Pergamon

Biochemical Pharmacology, Vol. 47, No. 2, pp. 203–208, 1994.

0006-2952/94 $6.00 + 0.00

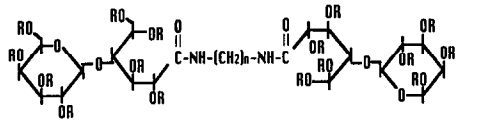
THE *IN VITRO* INFLUENCE OF SULFATED BIS-LACTOBIONIC ACID AMIDES ON O^6-ALKYLGUANINE-DNA ALKYLTRANSFERASE, DNASE I, NUCLEIC ACID SYNTHESIS AND CHROMATIN STRUCTURE

ANITA IGNATIUS,* EIKE MEINETSBERGER, WOLFRAM RAAKE and KARLHEINZ TEMPEL†
Institute of Pharmacology, Toxicology and Pharmacy, University of Munich, Königinstraße 16, D-80539 München, F.R.G.‡

(*Received* 10 *May* 1993; *accepted* 4 *October* 1993)

Abstract—The influence of four sulfated bis-lactobionic acid amides (BLAA) of molecular weights between 2388 and 2514 on O^6-alkylguanine-DNA alkyltransferase (AT), DNase I and nucleic acid synthesis as well as on nucleoid sedimentation and the viscosity of alkaline lysates of chicken embryo cells was studied *in vitro*. The activities of AT and DNase I were inhibited by BLAA in a dose-dependent manner. Depending on the polyanion used, concentrations depleting AT activity by 50% ranged between 3.5 and 7.0 μM, whereas BLAA concentrations of almost 250–320 μM were needed to halve DNase I activity. At concentrations above 8 μM, BLAA decreased scheduled DNA synthesis in a dose-dependent fashion whereas RNA synthesis remained unchanged even at the highest BLAA concentrations used (2 mM). In chicken embryo brain cells BLAA exerted a biphasic effect on the nucleoid sedimentation and the viscosity of alkaline cell lysates reflecting a decrease in chromatin compactness at lower BLAA concentrations (10–100 μM) and an increase in chromatin compactness at higher polyanion concentrations (≥200 μM). The remarkably high sensitivity of the nuclear enzyme AT deserves further investigation in regard to the fate of the polyanions within cells and tissues.

In the course of the search for new antithrombotic substances, a series of sulfated bis-lactobionic acid amides (BLAA§) (Fig. 1) were synthesized. The compounds are highly charged polyanions of low molecular weight [1, 2]. Aside from the anticoagulant activity of heparin and heparinoids, polyanions exhibit a number of other biological effects *in vitro* and *in vivo* some of which may be of therapeutic and/or toxicological interest [3], especially in relation to antiproliferative/antitumor activity [4–6]. Recent investigations focused on a polysulfated naphthylurea, suramin, possessing antitumor activity in some advanced cancers [7–9]. Its mechanism(s) of action, however, is/are not understood. In addition to the generally accepted binding of the polyanion to many growth factors [10], its antiproliferative action, when the compound is used at clinically relevant concentrations, may include the inhibition of critical enzymes [9, 11–13]. Because the BLAA, like suramin, are characterized by a high negative charge and low molecular weight, the possible interaction of these polyanions with some DNA-directed enzyme systems and nuclear structures was investigated. The enzyme systems comprised O^6-alkylguanine-DNA alkyltransferase (AT) and neutral deoxyribonuclease (DNase I) as well as scheduled DNA synthesis and RNA synthesis. Nuclear structures were examined on the basis of nucleoid sedimentation and the viscosity of alkaline cell lysates.



Substance	n	Molecular weight
LW 10082	3	2388
LW 10078	6	2430
LW 10086	9	2472
LW 10079	12	2514

Fig. 1. Chemistry of BLAA.

MATERIALS AND METHODS

Chemicals. The BLAA were synthesized by standard chemical methods [14] and purified to >99.5% by differential precipitation and chromatographic procedures. A series of analytic methods, including elementary analysis, ^{13}C-NMR spectroscopy, ^{1}H-NMR spectroscopy, cellulose acetate electrophoresis and HPLC, were used to establish the identity and purity of the substances.

