Biochemical Pharmacology, Vol. 42, No. 9, pp. 1705–1710, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

THE REPAIR OF DNA DAMAGE INDUCED IN V79 MAMMALIAN CELLS BY THE NITROIMIDAZOLE-AZIRIDINE, RSU-1069

IMPLICATIONS FOR RADIOSENSITIZATION

TERRY J. JENNER, PETER O'NEILL,* PAUL W. CRUMP, E. MARTIN FIELDEN, ORAZIO SAPORA† and LAURA SANTODONATO†

MRC Radiobiology Unit, Chilton, Didcot OX11 0RD, U.K. and † Istituto Superiore di Sanita, Viale Regina Elena 299, Rome, Italy

(*Received* 15 *April* 1991; *accepted* 25 *June* 1991)

Abstract—The induction and repair of single (ssb) and double (dsb) strand breaks in DNA under aerobic or hypoxic conditions have been determined using sucrose sedimentation techniques following incubation of V79 mammalian cells with RSU-1069 or misonidazole, representative of a conventional 2-nitroimidazole radiosensitizer, for 1–1.5 hr at either 293 or 277°K and subsequent irradiation at 277°K. In all cases, the dose dependences for the induction of strand breaks are linear and consistent with an enhancement in the yield of DNA damage induced by the 2-nitroimidazoles under hypoxic conditions. With RSU-1069 at 293°K, the dose dependence of ssb is displaced reflecting DNA damage induced during pre-incubation. From these dependences, it is evident that the enhanced radiosensitization by RSU-1069 may not be accounted for in terms of accumulation of the agent at DNA. From the repair studies, DNA breaks induced by RSU-1069 in the absence of radiation have been shown to persist for at least 3 hr. With a combination of RSU-1069 and radiation under hypoxic conditions, the repair timescale of the induced breaks is significantly longer and an increase in the residual yields of both ssb and dsb (at 2–3 hr) was observed when compared with the observation in the presence of misonidazole or oxygen. From these studies, it is inferred that the enhanced radiosensitization of RSU-1069 at 293°K is a consequence of the formation of non-repairable DNA damage together with a modification of the repairability of the radiation-induced DNA breaks.

The compound RSU-1069 (1-(2-nitro-1-imidazolyl)-3-(1-aziridinyl)-2-propanol) is considerably more cytotoxic towards hypoxic cells relative to aerobic cells *in vitro* [1–3] and is a more efficient hypoxic cell radiosensitizer than misonidazole [4, 5]. Chemopotentiation [5, 6] and potentiation of tumour cytotoxicity and radiosensitization *in vivo* [3, 4, 7–9] have been demonstrated to be more effective with RSU-1069 than with misonidazole.

Cellular and molecular studies [1, 2, 10–15] have shown that RSU-1069 acts as a monofunctional agent under aerobic conditions whereas upon reduction it becomes bifunctional in character. Studies with plasmid DNA [10–14] have shown that the major types of DNA damage are single strand breaks (ssb), base adducts and, under reducing conditions, crosslinks. Recently, it has been shown that DNA damage induced in mammalian cells by RSU-1069 is substantially increased under hypoxia with the formation of double strand breaks (dsb), an indication of the compound's bifunctional character [16]. The induction of dsb and crosslinks probably plays a major role in determining the ability of RSU-1069 to act as a hypoxia-selective cytotoxin [17]. Further, RSU-1069 induces cellular DNA adducts which are converted into ssb under conditions of high alkalinity [18].

The present study was undertaken to investigate the repair (rejoining) of DNA strand breaks induced within mammalian cells following incubation with RSU-1069 under aerobic and hypoxic conditions. The DNA strand breaks were determined using either neutral sucrose sedimentation techniques or neutral filter elution where the lysis conditions are less harsh. Further, since DNA damage produced by RSU-1069 during pre-irradiation contact has been suggested to be responsible for its enhanced radiosensitizing efficiency [16, 19], DNA damage and its repair have been assessed subsequent to irradiation following a pre-irradiation contact with RSU-1069 or misonidazole of 1–1.5 hr. Misonidazole was chosen for comparison, as a representative example of a mono-functional 2-nitroimidazole with a one-electron reduction potential similar to that of RSU-1069.

