyclic drugs can induce both peripheral [12–14] and central neuropathy [15] and it is possible that the nervous system will be the critical tissue for using radiosensitizers in combination chemotherapy.

When considering the potentiating effect of radiosensitizers towards other chemotherapeutic agents it may not be justifiable to compare results from *in vitro* with *in vivo*. *In vitro* the degree of potentiation is dependent on the culture history of the cells, a long cell-radiosensitizer contact time in hypoxia, pH, temperature and the electron affinity and concentration of the sensitizer [16, 17]. *In vivo* maximum potentiation is usually achieved when the

BP 31-15 - A

sensitizer is given a short time before (30 min) [11] or concommitantly [10, 18] with the challenge drug, and there is also evidence for enhanced cytotoxicity if the sensitizer is given after the cytotoxic agent [10].

Reduction of nitroheterocyclic compounds in vitro

Nitroheterocyclic compounds are metabolized in anaerobiosis by enzymes acting as nitroreductases [19]. Furthermore, the chronic aerobic toxicity [20], hypoxic cell toxicity [21], radiosensitization [22] and possibly mutagenicity [23] of these compounds appear to correlate with the electron affinity of their respective nitro groups. Early evidence that reduced free radical intermediates may be responsible for the cytotoxic properties of nitroheterocyclics came from microbial systems. Metronidazole was shown to inhibit hydrogen evolution by *Trichomonas vaginalis* [24] and *Clostridium acetylbutyricum* [25] and it was proposed that the drug was acting as a preferential electron acceptor from reduced ferridoxin [25]. There is now considerable evidence that certain enzymes in mammalian cells can behave as nitroreductases in an hypoxic environment. These include NADPH-cytochrome *c* reductase [26], DT-diaphorase [27], xanthine oxidase [28], aldehyde oxidase [29], lipoxyl dehydrogenase [30] and cyclooxygenase (B. C. Millar, unpublished observation). Misonidazole has been shown to undergo similar metabolism in anaerobic caecal contents and in hypoxic cells *in vitro* [31]. Both systems form the amino derivative of misonidazole 1-(2-aminoimidazole-1-yl)-3-methoxypropan-1-ol (AIM) and urea as well as a metabolite (2-hydroxy-3-methoxypropanyl) guanidine. In both bacterial [32] and mammalian cells [33] and in animals [34], intermediates in this degradative pathway have been shown to bind to cellular macromolecules, whereas there are no reports of binding of the parent compounds [35]. In bacterial systems a urinary excreted hydroxylated product of metronidazole has been shown to be ten times more mutagenic than the parent drug [36]. Additionally, in nitrofurazone-sensitive (wild type) bacteria containing oxygen-insensitive nitrofuran nitroreductase a 10-fold increase in drug resistance is seen after loss of this enzyme [37]. These mutants contain the oxygen sensitive nitroreductase and become as sensitive as the wild type when incubated with nitrofurazone under anaerobic conditions.

The oxygen inhibition of microsomal nitroreductase has been shown to be the result of air oxidation of the first product of reduction [38, 39]. An electron, transferred from flavoprotein reductase to the nitroaromatic substrate, produces the nitro radical anion. This radical reacts rapidly with oxygen to form superoxide and regenerates the parent compound. Thus, in aerobic conditions there is no net reduction. Nitroheterocyclics may enhance superoxide production which in aerobic cells is detoxified by superoxide dismutase. Further evidence that nitro reduction is important in determining the differential cytotoxicity of radiosensitizers is provided from experiments showing that modest hyperthermia enhances toxicity *in vitro* [17, 40] and that the greatest thermal enhancement occurs with compounds of least electron affinity [17]. This is consistent with the proposal that the activation energy for the reduction process decreases with increasing electron affinity. Whilst hypoxia-mediated nitro reduction could account for the direct cytotoxicity of nitroheterocyclic compounds and potentiation of other agents *in vitro* it cannot totally explain potentiation *in vivo* since a major percentage of most drugs is excreted unchanged or as the demethylated metabolite [41].

The role of sulfhydryls in determining the toxicity of nitroheterocyclics in vitro

Both nitrofurans and nitroimidazoles have been shown to deplete cells of endogenous sulfhydryls, in particular glutathione, either by the formation of thiol reactive intermediates (e.g. misonidazole) or by acting as substrates for glutathione-*S*-transferase (e.g. nitrofurans) [42].

Cysteamine, a free radical scavenger, protects against the cytotoxicity of misonidazole in hypoxia [40, 43] and this is seen predominantly as a change in the shoulder of the toxicity versus time curve. Additionally, when hypoxic cells are treated with misonidazole and allowed to recover for several hours in air prior to a second treatment with the sensitizer in hypoxia, the shoulder of the toxicity curve is reconstituted [44]. This time scale has been shown to be sufficient to allow regeneration of endogenous sulfhydryl in cells after depletion by diethylmaleate [45] and is consistent with the suggestion that the shoulder of toxicity versus time curves represents the time required for toxic products of the sensitizer to accumulate at sufficient concentrations to produce exponential cell killing [43].

