Biochemical Pharmacology, Vol. 35, No. 1, pp. 97–103, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

THE BIOLOGICAL PROPERTIES OF REDUCED NITROHETEROCYCLICS AND POSSIBLE UNDERLYING BIOCHEMICAL MECHANISMS

G. F. WHITMORE and A. J. VARGHESE
Physics Division, Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ontario M4X 1K9, Canada

Much of the current interest in nitroheterocyclic compounds focuses on their potential role in the treatment of malignant disease. Originally this interest centred on their ability to sensitize normally radioresistant hypoxic cells to the lethal effects of radiation with little or no sensitizing effect on aerobic cells [1, 2]. In this situation the agents were assumed to mimic the electron-affinic properties of oxygen and radiosensitizing efficiency showed a strong correlation with electron affinity [3]. Such sensitization, which may be described as oxygen-mimic radiosensitization or dose-multiplication sensitization, can be detected within milliseconds of mixing drugs and hypoxic cells [4] and therefore almost certainly arises from the interaction of the unaltered parent compound with radiation induced products or lesions within the cell.

While oxygen-mimic radiosensitization arises directly from the parent compound there are a variety of biological properties which arise from drug metabolism, almost certainly reductive metabolism. In addition to oxygen-mimic radiosensitization, the biological properties which are of most interest to cancer diagnosis and therapy are:

(1) cytotoxicity under aerobic and hypoxic conditions;
(2) dose additive radiosensitization;
(3) chemopotentiation;
(4) loss of intracellular thiols;
(5) binding to cellular constituents;
(6) normal tissue toxicity.

Each of these phenomena will be discussed briefly with reference to pertinent publications and recent reviews. In general, these phenomena are preferential for hypoxic cells, are slow to develop, are often strongly correlated with electron-affinity, and are associated with the formation of a variety of reduction products. Consequently, it has become common to assume that they are related to reductive metabolism of the agents and we will discuss some of the reduction chemistry of 2-nitroimidazoles which may underlie these phenomena.

BIOLOGICAL EFFECTS OF NITROHETEROCYCLICS

Aerobic and hypoxic cytotoxicity

Early in the evaluation of nitroheterocyclics as possible clinical radiosensitizers it was recognized that cytotoxicity might prove to be a significant limitation. Initial studies were confined to examinations of cytotoxicity in aerobic cells but relatively early on it was shown that the agents demonstrated a marked preferential toxicity to hypoxic as compared to aerobic cells [5–7]. This observation, which has had a major impact on subsequent studies and interest in the nitroheterocyclics should not have been surprising since nitroheterocyclic compounds have played major roles in the treatment of anaerobic infections.

Dose additive radiosensitization

Once it became apparent that the nitroheterocyclics exhibited both radiosensitizing ability and preferential hypoxic cell toxicity it posed the question as to whether prolonged exposure to nitroheterocyclics under hypoxic conditions might affect the nature or degree of radiosensitization. In a series of experiments [8] it was shown that this was indeed the situation. If mammalian cells are exposed to nitroheterocyclics under hypoxic conditions for prolonged periods of time then a second type of radiosensitization is seen which manifests itself primarily as a reduction in the shoulder of the survival curve seen with low doses of radiation. This radiosensitization, which has been referred to as dose additive sensitization, may be of major clinical significance since its magnitude may be greater than that of the oxygen effect and since it is most pronounced at radiation doses typical of those used in conventional radiation therapy [9].

Chemopotentiation

Hypoxic cell populations within tumours, in addition to their intrinsic resistance to radiation damage, are often refractory to the cytotoxic actions of conventional chemotherapeutic agents [10]. Such resistance may arise from reduced drug penetration, reduced drug activation or a reduced response of non-cycling cells to the cytotoxic effects of agents which are specific to one phase of the cell cycle or which are preferentially toxic to rapidly proliferating cells. Given this situation an enhanced tumour response might be expected from combining the hypoxic cell killing ability of the nitroheterocyclics with agents known to be effective against non-hypoxic and accessible tumour cells. Beneficial effects of such drug combinations have been seen in experimental tumours [11, 12] but it now seems apparent that, although cell killing by nitroheterocyclics can be seen in the hypoxic regions of tumours, the benefits of combining nitroheterocyclics with other chemotherapeutic agents do not

arise solely from the cytotoxic effects of the nitroheterocyclics on hypoxic cells. It now seems clear that, while the presence of hypoxic cells may be necessary in order to detect any benefit, the benefits may arise from any one or a combination of factors including: hypoxic cell toxicity; enhanced total cellular damage; reduced repair capacity; removal of intracellular protective agents or altered pharmacokinetics of one or other agent, and indeed evidence for all of these phenomena has been observed [10]. Whatever the mechanism of the apparent chemopotentiation, its appearance has helped to prompt a growing interest in agents which might require bioreductive metabolism for activation and whose cytotoxic properties might be preferentially expressed in hypoxic and surrounding aerobic tumour cells.

