Biochemical Pharmacology, Vol. 37, No. 20, pp. 3837–3842, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

ENHANCEMENT OF DNA DAMAGE IN MAMMALIAN CELLS UPON BIOREDUCTION OF THE NITROIMIDAZOLE-AZIRIDINES RSU-1069 AND RSU-1131

TERRY J. JENNER, ORAZIO SAPORA,* PETER O'NEILL and E. MARTIN FIELDEN

Division of Molecular Processes, MRC Radiobiology Unit, Chilton, Didcot, Oxon, OX11 0RD, U.K., and *Instituto Superiore di Sanita, Viale Regina Elena 299, 161 Rome, Italy

(*Received* 9 *February* 1988; *accepted* 18 *April* 1988)

Abstract—The induction of DNA double-(dsb) and single-(ssb) strand breaks by RSU-1069, RSU-1131 and misonidazole in V79 mammalian cells has been investigated using sedimentation in isokinetic sucrose gradients after incubation for various times (1–3 hr) at 310 K under both hypoxic and aerobic conditions. Double strand breaks are produced by RSU-1069 and RSU-1131 predominantly under hypoxic conditions.

Comparison of the cellular DNA damage induced by these agents leads to the following facts: (1) the yield of ssb induced by these agents is substantially increased under hypoxia, (2) RSU-1069 and RSU-1131 are much more effective than misonidazole, on a concentration basis, at causing strand breakage both under hypoxic and aerobic conditions; and (3) RSU-1069 is more efficient on a concentration basis than RSU-1131 at inducing both ssb and dsb under both conditions. From these findings and molecular studies it is suggested that these 2-nitroimidazole aziridines act as monofunctional alkylating agents under aerobic conditions, a factor that governs their aerobic cytotoxicity. Under hypoxic conditions, it is suggested that the induction of dsb and crosslinks by these agents (bifunctional character) may play a major role in determining the ability of such agents to act as hypoxia-selective cytotoxins.

The compound RSU-1069 [NSC 347503, 1-(2-nitro-1-imidazoyl)-3-(1-aziridinyl)-2-propanol] is a more efficient chemopotentiator and hypoxic cell radiosensitizer than misonidazole [1–5]. Further, RSU-1069 is considerably more cytotoxic towards hypoxic relative to aerobic cells *in vitro* [6–8]. Potentiation of tumour cytotoxicity of RSU-1069 has been demonstrated *in vivo* with vasoactive drugs [9]. Monomethyl substitution of the aziridine moiety of RSU-1069, RSU-1131, results in decreased hypoxic and aerobic cytotoxicity [10, 11] and reduces the extent of unscheduled DNA synthesis [12].

Cellular and molecular studies indicate that both RSU-1069 and RSU-1131 act as monofunctional alkylating agents under aerobic conditions whereas upon reduction, they become bifunctional in character [6, 7, 11, 13–16]. Studies with plasmid DNA have shown [11, 13, 15, 16] that the major types of DNA damage produced by these agents are single-strand breaks (ssb), base adducts and, under reducing conditions, crosslinks. Furthermore the efficiency of RSU-1069 to produce such damage is greater than that of RSU-1131. Such studies have highlighted that these agents alkylate DNA at both phosphate and purine base sites [14–16], processes which may lead to strand breakage and/or alkaline-labile sites. The formation of such damage produced by RSU-1069 during pre-irradiation contact time has been suggested to be responsible for its enhanced radiosensitizing efficiency at 310 K [8, 17, 18].

The present study was undertaken to investigate the ability of RSU-1069 and RSU-1131 to produce cellular DNA damage within mammalian cells under aerobic and hypoxic conditions using sedimentation in isokinetic sucrose gradients. By comparing the agent-induced damage in cellular DNA and that produced in plasmid DNA to their relative cytotoxicities, it is our aim to identify those molecular aspects which are required for such compounds to act as hypoxia-selective cytotoxins.