Treatment of cells in hypoxia with cysteamine and misonidazole also protects against the enhanced toxicity produced by bleomycin, melphalan (L-phenylalanine mustard) and *cis*-platinum [8], suggesting that, like bleomycin, free radicals may be involved in the mechanism of potentiation of chemotherapeutic agents by radiosensitizers *in vitro*. Furthermore, if cells are allowed to recover in air for 2–4 hr after exposure to the sensitizer in hypoxia, the amount of enhanced toxicity produced by the second drug is reduced [46].

These data provide strong evidence that radiosensitizer-mediated sulfhydryl depletion is important in determining the enhanced toxicity of other drugs *in vitro*, especially as the efficacy of alkylating agents has been shown to depend on intracellular sulfhydryl levels [47]. Suzukake *et al.* [48] showed that murine L1210 cells resistant to melphalan toxicity can be completely sensitized to the drug by reducing the intracellular concentration of glutathione. The differential toxicity of melphalan in sensitive and resistant cells of this tumour is directly related to the cell glutathione levels. However, sulfhydryl removal can only partially explain the enhancement of melphalan toxicity by misonidazole. Depletion of endogenous sulfhydryl *in vitro* with diethylmaleate produces less potentiation of melphalan toxicity than that obtained by exposure to misonidazole in hypoxia prior to administering the challenge drug [49]. Additionally, Smith *et al.* [46] were unable to demonstrate any correlation

between sulfhydryl levels and the enhancement of melphalan toxicity, following hypoxic exposure to misonidazole, in plateau phase compared with exponential phase cells. Although the amount of cell killing by melphalan was similar for both plateau and exponential cells that had been treated with the sensitizer, there was less enhancement of melphalan toxicity in plateau phase cells. Since sulfhydryl levels vary throughout the cell cycle, reaching a maximum during *S* phase it would be predicted that the levels in plateau phase cultures would be low due to the large proportion of cells that are out of cycle. Since the potentiation of toxicity of other drugs is dependent on the age of the culture, the cell type and experimental growth conditions it seems probable that this will be reflected in different enzyme levels. The lack of correlation between sulfhydryl levels and enhancement of melphalan toxicity may reflect an increased complement of enzymes capable of nitroreduction in plateau phase cultures. Taylor and Rauth [43] attributed the differential toxicity of misonidazole towards hypoxic CHO cells compared with HeLa cells to differences in the rate at which they metabolise the drug.

Changes in ultrastructure and energy metabolism by radiosensitizers in vitro

Whilst nitro reduction and depletion of sulfhydryl levels are important in determining both direct cytotoxicity and enhancement of the toxicity of a second agent, other biochemical processes are clearly involved in hypoxia mediated toxicity *in vitro*. Since exposure of cells in hypoxia to misonidazole leads to changes in cellular ultrastructure, such as severe damage to the endoplasmic reticulum and mitochondria [50], it is arguable that this involves damage to glycolytic enzymes and thus inhibition of energy metabolism. Impairment of mitochondrial electron transport on return to aerobic conditions could also inhibit energy metabolism and render cells more susceptible to a second agent. In aerobic conditions both nitroimidazoles and nitrofurans have been shown to increase oxygen consumption in cyanide-inhibited cells, suggesting a direct effect on energy production [51]. However, it seems unlikely that this effect will be important *in vivo* and particularly in man since the excessively high drug concentrations used *in vitro* bear no relation to clinical levels of the drugs. Nevertheless *in vitro* it is feasible that binding of reduced products of radiosensitizers to macromolecules combined with depletion of sulfhydryl levels and impairment of cellular ultrastructure could inhibit the biosynthesis of nucleic acids and protein. Subsequent treatment with a second agent may result in enhanced cytotoxicity due to inhibition of repair processes and cell division. Even non-toxic doses of sensitizer may impair cellular functions sufficiently to inhibit repair following a challenge with a second agent.

Potentiation in vivo *by radiosensitizers*

Evidence for *in vivo* potentiation of the cytotoxic action of chemotherapeutic agents by radiosensitizers has come entirely from work using mouse models. The first studies by Rose *et al.* [10] and Clement [11] showed that misonidazole substantially enhanced the antitumour effects of melphalan, 5-FU, methyl-CCNU, cyclophosphamide and aziridinyl-benzoquinone. Although there was also an increase in normal tissue damage, the net result was a therapeutic gain. There have been many other examples of chemopotentiation *in vivo* since this report and most have indicated a selective advantage against the tumour. However, the degree of potentiation depends on several facts (1) the chemotherapeutic agent [52]; (2) tumour type [52]; (3) tumour size [53]; (4) the lipophilicity of the radiosensitizer [54]; (5) the dose of radiosensitizer and of the challenge drug [9, 55]; and (6) the sequencing of the radiosensitizer and the challenge drug [52].