Depletion of intracellular sulphydryls

It has been known since the early work of Patt [13] that sulfhydryl compounds such as glutathione, cysteine and cysteamine are capable of protecting biological systems from the damaging effects of radiation. It has also been established that these agents protect against a variety of chemotherapeutic agents [10]. Whether the sulfhydryl compounds act as radical scavengers, hydrogen donors or inhibit oxidative activation has not been clearly established. Prolonged exposure of mammalian cells to nitroheterocyclics under hypoxic conditions leads to marked reductions of intracellular sulfhydryl levels [14, 15] and there has been considerable interest in the possibility that such loss might play a major role in the radiosensitizing and chemopotentiating properties of the nitroheterocyclics. This is almost certainly not the explanation for the oxygen-mimic type of sensitization and cannot be the total explanation for dose additive radiosensitization and chemopotentiation. What is clear, however, is that because nitroheterocyclics do reduce intracellular sulfhydryl levels and because sulfhydryls do protect against several of the biological effects of the nitroheterocyclics the compounds do in fact modulate their own biological activities.

Binding to cellular macromolecules

Prolonged exposure of hypoxic cells to nitroheterocyclics containing radioactivity leads to marked binding of the radioactivity to various intracellular constituents including DNA, RNA, protein and non-protein sulfhydryl compounds [16, 17]. The biological consequences of such binding are unclear. However, because binding is so much more efficient in hypoxic cells it opens up the interesting possibility that, in combination with autoradiographic techniques the phenomenon can be used to locate regions of hypoxia within tumours [18]. Also, if appropriate γ-emitting isotopes can be incorporated into the nitroheterocyclics then the compounds might be used in the detection and evaluation of human tumours. However, because a variety of factors, including intracellular sulfhydryl levels may affect such binding and because potential binding agents may migrate from hypoxic regions, care must be exercised in interpreting the biological significance of such observations.

Normal tissue toxicity

Current clinical interest in the nitroheterocyclics as radiation sensitizers has focused on the 2-nitroimidazoles because of their greater radiosensitizing ability *vis à vis* other 4- or 5-nitroimidazoles. With misonidazole, the prototype 2-nitroimidiazole, drug dosage in the human was limited by the onset of peripheral neurotoxicity [19] and it has been common to assume that neurotoxicity may be the major clinical limitation to the use of all hypoxic cell sensitizers. However, with metronidazole, a 5-nitroimidazole, the limiting toxicity was seen in the gut [20] and there is evidence that this may also be true with some of the newer 2-nitroimidazoles currently undergoing limited chemical evaluation.

It must be remembered that any nitroheterocyclic compound of biological interest basically consists of two major components, the nitroheterocyclic moiety which is the major determinant of electron affinity and a side chain which is a major determinant of various pharmacological and pharmacokinetic properties such as tissue distribution, excretion rate, etc. It may be that neurological symptoms are characteristic responses to the 2-nitroimidazoles but not nitroheterocyclics. It may also be that the introduction of various active functions, e.g. alkylating groups, into the side chain or modification of the side chain to alter lipid solubility or acid-base properties will play a major role in determining the site of limiting clinical toxicity or the drug doses at which such toxicities become important [21]. In the light of the very limited clinical data currently available it seems premature to assume that neurotoxicity will be the major limitation to the clinical use of all nitroheterocyclics.

NITRO REDUCTION AND BIOLOGICAL EFFECTS

It is apparent that the nitroheterocyclic compounds are capable of inducing a variety of biological effects and that for the majority of the phenomena discussed above the effects are exacerbated in hypoxic cells. Since reduction of the nitro group is a major pathway of metabolism in hypoxic cells [22], it seems probable that the effects arise as a result of the reduction *per se* or the formation of toxic products. It may be tempting to assume that reduction and biological effect will occur within the same cell. This need not always be the case since there is evidence with 2-nitroimidazoles that activation within hypoxic cell populations can be followed by toxicity in adjacent non-hypoxic cell populations [23, 24]. This contrasts with results seen with most chemotherapeutic agents and leads to the observation of drug-induced tumour destruction extending away from sites of necrosis [24] or out from the centres of spheroids [23] rather than being most pronounced on the outer spheroid surface or adjacent to blood vessels. These observations also suggest that toxicity and perhaps other biological phenomena arise as a result of the formation of reactive and potentially diffusible toxic products formed during the course of reduction. The question now arises as to the nature of the reduction process,

the chemical species formed and the ability of such species to account for the various biological effects observed.

