Anti-Cancer Drugs, 3, pp. 499–505

Influence of suramin on some DNA-directed enzymes and primary cultures of chicken embryo and rat cells

Anita Ignatius and Karl-Heinz Tempel[CA]
The authors are at the Institute of Pharmacology, Toxicology and Pharmacy, Faculty of Veterinary Medicine, University of Munich, Königinstrasse 16, D-8000 Munich 22, Germany. Tel: 089-21802667. Fax: 089-342316.

The influence of suramin on O^6-alkylguanine-DNA alkyltransferase (AT), DNase I and poly(adenosine diphosphate ribose)polymerase (PADPR) as well as on primary cultures of rat and chick embryo cells was examined by using some short-term tests. AT and DNase I were inhibited by suramin in a dose-dependent manner (DE_{50} = 65 and 100 μg/ml, respectively). PADPR activity was increased over a concentration range of 40–320 μg/ml. At concentrations above 40 μg/ml suramin decreased scheduled and unscheduled DNA synthesis. At doses of below 20 μg/ml the substance slightly stimulated unscheduled DNA synthesis in embryonic cells. Suramin enhanced nucleoid sedimentation and diminished the viscosity of alkaline cell lysates. From the present results it is concluded that suramin, at clinically relevant concentrations, is able to interact with enzyme systems which are critical to important nuclear functions and to interfere—in a cell specific manner—with histones and/or matrix proteins, resulting in greater chromatin compactness.

Key words: Chick embryo cells, DNA-directed enzymes, DNA synthesis, nucleoid sedimentation, rat thymocytes, suramin.

Introduction

Suramin, a polysulfonated naphthylurea, is used in the treatment of African trypanosomiasis and onchocerciasis.[1] The substance was found to inhibit reverse transcriptase of RNA tumor viruses[2] and, therefore, has been tested in the therapy of acquired immunodeficiency syndrome (AIDS).[3] Because of important toxic reactions the drug was abandoned in the treatment of AIDS;[4] however, it has recently been reported to exhibit antitumor activity in some systems of advanced cancer.[5–7]

Suramin exerts a variety of biological effects. For example, it binds several growth factors *in vitro*[8] and inhibits many different enzyme proteins such as protein kinase C, various ATPases, lysosomal enzymes, and enzymes concerned with DNA and RNA metabolism.[5,9–11] However, it is difficult to evaluate which, if any, of these targets is involved in the antitumor activity. One important mechanism could be the nucleotoxic activity of the drug by interfering with DNA-related enzymes such as DNA polymerases[9] or with chromatin structure.

Our principal approach has been, therefore, to obtain more detailed information about the possible nucleotoxicity of suramin by biochemical and physico-chemical methods. In a first series of experiments three DNA-directed enzymes, i.e. O^6-alkylguanine-DNA alkyltransferase (AT), DNase I and poly(adenosine diphosphate-ribose)-polymerase (PADPR), were examined. In a second series of experiments, the influence of suramin on scheduled DNA synthesis, unscheduled DNA synthesis and RNA synthesis was investigated. Additional investigations focussed on possible suramin–chromatin interactions studied by nucleoid sedimentation and viscometry of alkaline cell lysates (ALV). Finally, the effect of suramin on DNA repair induced by UV light or X-irradiation was examined. The experiments were performed in liver and brain cells of the chicken embryo and in thymic cells of the rat in order to compare different cell types and various developmental stages.

Materials and methods

Chemicals

[5,6-^{3}H]Uridine ([^{3}H]U, specific activity 1.48 TBq/mmol) and nicotinamide-[U-^{14}C]adenine dinucleotide ([^{14}C]NAD, ammonium salt, specific activity

[CA] Corresponding Author

11.0 GBq/mmol) were purchased from Amersham Buchler (Braunschweig, Germany). [^{3}H]methylthymidine ([^{3}H]dT, specific activity 1.85–2.96 TBq/mmol) was from New England Nuclear (Boston, MA, USA). Suramin was a generous gift of the Bayer AG (Leverkusen, Germany). DNase I from bovine pancreas was obtained from Sigma (no. D-0876, 550 Kunitz units/mg protein, EC 3.1.21.1., Munich, Germany). The other substances were bought from Merck (Darmstadt, Germany), Serva (Heidelberg, Germany) and Sigma.