* Present address: Department of Trauma Surgery Research and Biomechanics, University of Ulm, Helmholtzstraße 14, D-89081 Ulm, F.R.G.

† Corresponding author: Prof. Dr K. Tempel, Institut für Pharmakologie, Toxikologie und Pharmazie der Universität München, Königinstraße 16, D-80539 München, F.R.G. Tel (49) 89 21802667; FAX (49) 89 342316.

‡ In cooperation with the Departments of Pharmacology and Chemistry, Luitpold, Pharma GmbH, Sankyo Group, Zielstattstraße 9, D-81379 München, F.R.G.

§ Abbreviations: AT, O^6-alkylguanine-DNA alkyltransferase; BLAA, sulfated bis-lactobionic acid amide(s); LW, Luitpold Werk; PCA, perchloric acid.

Their molecular weights are 2388 (LW 10082), 2430 (LW 10078), 2472 (LW 10086), and 2514 (LW 10079). [5.6-^{3}H]Uridine ([^{3}H]U, sp. act. 1.48 TBq/mmol) was purchased from Amersham Buchler (Braunschweig, Germany). [^{3}H]Methyl-thymidine ([^{3}H]dT, sp. act. 1.85–2.96 TBq/mmol) was obtained from New England Nuclear (Boston, MA, U.S.A.). DNA treated with *N*-[^{3}H]methyl-*N*-nitrosourea (7.7 μg containing 100 fmol O^6-methylguanine, substrate of AT) had been prepared previously [15].

The specific activity for O^6-methylguanine was considered to be the same as for the methylation of the substrate DNA with [^{3}H]methylnitrosourea (94.7×10^{10} Bq/mmol). An enzyme extract of chicken embryo liver and/or brain [15] was used for AT. The specific AT activity expressed in fmol of transfer of O^6-[^{3}H]methylguanine per 250 μg extract protein was 43.8 ± 4.2 (liver) and 28.2 ± 3.6 (brain). DNase I from bovine pancreas (EC 3.1.21.1) was obtained from Sigma (Deisenhofen, Germany; no. D-0876, 550 Kunitz units/mg protein). The other substances were bought from Merck (Darmstadt, Germany), Serva (Heidelberg, Germany), Sigma and Aldrich (Steinheim, Germany).

Animal and cell preparation. Fertile eggs from White Leghorn chickens from a conventional breeding farm were used. The embryos were removed from the eggs and killed by decapitation at the developmental stage of 15 days. Liver and/or brain cell suspensions were prepared as described previously [16] and adjusted to final concentrations of 1×10^6–12.5×10^6 cells/mL of Hanks solution (Ca^{2+}, Mg^{2+}-free). Cell viability was assessed by Trypan blue dye exclusion.

Treatment of the cells and enzymes. Freshly prepared polyanionic stock solution (0.1 mL), containing the substances at 11 times the concentrations needed in the test assays, was added to 1 mL of the cell suspension; this was followed by a 30 min preincubation period at 37°. When examining the polyanionic effects on the enzymes, the stock solutions were first mixed with the substrate solution. After a 30 min preincubation period at 37°, DNase I solution or protein extract (AT) was added to the enzyme assays.

Analytical procedures. The AT assay was performed as described [15]. [^{3}H]*N*-Methyl-*N*-nitrosourea-treated DNA (7.7 μg containing 100 fmol O^6-methylguanine, ~2000 cpm) was incubated with increasing concentrations of protein extract (100–400 μg) at 37° for 15 min in a buffer containing 50 mM Tris, 1 mM EDTA and 6 mM β-mercaptoethanol (pH 8.3) giving a total volume of 1.1 mL. The reaction was stopped by adding 1 mL 4 M perchloric acid (PCA) to precipitate both DNA and protein. The DNA was hydrolysed by heating at 70° for 30 min. The samples were centrifuged (10 min at 7000 *g*) and the washed protein pellet (twice in 1 M PCA) resuspended in 0.1 mL 0.01 M NaOH. The amount of [^{3}H]methyl transferred to the acceptor protein (AT) was given by the amount of radioactivity in the protein pellet. This was quantified by liquid scintillation counting in Beckman Ready-Solv Protein^{+TM}. The test proved linear between 100 and 400 μg protein/assay.