Radiosensitizer-induced potentiation of toxicity by nitrosoureas correlates with the carbamoylating activity of these compounds [56]. In mice bearing the KHT sarcoma the toxicity of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), which has strong carbamoylating properties, is enhanced by misonidazole whereas there is no potentiation of 2,3-(2-chloroethyl)-3-nitrosoireido-D-glucopyranose (chlorozotoxin) toxicity which does not have this property. Furthermore, potentiation of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) toxicity *in vivo* by nitroimidazoles of similar electron affinity has been shown to depend upon the lipophilicity of the sensitizers. More lipophilic agents show greater effects than hydrophilic compounds [54]. However, the enhanced effectiveness produced by increased lipophilicity decreases when the octanol:water partition coefficient of the sensitizer is in excess of 20. This may reflect both uptake into cells and subsequent metabolism. Most hydrophilic compounds are excreted through the kidneys whereas more lipophilic compounds undergo demethylation by the P-450 enzyme system in the liver to produce more polar metabolites. It is arguable that more lipophilic compounds may be metabolised less rapidly by the liver due to their affinity for cellular lipids, and consequently may be more susceptible to nitro reduction in anaerobic sites in tumours. The possibility that lipophilic compounds may be of greater advantage in mediating chemopotentiation is in contrast to the preference for hydrophilic compounds as adjuncts to radiotherapy, where rapid clearance has been considered a possible means of overcoming the neurotoxic side effects of these drugs.

Whilst most investigators report a therapeutic gain when misonidazole is used in combination with nitrosoureas this may be due to the tumour types selected for these studies relative to the sensitivities of the normal tissue endpoint towards the chosen chemotherapeutic agent. For example, potentiation of CCNU toxicity by misonidazole in the SQ-carcinoma, which is resistant to CCNU, was only demonstrable using high doses of CCNU [9]. Furthermore, at these high doses normal tissue toxicity, measured by the CFUs compartment of the haematopoietic system, was equally increased. Also, the same reporters noted that in animals bearing the EMT6 or KHT tumours, which are sensitive to CCNU, spermatogonia were sensitized by misonidazole to

almost the same extent as were tumours, at all CCNU doses.

Siemann [57] showed that there is a direct relationship between the dose of misonidazole and the enhancement of toxicity to both tumour and normal tissue in animals bearing the KHT sarcoma, given simultaneously with 20 mg/kg CCNU. However, at higher doses of CCNU there was a decrease in the enhancement of toxicity following misonidazole administration [57]. Not only are the doses of the two agents important when considering potentiation but also the scheduling of the two drugs. Administration of misonidazole 3 hr prior to BCNU reduced the growth delay of the KHT tumour compared with that seen in animals given a similar dose of BCNU alone, whereas simultaneous administration of the two agents or administration of the sensitizer up to 12 hr after the nitrosourea markedly enhanced the growth delay of the tumour [58]. A similar enhancement of BCNU toxicity towards the KHT tumour was seen when desmethylmisonidazole was administered up to 3 hr after BCNU [58]. The complexity of the potentiating effect of radiosensitizers *in vivo* is further exemplified from work showing that SR-2555, a hydrophilic nitroimidazole, did not potentiate the toxicity of CCNU in KHT tumours, whereas it was effective in enhancing the toxicity of CCNU in RIFI tumours [59].

At least part of the enhancement of toxicity of nitrosoureas by sensitizers may be due to changes in pharmacokinetics. Workman [54] demonstrated excellent enhancement of CCNU toxicity in the KHT tumour with the non-electron affinic agent 2-diaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525A), whereas there was no increased toxicity when animals were given imidazole with CCNU. SKF 525A is a potent inhibitor of drug metabolism [60]. Changes in pharmacokinetics of the challenge drug have also been shown in animals treated with alkylating agents. Clutterbuck *et al.* [61] have shown that both misonidazole and metronidazole decrease the clearance of [^{14}C]melphalan in blood and cause substantially higher peak plasma levels of [^{14}C]melphalan in mice. Tannock proposed similar pharmacokinetic changes to account for the increased toxicity of cyclophosphamide following administration of misonidazole to animals bearing the KHT tumour [62]. This change may be due to lowered body temperature following administration of a sensitizer. Haynes and Inch [63] noted a 5° temperature decrease and 35% decrease in heart rate after administration of metronidazole to C3H mice. A similar temperature decrease has been seen in the same mouse strain after treatment with misonidazole (1 mg/g) [64]. Furthermore, if body temperature is maintained at normal during treatment with misonidazole the toxicity of the sensitizer is significantly increased. Gomer and Johnson [64] reported a decrease in LD_{50} from 1.48 mg/g in cold mice to 0.77 mg/g in animals maintained at constant temperature. Recently Pederson [65] has shown that when misonidazole is combined with cyclophosphamide there is a decrease in the LD_{50} of cyclophosphamide from 360 mg/kg to 177 mg/kg when misonidazole is administered simultaneously. Delayed clearance mediated by lowered body temperature could decrease glomerular filtration of the challenge drug and thus permit more tumour cells to be exposed to its toxic effect. This would be particularly important in large tumours, where it has been shown that the uptake of melphalan into human tumour xenografts is markedly reduced in tumours larger than 1 g [66].