Animals and cell preparation

We used fertile eggs from White Leghorn chickens and female Wistar rats (180–200 g body weight). The embryos were removed from the eggs and sacrificed by decapitation at the stage of 15 days after beginning of incubation. Liver cells were prepared as described previously,[12] tissue solution being performed by collagenase (0.1% in Hank's solution, Ca^{2+} and Mg^{2+} free). Brain cell suspensions were obtained by the same procedure without collagenase. The cell suspensions were adjusted to final concentrations of 1–12.5 × 10^6 cells/ml, providing comparable RNA or DNA contents. Cell viability was determined by trypan blue dye exclusion. Thymic cells of the rat were prepared as described previously.[13]

Treatment of the cells

Suramin stock solutions were freshly prepared in Hank's medium at 11 times the concentrations needed in the cell suspensions. In general, 0.1 ml of the stock solution was added to 1 ml of the cell suspensions. The addition of the test substance was followed by a 30 min incubation of the cells at 37°C prior to analytical procedures. When examining the effects of suramin on DNase I or AT, the stock solutions were first mixed with the substrate. Following a 30 min preincubation period at 37°C, DNase I solution or protein extract (AT) was added to the DNase I and AT assays, respectively.

X-irradiation. For X-irradiation *in vitro*, the Kristalloflex 710H X-ray machine (Siemens, Erlangen, Germany) was applied (55 kV, 39.9 mA, half value layer 0.16 mm Cu, filtration with 0.16 mm Cu) at a dose rate of 1 Gy/min (calibrated with a Siemens dosimeter in the center of the useful X-ray beam). During the exposure the cell suspensions remained within micro test tubes (Eppendorf 3812) placed on ice at the onset of irradiation. The control preparations were sham-irradiated.

UV irradiation. For UV irradiation, the cell suspensions were spread over ice-cooled Petri dishes (0.045 ml/cm^2) and exposed or sham-exposed with a germicidal tube (Phillips TUW 30W, delivering its most energy at 254 nm at an incident fluence of 4 J/m^2/s).

Analytical procedures

AT activity was determined in tissue extracts of the chick embryo liver as described previously.[14] Briefly, the method was based on the transfer of ^{3}H-labelled methyl groups from the O^6-position of guanine in the substrate DNA to the acceptor protein of the tissue extract. Enzyme activity was represented by the amount of radioactivity in the unsoluble protein which was determined by liquid scintillation counting.

The criterion for *DNase I* activity was the hyperchromic effect of the perchloric acid (6%)-soluble fraction of the assay.[14] Calf thymus DNA (Na salt, M_r = 8 900 000, minimum 95% DNA, Aldrich) was used as the substrate.

PADPR activity was assessed in permeabilized cells by the incorporation into the acid-insoluble precipitates of the ADP-ribose portion of [^{14}C]NAD.[15]

Scheduled DNA synthesis and *RNA synthesis* were measured *in vitro* by the incorporation of [^{3}H]dT and [^{3}H]U, respectively, into the perchloric acid precipitate (6%) of the cells.[12,16] In order to determine *unscheduled DNA synthesis,* scheduled DNA synthesis was suppressed by hydroxyurea (10^{-1} M) which was added to the Hank's medium during the cell preparation and the following incubations.

The DNA and RNA content of the assays was quantified from UV absorbance (wavelength 260 nm) using calf thymus DNA and yeast RNA, respectively, as standards. Radioactivity was determined by liquid scintillation counting in Ready solv MP® (Beckman).