Indeed previous studies have shown that radiation-induced DNA damage in the presence of misonidazole under hypoxic conditions is not readily repaired [20, 21]. From these studies, it is our aim to shed more light upon the potential involvement of "sub-toxic damage" formed during pre-irradiation contact with RSU-1069 in contributing to its enhanced radiosensitizing efficiency compared with that of misonidazole.

METHODS AND MATERIALS

Chemicals. The synthesis, purification and physico-chemical properties of RSU-1069 have been

* To whom correspondence should be addressed.

reported [4, 10]. Misonidazole was supplied by Dr C. E. Smithen (Roche Products Ltd). Sarkosyl and proteinase K were obtained from Sigma. All other chemicals were of analytical reagent grade and used as supplied. In order to minimize degradation of the 2-nitroimidazoles in aqueous medium, the solutions were freshly prepared as required.

DNA strand breaks. Chinese hamster cells, line V79-379A, were used throughout this work and grown in monolayer by methods described previously [22]. The procedures for labelling the cells and the technique of sucrose sedimentation for determination of ssb and dsb have been described previously [16, 23, 24]. The resultant activity profiles of the gradients after centrifugation were analysed as described previously [16], based upon the assumption that DNA undergoes scission at random on the DNA strand. A typical experimental dsb sedimentation profile is shown in Fig 1. For calculations of the number of dsb/genome it has been assumed that the molecular weight of untreated DNA is 3×10^{10} Daltons [16]. The data are generally presented as the reciprocal of the weight-averaged molecular weight (1/Mw) as a function of radiation dose. The experimental points in Figs 2–7 represent the average obtained from at least three replicate experiments.

For experiments to determine repair of ssb or dsb induced by RSU-1069, the cell suspension (10^6 cell/mL) was equilibrated with $N_2 + 5\%$ CO_2 or maintained aerobically (air + 5% CO_2) for 15 min prior to addition of the agent. Following incubation for 2 hr at 310°K under the relevant gassing conditions, the agent was removed followed by resuspension and incubation of the cells at 310°K under aerobic conditions for a further 0–180 min to allow time for repair. Aliquots of cell suspension were subsequently placed on ice to minimize further repair, centrifuged at 2000 rpm for 10 min followed by resuspension of the pellet in ice-cold 0.2 cm^3 of 0.01 mol/dm^3 Tris–1 mmol/dm^3 EDTA–0.15 mol/dm^3 NaCl at pH 7.5. The yields of ssb and dsb were subsequently determined using sucrose sedimentation technique.

Irradiations. Following pre-incubation of cellular suspension in the absence or presence of the 2-nitroimidazoles in $N_2 + 5\%$ CO_2 at 293°K for 1 hr, the suspension was placed on ice to minimize further damage and/or repair. The suspension was X-irradiated under either hypoxic or aerobic conditions at a dose rate of 3.8 Gy/min to deliver total doses of 30–250 Gy. Similar experiments were performed but with the pre-incubation being carried out at 277°K in order to minimize the chemical activity of RSU-1069. Following irradiation, the drug was removed and the initial yields of ssb and dsb were determined as described above. Under these conditions, but in the absence of radiation, the plating efficiency at the highest concentration of agent used is reduced by ~20%. The time course of repair of the chemical/radiation-induced ssb and dsb were determined under aerobic conditions at 310°K, a temperature required to optimize repair, using the procedure

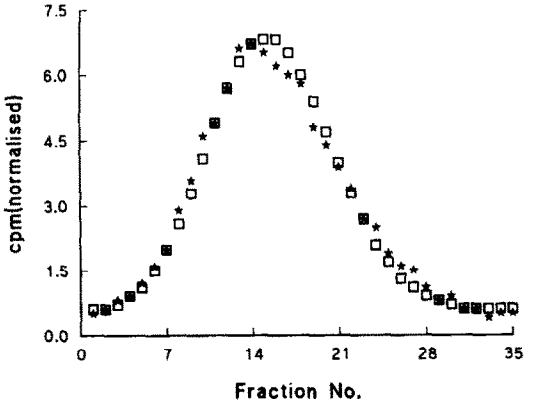

Fig. 1. A typical dsb sedimentation profile obtained following irradiation of V79 cells under aerobic conditions with a dose of 120 Gy and fitted to a Poisson distribution; (★) experimental data, (□) calculated values.