MATERIALS AND METHODS

Chemicals. The synthesis, purification and physico-chemical properties of RSU-1069 and RSU-1131 have been reported [1, 11]. Misonidazole was supplied by Dr. C. E. Smithen (Roche Products Ltd., Welwyn, U.K.). Sarkosyl and proteinase K were obtained from Sigma. All other chemicals were of AnalaR grade and used as supplied. All solutions containing the 2-nitroimidazoles were freshly-prepared as required in order to minimise their degradation in aqueous media [11, 15, 16].

DNA strand breaks. Chinese hamster cells, line V79-379A, were used throughout this work and grown in monolayer by methods described previously [19]. The procedure for labelling the cells and preparation of sucrose density gradients have been described previously [20, 21].

Briefly, cells ($1.2–1.5 \times 10^6$) were seeded into 50 ml Falcon flasks containing growth medium. After attachment the medium was exchanged for similar medium containing 0.5 μCi/mmol [^{3}H] thymidine (25 mCi/mmol—Amersham Int.) to label the cells. Cultures were incubated for about 24 hr at 310 K. Then the active medium was replaced with fresh medium supplemented with cold thymidine (0.1 mg/ml final concentration), and cells incubated for 1 hr. The cells were trypsinized and resuspended in label-free medium (MEM modified for suspension

culture). Prior to agent treatment the cell suspension was equilibrated with N_2 + 5% CO_2 (<10 ppm O_2) or maintained aerobically (air + 5% CO_2) and after 15 min an aqueous solution of the agent was added and incubated for fixed times at 310 K under the relevant gassing condition. After the incubation period, the cell suspension containing the agent was placed on ice to minimise further damage and/or repair. The agent was removed by spinning at 3000 rpm for 10 min followed by resuspension of the pellet in 0.2 ml of 0.01 mol dm^{-3} Tris/1 mmol dm^{-3} EDTA/0.15 mol dm^{-3} NaCl at pH 7.5.

The DNA molecular weight distribution in treated cells was determined by sedimentation in isokinetic sucrose gradients following cell lysis and DNA release. The gradients were prepared as described previously [20] using polycarbonate tubes. For single-strand break damage, 120 μl of the cell lysate (0.5 mol dm^{-3} NaOH, 10 mmol dm^{-3} EDTA, 1% sarkosyl at pH 12.7) was layered on the top of the alkaline sucrose gradients (5–20% w/v in 0.3 mol dm^{-3} NaOH, 0.1% sarkosyl, 1 mmol dm^{-3} EDTA, 0.9 mol dm^{-3} NaCl) followed by 30 μl of cell suspension. After standing for 4 hr at room temperature to ensure dissociation and denaturing of the DNA, the gradients were spun at 293 K for 14 hr at 10,000 rpm using a 6 × 5.5 ml swing-out rotor in a centrifuge. After centrifugation the gradient was fractionated and further processed as described previously [20].

For double-strand break (dsb) determinations [21], 30 μl of drug-treated cell suspension was added to 120 μl of lysis solution (0.2% proteinase K, 1% sarkosyl, 1% sodium dodecyl sulphate, 0.5% sodium deoxycolate, 0.01 mol dm^{-3} Tris, 0.02 M EDTA pH 8.7) and kept on ice for 30 min. Following a 30-min lysis at 343 K, the cells were left to lyse for about 18 hr at 323 K. The lysed solution was transferred to the top of the sucrose gradient (5–20% w/v sucrose in 0.01 mol dm^{-3} Tris, 1 mmol dm^{-3} EDTA, 0.1 mol dm^{-3} NaCl pH 7.5), left for 4 hr and finally spun for 65 hr at 3000 rpm at 293 K. The gradients were processed as previously described [20].

For both ssb and dsb determinations the density gradients were linear based upon refractive index measurements of the fractions. The resultant activity profiles of the gradients after centrifugation were analysed as previously described [20] based upon the assumption that DNA undergoes scission at random on the DNA strand. For calculation of the number of ssb and dsb/genome it has been assumed for convenience that the mean molecular weight of untreated DNA is 1×10^{10} and 3×10^{10} respectively.

The molecular weight standard used to calibrate the system was T_4 phage (molecular weight of 1.1×10^8 as single-stranded DNA). Using a sedimentation coefficient of 56.7 [22], the molecular weight (M) of DNA was determined from the sedimentation coefficient using $S = 0.0355\ M^{0.3978}$ for ssb [22].