For *viscometry of alkaline cell lysates*, the device of Marshall and Ralph[17] was slightly modified.[18] To 3 ml of lysis medium, 0.3 ml of the cell suspensions (about 10^7 cells) was added.

The *nucleoid sedimentation* technique was performed as described for thymic and splenic cells of the rat.[19]

Results

Biochemical investigations

The first series of experiments was aimed at testing the influence of suramin on the activities of some DNA-directed enzymes, and on scheduled and unscheduled DNA synthesis and RNA synthesis. The results are presented in Table 1 and Figure 1. Suramin inhibited AT as well as the DNase I reaction in a dose-dependent fashion, the extrapolated DE_{50} values (concentration depleting activity by 50%) being about 65 and 100 μg/ml, respectively. For determination of PADPR activity we used thymic cells because of their high potential of PADPR-induction following DNA damage as compared with chick embryo cells.[20] Suramin increased PADPR activity over a concentration range of 40–320 μg/ml. At higher doses, the enzyme activity returned to the control level (Table 1).

As presented in Figure 1, scheduled DNA synthesis was inhibited by suramin in the embryonic cells, liver cells being more sensitive than brain cells. Thymic cells behaved like brain cells (results not shown in Figure 1 because the plots of thymic and brain cells were almost identical). The DE_{50} values for liver cells were 80 μg/ml, and for brain and thymic cells about 320 μg/ml. Unscheduled DNA synthesis was also depleted by suramin in a dose-dependent manner, 50% inhibition being

Table 1. AT, DNase I and PADPR activity following suramin treatment expressed as percent of untreated controls (data points represent the mean of two to three experiments which were done in triplicates ±SD)

Suramin (μg/ml)	DNA related enzymes		
	AT	DNase I	PADPR
10	105 ± 5	100 ± 5	ND
20	101 ± 7	97 ± 7	97 ± 13
40	85 ± 1	95 ± 4	110 ± 2
80	45 ± 8	66 ± 8	173 ± 20
160	19 ± 2	2 ± 1	187 ± 7
320	6 ± 2	ND	140 ± 40
640	2 ± 3	ND	90 ± 13

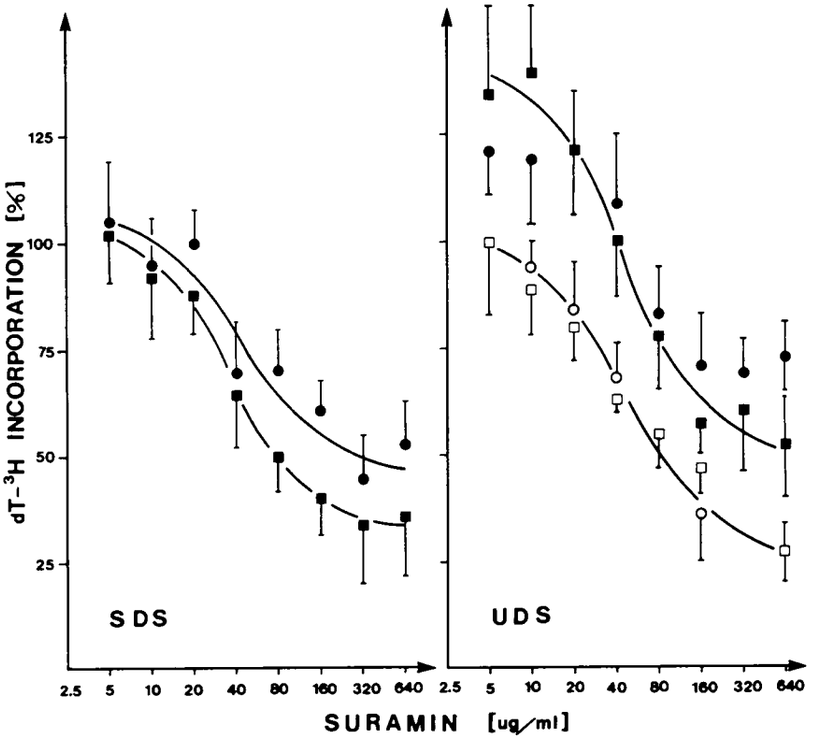

Figure 1. Influence of suramin on scheduled DNA synthesis (SDS) and unscheduled DNA synthesis (UDS) of brain (●) and liver (■) cells measured by [^{3}H]dT incorporation. (○) UV-stimulated UDS of brain cells, (□) UV-stimulated UDS of liver cells. The values represent the mean of three experiments which were done in triplicate (per cent of untreated controls ±SD).