REDUCTION CHEMISTRY OF NITROHETEROCYCLIC COMPOUNDS

By analogy with the nitrobenzenes and based on evidence obtained from a variety of chemical, radiolytic, electrolytic and enzymatic reduction systems, it is apparent that reduction of nitroheterocylic compounds proceeds stepwise and involves the formation of three relatively stable reduction species, the nitroso, hydroxylamine and amine derivatives corresponding to two, four and six electron reductions, respectively. In addition reduction also leads to the formation of a radical anion, $RNO_2^{\cdot-}$ and possibly two other radical species corresponding to three and five electron reductions [25]. A simplified reduction scheme is illustrated in Fig. 1. While it would appear that, under appropriate conditions, all of the reduction species illustrated in Fig. 1 may be formed, all species may not be formed in all reduction systems and because of their reactivity, the amounts and types of products formed and their subsequent lifetimes may be highly dependent upon the conditions of reduction. Oxidation reactions, which at least partially reverse the whole process, are also possible.

Figure 1 indicates that, in the presence of oxygen, formation of the radical anion, $RNO_2^{\cdot-}$, leads to a futile recycling which prevents further reduction and leads to the formation of superoxide. If the biological effects referred to earlier result from reduction processes, then the futile recycling will prevent their occurrence. Whether or not the limited toxicity seen in aerobic systems results from the formation of active oxygen species during the futile reduction or from inefficient reduction has not been determined. If, however, aerobic toxicity results from the formation of active oxygen species the relatively low level of aerobic toxicity would suggest that the active oxygen species are relatively non-toxic, compared to the process or products of reduction.

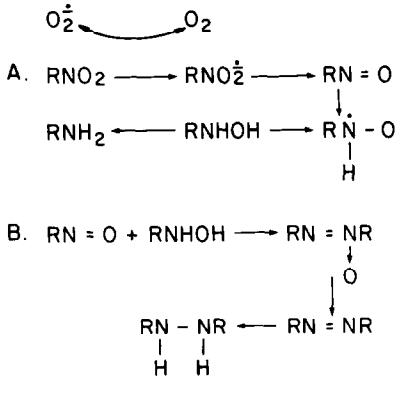


Fig. 1. (a) Postulated pathway for the reduction of nitroheterocyclic compounds and (b) for the formation and subsequent reduction of the bimolecular, azoxy, azo and hydrazo derivatives. Figure 1(a) also shows the "futile reduction" reaction.

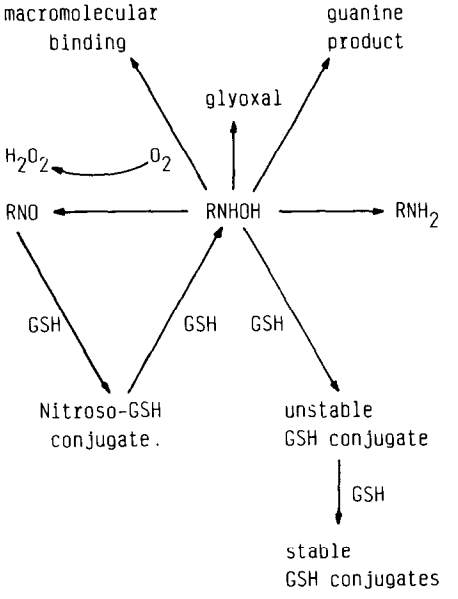


Fig. 2. Schematic representation of possible biologically important reactions of 2-hydroxylaminoimidazoles.

REACTIONS OF 2-HYDROXYLAMINOIMIDAZOLES

On the assumption that the biological phenomena described earlier arise as a result of the formation of reactive species during the process of reduction, we chose to focus on reactions of the hydroxylamine. This choice was predicted on several factors. The hydroxylamine is known to be reactive whereas the amine has proven to be relatively inactive. The nitroso derivative is highly electron-affinic and therefore in most reducing situations will be rapidly reduced to the hydroxylamine. For these reasons we have chosen to investigate the reactions of 2-hydroxylaminoimidazoles, and Fig. 2 summarizes the types of reactions investigated and described below. In situations where the reactions appeared to be of possible biological significance, we have looked for evidence of similar reactions in biological systems, *in vitro*, *in vivo* and in clinical situations.