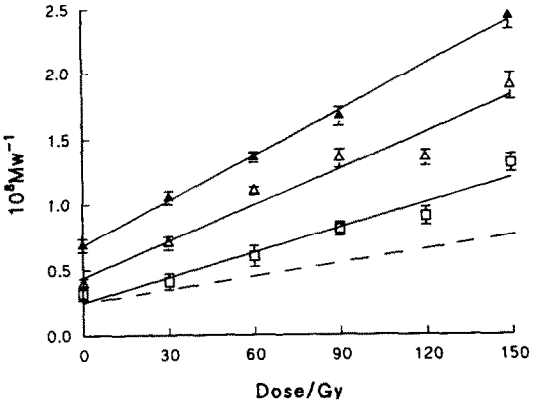

Fig. 2. The dependence of the initial yield of ssb on radiation dose (hypoxic conditions) following incubation of V79 mammalian cells in the presence of: 0.5 mmol/dm^3 RSU-1069 (▲) or misonidazole (□) for 1 hr at 293°K under hypoxia; (△) 0.5 mmol/dm^3 RSU-1069 for 1 hr under hypoxia at 277°K. Dashed line represents the hypoxic control in the absence of the agents. The solid lines are the best fits to the data based upon unweighted least squares analysis.

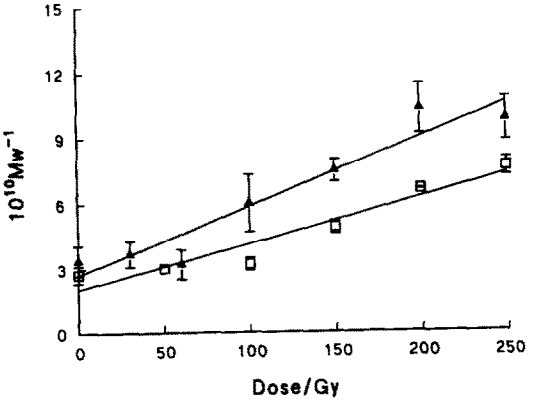

Fig. 3. The dependence of the initial yield of dsb on radiation dose (hypoxic irradiation) for a pre-irradiation contact period of 1 hr under hypoxia with either 0.5 mmol/dm^3 RSU-1069 (▲) or misonidazole (□) at 293°K. The solid lines are the best fits to the data based upon unweighted least squares analysis.

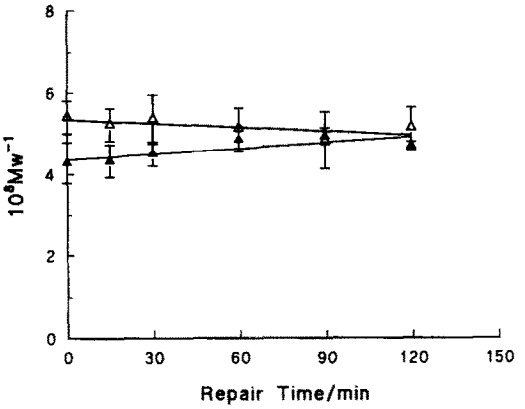

Fig. 4. The kinetics of rejoining of ssb induced following incubation of V79 cells for 2 hr at 310°K with RSU-1069 under aerobic (1 mmol/dm^3, △) and hypoxic (0.5 mmol/dm^3, ▲) conditions.