Law *et al.* [55] proposed that the enhancement of cyclophosphamide toxicity by misonidazole in mice bearing the RIFI tumour was due to the inhibition of potentially lethal damage (PLD). Although there was no difference in the number of clonogenic tumour cells recoverable immediately after treatment with misonidazole and cyclophosphamide compared with cyclophosphamide alone, the number of clonogenic cells declined during the subsequent 24 hr in animals given the sensitizer. Inhibition of PLD has been implicated as an important factor in determining the enhanced toxicity produced by misonidazole in combination with cyclophosphamide in WHF1B fibrosarcoma [67] and with melphalan in the KHT sarcoma [68]. The inference from these data is that radiosensitizers may inhibit the synthesis of repair enzymes or enhance the number of inter/intra strand cross-links in DNA. Thus inhibition of PLD cannot be considered a mechanism but a phenomenon resulting from underlying biochemical events. Additionally, not all workers report inhibition of PLD as a possible explanation for potentiation, for example, the enhanced toxicity of melphalan by misonidazole in the MT tumour [69] and WHF1B fibrosarcoma [67] cannot be explained by this mechanism.

The *in vivo* toxicity of both alkylating agents and nitrosoureas is thought to be mediated by events resulting from binding of the chemically reactive electrophilic drug or metabolites to macromolecules. Glutathione is involved in the detoxification of drug-induced toxicity by providing nucleophiles for conjugation with toxic electrophilic species. Depletion of endogenous glutathione with diethylmaleate increases the toxicity of the alkylating agent 4-ipomeanol *in vivo*, whereas cysteine and cysteamine decrease both the covalent binding of the activated drug to macromolecules and toxicity [70]. Simultaneous administration of an alkylating agent or a nitrosourea with a radiosensitizer may result in competition between reactive metabolites of the two drugs for endogenous glutathione. If sulfhydryl reacts more rapidly with the sensitizer than with the second drug, this may facilitate alkylation or carbamylation (in the case of the nitrosoureas). However, this mechanism cannot explain the enhanced toxicity that is produced when radiosensitizers are given to animals several hours after CCNU [58]. Neither can this mechanism explain why potentiation of melphalan toxicity is diminished when misonidazole is given to animals several hours before the challenge drug [10]. If depletion of sulfhydryl is involved in potentiation, further enhancement of toxicity would be expected by delaying the time interval between the radiosensitizer and melphalan, similar to the results of prolonged contact experiments *in vitro*.

Since chemopotentiation by radiosensitizers is usually greater in large tumours, where a fraction

of hypoxic cells may be expected, it has been suggested that hypoxia-mediated nitroreduction may be responsible for the differential effects against tumour tissue. Although most authors do not describe any toxicity from radiosensitizers *per se* in situations where the toxicity of other agents is enhanced *in vivo*, it should be remembered that *in vitro* potentiation can also occur when cells are pretreated in hypoxia with non-toxic concentrations of these drugs. Since most radiosensitizers are slowly metabolised, their penetration into tissue is less dependent on the capillary network, than that of other drugs with a shorter half-life [66]. Nitro reduction could account for the enhancement of toxicity when radiosensitizers are given simultaneously with a second agent. However, if this mechanism is applicable there should be greater enhancement of toxicity if the time interval between administration of the radiosensitizer and the second drug are increased. This would allow toxic metabolites of the radiosensitizer to accumulate in hypoxic regions of the tumour and to diffuse into adjacent aerobic cells. Since the amount of potentiation diminishes when the time interval between administration of the sensitizer and the second drug is increased it seems unlikely that nitro reduction is the principal mechanism for the enhanced tumour toxicity *in vivo*. Additionally, in animals bearing the RIFI tumour, potentiation of cyclophosphamide toxicity by 1 g/kg of misonidazole diminishes at doses greater than 75 mg/kg of the alkylating agent [71]. This could also be used as an argument against both nitro reduction and sulfhydryl depletion since maximum potentiation must be occurring in cells that are easily accessible to the alkylating agent and therefore presumably oxygenated. If potentiation of drug toxicity is mediated by the hypoxic metabolism of radiosensitizers or depletion of sulfhydryl, greater enhancement of toxicity would be expected at higher concentrations of cyclophosphamide where enhanced tumour penetration of the alkylating agent may be expected, thereby permitting greater interaction with the sensitizers.