obtained at about 300 μg/ml in brain, liver and thymic cells. A significant stimulation of unscheduled DNA synthesis was observed at low concentrations of suramin (below 20 μg/ml) in the embryonic cells (Figure 1) but not in thymic cells (results not shown). When unscheduled DNA synthesis was stimulated by UV irradiation, the cells responded to suramin by a significantly greater inhibition of [^{3}H]dT incorporation; no increase in unscheduled DNA synthesis could be observed at low concentrations (Figure 1). RNA synthesis was not influenced even at high concentrations of suramin (1000 μg/ml) (results not shown).

Physico-chemical investigations

In order to investigate possible chromatin interactions, a further series of experiments comprised nucleoid sedimentation and viscometry of alkaline cell lysates following suramin treatment. The results are presented by Figure 2. Suramin increased the sedimentation distance in brain, liver and thymic cells in a dose-dependent fashion with remarkable cell specific peculiarities. In liver cells about 200% of the sedimentation distance of untreated controls was reached at 240 μg/ml suramin, whereas thymic cells exhibited only 155% and brain cells 140% at the same concentration. In the presence of proteinase K (1.0 mg/ml lysis medium) the effect was abolished (Figure 2). When DNA damage was measured by the viscosity test, the viscometry of the alkaline lysates decreased in a strictly dose-dependent manner ($DE_{50} \sim 120$ μg/ml) (results not shown).

Additional investigations focused on the effects of suramin on X-ray- or UV-light-induced DNA repair reactions measured by nucleoid sedimentation. The investigations were performed in thymic cells because of their substantially higher post-irradiation DNA repair as compared with chicken embryo cells.[20] The repair process was studied by incubating UV- (64 J/m^2) or X- (8 Gy) irradiated cells at 37°C for 30 min after exposure in the presence of suramin. Control cells were kept at 4°C during the 30 min repair period. As presented in Figure 3, a 30 min repair period increased the sedimentation distance in X-irradiated cells to the same extent at each tested suramin concentration.

When the cells were exposed to UV light, a 30 min post-incubation period reduced sedimentation distance reflecting endonucleolytic DNA degradation during long patch repair.[21] As shown in Figure 3, suramin increased sedimentation distance of UV-irradiated cells both in controls (4°C) and post-incubated cells (37°C), the dose-