RESULTS

Single-strand breakage of DNA

The effects of incubation time on changes of the molecular weight of DNA from V79 mammalian cells (ssb) after incubation in suspension at 310 K with various concentrations of RSU-1069 are shown in Fig. 1 for aerobic conditions. The data are presented as the reciprocal of the mean molecular weight ($1/M_n$) as a function of incubation time. From Fig. 1 the relative number of ssb is approximately linear with time. Typical sedimentation profiles comparing the effects of different concentrations for a given incubation time of RSU-1131 are shown in Fig. 2. It should be noted that the yields of ssb include those arising from "alkali-labile" sites.

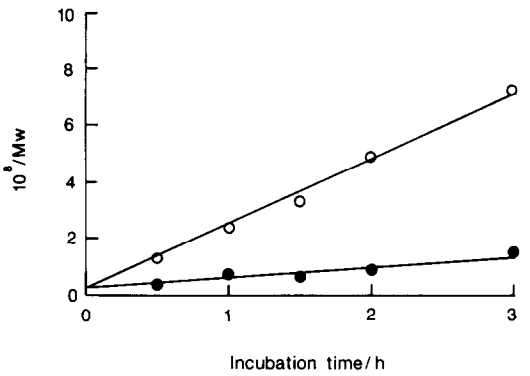

Fig. 1. The dependence of the yield of ssb on contact time in air following incubation of (●) 0.2 mmol dm^{-3} and (○) 1.0 mmol dm^{-3} RSU-1069 with V79 mammalian cells.

In order to determine the concentration-dependence of ssb yields for a fixed time in the presence of the agents, an incubation period of 2 hr under aerobic or hypoxic conditions was chosen. Such dependences following 2 hr incubation with RSU-1069 or RSU-1131 of V79 mammalian cells under aerobic and hypoxic conditions are shown in Fig. 3. The corresponding concentration-dependence determined for misonidazole under hypoxic conditions is also shown in Fig. 3. In order to compare the different compounds and conditions, the reciprocal molecular weight of 2×10^{-8} has been chosen as an arbitrary value; this value corresponds to the "yield" of ssb following a radiation dose of about 110 Gy to V79 cells under aerobic conditions. From calibration of the gradients, it is calculated that this value corresponds to about 125 ssb/10^{10} dt. In Table 1 are presented the concentrations of RSU-1069, RSU-1131 and misonidazole required to produce equivalent levels of DNA ssb yields (125 ssb/10^{10} dt) under the various conditions. The cytotoxicity data [7, 11] for these 2-nitroimidazoles is also presented in Table 1. The differential effects of hypoxic to aerobic conditions for cytotoxicity [7, 11], ssb in cellular DNA and strand breakage in plasmid DNA are presented in Table 2 for RSU-1069, RSU-1131 and misonidazole.

Double-strand breakage of DNA

The effect of incubation time upon changes of the molecular weight of DNA for V79 cells (dsb) incubated at 310 K in suspension with various concentrations of RSU-1069 under hypoxic conditions is shown in Fig. 4. In contrast to the linear