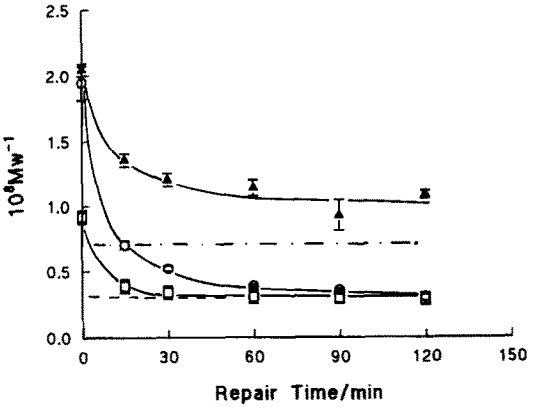

Fig. 6. The kinetics of rejoining of radiation induced ssb (following incubation of V79 cells for 1.5 hr at 293°K under hypoxic conditions) in the presence of 0.5 mmol/dm^3 RSU-1069 (▲) or 2 mmol/dm^3 misonidazole (□) and irradiation with 120 Gy under hypoxic conditions. (○) Rejoining of ssb induced under aerobic conditions following aerobic X-irradiation with a dose of 125 Gy. The line (—·—) represents the level of damage induced by RSU-1069 in the absence of radiation. The line (— —) represents the control level in the absence of radiation or agent.

described above for chemically-induced DNA damage.

RESULTS

Initial yields of ssb and dsb induced by RSU-1069 and radiation

ssb. The initial yield of DNA ssb determined by sucrose sedimentation increases linearly with radiation dose as shown in Fig. 2, following incubation of V79 mammalian cells in the presence of 0.5 mmol/dm^3 RSU-1069 or misonidazole for 1 hr at 293°K under hypoxic conditions and subsequent irradiation at 277°K under hypoxia. The concentration of the 2-nitroimidazoles chosen was based upon the maximum concentration previously [2] used for RSU-1069 yielding a sensitizer enhancement ratio of ~2.8 for survival of V79 mammalian cells under hypoxia. At greater concentrations, the cytotoxicity of RSU-1069 for 1 hr incubation under hypoxia results in significant cell lethality.

With misonidazole, the dose dependence extrapolates to the control value for zero radiation dose in contrast to the displacement observed with RSU-1069. This displacement is equivalent to the yield of ssb induced during the 1 hr pre-irradiation contact period. In Fig. 2, the dependence of the yield of ssb on radiation dose is also shown for a pre-irradiation contact period of 1 hr with RSU-1069 at 277°K, a procedure which minimizes the chemical activity of RSU-1069. This dependence, essentially parallel to that determined at 293°K, extrapolates, within experimental error, to the value observed with misonidazole (Fig. 2) and is, therefore, indicative of little or no DNA damage induction during the pre-

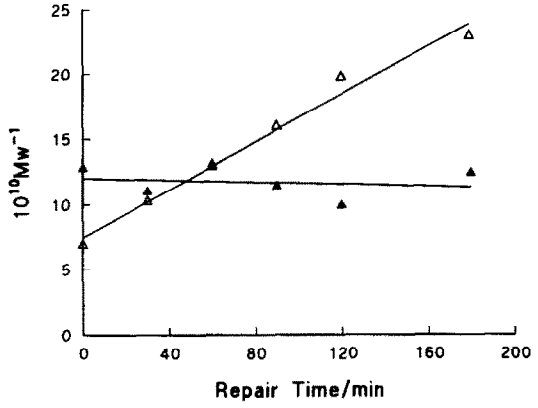

Fig. 5. The kinetics of rejoining of dsb induced following incubation of V79 cells for 2 hr at 310°K with RSU-1069 under aerobic (2 mmol/dm^3, △) and hypoxic (0.4 mmol/dm^3, ▲) conditions.

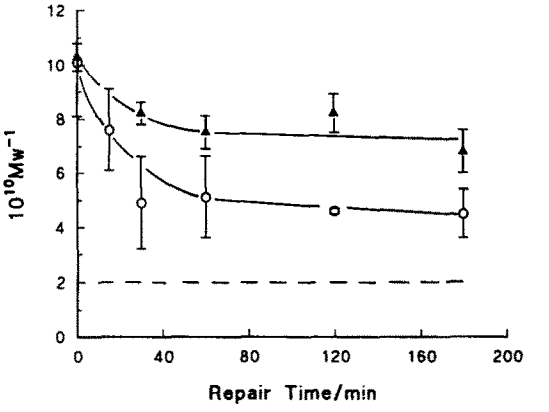

Fig. 7. The kinetics of rejoining of radiation induced dsb following incubation of V79 cells for 1 hr at 293°K under hypoxic conditions in the presence of 0.5 mmol/dm^3 RSU-1069 (▲) and irradiation with 200 Gy under hypoxic conditions. (○) represents rejoining of dsb induced following X-irradiation with a dose of 125 Gy under aerobic conditions. The dashed line represents the control level in the absence of radiation or agent. The radiation doses were chosen so that the initial yields of dsb are equivalent.