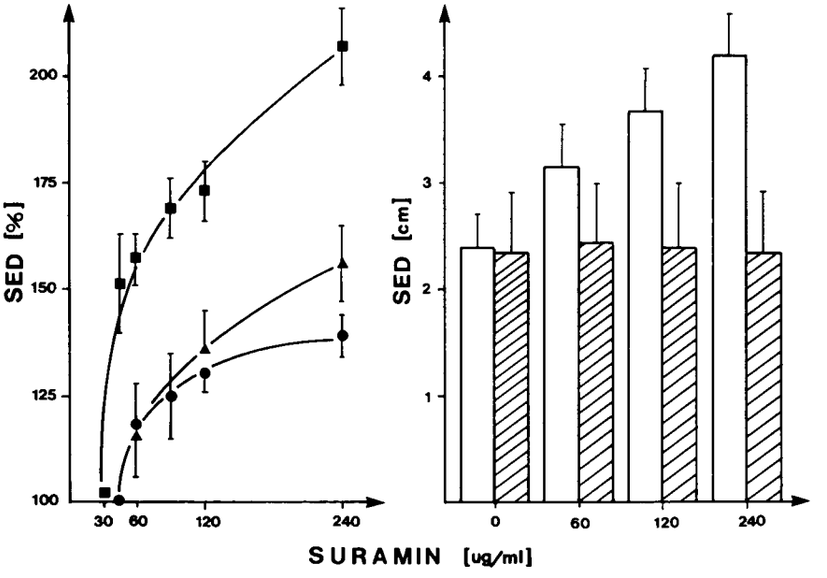

Figure 2. Influence of suramin on nucleoid sedimentation (SED). Left side: (●) brain cells, (■) liver cells and (▲) thymic cells. The values represent the mean of three experiments in per cent of untreated controls ±SD. Right side: sedimentation distance (cm) of liver cells. White columns: without proteinase K, shaded columns: with proteinase K.

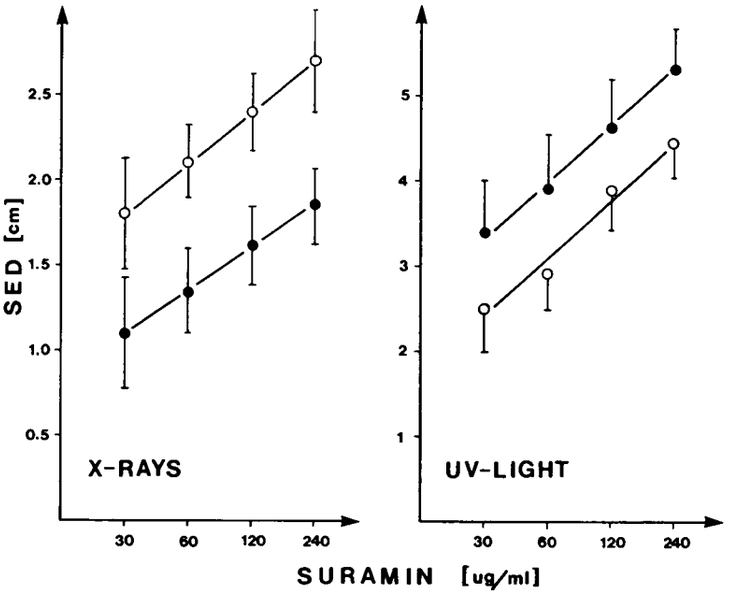

Figure 3. Influence of suramin on nucleoid sedimentation (SED) of thymic cells immediately following exposure to X-rays or UV light (●) and after a repair period of 30 min (○). The values represent the mean of two experiments (cm) ±SD.

response curve being almost identical. Therefore, repair processes measured by nucleoid sedimentation were not influenced by suramin.

Discussion

A variety of biological systems have been shown to be affected by suramin, the molecular basis of the antitumor activity being hitherto unknown.[5,6] Mainly, its interference with a wide range of growth factors is to be considered as an important mechanism of anticancer activity; however, interaction with other cell compartments might be equally important.

Suramin is taken up into the cell by endocytosis and penetrates to the nucleus.[11] Bojanowski *et al.*[11] found a 3 times higher concentration of radiolabeled suramin in the nucleus than in the cytoplasma. Therefore, and because of the high sensitivity of some DNA related enzymes *in vitro*, e.g. DNA and RNA polymerases[9,10] and DNA topoisomerase II,[11] the effects of suramin on nuclear functions should be studied more extensively.