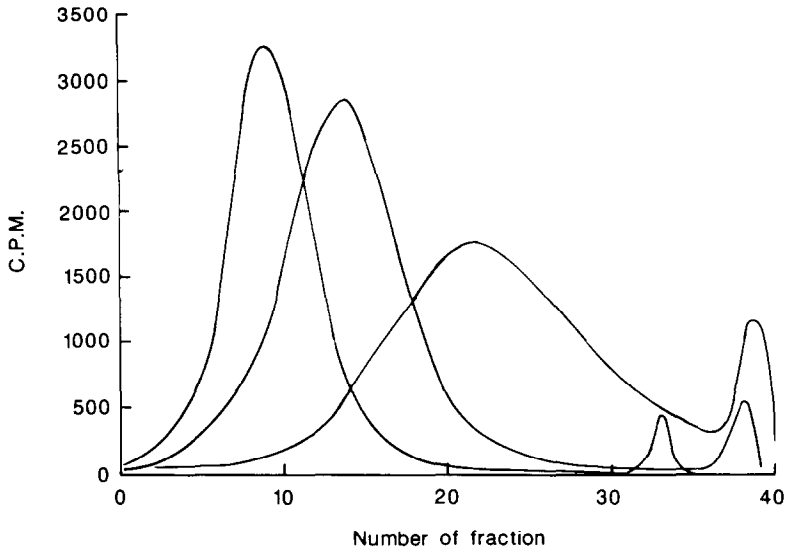

Fig. 2. Typical experimental ssb sedimentation profiles following incubation of RSU-1131 with V79 cells in air.

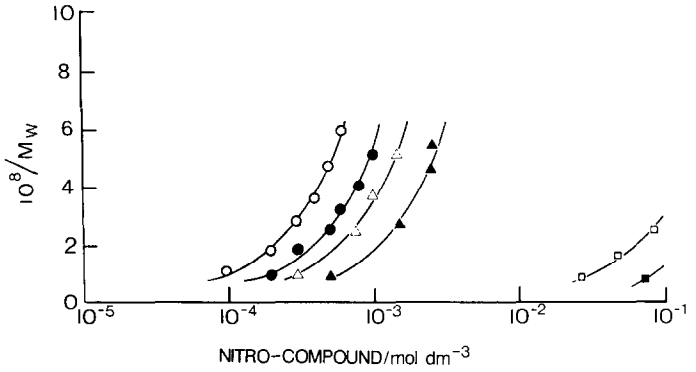

Fig. 3. Dependence of the yield of ssb on concentration of RSU-1069 (○, ●), -1131 (△, ▲) and misonidazole (□, ■) following a 2-hr incubation with V79 cells in air (solid symbols) and N_2 (open symbols).

Table 1. Concentrations (in μmol dm^{-3}) of a series of 2-nitroimidazoles require to induce DNA strand breakage* and reduce cell viability to 10%†

Compound	ssb		dsb		Toxicity	
	N_2	Air	N_2	Air	N_2	Air
RSU-1069	210	360	65	1000	3.3 (10)§	340
RSU-1131	505	1150	330	1550	65	790
Misonidazole	≥60,000	NO‡	NO‡	NO‡	5500	≥50,000

* Related to a radiation dose equivalence of 110 Gy.
† From Ref. [11].
‡ NO = not observed.
§ This value relates to exponentially growing cells [7].

Table 2. Differential between the effects of hypoxic and aerobic treatments with a series of 2-nitroimidazoles

Compound	Strand breakage* plasmid DNA	Cellular DNA ssb	Cellular DNA dsb	Cytotoxicity*
RSU-1069	3.2	1.7	15	103 (34)§
RSU-1131	4.8	2.3	4.7	12.2
Misonidazole	†	NO‡	NO‡	≥9.1

* From Ref. [11].
† No breaks observed at pH 7.0 [25].
‡ NO = not observed under aerobic conditions.
§ Relates to exponentially-growing cells [7].

dependence for ssb (Fig. 1), the dsb dependence is non-linear with time for the concentration studied.

The concentration dependences for dsb formation by RSU-1069 and RSU-1131 following 2 hr incubation with V79 cells under both aerobic and hypoxic conditions are shown in Fig. 5. The concentrations of RSU-1069 and RSU-1131 to produce dsb under hypoxic conditions are displaced to lower concentrations as compared to that required under aerobic conditions. In order to quantify these differences and to compare the concentrations with those for ssb formation, the concentration to reduce the reciprocal of the mean molecular weight to 5.8×10^{-10} following a 2-hr incubation has been chosen. This value corresponds to that for dsb produced after irradiation of V79 cells in air with a dose of 110 Gy. The concentrations of RSU-1069 and RSU-1131 to produce corresponding levels of dsb under the different conditions are shown in Table 1. Under these conditions, dsb are not evident following incubation with misonidazole up to a concentration of 100 mmol dm^{-3}.

The differential effect of hypoxic and aerobic conditions for formation of dsb in cellular DNA by RSU-1069 and RSU-1131 are presented in Table 2. In order to compare the increased effectiveness of RSU-1069 to that of RSU-1131, both molecular and cellular data are presented in Table 3.