irradiation period. Using a higher concentration of 1 mmol/dm^3 RSU-1069 at 277°K does not result in the induction of measurable yields of ssb during pre-incubation. These observations, are consistent with RSU-1069 acting as a hypoxic cell radiosensitizer under conditions where its bioreductive and alkylating actions are minimized. The enhancement in the yield of ssb under hypoxic conditions is greater with RSU-1069 (at 277°K) than with misonidazole, consistent with the greater enhancement of hypoxic cell killing seen with RSU-1069 under equi-concentration conditions [3, 25].

dsb. The linear dependence of the yield of dsb on radiation dose, as determined using the sucrose sedimentation technique, is shown in Fig. 3 for a pre-irradiation contact period of 1 hr with 0.5 mmol/dm^3 RSU-1069 or misonidazole under hypoxia at 293°K followed by irradiation at 277°K under hypoxia. The enhancement of the yield of dsb under hypoxic conditions (Fig. 3) is greater with RSU-1069 than with misonidazole and is compatible with the enhancements observed for ssb. Further, this dependence on radiation dose is not significantly affected for pre-irradiation contact with either RSU-1069 or misonidazole at 277°K. Incubation of V79 cells with 0.5 mmol/dm^3 RSU-1069 under hypoxia for 1 hr at 293°K does not result in significant numbers of dsb, produced during this pre-irradiation period, above that observed for the control. As reported previously [16], incubation with RSU-1069 under these conditions but at 310°K results in dsb.

Repair of strand breaks chemically induced by RSU-1069

Since DNA ssb and dsb are induced in V79 cells upon incubation with RSU-1069 under both aerobic and hypoxic conditions [16], the kinetics and extent of rejoining of such lesions have been determined using sucrose sedimentation techniques following incubation of V79 cells with 0.4–2 mmol/dm^3 RSU-1069 at 310°K for 2 hr under both gassing conditions. As shown in Figs 4 and 5, insignificant rejoining of both DNA ssb and dsb induced by RSU-1069 occurs within 120 and 180 min, respectively, the period for which repair was assessed. Under aerobic conditions, the yield of dsb induced by RSU-1069 in fact increases with time, from which it is tentatively suggested that enzymatic processes may, in part, be responsible for these increases.

Repair of strand breaks induced by RSU-1069 and radiation

Since DNA strand breaks induced by RSU-1069 during pre-irradiation contact are not readily rejoined (see above) it is assumed that RSU-1069 induced DNA damage at 293°K, may still persist at and during the time of X-irradiation. Therefore, the kinetics and extent of rejoining of DNA ssb and dsb induced upon irradiation following pre-irradiation contact with RSU-1069 were determined using sucrose sedimentation techniques for repair periods up to 120 and 180 min at 310°K for ssb and dsb, respectively.

ssb. The repair kinetics of ssb following pre-incubation of V79 cells under hypoxia with 0.5 mmol/dm^3 RSU-1069 for 1.5 hr at 293°K and subsequent irradiation under hypoxia at 277°K with a dose of 120 Gy are shown in Fig. 6. Up to 120 min, ~60% of the ssb produced by the combination of RSU-1069 and radiation are rejoined, a value which is less than would be expected on the basis of anaerobic radiosensitization and assuming that the ssbs induced prior to irradiation by RSU-1069 are not rejoined. Taking into account the yield of pre-irradiation-induced breaks, the extent of ssb rejoining is ~75%. The kinetics for repair of these ssb have been interpreted in terms of two exponential components (value in parentheses represents proportion of repaired breaks) with half-lives of <6 min, (50%) and ~80 min (~50%). These findings contrast with those under aerobic conditions whereby >95% of the ssb are rejoined with half-lives of ≤6 min (62%) and ~21 min (38%) treating the repair kinetics in terms of two exponential components (Fig. 6). As shown in Fig. 6, using misonidazole, a conventional 2-nitroimidazole radiosensitizer, the majority of the ssb induced under hypoxic conditions are rejoined by a first-order process with a half-life of ~7 min (~85%). The residual yield of unrejoined ssb at 120 min is <5% of the initial yield, similar to aerobic conditions.