DNase I activity was estimated spectrophotometrically at 260 nm on the basis of the hyperchromic effect of the fraction soluble with 1 M PCA (precipitation assay) as well as by the increase in absorbance of the whole reaction mixture at 260 nm during an incubation period of 20 min (kinetic assay) [15]. The DNase I assay consisted of 800 μL Tris–HCl, pH 7.4, 100 μL DNase I solution, 50 μL DNA (calf thymus DNA, Na salt, molecular weight 9×10^6, Aldrich) solution containing 250 μg DNA and 50 μL 4 M $MgCl_2$. By incubating this assay at room temperature, a linear increase in the extinction of the reaction mixture measured automatically at 260 nm was produced for at least 2.5 min (kinetic assay). Under the same conditions a linear increase in UV extinction of the PCA-soluble DNA fraction could be observed for almost 20 min (precipitation assay).

Nucleic acid synthesis was measured *in vitro* by the incorporation of [^{3}H]dT and [^{3}H]U, respectively, in the PCA-insoluble cell fraction [16, 17]. To measure scheduled DNA synthesis, [^{3}H]dT was added to the cell suspension (50 μL with an activity of 46.25 kBq to 500 μL cell suspension), and the cells were incubated for 90 min at 37°. The incorporation of [^{3}H]dT was stopped by the addition of cold PCA to a final concentration of 1.0 M.

The samples were cooled for 15 min and then centrifuged (5 min at 6500 *g*). The washed pellet (twice in 1 M PCA) was solubilized by heating in 1 M PCA to 70° for 20 min and, after cooling of the samples to −15° for 20 min, centrifuged at 6500 *g* for 10 min at room temperature. The DNA content of the supernatant was determined from UV absorbance (wavelength 260 nm) using calf thymus DNA as a standard, and radioactivity was quantified by liquid scintillation counting in Ready Solf MPR (Beckman). The radioactivity was related to the DNA content of the assays.

When determining RNA synthesis, to 500 μL of the cell suspensions [^{3}H]U was added (50 μL with an activity of 37 kBq), and the cells were incubated for 45 min at 37°. The incorporation of [^{3}H]U was stopped by the addition of cold PCA to a final concentration of 0.125 M. After centrifugation (5 min at 9000 *g*, 4°) and washing (three times in 0.125 M PCA, 4°), the pellet was dissolved in 1.5 mL 1 M NaOH at room temperature. After 1 hr 0.5 mL 4 M HCl was added, and then the samples were centrifuged (10 min at 900 *g*). The supernatant (hydrolysed [^{3}H]U-RNA) was saved to quantify [^{3}H]U incorporation by liquid scintillation counting in Ready-Solv EP (Beckman). RNA in this supernatant was read at 260 nm against hydrolysed yeast RNA standard.

Nucleoid sedimentation reflects DNA supercoils, i.e. the three-dimensional organization of DNA. Changes in nucleoid compactness are determined by low speed sucrose gradient sedimentation [18, 19]. The sedimentation assay was carried out in chicken embryo brain cells as described earlier for thymic and splenic cells of the rat [20]: in order to obtain the nucleoid fraction for sedimentation, the lysis medium on the top of a sucrose gradient (15–30% (w/w), pH 8.0) contained 1.5 M NaCl, 100 mM Na_2EDTA and 7 g Triton X-100 per liter. The sucrose gradients contained 1 M NaCl, 0.02 M Tris,

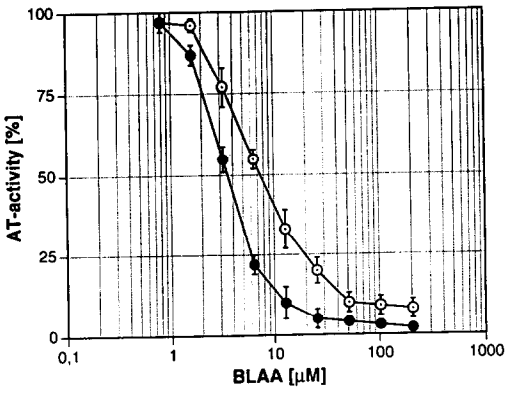


Fig. 2. AT activity after exposure to BLAA. AT activity expressed as per cent of BLAA-free controls; actual values: 43.8 ± 4.2 fmol of O^6-[^{3}H]methylguanine transfer per 250 μg extract protein (liver). (●) LW 10078, LW 10079, LW 10086, (○) LW 10082. BLAA concentrations on a log scale.