DISCUSSION

Previous studies of the *in vitro* cytotoxicity [7, 11] have shown that the large hypoxic:aerobic differential cytotoxicity ratio using RSU-1069 is greatly reduced with both RSU-1131 and misonidazole (Table 2). The differential cytotoxicity ratio for RSU-1069 has been taken from the studies with cells in log phase [7]. RSU-1131 is, however, more toxic than misonidazole, on a concentration basis (Table 1), under either aerobic or hypoxic conditions [11]. The aim of the present study was to compare the relative cytotoxic effects of these 2-nitroimidazoles with their ability to induce various classes of DNA damage at both the cellular and molecular [6, 7, 11–16] level.

Such comparisons lead to the following facts: (1) The yields of dsb and ssb induced by both RSU-1069 and RSU-1131 at a given concentration are increased under hypoxic conditions (Figs 3 and 5); (2) Under both hypoxic and aerobic conditions, the nitroimidazole aziridines are more efficient than misonidazole, on a concentration basis, at inducing ssb; (3) RSU-1069 and RSU-1131 induce dsb under aerobic and hypoxic conditions, whereas misonidazole does not induce dsb up to concentrations used of 100 mmol dm^{-3}; (4) RSU-1069 is more efficient on a concentration basis than RSU-1131 at inducing both ssb and dsb in cellular DNA under both aerobic and hypoxic conditions; (5) The yield of ssb but not dsb is linearly dependent upon contact time for fixed concentrations of the 2-nitroimidazole-aziridines.

The concentrations of RSU-1069 and RSU-1131 required to produce significant yields of ssb in air are similar to those required to reduce cell viability to 10% in air (Fig. 3 and Table 1). In contrast, higher concentrations of these 2-nitroimidazoles are required to induce ssb in hypoxia and dsb in both air or hypoxia when compared to those required to reduce cell viability to 10%. Since the concentrations of 2-nitroimidazole aziridines to produce a given amount of DNA damage are related to an arbitrary radiation dose equivalence of 110 Gy, it is expected that at lower concentrations, similar to those inducing cytotoxicity in hypoxia (Table 1), significant yields of dsb will still be produced in cellular DNA.

The increase in the yield of cellular DNA damage induced by these 2-nitroimidazoles under hypoxic relative to aerobic conditions (Table 2) is consistent with their bioreductive activation to yield metabolites with increased cytotoxicity. Indeed, previous studies [23, 24] have highlighted the induction of DNA damage by misonidazole under hypoxic

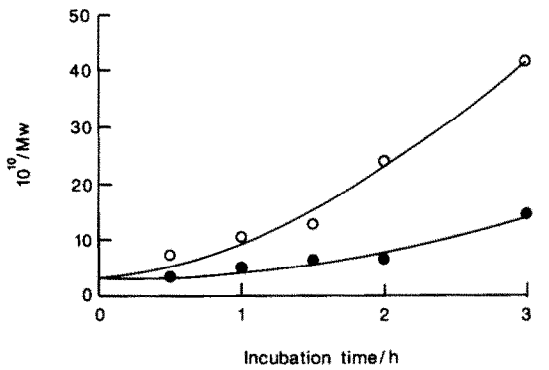

Fig. 4. The dependence of the yield of dsb induced in N_2 on contact time following incubation of (●) 0.2 mmol dm^{-3} and (○) 0.5 mmol dm^{-3} RSU-1069 with V79 cells.