Under conditions whereby induction of ssb by chemical action of RSU-1069 is minimized (incubation at 277°K, data not shown), the yield of ssb rejoined is ~75% ($T_{\frac{1}{2}}$ ~ 7 min) following incubation of V79 cells with 0.5 mmol/dm^3 RSU-1069 under hypoxic conditions for 1.5 hr followed by irradiation at 277°K with a dose of 120 Gy under hypoxia. Indeed the initial yield of ssb under these conditions did not reflect the presence of any agent-induced lesions. These residual breaks are not as a result of residual RSU-1069 since varying the number of wash-outs does not influence the findings.

dsb. The repair kinetics of dsb induced following incubation of V79 cells with 0.5 mmol/dm^3 RSU-1069 for 1 hr under hypoxic conditions at 293°K and subsequently irradiated at 277°K with a dose of 200 Gy under hypoxic conditions are shown in Fig. 7. A residual yield of 59% of the initial yield is observed at 180 min. In order to compare the extent of repair of dsb induced by irradiation, a radiation dose of 125 Gy under aerobic conditions was required to produce an equivalent yield of initial dsb as observed with the conditions used for RSU-1069. The repair time for radiation-induced dsb in the presence of RSU-1069 are similar to the radiation only situation under aerobic conditions. In contrast to the residual yield of dsb with RSU-1069 after 180 min, only ~35% of the yield of dsb remain under aerobic conditions.

DISCUSSION

Dsb induced by RSU-1069 prior to irradiation at 310°K were observed using the technique of sucrose sedimentation. Parallel studies using the technique of neutral elution [26] to determine dsb induced by RSU-1069 were without success since chemically-induced dsb were not detected. Differences between the techniques for the determination of cellular ssb induced by RSU-1069 have already been highlighted [18] and emphasized that the majority of ssb pro-

duced in cellular DNA by RSU-1069 [18] result from alkali- or heat-labile sites [27]. It is inferred that RSU-1069 adducts are only converted into breaks during the cell lysis and subsequent treatment of the DNA using the harsher conditions associated with sucrose sedimentation. With the latter technique, a 30-min lysis is performed at 343°K compared to the lower temperature conditions used in the elution technique. Differences in the ability of alkylating agents to induce dsb and crosslinks have been noted when comparing DNA precipitation and unwinding assays [28], whereby the former technique also involves a heat treatment. The following discussion therefore concentrates on the results obtained using the sucrose sedimentation technique.

It has previously been shown that RSU-1069 induces DNA dsb and ssb in V79 mammalian cells after incubation at 310°K under both hypoxic and aerobic conditions [16] in support of the mechanistic implications drawn from molecular studies [10–18, 27]. Further the concentrations of RSU-1069 used to induce DNA damage correspond to those used in the cytotoxicity studies [10]. From the previous study [16] it was shown that the rate of production of DNA strand breaks exceeds that of their potential rejoining by the various cellular repair processes resulting in the accumulation of DNA damage. From the time courses for rejoining of DNA strand breaks induced by RSU-1069 at 310°K under either aerobic or hypoxic conditions (Figs 4 and 5) it is demonstrated that the DNA damage is persistent for at least 2 and 3 hr for ssb and dsb, respectively. It is not as yet known whether this DNA damage represents permanent non-repairable damage. Further, the inability of the cells to repair the observed DNA damage is independent of whether the incubation with the RSU-1069 is carried out under oxic or hypoxic conditions. From this apparent lack of repair, it is inferred that both monofunctional and bifunctional action of RSU-1069 results in persistent DNA damage. Such damage could result from inefficient removal of RSU-1069 from the cells, however the results of Hill *et al.* [3] and Walling *et al.* [25] and the invariance with number of washouts argue for effective removal of RSU-1069. Previous studies with misonidazole under hypoxic conditions have also indicated the lack of effective rejoining of chemically-induced DNA damage [20, 21].