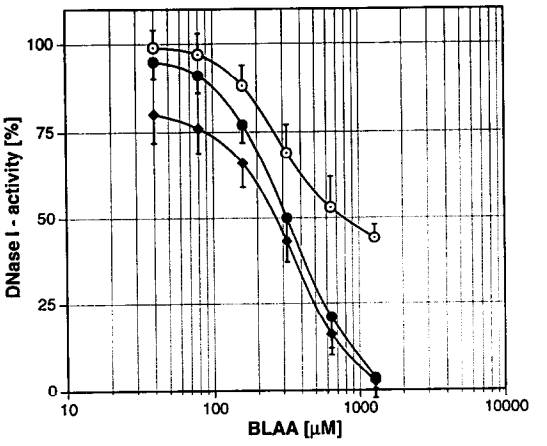


Fig. 3. DNase I activity after exposure to BLAA. DNase I activity expressed as per cent of BLAA-free controls; actual values: 1.375 Kunitz units per assay, resulting within 8 min in an increase in extinction of 0.45 ± 0.05 (kinetic assay, 22°). (○) Precipitation assay (LW 10082), (●) kinetic assay (LW 10082), (◆) kinetic assay (LW 10078, LW 10079, LW 10086).

0.001 M Na_2EDTA and 1 mg Hoechst 33258 per liter. Thirty minutes after the addition of the cells (250 μL containing ~ 5×10^6 cells) to the lysis medium (340 μL) on the top of the gradient, the tubes (HT 90426 Kontron) were centrifuged in the SW41 rotor (Beckman ultracentrifuge L2) at 20° and 25,000 rpm for 90 min. Thereafter, the gradients were illuminated by UV light to visualize the DNA–Hoechst 33258 complex. The sedimentation distance was measured from the top of the gradient–lysis mixture to the center of the nucleoid band.

To measure the viscosity of alkaline cell lysates, the method of Marshall and Ralph [21] was slightly modified [22].

In general each data point represents the mean of at least two to three independent experiments which were carried out in triplicate.

RESULTS

BLAA inhibited AT (Fig. 2) and the DNase I reaction (Fig. 3) in a dose-dependent manner. The ED_{50} values, however, differed by one to two orders, i.e. AT: 3.5–7.0 μM, DNase I: 250–320 μM. With respect to AT, LW 10082 seemed to be slightly less active than the other polyanions (Fig. 2).

As to the DNase I activity, the shapes of the dose–effect relationships of the four polyanions were similar (Fig. 3). The kinetic assay was more sensitive than the precipitation method, especially at higher BLAA concentrations (Fig. 3). As was the case with AT, LW 10078, LW 10079 and LW 10086 showed a significantly higher DNase I inhibiting activity than the polyanion with the lowest molecular weight (LW 10082). At DNA concentrations of 31.25–125.0 μg/assay (1.1 mL), the Lineweaver–Burk plot revealed competitive DNase I inhibition by BLAA (Fig. 4).

Whereas RNA synthesis remained unchanged, DNA synthesis decreased under the influence of BLAA in a concentration-dependent manner with ED_{50} being about 500–1000 μM. Liver cells seemed

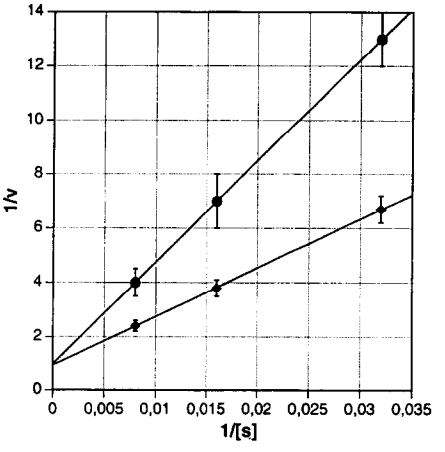


Fig. 4. DNase I activity after exposure to the BLAA LW 10082 (kinetic assay). Lineweaver–Burk plot with an enzyme activity of 1.357 Kunitz units and DNA concentrations of 125, 62.5 and 31.25 μg/assay (1.1 mL). (◆) Control assay, (●) 320 μM LW 10082.