There have been no reports describing the enhancement of drug toxicity using clinically relevant concentrations of radiosensitizers and a similar paucity of data describing the pharmacokinetics of the challenge drug. In the RIFI tumour, potentiation of cyclophosphamide toxicity by misonidazole is diminished using a low dose of the sensitizer (0.33 mg/kg) even though this would be higher than clinically acceptable levels (0.1 mg/ml in man) [71]. One explanation for this diminished effect has been that serum and tumour levels of the sensitizer may be low due to the short half-life of the sensitizer in mice (~1 hr) compared with man (~12 hr). In an attempt to mimic the human situation Law *et al.* [55] gave repeated doses of misonidazole (300 mg/kg) and cyclophosphamide to mice, bearing the RIFI tumour, on a daily basis for 5 days. Potentiation increased sharply as the dose of cyclophosphamide was increased to 20 mg/kg/day. However, there was no significant difference in the amount of tumour growth delay in animals given a fractionated schedule of misonidazole (1500 mg/kg total dose) in combination with 175 mg/kg (total dose) cyclophosphamide and a single dose of 750 mg/kg misonidazole with 150 mg/kg cyclophosphamide. Since there was no comparison of normal tissue toxicities in the two drug schedules, it is questionable whether the fractionated regime offers any therapeutic advantage as the animals given repeated doses received more chemotherapy. Stephens *et al.* [52] showed that misonidazole could potentiate the toxicity of vincristine, an antimitotic agent, and 5-fluorouracil, an antimetabolite, and concluded that the mechanism of misonidazole potentiation may be nonspecific since the toxicity of so many agents with diverse mechanism can be enhanced. Whilst this may be an over-simplification it cannot be dismissed in view of the change in pharmacokinetics of melphalan [61] and cyclophosphamide [62] in mice given misonidazole, or the enhancement of CCNU toxicity by the non-electron affinic agent SKF 525A [54]. The lack of potentiation using low doses of radiosensitizers may indicate that at these doses the pharmacokinetics of the challenge drug are unaltered.

Whether radiosensitizers delay the clearance of other drugs in man may be particularly important in assessing their usefulness as adjuncts to chemotherapy. Several centres have initiated phase I clinical trials using misonidazole in combination with other agents, including BCNU [72], 5-fluorouracil [73], cyclophosphamide [74] and melphalan (T. J. McElwain, personal communication). There have been no reports of changes in peak plasma levels or serum half-lifes of these agents by including misonidazole in the drug schedules. Furthermore, since there has been no increase in adverse effects to normal tissue, particularly neuropathy, clinicians have been encouraged to continue testing.

Neuropathy

As mentioned earlier, the principal adverse effect seen in patients given nitroheterocyclic drugs is neurotoxicity. There are several reports in the literature that patients receiving nitrofurans [14] at bacteriacidal doses exhibit peripheral neuropathy and that this subsides upon cessation of drug treatment. Similar deleterious effects have been seen in patients given metronidazole [13], misonidazole and nimorazole [14, 75] for the treatment of anaerobic infections, and in clinical trials using misonidazole as an hypoxic cell radiosensitizer, neurotoxicity has limited the total dose to 12 g/m^2. Various workers have suggested that neurotoxicity is dependent upon cells' exposure to the sensitizer (viz. concentration × time). Workman and coworkers have shown that phenytoin and phenobarbitone shorten the half-life of misonidazole in mice, dogs and man [76–78] by increasing the oxidative metabolism of misonidazole to produce the polar metabolite desmethylmisonidazole. However, in none of these reports is there any concomitant measurement of effects on neurotoxicity. The possibility that polar compounds may be less neurotoxic due to their more rapid elimination from the body has led to the development of hydrophilic molecules as possible radiosensitizers. The demethylated product of misonidazole, Ro.03-9963, has a half-life of 4 hr in man compared with 12 hr for the parent compound [79]. Despite the predicted reduced toxicity, des-

methylmisonidazole is proving to be as equally neurotoxic as misonidazole in current clinical trials [79]. Although most workers agree that less neurotoxic compounds should be synthesized, it seems unlikely that this problem will be overcome until the underlying mechanism of this toxicity has been found.