Our aim was, at first, to investigate the influence of suramin on some nuclear enzymes, which, to our knowledge, have not been examined before. As presented in Table 1, the enzyme activities are influenced by suramin at concentrations easily achievable *in vivo*.[23]

O^6-alkylguanine is known to be mainly responsible for mutagenic and carcinogenic lesions of a number of alkylating agents.[24] The repair of O^6-alkylguanine is achieved by an alkyl acceptor protein, AT, which carries the alkyl group from the O^6-position of the guanine to a cysteine residue of the same AT protein.[25,26] Suramin inhibited AT reaction in a concentration-dependent manner, the DE_{50} being about 65 μg/ml. Under the experimental conditions used, effects of potentially suramin-modified DNA on AT cannot be distinguished from direct interactions of suramin with the AT protein, but this question might be considered as secondary to possible *in vivo* effects. With respect to clinical oncology, direct and/or indirect inhibition of AT activity by suramin may provide a basis to potentiate the antineoplastic activity of alkylating chemotherapeutic agents.[27,28] The reaction of DNase I, a DNA degrading enzyme,[29] was nearly abolished at 160 μg/ml suramin. The Michaelis–Menten plot revealed competitive inhibition (results not shown) suggesting polyanionic interactions.[5] In spite of the fact that the results can only be interpreted in terms of a model system, the possibility should be considered that other neutral endonucleases are also inhibited by suramin.

PADPR, which plays a fundamental role in a number of nuclear functions such as DNA repair, differentiation and gene expression, is tightly bound to chromatin and catalyzes the poly(ADP-ribosyl)-ation of structural chromosomal proteins and nuclear enzymes.[30] It was activated by suramin when measured by the incorporation of the ADP-ribose portion of [^{14}C]NAD. Although the enzyme activity is increased by single- and double-strand breaks and by agents which provoke DNA breaks,[30,31] the alkaline elution assay exhibited no evidence for suramin-induced strand breaks.[11,32] Therefore, further investigations should be performed to elucidate the mechanism of PADPR stimulation by suramin.

The strong but not complete inhibition of DNA synthesis (Figure 1) is in agreement with the investigations of Jindal *et al.*[9] who demonstrated complete depression just at long incubation periods (over 2 days). Therefore, the uncomplete inhibition of [^{3}H]thymidine incorporation in the present study could be explained by short-time exposure (30 min) of the primary cell cultures to suramin. Unscheduled DNA synthesis, as well as UV-stimulated unscheduled DNA synthesis, involving mainly DNA polymerase α were also decreased, supporting the results of other authors who found the inhibition of DNA synthesis to be due to a direct action on DNA polymerases, predominantly polymerase α.[9,10] The slight stimulation of unscheduled DNA synthesis in the embryonic cells at suramin concentrations below 20 μg/ml might be explained in terms of a stimulation of DNA polymerase α which has been shown by Jindal *et al.*[9] at concentrations below 30 μg/ml.

The decrease in *viscometry of alkaline cell lysates* and the increase in *nucleoid sedimentation distance* (Figure 2) by suramin reflect suramin–chromatin interactions resulting in greater nucleoid compactness. Since the effect on nucleoid sedimentation could be abolished by treatment with proteinase K (Figure 2), it is concluded that suramin interferes with histones and/or matrix proteins probably because of its polyanionic character.[5] The different sensitivity of liver, brain and thymic cells might reflect, therefore, a different constitutive chromatin compactness and/or a different histone- and/or matrix protein–nucleic acid relation.

In order to investigate the influence of suramin on DNA repair processes, nucleoid sedimentation was assessed after a 30 min post-exposure period. Following X-irradiation, DNA repair ('short patch repair')[33] is characterized by an increase in the sedimentation distance (e.g. Tempel[34] and Snyder[35]), whereas in UV-irradiated cells ('long patch repair')[21,33] only the endonucleolytic DNA degradation can be measured during the short post-exposure period by a decrease in nucleoid sedimentation. Both repair phenomena were not influenced by suramin (Figure 3).

Conclusion

One cannot ignore the possibility that suramin binds to many proteins in a non-specific fashion. Therefore, it might be very difficult to relate antitumor activity of suramin to a small number of effects, e.g. interaction with single enzymes. Despite this fact, the observed high sensitivity of enzyme systems which are critical to essential nuclear functions could play an important role in the cytotoxicity of suramin. With respect to possible suramin effects on nuclear functions *in vivo*, i.e. under clinical conditions, the fate of the polyanion within the cell has to be followed in a more quantitative manner. Cell-specific chromatin condensation may be a further significant determinant with regard to the tissue specificity of the drug.