to be slightly more sensitive than brain cells (Fig. 5). No significant differences were observed between the four polyanions used. At a concentration of 320 μM, for example, LW 10082, LW 10078, LW 10086 and LW 10079 inhibited DNA synthesis by 34 ± 5, 38 ± 10, 35 ± 9 and 37 ± 6%, respectively.

With respect to nucleoid sedimentation and viscosity of alkaline cell lysates, the addition of BLAA to chicken brain cells resulted in a biphasic effect: at 12–96 μM nucleoid sedimentation decreased by about 10–20%. Within the same concentration range, the viscosity of alkaline cell lysates increased

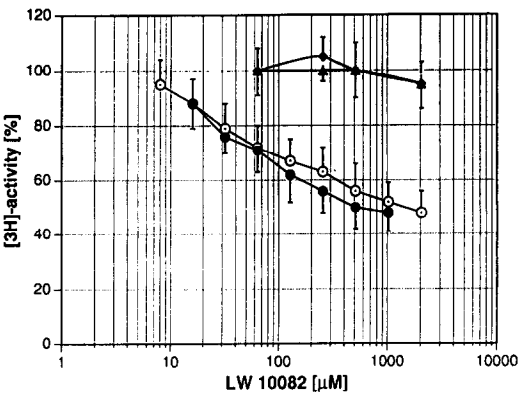


Fig. 5. Nucleic acid synthesis of chicken embryo cells after exposure to the BLAA LW 10082. (○) DNA synthesis in brain cells, (●) DNA synthesis in liver cells, (▲) RNA synthesis in brain cells, (◆) RNA synthesis in liver cells. LW 10082 concentrations on a log scale.

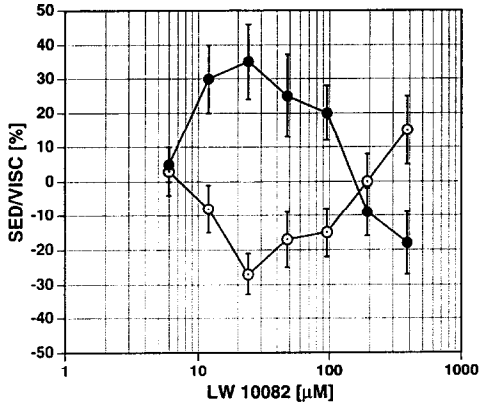


Fig. 6. Viscosity of alkaline lysates (VISC) and nucleoid sedimentation (SED) of chicken embryo cells after exposure to the BLAA LW 10082 (LW 10078, LW 10079 and LW 10086 with almost identical results). Values expressed as per cent of BLAA-free controls; (●) VISC, (○) SED. Control values: SED, brain cells: 1.7 ± 0.3 cm; VISC, brain cells: 12 ± 3 sec.

by almost 20–40% (Fig. 6). At higher polyanion concentrations (>96 μM), the values returned to the control level, and at very high BLAA additions (>192 μM), nucleoid sedimentation tended to increase whereas the viscosity of alkaline cell lysates diminished (Fig. 6).

DISCUSSION

A polyanionic drug, suramin, appears to be a new therapeutic concept in the treatment of some tumors [7–9], though the molecular basis of its anticancer activity remains unclear. In addition to the well-documented interference of the drug with a wide range of growth factors [10], further effects on DNA-related enzymes, such as DNA and RNA polymerases [12, 23, 24] or topoisomerase II [11], are seen *in vitro* at clinically relevant concentrations (<210 μM). The synthetic BLAA LW 10082 prolongs thrombin clotting times, when induced by human thrombin, in a concentration range of about 0.8–4 μM [25]. Whereas DNase I activity (Fig. 3), DNA synthesis (Fig. 5) as well as nucleoid sedimentation and viscosity of alkaline cell lysates (Fig. 6) remained unchanged at these concentrations, BLAA concentrations as low as 3.5–7.0 μM were able to inhibit AT by about 50% (Fig. 2).