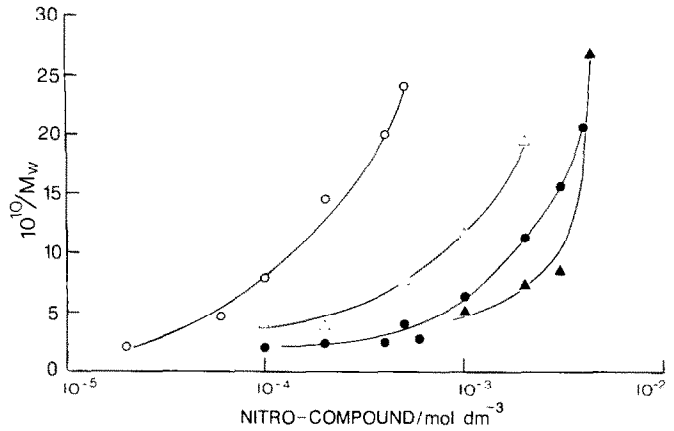

Fig. 5. The dependence of the yield of dsb on the concentration of RSU-1069 (○, ●) and -1131 (△, ▲) following a 2-hr incubation with V79 cells in air (solid symbols) and N_2 (open symbols).

conditions. Furthermore the increased efficiency of both RSU-1069 and RSU-1131 to induce DNA damage under hypoxia at much lower concentrations than that of misonidazole is related to the additional alkylating activity of the aziridine moiety. Such alkylating action together with reductive activation of RSU-1069 and RSU-1131 results in these compounds having bifunctional character under hypoxic conditions as emphasised in the plasmid DNA systems whereby crosslinks are evident [16]. It is expected that the rate of bioreduction of both these 2-nitroimidazoles will be similar, based upon the similarity of their redox potentials ($E_{\frac{1}{7}}$-values) and cell-uptake [11, 18]. However, the above arguments do not take into account the subsequent biochemical repairability of the DNA lesions or the relative distribution of reaction(s) with the various sites of the DNA.

From a comparison of the cellular DNA damage (ssb and dsb) and the chemical activity of RSU-1069 and RSU-1131, the increased efficiency of RSU-1069 compared to that of RSU-1131 under aerobic conditions is consistent with the approximately 2-fold decrease in reactivity of the aziridine moiety upon monomethyl substitution as reflected by their phosphate reactivities (Table 3). The slightly higher value of this ratio for ssb formation is consistent with the increased ability of RSU-1069 compared to that of RSU-1131 to induce alkali-labile sites in plasmid DNA [16]. Such sites are minimised when determining dsb yields so that the increased efficiency of RSU-1069 compared with RSU-1131 to induce dsb in air is comparable with their differences in phosphate reactivities. A similar decrease in efficiency of RSU-1131 compared with that of RSU-1069 in air was also observed for cytotoxicity and strand breakage induced in plasmid DNA. It is proposed that the difference in the aerobic cytotoxicity of these 2-nitroimidazole aziridines is a reflection of the difference in monofunctional alkylating efficiency of the respective aziridine moieties. Even under hypoxic conditions, the relative efficiencies of ssb induced by RSU-1069 and RSU-1131 (Table 3) and the differential hypoxic:oxic effects for ssb induced by both RSU-1069 and RSU-1131 and for dsb induced by RSU-1131 (Table 2) still reflect mainly the reactivity of the aziridine moiety consistent with molecular data on plasmid DNA damage. On the other hand, the increase in dsb yield on changing from aerobic to hypoxic conditions for RSU-1069 of about 15 is of the same order to that for the differential hypoxic: aerobic cytotoxicity of about 34 for log phase cells. Furthermore, the increased effect of RSU-1069:RSU-1131 of about 5 for dsb formation under hypoxia together with the equivalent ratio for crosslinkage in plasmid DNA is a reasonable reflection of the relative hypoxic cytotoxicities of these compounds (Table 3). The contribution from crosslinks, as demonstrated in the *in vitro* DNA studies [16], is not assessed in this study since determination of crosslinks in the presence of ssb DNA damage is difficult using sedimentation analysis. Under hypoxic conditions it is proposed that the difference in the cytotoxicity of these 2-nitroimidazoles aziridines is to a large extent a reflection of their differences to act as bifunctional agents following bioreduction.

Table 3. The increased effectiveness of RSU-1069 to RSU-1131 on a concentration basis based upon different endpoints

Property	RSU-1069:RSU-1131 Air	N_2
Cytotoxicity*	2.3	~20
Cellular DNA		
ssb	3.2	2.4
dsb	1.6	5.0
Plasmid DNA§		
ssb*	3.4	2.3
Crosslinks†	not observed	~15–20
Phosphate reactivity†	~2.0	ND‡

* From Ref. [11].
† From Ref. [16].
‡ ND = not determined.
§ With plasmid DNA—Air ≡ exposure to parent compounds, N_2 ≡ exposure to radiation-reduced compounds (see Ref. 13).