Acknowledgments

The authors are indebted to Mrs H. Kortenbeutel for her excellent technical assistance. The study was partly sponsored by the Ernst und Berta Grimmke-Stiftung, Düsseldorf, Germany. Preliminary results have been presented at the *33rd Spring Meeting of the Deutsche Gesellschaft für Pharmakologie und Toxikologie*, 10–12 March 1992, Mainz.

References

1. Hawking F. Suramin: with special reference to onchocerciasis. *Adv Pharmacol Chemother* 1978; **15**: 289–322.
2. De Clercq E. Suramin: a potent inhibitor of the reverse transcriptase of RNA tumor viruses. *Cancer Lett* 1979; **8**: 9–22.
3. Broder S, Collins JM, Markham PD, *et al.* Effects of suramin on HTLV-III/LAV infection presenting as Kaposi's sarcoma or AIDS-related complex: clinical pharmacology and suppression of virus replication *in vivo*. *Lancet* 1985; **ii**: 627–30.
4. Cheson BD, Levine AM, Mildvan D, *et al.* Suramin therapy in AIDS and related disorders. Report of the US suramin working group. *J Am Med Ass* 1987; **258**: 1347–51.
5. La Rocca RV, Stein CA, Myers CE. Suramin: prototype of a new generation of antitumor compounds. *Cancer Cells* 1990; **2**: 106–15.