The mutagenic, carcinogenic and cytotoxic properties of alkylating agents, such as alkylnitrosamines, are largely ascribed to the introduction and persistence of O^6-alkylguanine in the DNA. This lesion is normally removed from DNA by AT [26–29]. AT is an alkyl acceptor protein which carries the alkyl group from the O^6-position of the guanine to a cysteine residue of the same AT protein via a suicidal mechanism. The mechanism, however, by which the alkyl group is transferred is not fully understood [28, 29]. It is suggested that a basic residue of the AT protein, e.g. arginine, interacts with the acceptor protein. This interaction probably leads to the generation of a thiolate anion that attacks the alkyl group [28, 29]. Like nucleic acids, polyanions in general may be non-specifically bound to the AT protein which interferes with its access to the alkylated base, thus inhibiting AT activity [30]. From a clinical point of view, the possibility has to be considered that inhibition of AT activity may potentiate the antineoplastic effectivity [31, 32] and/or enhance the carcinogenic/mutagenic [33] activity of alkylating agents. Because AT is a nuclear enzyme [26–29], investigations are needed on the distribution of BLAA within the cells. Suramin is taken up into the cell by endocytosis and concentrated to some extent (about three times) within the nucleus [11]. By using [^{3}H]polypentose sulfate and autoradiography, Benes and Tallova [34] demonstrated that the polyanion, *in vitro*, reaches the nuclei of bone marrow cells. Therefore, an intracellular/intranuclear penetration of BLAA might also be possible.

The inhibition of DNA synthesis by polyanions [12, 13, 35] is due to a direct action on DNA polymerases, mainly polymerase α; the polyanionic negative charges are "assumed to interact electrostatically with positively charged side chains in the DNA-binding site" of the enzyme [36]. For a number of synthetic polyanions it could be shown that the extent of inhibition depends on the molecular distance between neighbouring charges [36]. The BLAA, like other polyanions [37, 38], are able to inhibit DNases. However, concentrations of at least >40–80 μM were needed to inhibit DNase I significantly (Fig. 3), which reflects a rather low specificity. As in the case of DNA polymerase α [36], polyanionic interaction is suggested by the competitive character of the enzyme inhibition. As shown by various investigators (see e.g. [33]), polyanions bind to histones thereby diminishing the DNA attachment sites. A decrease in DNA attachment sites would reduce DNA compactness.

Table 1. Comparison of LW 10082 and suramin with respect to their effects on some DNA-related enzymes, DNA and RNA synthesis, nucleoid sedimentation, and viscosity of alkaline cell lysates

Parameter	LW 10082	Suramin
Molecular weight	2388	1429
Number of SO_3^- Na^+ groups	16	6
Plasma concentration	≤4.2*	≤210 μM†
AT inhibition (ED_{50})	~7.0	~45 μM
DNase I inhibition (ED_{50})	~320	~70 μM
RNA synthesis	No effect	No effect
SDS inhibition (ED_{50})	~1000	~56 μM
SED increase (ED_{50}	≥1000	~85 μM
VISC decrease (ED_{50})	≥1000	~85 μM

* Klauser [25], † Scher [8], Stein [9].
SDS, scheduled DNA synthesis; SED, nucleoid sedimentation; VISC, viscosity of alkaline cell lysates.

This is confirmed in the present investigation by the decrease in nucleoid sedimentation and the increase in the viscosity of alkaline lysates of brain cells at BLAA concentrations of 12–96 μM (Fig. 6). The decrease in viscosity of alkaline cell lysates and the increase in nucleoid sedimentation at very high BLAA concentrations (Fig. 6) reflect greater nucleoid compactness. Because various—enzymatic and non-enzymatic—mechanisms are implicated in regulating chromatin structure (see e.g. [39, 40]), further investigations are needed with regard to the mechanisms of chromatin–polyanion interactions.