In agreement with the *in vitro* DNA studies [11–16], it is inferred that RSU-1069 and RSU-1131 act as monofunctional agents in air and upon bioreduction under hypoxic conditions these compounds are converted into bifunctional agents. In support of their mode of action, the formation of ssb in air is almost linearly dependent upon contact time as expected for single-hit damage (monofunctional action). From the concentrations required to induce ssb and dsb (Figs 3 and 5) and their time dependences (Figs 1 and 4), it is inferred that dsb induced by RSU-1131 predominantly arise via 2 independently-produced ssb. On the other hand, the dependence of dsb on concentration of RSU-1069 represents not only a dual-hit mechanism whereby a dsb is produced by 2 independently-produced ssb but also "true" dsb.

In conclusion it is emphasised that these 2-nitroimidazole aziridines act as monofunctional alkylating agents under aerobic conditions, a factor that governs their aerobic cytotoxicity. Under hypoxic conditions, the induction of "true" dsb and DNA crosslinks [16] by agents which are converted to metabolites with bifunctional character may play a predominant role in their hypoxic cytotoxicity. Furthermore the induction of such lesions (dsb and crosslinks) probably play a major role in determining the ability of such agents to act as hypoxia-selective cytotoxins.

Acknowledgements—The authors wish to thank Prof. G. E. Adams and Drs I. J. Stratford and T. C. Jenkins for helpful comments. We thank Drs T. C. Jenkins and I. Ahmed for the synthesis of RSU-1069 and RSU-1131.