Preparation

Rapid reduction of 2-nitroimidazoles in dilute solution by zinc dust in the presence of ammonium chloride provides almost quantitative yields of the 2-hydroxylaminoimidazoles [26]. Radiolytic [27], electrolytic [28] and enzymatic [29] reductions are also capable of producing the hydroxylamine derivatives. However, because of the extreme reactivity of 2-hydroxylaminoimidazoles, there may be very marked loss of the product during the course of reduction. In general, the hydroxylamines are more stable as the hydrochlorides [30].

Early studies with ^{14}C-misonidazole labelled at the 2 position indicated that, upon reduction by zinc and ammonium chloride, incubation with DNA, RNA or protein led to macromolecular binding of radioactivity [16, 31]. The precise chemical nature of this covalent binding, especially to nucleic acid, remains unknown.

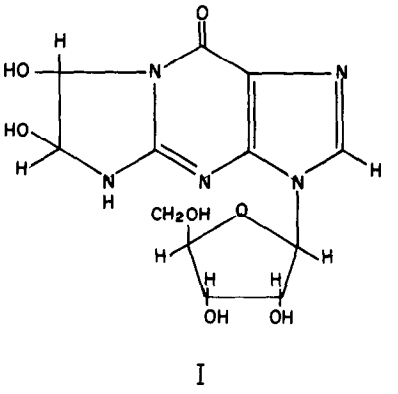
Oxidation

When aqueous solutions of 2-hydroxylaminoimidazoles are exposed to air under neutral conditions, formation of the azoxy derivative is detected [30]. The azoxy derivative almost certainly arises from an initial oxidation of the hydroxylamine to the nitroso derivative followed by a condensation of nitroso with hydroxylamine (see Fig. 1B). During the course of oxidation to the nitroso, there is concomitant formation of H_2O_2, itself a potentially biological reactive species. Therefore, should the hydroxylamine find itself in an aerobic (non-reducing) environment, some of the biological consequences might arise from the formation of H_2O_2, an excellent source of hydroxyl radicals.

Reactions in aqueous solution

As mentioned earlier, the hydroxylamines are relatively stable in dilute HCl. Presumably at low pH the imidazole nitrogen is protonated (**II**; Fig. 3) and this prevents molecular rearrangement. However, at neutral pH the formation of a stabilized nitrenium ion (**III**, **IV**) permits nucleophilic attack on the imidazole ring resulting in the formation of a Bamberger type rearrangement (**V**, **VII**) which on addition of water yields the dihydroxy derivative (**VI**) [32]. The dihydroxy derivative in turn may undergo fragmentation to yield glyoxal (**IX**) and a guanidine derivative (**VIII**) although the equilibrium of this reaction favours the dihydroxy form. The time scales of the reactions shown in Fig. 3 are highly pH dependent and at neutral pH the lifetime of the 2-hydroxylaminoimidazole is likely to be of the order of minutes or less and may vary somewhat with the nature of the N_1 substitution [30, 32].

Evidence for the formation of the glyoxal derivative came first from reactions of reduced 2-nitroimidazoles with guanine derivatives [33]. Such reaction produced a two carbon addition fragment across



I

Fig. 4. Structure of the guanine-glyoxal adduct.

the N_1 and N^2 positions of guanine (Fig. 4), a product identical to that produced by reaction of glyoxal with guanine [34]. Evidence for a glyoxal-like reaction has been seen with all 2-nitroimidazoles tested and in all types of reduction systems including homogenates of cells exposed to misonidazole under hypoxic conditions [35]. The product has also been seen in cellular nucleic acids following misonidazole exposure [36]. While the ultimate product of the reaction with guanine is similar to that produced by glyoxal and there is some evidence of free glyoxal [37] in all reduction systems the largest part of the reaction with guanine occurs slowly and must involve a precursor of glyoxal [35]. It should also be stressed that a glyoxal-like reaction cannot account for the binding of 2-[^{14}C] referred to earlier.