Based upon this stability of DNA damage induced by RSU-1069 (Figs 4 and 5), it is implied that DNA damage accumulates over the time period generally reported for *in vitro* cytotoxicity studies [10]. Further, from the *in vitro* hypoxic radiosensitization witnessed with RSU-1069 at 293°K, it is apparent that any DNA damage induced by RSU-1069 during pre-incubation contact under hypoxia will persist at the time of irradiation. Indeed, previous studies have shown that RSU-1069 is only slightly more effective than misonidazole as a hypoxic cell radiosensitizer (SER = 1.9 compared with 1.55 at 0.5 mmol/dm^3 for misonidazole) under conditions whereby the reactivity of RSU-1069 is minimized e.g. lower temperature [3, 25] or short pre-irradiation contact periods [18]. The observed linear dependence of the yield of ssb and dsb on radiation dose for pre-irradiation contact of RSU-1069 or misonidazole at 277°K under hypoxia reflect the slight differences observed for their radiosensitizing efficiencies for cell killing [25]. These dependences are consistent with RSU-1069 acting predominantly as an oxygen-like "electron-affinic" radiosensitizer at 277°K. With RSU-1069 (0.5 mmol/dm^3), the SER increases from 1.9 up to >3.1 on increasing the temperature of the pre-irradiation contact from 277°K up to 293°K.

The question arises as to whether DNA damage induced at 293°K during pre-irradiation incubation under hypoxia is important for the enhanced radiosensitization observed with RSU-1069. Indeed, pre-irradiation incubation of cells with RSU-1069 at 293°K under hypoxia results in the formation of ssb (Fig. 2) in contrast to the negligible yield of ssb induced for pre-incubation at 277°K. The subsequent dependence of the yield of ssb on radiation dose for pre-incubation of RSU-1069 under hypoxia at 293°K is parallel to that determined for pre-incubation at 277°K. It is apparent that the yield of ssb produced by a combination of radiation and RSU-1069 is additive. For misonidazole, a conventional radiosensitizer, the determined dependences of ssb are independent of temperature consistent with the small temperature coefficient for its radiosensitization [3, 25].

Based upon the yields of residual ssbs it is apparent that a synergistic effect occurs upon the repair of breaks produced in the treatment with RSU-1069 and X-irradiation. This synergism contrasts with the apparent additivity of the initial yield of ssb breaks. The repair of ssb appears to be biphasic as previously shown for the repair of radiation-induced breaks alone [29–31]. Following the initial rapid rejoining of ssb witnessed in all cases over the initial 15 min (Fig. 6), the subsequent repair processes are significantly slower for those ssb induced by RSU-1069 in conjunction with radiation compared to the radiation only condition. Similarly, differences in the extent of repair of dsb damage were observed when comparing radiation only and radiation in combination with RSU-1069. Such gross changes in the extent of repair and their kinetics are not observed using misonidazole, a conventional electron affinic radiosensitizer. Although, at reduced temperatures, RSU-1069 still produces considerably more non-repairable breaks compared with misonidazole.

It is inferred from these studies that the enhanced radiosensitization of RSU-1069 at 293°K is a consequence of the formation of persistent DNA damage formed during pre-irradiation incubation. Further, the extent of repair of radiation-induced DNA damage is also modified in the presence of chemically-induced damage by RSU-1069.

Acknowledgements—We thank P. Webb for the synthesis of RSU-1069 and Professor G. E. Adams for helpful discussions.