A comparison between the BLAA, e.g. LW 10082, and suramin (Table 1) shows that both classes of compounds, like other polyanions, are able to interfere with many proteins *in vitro*, including nuclear enzymes and histones. When compared to DNase I, DNA synthesis and chromatin–polyanion interactions, a remarkably high inhibition by BLAA is seen for the repair enzyme O^6-alkylguanine-DNA alkyltransferase. Their potential *in vivo* awaits analysis of the distribution of the BLAA within tissues and cells.

Acknowledgements—The authors are indebted to Mrs H. Kortenbeutel for her excellent technical assistance. The study was partly sponsored by the Ernst and Berta Grimmke-Stiftung, Düsseldorf, F.R.G.

REFERENCES

1. Raake W, Klauser RJ, Meinetsberger E, Zeiller P and Elling H, Pharmacological profile of the antithrombotic and bleeding actions of sulfated lactobionic acid amides. *Semin Thromb Hemostas* **17** (Suppl): 129–135, 1991.
2. Klauser RJ, Meinetsberger E and Raake W, Biochemical studies on sulfated lactobionic acid amides. *Semin Thromb Hemostas* **17** (Suppl): 118–125, 1991.
3. Donaruma LG, Ottenbrite RM and Vogl O (Eds.), *Anionic Polymeric Drugs*. John Wiley & Sons, New York, 1980.
4. Regelson W, The antimitotic activity of polyanions (antitumor, antiviral, and antibacterial action of heparin, heparinoids, anionic dyes, and synthetic polymers). *Adv Chemother* **3**: 303–371, 1968.
5. Regelson W, Heparin, heparinoids, synthetic polyanions, and anionic dyes. Opportunities and new developments. *ACS Symp* **467**: 367–393, 1991.
6. Kaplan A, Antitumor activity of synthetic polyanions. In: *Anionic Polymeric Drugs* (Eds. Donaruma LG, Ottenbrite RM and Vogl O), pp. 227–253. John Wiley & Sons, New York, 1980.
7. LaRocca RV, Stein CA and Myers CE, Suramin: prototype of a new generation of antitumor compounds. *Cancer Cells* **2**: 106–115, 1990.
8. Scher HI, Suramin: here to stay!? *J Natl Cancer Inst* **85**: 594–597, 1993.
9. Stein CA, Suramin: a novel antineoplastic agent with multiple potential mechanisms of action. *Cancer Res* **53**: 2239–2248, 1993.
10. Middaugh CR, Mach H, Burke CJ, Volkin DB, Dabora JM, Tsai PK, Bruner MW, Ryan JA and Marfia KE, Nature of the interaction of growth factors with Suramin. *Biochemistry* **31**: 9016–9024, 1992.
11. Bojanowski K, Lelievre S, Markovits J, Couprie J, Jacquemin-Sablon A and Larsen AK, Suramin is an inhibitor of DNA topoisomerase II *in vitro* and in Chinese hamster fibrosarcoma cells. *Proc Natl Acad Sci USA* **89**: 3025–3029, 1992.
12. Ignatius A and Tempel K, Influence of suramin on some DNA-directed enzymes and primary cultures of chicken embryo and rat cells. *Anti-cancer Drugs* **3**: 499–505, 1992.
13. Tempel K, Nucleinsäure- und Proteinsynthese in Milzlymphozyten der Ratte unter dem Einfluß von Mucopolysaccharidpolyschwefelsäureestern. *Arzneim Forsch Drug Res* **30**: 1119–1123, 1980.
14. Eur. Patent 0312 086 A2, Polyschwefelsäureester von Bis-lactobionsäureamiden und deren Derivaten. Verfahren zu ihrer Herstellung und Arzneimittel.
15. Link A and 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* **117**: 549–555, 1991.
16. Stammberger I, Schmahl W and 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* **56**: 325–333, 1989.
17. Link A, Tempel K and Hund M, RNA metabolism, DNA damage and cellular resistance to X-rays: Investigations in chick embryo and rat cells. *Z Naturforsch* **47c**: 249–254, 1992.
18. Cook PR and Brazell IA, Supercoils in human DNA. *J Cell Sci* **19**: 261–279, 1975.
19. Mattern M, Kerrigan DJ and Pommier Y, Nucleoid