Reactions with GSH

We have mentioned earlier that the presence or absence of glutathione modulates the toxic effects of 2-nitroimidazoles [10] and also that prolonged exposure of mammalian cells to 2-nitroimidazoles

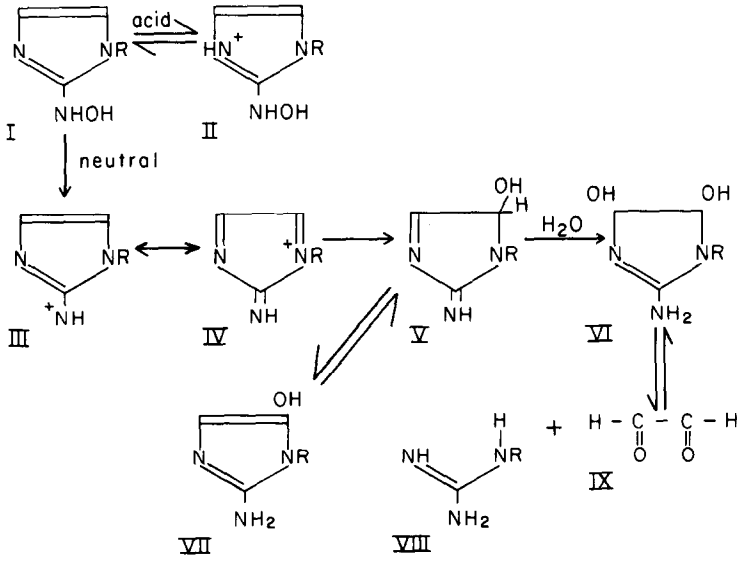


Fig. 3. Postulated reaction scheme illustrating reaction of 2-hydroxylaminoimidazoles in aqueous solutions under hypoxic conditions.

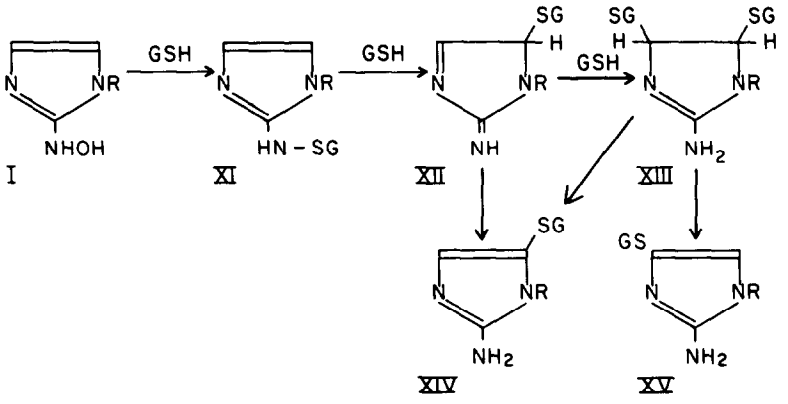


Fig. 5. Postulated reaction scheme illustrating reactions of 2-hydroxylaminoimidazoles with glutathione.

under hypoxic conditions reduces the intracellular level of glutathione [14, 15]. In an attempt to understand these phenomena better we have investigated the reactions of reduced 2-nitroimidazoles with glutathione [38, 39]. Our conclusions as to the likely interactions of the hydroxylamine with glutathione are summarized in Fig. 5. A comparison of Figs 3 and 5 suggests that in many respects GSH mimics the reactions of the hydroxyl group. Initially the GSH reacts with the hydroxylamine to produce an unstable GS conjugate. In the presence of excess GSH, this product undergoes further reactions to yield two stable GS adducts (**XIV**, **XV**) and a saturated product (**XIII**) which may also undergo loss of GSH to produce one of the two stable amino products containing a GS adduct on either the 4 or 5 position of the imidazole ring. While it is likely that these latter two products constitute effective means for detoxifying the hydroxylamine the initial hydroxylamine-GS adduct (**XI**) is a reactive species which may be capable of transferring toxic species from a site of origin to a site of action. Certainly this species, on exposure to air under neutral conditions, forms the azoxy derivative, and, on reacting with guanine, forms the guanine-glyoxal adduct (Fig. 4).

REACTIONS OF HYDROXYLAMINES IN BIOLOGICAL SYSTEMS

Up to this point we have presented evidence that reduction of 2-nitroimidazoles is similar to that of the nitrobenzenes and follows the general reduction scheme shown in Fig. 1. We have also shown that, once formed, the hydroxylamines are highly reactive and can undergo a complex variety of reactions and give rise to a variety of potentially reactive derivatives. In particular we have attempted to describe derivatives and reactions which might be of potential biological significance. However, in order to establish better the potential biological importance of the hydroxylamine, it seemed important to demonstrate that the reactions which we have associated with the hydroxylamine can be observed in biological systems. In order to do this, we have carried out studies in mammalian cells *in vitro*, in experimental animals and in patients undergoing radiation therapy in combination with misonidazole. Perhaps the earliest indication that reduction of 2-nitroimidazoles did occur in biological systems was the finding of the amine derivative in homogenates of cells exposed to misonidazole under hypoxic conditions [22]. Later experiments demonstrated the existence of the amine in the urine of animals [22] and patients [40, 41] following exposure to the same agent. Because the amine is the terminal step of the reduction reaction, its formation can be taken as an indication of the formation of its precursor, the hydroxylamine.