REFERENCES

1. 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.
2. Stratford IJ, Walling JM and Silver ARJ, The dif-

ferential cytotoxicity of RSU-1069: cell survival studies indicating interaction with DNA as a possible mode of action. *Br J Cancer* **53**: 339–344, 1986.
3. 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.
4. Hill RP, Gulyas S and Whitmore GF, Toxicity of RSU-1069 for KHT cells treated *in vivo* or *in vitro*: evidence for a diffusible toxic product. *Int J Radiat Oncol Biol Phys* **16**: 1111–1114, 1989.
5. 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.
6. 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.
7. Chaplin DJ, Durand RE, Stratford IJ and Jenkins TC, The radiosensitivity and toxic effects of RSU-1069 on hypoxic cells in a murine tumour. *Int J Radiat Oncol Biol Phys* **12**: 1091–1095, 1986.
8. Chaplin DJ, Potentiation of RSU-1069 tumour cytotoxicity by 5-hydroxytryptamine (5-HT). *Br J Cancer* **54**: 727–732, 1986.
9. Stratford IJ, Adams GE, Godden J and Howells N, Induction of tumour hypoxia post-irradiation: a method for increasing the sensitizing efficiency of misonidazole and RSU-1069 *in vivo*. *Int J Radiat Biol* **55**: 411–422, 1989.
10. 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.
11. 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.
12. 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 Biol* **12**: 1203–1206, 1986.
13. 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.
14. O'Neill P, McNeil SS and Jenkins TC, Induction of DNA crosslinks *in vitro* upon reduction of the nitroimidazole-aziridines RSU-1069 and RSU-1131. *Biochem Pharmacol* **36**: 1787–1792, 1987.
15. O'Neill P and Cunniffe SMT, Assessment of the repair and damage of DNA induced by parent and reduced RSU-1069, a 2-nitroimidazole-aziridine. *Int J Radiat Oncol Biol Phys* **16**: 963–966, 1989.
16. Jenner TJ, Sapora O, O'Neill P and Fielden EM, Enhancement of DNA damage in mammalian cells upon bioreduction of the nitroimidazole-aziridines RSU-1069 and RSU-1131. *Biochem Pharmacol* **37**: 3837–3842, 1988.
17. O'Neill P, Jenner TJ, Sapora O, Crump PW, Cunniffe SMT and Fielden EM, The role of DNA damage in the bioreductive action of 2-nitroimidazoles. In: *Selective Activation of Drugs by Redox Processes* (Eds. Adams GE, Breccia A, Fielden EM and Wardman P), Vol. 198, pp. 53–61. Plenum Press, New York, 1990.
18. Crump PW, Fielden EM, Jenner TJ and O'Neill P, A comparison of the techniques of alkaline filter elution and alkaline sucrose sedimentation used to assess DNA damage induced by 2-nitroimidazoles. *Biochem Pharmacol* **40**: 621–627, 1990.
19. 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.
20. Palcic B and Skarsgard LD, Cytotoxicity of misonidazole and DNA damage in hypoxic mammalian cells. *Br J Cancer* **37** (Suppl III): 54–59, 1978.
21. 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.
22. Stratford IJ and Adams GE, Effects 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.
23. 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, 1981.
24. 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.
25. 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.
26. Bradley MO and Kohn KW, X-Ray induced DNA double strand break production and repair in mammalian cells as measured by neutral filter elution. *Nucl Acid Res* **7**: 793–804, 1979.
27. Silver ARJ, McNeil SS, O'Neill P, Jenkins TC and Ahmed I, Induction of DNA strand breaks by reduced nitroimidazoles. *Biochem Pharmacol* **35**: 3923–3928, 1986
28. Olive PL, Chan APS and Cu CS, Comparison between DNA precipitation and alkali unwinding assays for detecting DNA strand breaks and cross-links. *Cancer Res* **48**: 6444–6449, 1988.
29. Dikomey E and Franzke J, DNA repair kinetics after exposure to X-irradiation and to internal β-rays in CHO cells. *Radiat Environ Biophys* **25**: 189–194, 1986.
30. Dikomey E and Franzke J, Three classes of DNA strand breaks induced by X-irradiation and internal β-rays. *Int J Radiat Biol* **50**: 893–408, 1986.
31. Frankenberg-Schwager M, Review of repair kinetics for DNA damage induced in eukaryotic cells *in vitro* by ionizing radiation. *Radiother Oncol* **14**: 307–320, 1989.