One area of approach that has emerged from the clinic is that patients receiving dexamethasone, a steroidal anti-inflammatory agent, in combination with misonidazole, exhibit less neurotoxicity than patients given misonidazole alone [80]. Experiments *in vitro* have shown that dexamethasone protects against hypoxia-mediated misonidazole toxicity [81]. However, this protective effect is accompanied by an increase in the radiation resistance of cells [81], which could also have serious clinical implications. Cells pretreated with certain non-steroidal anti-inflammatory agents (NSAIA), such as flurbiprofen, aspirin or indomethacin, are also protected against sensitizer-induced toxicity *in vitro* [82, 83]. In this instance there is no effect on the radiation response of cells. Both steroidal and NSAIA appear to exert their effects *in vitro* by inhibiting the reduction of the radiosensitizers' nitro group in hypoxia by the cyclooxygenase system responsible for prostaglandin biosynthesis from arachidonic acid (B. C. Millar, unpublished observation). Since reduced intermediates of radiosensitizers are strongly implicated as the toxic species, it seems reasonable to suggest that certain common intermediates may be produced in both tumours and normal tissue. The possibility that reductive processes occur in normal tissue is purely speculative. Nitro reduction will depend upon the oxygen tensions in individual tissues and on the availability of enzymes capable of carrying out nitro reduction. *In vitro* misonidazole toxicity is enhanced at intermediate oxygen tensions [84] and this may be particularly important with regard to man since some normal tissues such as cartilage (N. Kember, personal communication), liver, kidney and brain [85] have low oxygen levels. Whether non-steroidal anti-inflammatory agents will protect against neurotoxicity *in vivo*, especially in man, has yet to be investigated. NADPH cytochrome P-450 reductase has been shown to mediate the nitro reduction of nitrofurantoin *in vitro* using lipid as substrate [86]. If nitro reduction involving lipid metabolism is important in determining neurotoxicity the relative amounts of enzymes for both the prostaglandin and P-450 system may determine whether NSAIA offer any protection in man.

Future prospects

The value of adding radiosensitizers to chemotherapy regimes for man is still open to speculation. Whilst there is undoubtedly evidence from mouse models that sensitizers can potentiate the toxicity of other drugs and in general favours a therapeutic gain there are several important questions to be answered before their use in man can be justified. On the basis of available data, particularly the limited pharmacokinetic studies, the mouse may not be a good model. More pharmacokinetic studies on the clearance of drugs following treatment with radiosensitizers are required. This will enable evaluation of whether potentiation occurs in situations where there is no change in the pharmacokinetics of the second drug and whether potentiation can be measured using clinically relevant doses of sensitizers. If these problems can be solved it will be possible to investigate whether similar mechanisms are applicable *in vivo* as *in vitro*, for example, the role of sulfhydryls and nitro reduction. Perhaps the most important consideration, however, will be concerning the effects of radiosensitizers in combination with other agents on neurotoxicity. If similar doses of sensitizer are required to produce chemopotentiation as are required to produce radiosensitization then it is likely that neurotoxicity will remain a problem. It is unlikely that the mouse will provide an adequate model to assess this possibility and recourse to large mammals will be necessary.

If non-steroidal anti-inflammatory agents prove to be successful in protecting against neurotoxicity, this may result in loss of chemopotentiation, such an effect will depend on whether similar biochemical events are required to induce neurotoxicity and potentiation. Further studies are required to investigate toxicity to tissues which are critical for the use of the challenge drug in any assessment of a potential therapeutic gain. In conclusion, whilst more data are needed, it must be emphasized that the interest in using sensitizers in combination with other agents has gained impetus in a very short time so that perhaps it will be possible to give a more positive assessment in the near future.

Acknowledgements—I would like to thank Professors G. E. Adams and P. Alexander and Drs. E. M. Fielden, J. L. Millar and I. J. Stratford for their helpful advice and discussion.

REFERENCES

1. G. E. Adams and D. L. Dewey, *Biochem. biophys. Res. Commun.* **12**, 473 (1963).
2. R. M. Sutherland, *Cancer Res.* **34**, 3501 (1974).
3. E. J. Hall and L. A. Roizin-Towle, *Radiology* **117**, 453 (1975).
4. C. West, I. J. Stratford, N. Barrass and E. Smith, *Br. J. Cancer* **44**, 798 (1981).
5. E. Smith, I. J. Stratford and G. E. Adams, *Br. J. Cancer* **41**, 568 (1980).
6. I. S. Stratford, C. Williamson and G. E. Adams, *Br. J. Cancer* **41**, 517 (1980).
7. E. Smith, C. E. Lumley, I. J. Stratford and G. E. Adams, *Int. J. Radiat. Oncol. Biol. Phys.* **8**, in press.
8. L. A. Roizin-Towle, E. J. Hall and L. Capuano, *Br. J. Cancer* **44**, 201 (1981).
9. D. G. Hirst, J. M. Brown and J. L. Hazlehirst, *Br. J. Cancer*, in press.
10. C. M. Rose, J. L. Millar, J. H. Peacock, T. A. Phelps and T. C. Stephens, *Cancer Mgmt* **5**, 250 (1981).
11. J. J. Clement, M. S. Gorman, I. Wodinsky, R. Catane and R. K. Johnson, *Cancer Res.* **40**, 4165 (1980).
12. S. Dische, M. I. Saunders, M. E. Lee, G. E. Adams and I. R. Flockhart, *Br. J. Cancer* **35**, 567 (1977).
13. R. C. Urtason, J. D. Chapman, M. L. Fieldstein, R. P. Band, H. R. Rabin, A. F. Wilson, B. Marynowski, E. Starreveld and T. Shnitka, *Br. J. Cancer* **37**, Suppl. III, 271 (1978).
14. J. C. Heffelfinger and R. J. Allen, *J. Pediat.* **65**, 611 (1964).
15. R. K. Kusumi, J. F. Pouffe, R. H. Wyatt and R. J. Foss, *Ann. intern. Med.* **93**, 59 (1980).