Reference has already been made to studies both by ourselves [16] and others [17] that demonstrate binding of radioactively labelled misonidazole to macromolecules in cells exposed to misonidazole *in vitro* and to tumours and normal tissues of animals. While binding of a glyoxal-like derivative to nucleic acids or proteins could explain the binding seen with non-specifically tritiated misonidazole, it could not explain the binding seen with 2-[^{14}C] since the radioactive carbon does not form part of the glyoxal adduct [34]. The precise chemical nature of the binding of 2-[^{14}C] to nucleic acids and proteins remains to be further elucidated but, since similar binding to macromolecules in solution can be seen with misonidazole reduced to the hydroxylamine [16], it seems likely that the intracellular binding may be attributed to reactions of the hydroxylamine.

Additional evidence for the formation and possible importance of the hydroxylamine in *in vivo* situations comes from attempts to look for glyoxal or its precursor in cells, animals and humans exposed to misonidazole. These studies have indicated the presence in cellular homogenates [36] and in the urine of patients [42] exposed to misonidazole of a product which, on subsequent addition of guanine derivatives, gives rise to the guanine-glyoxal product of Fig. 4. We have also seen evidence for the existence of the guanine adduct in the nucleic acids of cells exposed to misonidazole under hypoxic conditions [36]. In addition to providing strong evidence for the initial formation of the hydroxylamine in these situations and for formation of a DNA adduct, the urine studies provide evidence that potentially toxic products once formed are capable of migration far from their initial site of formation.

In an attempt to evaluate the possible biological significance of the glyoxal derivative on its precursors, we have attempted to determine to what extent a glyoxal-like reduction product could mimic the

biological effects of the 2-nitroimidazoles. These investigations [36] indicate that glyoxal is toxic to both aerobic and hypoxic cells and that the pattern of toxicity as a function of age in the cell cycle mimicked that of misonidazole. Glyoxal also removes the shoulder on radiation survival curves and DNA repair-deficient mutant cells show enhanced sensitivity to both misonidazole and glyoxal. These observations suggest that a reduction product with the properties of glyoxal could account for all of the biological properties seen with exposure to 2-nitroimidazoles under hypoxic conditions. They do not, however, establish the biological importance of reactions of this type.

Reference has already been made to the fact that the presence or absence of GSH profoundly modifies the biological activity of the 2-nitroimidazoles and that hypoxic incubation of mammalian cells with misonidazole reduces the level of intracellular GSH. Both of these observations are consistent with the formation of hydroxylamine-GSH conjugates. Analysis of the homogenates of CHO cells exposed to misonidazole indicates the presence of the stable GS conjugates **XIV** and **XV** of Fig. 5 [30]. Addition of increasing amounts of GSH to the cells prior to incubation with misonidazole also increases the formation of these stable conjugates, an observation which is consistent with the concept that reaction with GSH and the formation of stable conjugates is an effective means of detoxifying the reduction products of the 2-nitroimidazoles. In addition to the formation of the stable GSH conjugates, there is also evidence for the existence in cell homogenates of an unstable GSH conjugate, probably **XI** of Fig. 5. This evidence comes from the fact that if homogenates of cells exposed to misonidazole are subsequently incubated in the presence of GSH, increased formation of the stable conjugates **XIV** and **XV** is observed. Since there is unlikely to be free hydroxylamine in such situations, the likely precursor of the reaction would appear to be structure **XI**.

If we return now to Fig. 2, which summarizes known reactions of the 2-hydroxylaminoimidazoles in chemical systems, it now appears that all of the reactions summarized there, with the exception of oxidation to the nitroso derivative, have been identified in biological systems exposed to 2-nitroimidazoles under hypoxic conditions. Overall, this confirms that reduction is an important metabolic pathway for 2-nitroimidazoles and that reduction proceeds at least to the hydroxylamine and sometimes to the amine stage. Once formed, the hydroxylamine, because of its reactivity, is capable of reaction with a variety of biologically important molecules, including nucleic acids, proteins and various sulfhydryl compounds.

