



Enhancement of the activity of bleomycin by cysteamine in a micronucleus assay in G₀ human lymphocytes

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Abstract

The aminothiol cysteamine enhances the induction of micronuclei by bleomycin in G₀ human lymphocytes. The potentiation of bleomycin (12.5, 25, 50, or 100 µg/ml) increased with cysteamine concentration from 5 to 20 mM in a 2-h treatment before culturing the cells for the cytokinesis-block assay. The maximum clastogenic activity of bleomycin in the presence of cysteamine was more than 10-fold greater than that of the same dosage of bleomycin alone. Both the thiol and amine functions of aminothiols seem to contribute to the potentiation of bleomycin.

Keywords: Bleomycin; Cysteamine; WR-1065; Aminothiols; Radioprotectors; Micronucleus

1. Introduction

Bleomycin (BLM) is mutagenic in diverse genetic assays [1]. It is considered to be a radiomimetic chemical because, like ionizing radiation, it is clastogenic at all stages of the cell cycle, and its clastogenicity does not depend on passage of the chemical lesions through replication. BLM is an effective inducer of chromosome aberrations [2] and micronuclei [3] in G₀ human lymphocytes, and its mode of action has been thoroughly reviewed [1].

The radioprotectors dimethylsulfoxide (DMSO) [4] and 2-[(aminopropyl)amino] ethanethiol (WR-

1065) [5] reduce the effectiveness of ionizing radiation in inducing chromosome aberrations and micronuclei in human lymphocytes. Radioprotective effects of WR-1065 have also been reported in other assays for mutagenesis and DNA damage (reviewed in [6]). DMSO is radioprotective because it acts as a specific scavenger of hydroxyl radicals [4]. The mechanism of radioprotection by WR-1065 involves scavenging hydroxyl radicals, transferring hydrogen to DNA radicals, and creating an anoxic state near DNA [7].

Since BLM is a radiomimetic compound, we undertook studies of effects of radioprotectors on its genetic activity. Using the cytokinesis-block micronucleus assay [8] and metaphase analysis, we found that both DMSO and WR-1065, unlike their protective effects with ionizing radiation,

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potentiate the clastogenic activity of BLM in G₀ human lymphocytes [6,9]. Potentiation by DMSO occurs only at high concentration (≥ 1 M) and is apt to be caused by effects of DMSO on cell permeability [9]. WR-1065, however, potentiates BLM at much lower concentrations (1-10 mM). While the mechanism of potentiation by WR-1065 is not established, both a thiol-mediated increase in the activation of BLM and an amine-mediated conformational change that makes the target site in DNA more accessible to BLM have been suggested [6]. Possible clinical implications of interactions between BLM and aminothiols have also been discussed [6].

In this paper, we extend our study of interactions between BLM and radioprotectors to another aminothiol — cysteamine (CSM). Like WR-1065, CSM is an effective radioprotector [10]. We have used a micronucleus assay in G₀ human lymphocytes to measure effects of CSM on the induction of chromosome damage by BLM.

2. Materials and methods

2.1. Chemical sources

BLM (bleomycin sulfate; Chemical Abstracts Service (CAS) Number 9041-93-4) was obtained from Dr. Russell DuFrain, Bristol-Myers Company, Syracuse, NY. CSM (2-mercaptoethylamine hydrochloride; CAS No. 156-57-0) and cytochalasin B were purchased from Sigma Chemical Company, St. Louis, MO; phytohemagglutinin (PHA) from Murex Diagnostics, Inc., Norcross, GA; and penicillin-streptomycin, RPMI Medium 1640, and fetal bovine serum (FBS) from GIBCO, Grand Island, NY.

2.2. Lymphocyte techniques

The cytokinesis-block micronucleus assay was performed largely as described by Fenech and Morley [8], incorporating minor modifications as noted in Littlefield et al. [11] and Hoffmann et al. [3,9]. Briefly, blood was collected from a healthy adult male by venipuncture, and a lymphocyte-rich fraction (buffy coat) was prepared. A pretreatment with CSM was begun by mixing 1 ml buffy coat with 0.8 ml RPMI containing 10% FBS and the appropriate concentration of CSM. After

30 min incubation at 37°C, BLM was added in 0.2 ml RPMI + 10% FBS to give final BLM concentrations of 0, 12.5, 25, 50, and 100 $\mu\text{g/ml}$. Treatments (2 h at 37°C) were quenched by adding 12 ml RPMI + 10% FBS and washing 3 times. Cultures were started by suspending the cells in 10 ml RPMI + 15% FBS, 1% penicillin-streptomycin, and 2% PHA. Cytochalasin B (6 $\mu\text{g/ml}$) was added to cultures after 42 h at 37°C, and cells were harvested to prepare slides 24 h later.

2.3. Preparation and scoring of slides

Cultures were terminated after 66 h incubation by adding 4 ml cold fixative (3 methanol:1 acetic acid) per flask. The cells were harvested by centrifugation, washed in fixative, and dropped onto slides. Slides were stained for 12 min with 10% Giemsa. Micronuclei were counted in 1000 binucleate cells per treatment on coded slides, and a proliferation index (PI) was calculated from a sample of 300 cells: $\text{PI} = (1 \times \text{frequency of mononucleate cells}) + (2 \times \text{frequency of binucleate cells}) + (3 \times \text{frequency of cells with } > 2 \text{ nuclei})$.