REFERENCES

1. Adams GE, Ahmed I, Sheldon PW and Stratford IJ, Radiation sensitization and chemopotentiation: RSU-1069, a compound more efficient than misonidazole *in vitro* and *in vivo*. *Br J Cancer* **49**: 571–577, 1984.
2. Adams GE, Ahmed I, Sheldon PW and Stratford IJ, RSU-1069, a 2-nitroimidazole containing an alkylating group: high efficiency as a radio- and chemisensitizer *in vitro* and *in vivo*. *Int J Radiat Oncol Biol Phys* **10**: 1653–1656, 1984.
3. Siemann DW, Maddison K and Wolf K, Potentiation of melphalan activity in the KHT sarcoma by the radiosensitizer RSU-1069. *Int J Radiat Oncol Biol Phys* **10**: 1657–1660, 1984.
4. Chaplin DJ, Durand RE, Stratford IJ and Jenkins TC, The radiosensitizing and toxic effects of RSU-1069 and hypoxic cells in murine tumor. *Int J Radiat Oncol Biol Phys* **12**: 1091–1095, 1986.
5. Adams GE and Stratford IJ, Hypoxia-mediated nitroheterocyclic drugs in the radio- and chemotherapy of cancer. *Biochem Pharmacol* **35**: 71–76, 1986.
6. Stratford IJ, O'Neill P, Sheldon PW, Silver ARJ, Walling JM and Adams GE, RSU-1069, a nitroimidazole containing an aziridine group: bioreduction greatly increases cytotoxicity under hypoxic conditions. *Biochem Pharmacol* **35**: 105–109, 1986.
7. Stratford IJ, Walling JM and Silver ARJ, The differential cytotoxicity of RSU-1069: cell survival studies indicating interaction with DNA as a possible mode of action. *Br J Cancer* **53**: 339–344, 1986.
8. Hill RP, Gulyas S and Whitmore GF, Studies of the *in vivo* and *in vitro* cytotoxicity of the drug RSU-1069. *Br J Cancer* **53**: 743–751, 1986.
9. Chaplin DJ, Potentiation of RSU-1069 tumour cytotoxicity by 5-hydroxytryptamine (5-HT). *Br J Cancer* **54**: 727–732, 1986.
10. Ahmed I, Jenkins TC, Walling JM, Stratford IJ, Sheldon PW, Adams GE and Fielden EM, Analogues of RSU-1069: radiosensitization and toxicity *in vitro* and *in vivo*. *Int J Radiat Oncol Biol Phys* **12**: 1079–1081, 1986.
11. O'Neill P, Jenkins TC, Stratford IJ, Silver ARJ, Ahmed I, McNeil SS, Fielden EM and Adams GE, Mechanism of action of some bioreducible 2-nitroimidazoles: comparison of *in vitro* cytotoxicity and ability to induce DNA strand breakage. *Anti-Cancer Drug Design* **1**: 271–280, 1987.
12. Suzanger M, White INH, Jenkins TC and Connors TA, Effects of substituted 2-nitroimidazoles and related compounds on unscheduled DNA synthesis in rat hepatocytes and in non-transformed (BL8) and transformed (JB1) rat liver epithelial-derived cell lines. *Biochem Pharmacol* **36**: 3743–3749, 1987.
13. Silver ARJ, O'Neill P and Jenkins TC, Induction of DNA strand breaks by RSU-1069, a nitroimidazole-aziridine radiosensitizer. *Biochem Pharmacol* **34**: 3537–3542, 1985.
14. Silver ARJ, O'Neill P, Jenkins TC and McNeil SS, The phosphate-group of DNA as a potential target for RSU-1069, a nitroimidazole-aziridine radiosensitizer. *Int J Radiat Oncol Biol Phys* **12**: 1203–1206, 1986.
15. Silver ARJ and O'Neill P, Interaction of the aziridine moiety of RSU-1069 with nucleotides and inorganic phosphate. *Biochem Pharmacol* **35**: 1107–1112, 1986.
16. O'Neill P, McNeil SS and Jenkins TC, Induction of DNA crosslinks *in vivo* upon reduction of the nitroimidazole-aziridines RSU-1069 and RSU-1131. *Biochem Pharmacol* **36**: 1787–1792, 1987.
17. Austen KRJ, Jenner TJ, O'Neill P and Fielden EM, Variation of the radiosensitizing efficiency of RSU-1069 with pre-irradiation contact times: a rapid mix study. *Int J Radiat Biol* **52**: 281–288, 1987.
18. Walling JM, Stratford IJ, Adams GE, Silver ARJ, Ahmed I, Jenkins TC and Fielden EM, Studies on the mechanisms of the radiosensitizing and cytotoxic properties of RSU-1069 and its analogues. *Int J Radiat Oncol Biol Phys* **12**: 1083–1086, 1986.
19. Stratford IJ and Adams GE, Effect of hyperthermia on differential cytotoxicity of a hypoxic cell radiosensitizer, Ro-07-0582, on mammalian cells *in vitro*. *Br J Cancer* **35**: 307–313, 1977.
20. Millar BC, Sapora O, Fielden EM and Loverock PS, The application of rapid-lysis techniques in radiobiology. IV. The effect of glycerol and DMSO on Chinese hamster cell survival and DNA single-strand break production. *Radiat Res* **86**: 506–514, 1987.
21. Blöcher D, DNA double strand breaks in Ehrlich ascites tumour cells at low doses of X-rays. I. Determination of induced breaks by centrifugation at reduced speed. *Int J Radiat Biol* **42**: 317–328, 1982.
22. Clarke RW and Lange CS, The sucrose gradient and native DNAs, an examination of measurement problems. *Biochim biophys Acta* **454**: 567–577, 1976.
23. Palcic B and Skarsgard LD, Cytotoxicity of misomidazole and DNA damage in hypoxic mammalian cells. *Br J Cancer* **37**: (Suppl. III), 54–59, 1978.
24. Taylor YC, Evans JW and Brown JM, Radiosensitization by hypoxic pretreatment with misonidazole: an interaction of damage at the DNA level. *Radiat Res* **109**: 364–373, 1987.
25. Silver ARJ, McNeil SS, O'Neill P, Jenkins TC and Ahmed I, Induction of DNA strand breaks by reduced nitroimidazoles. Implications for DNA base damage. *Biochem Pharmacol* **35**: 3923–3928, 1986.