CONCLUSIONS

If we attempt to summarize briefly, 2-nitroimidazoles, under conditions of hypoxic metabolism, are capable of producing a variety of biological effects, some of which have been listed earlier. Since under aerobic conditions the parent nitro compounds are relatively inert biologically, it appears that the compounds require bioactivation. Since bioactivation occurs preferentially under reducing conditions and since the available evidence overwhelmingly indicates the presence of reductive metabolism, it seems likely that the activation results from a bioreductive pathway leading to one or more reactive species. Since the amine derivative has proven to be relatively inert in most chemical tests and, in any reducing situation, the nitroso derivative is likely to be rapidly converted to the hydroxylamine, the hydroxylamine would appear to be the most likely candidate for the active species. This conclusion is supported by the data presented here which indicate that the hydroxylamine is capable of reaction with a variety of biologically important molecules in chemical systems and that the existence of a variety of these same reactions can be demonstrated in cell systems, in whole animals and in human patients. Furthermore, these reactions can easily account for the observed binding to intracellular macromolecules and the loss of intracellular thiols. Since exposure to the 2-hydroxylaminoimidazoles leads to product formation with both nucleic acids and proteins, it seems reasonable that cytotoxicity, dose additive repair and chemopotentiation could arise either from the production of DNA lesions or by inhibition of the repair of lesions formed by other agents. This conclusion is supported by the evidence that glyoxal, which reacts with DNA and proteins and which is at least a minor product of the breakdown of hydroxylaminoimidazoles, is capable of inducing these biological phenomena. Further elucidation of the exact mechanisms underlying these biological phenomena is required and it seems likely that such studies may well make use of various purified reductive metabolites, repair-deficient mutant cells and techniques involving plasmids or other biologically active DNA species.

Up to this point, we have said little about the production of biological phenomena in normal tissues such as gut, brain and nervous tissue. At the present time it is not clear whether these toxicities have a common mechanism, whether they arise directly from the parent compounds or whether they arise as a result of bioactivation, reductive or otherwise, either in the target tissue or at a distance. In this latter connection, it is perhaps pertinent to point out that several of the reactions we have described for the 2-hydroxylaminoimidazole could give rise to chemical species capable of reaction at some distance from their cell of origin, either in neighbouring aerobic tumour cells or even in different organs. This may be true of the hydroxylamine itself or its RNH-SG derivative (**XI**) which, although reactive, may diffuse some distance from their cell of origin before reacting. It is certainly true of the precursor of the glyoxal-guanine adduct (Fig. 4) since this product can be detected in the urine of animals and patients and hence must circulate freely in the bloodstream.

In conclusion, it seems certain that nitroheterocyclic compounds do undergo bioreductive activation and at least in the case of the 2-nitroimidazoles it would appear that a major reactive product is the hydroxylamine. The hydroxylamine, either as such or as its reactive derivatives could account for many of the biological phenomena reported for the 2-nitroimidazoles. The exact mechanisms by which some of these occur has yet to be elucidated.

Acknowledgements—The authors wish to thank Drs A. M. Rauth and R. McClelland for helpful discussions during the course of this work. The financial support of the Ontario Cancer Treatment and Research Foundation and the National Cancer Institute of Canada is also gratefully acknowledged.