6. La Rocca RV, Stein CA, Danesi R, *et al.* Suramin, a novel antitumor compound. *J Steroid Biochem Mol Biol* 1990; **37**: 893–8.
7. Armand JP, Cvitkovic E. Suramin: a new therapeutic concept. *Eur J Cancer* 1990; **26**: 417–9.
8. Coffrey RJ, Leof EB, Shipley GD, *et al.* Suramin inhibition of growth factor receptor binding and mitogenicity in AKR-2B cells. *J Cell Physiol* 1987; **132**: 143–8.
9. Jindal HK, Anderson CW, Davis RG, *et al.* Suramin affects DNA synthesis in HeLa cells by inhibition of DNA polymerases. *Cancer Res* 1990; **50**: 7754–7.
10. Ono K, Nakane H, Fukushima M. Differential inhibition of various deoxyribonucleic and ribonucleic acid polymerases by suramin. *Eur J Biochem* 1988; **172**: 349–53.
11. Bojanowski K, Lelievre S, Markovits J, *et al.* Suramin is an inhibitor of DNA topoisomerase II *in vitro* and in Chinese hamster fibrosarcoma cells. *Proc Natl Acad Sci USA* 1992; **89**: 3025–9.
12. Stammberger I, Schmahl W, Tempel K. Scheduled and unscheduled DNA synthesis in chick embryo liver following X-irradiation and treatment with DNA repair inhibitors *in vivo*. *Int J Radiat Biol* 1989; **56**: 325–33.
13. Tempel K, Spath A. Stimulation of DNA repair synthesis of rat thymocytes by novobiocin and nalidixic acid *in vitro* without detectable DNA damage. *Arch Toxicol* 1987; **60**: 287–92.
14. Link A, Tempel K. Inhibition of O^6-alkylguanine-DNA alkyltransferase and DNase I activities *in vitro* by some alkylating substances and antineoplastic agents. *J Cancer Res Clin Oncol* 1991; **117**: 549–55.
15. Junod AF, Jornot L, Petersen H. Different effects of hyperoxia and hydrogen peroxide on DNA damage, polyadenosine diphosphate-ribose polymerase activity, and nicotinamide adenine dinucleotide and adenosine triphosphate contents in cultured endothelial cells and fibroblasts. *J Cell Physiol* 1989; **140**: 177–85.
16. Link A, Tempel K, Hund M. RNA metabolism, DNA damage and cellular resistance to X-rays: investigations in chick embryo and rat cells. *Z Naturforsch* 1992; **47c**: 249–53.
17. Marshall B, Ralph RK. A simple method for detecting drug effects on the DNA of mammalian cells. *Anal Biochem* 1982; **125**: 91–5.
18. Tempel K. Viscometry of alkaline cell lysates—the hitherto simplest short-term test for chromatin-interactive agents? Investigations in rat thymic and splenic cells. *Chem-Biol Interact* 1991; **77**: 25–37.
19. Tempel K, Heinzelmann R. Sedimentation von Nukleoiden aus Thymus- und Milzzellen der Ratte nach Ganzkörper-Röntgenbestrahlung. *Z Naturforsch* 1988; **43c**: 126–32.
20. Tempel K, Ignatius A, Hund M, DNA damage and repair in chick embryo cells following X-irradiation *in vitro* as compared to mammalian cells—biochemical and physicochemical investigations. *Radiat Environ Biophys* 1992; **31**: 91–100.
21. Bohr VA. Gene specific DNA repair. *Carcinogenesis* 1991; **12**: 1983–92.
22. Regelson W. Heparin, heparinoids, synthetic polyanions, and anionic dyes. Opportunities and new developments. *ACS Symp Ser* 1991; **467**: 367–93.
23. Brandsteterova E, Chovancova V, Koza I, *et al.* Determination of suramin in clinical samples using HPLC. *Neoplasma* 1991; **38**: 425–32.
24. Singer B, Grunberger D. *Molecular biology of carcinogens and mutagens*. New York: Plenum 1983.
25. Yarosh DR. The role of O^6-methylguanine-DNA methyltransferase in cell survival, mutagenesis and carcinogenesis. *Mutat Res* 1985; **145**: 1–16.
26. Pegg AE. Mammalian O^6-alkylguanine-DNA alkyltransferase: regulation and importance in response to alkylating carcinogenic and therapeutic agents. *Cancer Res* 1990; **50**: 6119–29.
27. Meer L, Schold SC, Kleihues P. Inhibition of the hepatic O^6-alkylguanine-DNA alkyltransferase *in vivo* by pretreatment with antineoplastic agents. *Biochem Pharmacol* 1989; **38**: 929–34.
28. Dolan ME, Moschel RC, Pegg AE. Depletion of mammalian O^6-alkylguanine-DNA alkyltransferase activity by O^6-benzylguanine provides a means to evaluate the role of this protein in protection against carcinogenic and therapeutic alkylating agents. *Proc Natl Acad Sci USA* 1990; **87**: 5368–72.
29. Sierakowska H, Shugar D. Mammalian nucleolytic enzymes. *Progr Nucl Acid Res Mol Biol* 1977; **20**: 60–130.
30. Mandel P. ADP-ribosylation—findings and perspectives. In: *Regulation of gene expression in the nervous system*. New York: Wiley-Liss 1990: 1–18.
31. Shall S. ADP-ribose in DNA repair: a new component of DNA excision repair. *Adv Radiat Biol* 1984; **11**: 1–69.
32. Stammberger I. personal communication Hoechst AG, Pharmaceutical Research Toxicology, D-6230 Frankfurt am Main, Germany, 1992.
33. Francis AA, Snyder RD, Dunn WC, *et al.* Classification of chemical agents as to their ability to induce long- or short-patch DNA repair in human cells. *Mutat Res* 1981; **83**: 159–69.
34. Tempel K. Changes in nucleoid viscosity following X-irradiation of rat thymic and splenic cells *in vitro*. *Radiat Environ Biophys* 1990; **29**: 19–30.
35. Snyder RD. Inhibition of X-ray-induced DNA strand break repair in polyamine-depleted HeLa cells. *Int J Radiat Biol* 1989; **5**: 773–82.

(Received 29 June 1992; accepted 14 July 1992)