16. R. M. Sutherland, *Int. J. Radiat. Oncol. Biol. Phys.* **8**, in press.
17. S. Rajaratnam, Ph.D. Thesis, University of London (1981).
18. D. W. Siemann, *Br. J. Cancer* **43**, 367 (1981).
19. R. P. Mason and J. L. Holtzman, *Biochemistry* **14**, 2184 (1975).
20. G. E. Adams, E. D. Clarke, P. Gray, R. S. Jacobs, I. J. Stratford, P. Wardman and M. E. Watts, *Int. J. Radiat. Biol.* **35**, 151 (1980).
21. G. E. Adams, I. J. Stratford, R. G. Wallace, P. Wardman and M. E. Watts, *J. natn. Cancer Inst.* **64**, 555 (1980).
22. G. E. Adams, I. R. Flockhart, C. E. Smithen, I. J. Stratford, P. Wardman and M. E. Watts, *Radiat. Res.* **67**, 9 (1976).
23. D. G. Lindmark and M. Muller, *Antimicrob. Agents Chemother.* **10**, 476 (1976).
24. R. W. O'Brien and C. J. Morris, *Arch. Mikrobiol.* **84**, 225 (1972).
25. D. I. Edwards and G. E. Mathison, *J. gen. Microbiol.* **63**, 297 (1970).
26. E. Perez-Reyes, B. Kalyanaraman and R. P. Mason, *Molec. Pharmac.* **17**, 239 (1980).
27. R. Kato, A. Takahashi and T. Oshini, *Biochem. Pharmac.* **19**, 45 (1970).
28. C. Y. Wang, B. C. Behreus, M. Ichikawa and G. T. Bryan, *Biochem. Pharmac.* **23**, 3395 (1975).
29. M. K. Wolpert, J. R. Althaus and D. G. Johns, *J. Pharmac. exp. Ther.* **185**, 202 (1973).
30. E. Bending and N. Jolliffe, *J. Pharmac. exp. Ther.* **88**, 300 (1946).
31. R. L. Koch, C. Rose, T. A. Rich and P. Goldman, *Biochem. Pharmac.* **31**, 411 (1982).
32. D. R. McCalla, A. Reuvers and C. Kaiser, *Cancer Res.* **31**, 2184 (1971).
33. P. L. Olive and D. R. McCalla, *Cancer Res.* **35**, 781 (1975).
34. W. G. Bradley, I. J. Karlsonn and C. G. Rassol, *Br. J. Med.* **2**, 610 (1977).
35. A. J. Varghese and G. F. Whitmore, *Cancer Res.* **40**, 2165 (1980).
36. T. H. Connor, J. Stoeckel, J. Evrard and M. S. Legator, *Cancer Res.* **37**, 629 (1977).
37. R. E. Asnis, F. B. Cohen and G. S. Gots, *Antibiot. Chemother.* **2**, 123 (1952).
38. R. P. Mason and J. L. Holtzman, *Biochemistry* **14**, 1626 (1975).
39. R. P. Mason and J. L. Holtzman, *Biochem. biophys. Res. Commun.* **67**, 1267 (1975).
40. E. J. Hall, M. Astor, C. Geard and J. Biaglow, *Br. J. Cancer* **35**, 809 (1977).
41. I. R. Flockhart, S. M. Malcolm, T. R. Marten, C. S. Parkins, R. J. Ruane and D. Troup, *Br. J. Cancer* **37**, Suppl. III, 264 (1978).
42. J. E. Biaglow, M. E. Varnes, M. Astor and E. J. Hall, *Int. J. Radiat. Oncol. Biol. Phys.* **8**, in press.
43. Y. C. Taylor and A. M. Rauth, *Br. J. Cancer* **41**, 892 (1981).
44. I. J. Stratford, *Br. J. Cancer* **38**, 130 (1978).
45. E. A. Bump and J. M. Brown, *Int. J. Radiat. Oncol. Biol. Phys.* **8**, in press.
46. E. Smith, I. J. Stratford and G. E. Adams, *Br. J. Cancer*, in press.
47. G. Calcutt and T. A. Connors, *Biochem. Pharmac.* **12**, 839 (1963).
48. K. Suzukake, B. J. Petro and D. T. Vistica, *Biochem. Pharmac.* **31**, 121 (1982).
49. Y. C. Taylor, E. A. Bump and J. M. Brown, *Int. J. Radiat. Oncol. Biol. Phys.* **8**, in press.
50. L. A. Roizin-Towle, L. Roizin, E. J. Hall and J. C. Liu, *Cancer Mgmt* **6**, 444 (1981).
51. J. E. Biaglow, M. E. Varnes, B. Jacobson and C. B. Schroy, *Cancer Mgmt* **6**, 127 (1981).