sedimentation analysis of DNA strand breaks induced in cells exposed to DNA intercalating agents. *Pharmacol Ther* **34**: 303–319, 1987.
20. Tempel K and Heizelmann R, Sedimentation von Nukleoiden aus Thymus und Milzzellen der Ratte nach Ganzkörper-Röntgenbestrahlung. *Z Naturforsch* **43c**: 126–132, 1988.
21. Marshall B and Ralph RK, A simple method for detecting drug effects on the DNA of mammalian cells. *Anal Biochem* **125**: 91–95, 1982.
22. 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* **77**: 25–37, 1991.
23. Jindal HK, Anderson CW, Davis RG and Vishwanatha JK, Suramin affects DNA synthesis in HeLa cells by inhibition of DNA polymerases. *Cancer Res* **50**: 7754–7757, 1990.
24. Ono K, Nakane H and Fukushima M, Differential inhibition of various deoxyribonucleic and ribonucleic acid polymerases by suramin. *Eur J Biochem* **172**: 349–353, 1988.
25. Klauser RJ, Interaction of the sulfated lactobionic acid amide LW 10082 with thrombin and its endogenous inhibitors. *Thromb Res* **62**: 557–565, 1991.
26. Singer B and Grunberger D, *Molecular Biology of Carcinogens and Mutagens*. Plenum Press, New York, 1983.
27. Yarosh DR, The role of O^6-methylguanine-DNA methyltransferase in cell survival, mutagenesis and carcinogenesis. *Mutat Res* **145**: 1–16, 1985.
28. Pegg AE, Properties of mammalian O^6-alkylguanine-DNA transferase. *Mutat Res* **233**: 165–175, 1990.
29. Pegg AE and Byers TL, Repair of DNA containing O^6-alkylguanine. *FASEB J* **6**: 2302–2310, 1992.
30. Bhattacharyya D, Foote RS, Boulden AM and Mitra S, Physicochemical studies of human O^6-methylguanine-DNA methyltransferase. *Eur J Biochem* **193**: 337–343, 1990.
31. Meer L, Schold SC and Kleihues P, Inhibition of the hepatic O^6-alkylguanine–DNA alkyltransferase *in vivo* by pretreatment with antineoplastic agents. *Biochem Pharmacol* **38**: 929–934, 1989.
32. Dolan ME, Moschel RC and 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* **87**: 5368–5372, 1990.
33. Bronstein SM, Hooth MJ, Swenberg JA and Skopek TR, Modulation of ethylnitrosourea-induced toxicity and mutagenicity in human cells by O^6-benzylguanine. *Cancer Res* **52**: 1851–1856, 1992.
34. Benes L and Tallova J, Interaction of polyanion polypentosesulphate with bone marrow cells *in vitro*. *Folia Biol (Praha)* **20**: 419–426, 1974.
35. Tempel K and Hollatz R, Wirkung von Polyanionen auf Nucleinsäure- und Protein-Synthese von Milzlymphozyten und Knochenmarkszellen der Ratte *in vitro*. *Zentralbl Veterinarmed [A]* **26**: 117–129, 1979.
36. Holler E, Achhammer G, Angerer B, Gantz B, Hambach C, Risner H, Seidel B, Weber C, Windisch C, Braud C, Guerin P and Vert M, Specific inhibition of Physarum polycephalum DNA-polymerase-α-primase by poly (L-malate) and related polyanions. *Eur J Biochem* **206**: 1–6, 1992.
37. Tunis M and Regelson W, A comparative study of the inhibiting effects of anionic polyelectrolytes on deoxyribonucleases. *Arch Biochem Biophys* **101**: 448–455, 1963.
38. Chen J, Herzenberg LA and Herzenberg LA, Heparin inhibits EcoRI endonuclease cleavage of DNA at certain EcoRI sites. *Nucleic Acids Res* **18**: 3255–3260, 1990.
39. Getzenberg RH, Pienta KJ, Ward WS and Coffey DS, Nuclear structure and the three-dimensional organization of DNA. *J Cell Biochem* **47**: 289–299, 1991.
40. Wolffe AP, New insights into chromatin function in transcriptional control. *FASEB J* **6**: 3354–3361, 1992.