3. Results and discussion

G₀ lymphocytes were treated for 2 h at 5 concentrations of BLM (0, 12.5, 25, 50, and 100 $\mu\text{g/ml}$) in combination with 5 concentrations of CSM (0, 5, 10, 20, and 40 mM). Table 1 shows frequencies of micronuclei in binucleate cells and proliferation indices resulting from these treatments. The proliferation indices indicate no toxicity associated with BLM alone and no toxicity of CSM alone up to 20 mM. There was a reduction in proliferation index at 10 and 20 mM CSM in the presence of BLM. At 40 mM, CSM alone reduced the proliferation index to 1.32, and the toxicity of the combined treatments was so great (proliferation indices < 1.2) that there were insufficient numbers of binucleate cells to determine micronucleus frequencies.

The data show no induction of micronuclei by CSM alone and a small increase in the frequency of micronuclei induced by BLM alone. The modest effect of BLM is not surprising, as previous studies have shown that the induction of micronuclei by 2-h treatments of G₀ lymphocytes

Table 1
Induction of micronuclei in G₀ human lymphocytes by bleomycin in the presence of cysteamine

BLM (μ g/ml)	CSM (mM)	Proliferation index	Cells with micronuclei ^a	Total micronuclei	Micronuclei per cell
0	0	1.76	5	5	0.005
0	5	1.73	9	10	0.010
0	10	1.69	8	8	0.008
0	20	1.69	8	10	0.010
12.5	0	1.77	9	11	0.011
12.5	5	1.73	35*	44	0.044
12.5	10	1.56	58*	68	0.068
12.5	20	1.61	186*	112	0.112
25	0	1.73	14	16	0.016
25	5	1.64	39*	50	0.050
25	10	1.61	95*	117	0.117
25	20	1.64	122*	163	0.163
50	0	1.73	9	10	0.010
50	5	1.61	51*	62	0.062
50	10	1.52	160*	232	0.232
50	20	1.59	175*	263	0.263
100	0	1.65	26	30	0.030
100	5	1.63	42	49	0.049
100	10	1.35	157*	237	0.237
100	20	1.33	279*	389	0.389

^aBased on 1000 binucleate cells per treatment.

*Highly significant difference from treatment without CSM (Fisher's exact test $P < 0.01$).

with BLM was most effective at concentrations from 150 to 600 μ g/ml; such high concentrations of BLM show little toxicity as reflected in proliferation indices [3].

Combined treatments with BLM and CSM lead to a greater clastogenic effect than that observed with BLM alone. Numbers of micronuclei per binucleate cell increased with the concentration of CSM at 5, 10, and 20 mM ($r^2 = 0.99, 0.95, 0.84$, and 0.94 at 12.5, 25, 50, and 100 μ g BLM/ml, respectively). The potentiating influence of CSM on the clastogenicity of BLM was reproducible in 3 independent experiments, and the maximum enhancement of BLM by CSM was more than 10-fold. The highest frequencies of micronuclei observed after treatments with BLM at 25, 50, and 100 μ g/ml in the presence of CSM were similar to those reported for BLM alone at 150, 300, and 450 μ g/ml, respectively [3].

Potential of BLM by aminothiols may involve enhanced generation of the active form of BLM through a redox mechanism [6,9]. The thiol may serve as an electron source for the activation of BLM in a process involving Fe(II)·bleomycin and oxygen. Thus, CSM may facilitate a process that occurs in its absence by means of an electron derived from an endogenous reducing agent or from another molecule of Fe(II)·bleomycin in a disproportionation reaction [12]. Alternatively, CSM may be involved in the reduction of inactive Fe(III)·bleomycin to Fe(II)·bleomycin. Other thiols, such as dithiothreitol and mercaptoethanol, which can similarly act as reducing agents, have been reported to potentiate BLM's ability to induce DNA damage [13].

Potential of BLM by CSM may also depend on CSM's amino group. The amines putrescine, spermidine, and spermine, none of which have a

thiol group, potentiate the degradation of DNA by bleomycin [14]. Binding of polyamines in the major groove of DNA seems to cause widening of the minor groove, which is the site of bleomycin action [14]. Aminothiols may be highly effective in potentiating BLM because they combine the postulated thiol and amine mechanisms or because they, as cationic thiols, concentrate the thiol mechanism near DNA. Both CSM [15] and WR-1065 [16] associate with DNA. In contrast to WR-1065 [6], higher concentrations of CSM are required to potentiate BLM, and the maximum micronucleus frequencies induced are smaller. CSM may be less effective than WR-1065 in enhancing BLM because it has one amino group, whereas WR-1065 has two. The diamine WR-1065 concentrates near DNA more than does the monoamine CSM [17]. Thus, CSM may potentiate BLM by similar means as WR-1065 but somewhat less effectively because of its less effective association with DNA.

CSM is the third aminothiol found to enhance the clastogenic action of BLM in G_0 lymphocytes; WR-1065 [6,9] and glutathione [18] have been shown to potentiate BLM in human and muntjac lymphocytes, respectively. In contrast, WR-1065 has been reported to protect against effects of BLM in cultured Chinese hamster cells [19].

Interactions between BLM and aminothiols may have implications for cancer chemotherapy. A phosphorylated form of WR-1065, called WR-2721, has been used to protect normal tissues in radiotherapy [20], and the possibility of using aminothiols as protective agents in combination with chemotherapy drugs [20], including BLM [19], has been suggested. The interaction that we have observed between BLM and aminothiols in G_0 human lymphocytes suggests that aminothiols may amplify, rather than protect against, the effects of BLM under some circumstances. However, speculation about possible hazards of the potentiation of BLM by aminothiols or about the potential for clinical exploitation of the enhancement [6] is limited by the inconsistency among biological systems [6,19] and the lack of data from intact mammals.

We are currently exploring the mechanism of potentiation of BLM by aminothiols and the basis for differences among biological systems in the

nature of the interaction. The qualitative similarity in the responses with CSM and WR-1065 should facilitate such studies because CSM, unlike WR-1065, is readily available from commercial sources.

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