52. T. C. Stephens, V. D. Courtenay, J. Mills, J. H. Peacock, C. M. Rose and D. Spooner, *Br. J. Cancer* **43**, 451 (1981).
53. D. Spooner, J. H. Peacock and T. C. Stephens, *Int. J. Radiat. Oncol. Biol. Phys.* **8**, in press.
54. P. Workman and P. Twentyman, *Int. J. Radiat. Oncol. Biol. Phys.* **8**, in press.
55. M. P. Law, D. G. Hirst and J. M. Brown, *Br. J. Cancer* **43**, 208 (1981).
56. R. T. Mulcahy, D. W. Siemann and R. M. Sutherland, *J. natn. Cancer Inst.* in press.
57. D. W. Siemann, *Br. J. Cancer* **43**, 367 (1981).
58. R. T. Mulcahy, D. W. Siemann and R. M. Sutherland, *Br. J. Cancer* **43**, 93 (1981).
59. D. W. Siemann, *Br. J. Cancer* **45**, 272 (1982).
60. M. W. Anders and G. J. Mannering, *Molec. Pharmac.* **2**, 319 (1966).
61. R. D. Clutterbuck, J. L. Millar and T. J. McElwain, *Cancer Clinical Trials*, in press.
62. I. F. Tannock, *Br. J. Cancer* **42**, 871 (1980).
63. M. J. Haynes and W. R. Inch, *Int. J. Radiat. Oncol. Biol. Phys.* **1**, 1125 (1976).
64. C. J. Gomer and R. J. Johnson, *Radiat. Res.* **78**, 329 (1979).
65. J. Pederspn, M. G. Barron and B. Meeker, *Int. J. Radiat. Oncol. Biol. Phys.* **8**, in press.
66. E. Wist, J. L. Millar and A. J. Shorthouse, *Br. J. Cancer* **43**, 458 (1981).
67. W. M. C. Martin, N. J. McNally and J. de Ronde, *Br. J. Cancer* **43**, 756 (1981).
68. D. W. Siemann and R. T. Mulcahy, *Int. J. Radiat. Oncol. Biol. Phys.* **8**, in press.
69. P. W. Sheldon and E. L. Batten, *Int. J. Radiat. Oncol. Biol. Phys.* **8**, in press.
70. M. R. Boyd, A. Stiko, C. N. Statham and R. B. Jones, *Biochem. Pharmac.* **31**, 1579 (1982).
71. P. R. Twentyman, *Br. J. Cancer* **43**, 745 (1981).
72. R. C. Urtason, H. Tanasichuk, D. Koziol, O. Agboola, G. Washer and J. Raleigh, *Int. J. Radiat. Oncol. Biol. Phys.* **8**, in press.
73. D. Spooner, R. Bugden, M. J. Peckham and E. Wist, *Int. J. Radiat. Oncol. Biol. Phys.* **8**, in press.
74. L. Klein, C. A. Presant, C. L. Vogel, R. Garns and R. Johnson, *Int. J. Radiat. Oncol. Biol. Phys.* **8**, in press.
75. R. D. Botero, *Trans. R. Soc. trop. Med. Hyg.* **67**, 145 (1973).
76. P. Workman, *Br. J. Cancer* **40**, 335 (1979).
77. R. A. S. White and P. Workman, *Br. J. Cancer* **41**, 268 (1980).
78. P. Workman, N. Bleehan and C. R. Wiltshire, *Br. J. Cancer* **41**, 302 (1980).
79. S. Dische, M. I. Saunders, P. Anderson, M. R. L. Stratford and A. I. Minchinton, *Int. J. Radiat. Oncol. Biol. Phys.* **8**, in press.
80. T. H. Wasserman, T. L. Phillips, G. Van Raalte, R. Urtason, J. Partington, D. Koziol, J. G. Schwade, D. Gangji and J. M. Strong, *Br. J. Radiol.* **53**, 736 (1980).
81. B. C. Millar and S. Jinks, *Br. J. Radiol.* **54**, 505 (1981).
82. B. C. Millar, S. Jinks and T. J. Powles, *Br. J. Cancer* **44**, 733 (1981).
83. B. C. Millar and S. Jinks, *Int. J. Radiat. Oncol. Biol. Phys.* **8**, in press.
84. Y. C. Taylor and A. M. Rauth, *Cancer Res.* **38**, 2745 (1978).
85. D. Jamieson and H. A. S. van den Brenk, *Nature, Lond.* **201**, 1227 (1964).
86. M. A. Trush, E. G. Mimnaugh, E. Ginsburg and T. E. Gram, *Biochem. Pharmac.* **31**, 805 (1982).