REFERENCES

1. G. E. Adams and M. S. Cooke, *Int. J. Radiat. Biol.* **15**, 457 (1969).
2. G. E. Adams, I. Ahmed, E. M. Fielden, P. O'Neill and I. J. Stratford, *Cancer Clin. Trials* **3**, 37 (1980).
3. G. E. Adams, I. R. Flockhart, C. E. Smithen and I. J. Stratford, *Radiat. Res.* **67**, 9 (1976).
4. D. W. Whillans, G. E. Adams and P. Neta, *Radiat. Res.* **62**, 407 (1975).
5. B. A. Moore, B. Palcic and L. D. Skarsgard, *Radiat. Res.* **67**, 459 (1976).
6. I. J. Stratford and G. E. Adams, *Br. J. Radiol.* **51**, 745 (1978).
7. R. M. Sutherland, B. J. Bareham and K. A. Reich, *Cancer Clin. Trials* **3**, 73 (1980).
8. T. W. Wong, G. F. Whitmore and S. Gulyas, *Radiat. Res.* **75**, 541 (1978).
9. B. Palcic, *Int. J. Radiat. Oncol. Biol. Phys.* **10**, 1185 (1985).
10. D. W. Siemann, *Int. J. Radiat. Oncol. Biol. Phys.* **10**, 1585 (1984).
11. J. J. Clement, M. S. Gorman, I. Wodinsky, R. Catane and R. K. Johnson, *Cancer Res.* **40**, 4165 (1980).
12. C. M. Rose, J. L. Millar, J. H. Peacock, T. A. Phelps and T. C. Stephens, in *Radiation Sensitizers; Their Use in the Clinical Management of Cancer* (Ed. L. W. Brady), p. 250. Masson, New York (1980).
13. H. M. Patt, *Physiol. Rev.* **33**, 35 (1953).
14. J. E. Biaglow, M. E. Varnes, M. Astor and E. J. Hall, *Int. J. Radiat. Oncol. Biol. Phys.* **8**, 719 (1982).
15. E. A. Bump, Y. C. Taylor and J. M. Brown, *Cancer Res.* **43**, 997 (1983).
16. A. J. Varghese and G. F. Whitmore, *Cancer Res.* **40**, 2165 (1980).
17. J. D. Chapman, K. Baer and J. Lee, *Cancer Res.* **43**, 1523 (1983).
18. J. D. Chapman, A. J. Franko and J. Sharplin, *Br. J. Cancer* **43**, 546 (1981).
19. R. C. Urtasun, C. N. Coleman, T. H. Wasserman and T. L. Philips, *Int. J. Radiat. Oncol. Biol. Phys.* **10**, 1691 (1984).
20. R. C. Urtasun, P. R. Band, J. D. Chapman, M. L. Feldstein, B. Mielke and C. Fryer, *New England J. Med.* **294**, 1364 (1976).
21. G. E. Adams, *Int. J. Radiat. Oncol. Biol. Phys.* **10**, 1181 (1984).
22. A. J. Varghese, S. Gulyas and J. K. Mohindra, *Cancer Res.* **36**, 3761 (1976).
23. R. Sridhar, C. Koch and R. Sutherland, *Int. J. Radiat. Oncol. Biol. Phys.* **1**, 1149 (1976).
24. J. M. Brown, *Int. J. Radiat. Oncol. Biol. Phys.* **8**, 675 (1982).
25. P. Wardman, *Curr. Top. Radiat. Res. Q.* **11**, 347 (1977).
26. A. J. Varghese and G. F. Whitmore, *Chem.-Biol. Interact.* **36**, 141 (1981).
27. D. W. Whillans and G. F. Whitmore, *Radiat. Res.* **86**, 311 (1981).
28. R. A. McClelland, J. R. Fuller, N. E. Seaman, A. M. Rauth and R. Battistella, *Biochem. Pharmac.* **33**, 303 (1984).
29. P. D. Josephy, B. Palcic and L. D. Skarsgard, *Biochem. Pharmac.* **30**, 849 (1981).
30. A. J. Varghese and G. F. Whitmore, *Chem.-Biol. Interact.*, submitted for publication.
31. A. J. Varghese and G. F. Whitmore, *Cancer Clin. Trials* **3**, 43 (1980).
32. R. A. McClelland, R. Panicucci and A. M. Rauth, *J. Am. chem. Soc.* **107**, 1762 (1985).
33. A. J. Varghese and G. F. Whitmore, *Cancer Res.* **43**, 78 (1983).
34. R. Shapiro and J. Hachman, *Biochemistry* **5**, 2799 (1966).
35. A. J. Varghese and G. F. Whitmore, *Radiat. Res.* **97**, 262 (1984).
36. G. F. Whitmore, A. J. Varghese and S. Gulyas, in *Proceedings of the Conference on the Role of Cyclic Nucleic Acid Adducts, Carcinogens and Mutagens.* World Health Organization, in press.
37. J. A. Raleigh and S. F. Liu, *Biochem. Pharmac.* **32**, 1444 (1983).
38. A. J. Varghese, *Biochem. biophys. Res. Commun.* **112**, 1013 (1983).
39. A. J. Varghese and G. F. Whitmore, *Int. J. Radiat. Oncol. Biol. Phys.* **10**, 1341 (1984).
40. I. R. Flockhart, S. L. Malcolm, T. R. Martin, C. S. Parkins, R. J. Ruane and D. Troup, *Br. J. Cancer* **37**, Suppl. III, 264 (1978).
41. A. J. Varghese, *Analyt. Biochem.* **110**, 197 (1981).
42. A. J. Varghese and G. F. Whitmore, *Int. J. Radiat. Oncol. Biol. Phys.* **10**, 1361 (1984).