Biochemical Pharmacology, Vol. 31, No. 2, pp. 273-276, 1982.
Printed in Great Britain.

0006-2952/82/020273-04$03.00/0

INHIBITORY EFFECTS OF PSEUDOURIDINEDICARBOXALDEHYDE ON TUBULIN METABOLISM

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(Received 8 August 1981; accepted 2 November 1981)

The antitumor activities of various dicarboxaldehydes are well documented [1-5]. A broad spectrum of enzymes and metabolic processes revolving about nucleic acid metabolism has been reported to be inhibited [1-7]. In addition, Plagemann et al. [7] reported that inox, the dicarboxaldehyde derivative of inosine, produces a primary blockade in the G_2 + M phase of the cell cycle–a finding that we have recently confirmed. In this preliminary communication, we report on the inhibitory effects on tubulin metabolism by pseudouridinedicarboxaldehyde (ψ-OX), the periodate oxidation product of pseudouridine.

ψ-OX is a C-nucleoside analog first synthesized about 20 years ago by Cohn [8] in his studies of pseudouridine. This dicarboxaldehyde is easily prepared by periodate oxidation of the nucleoside [3,6]. Like many dialdehydes, ψ-OX also has antileukemic activity. When BD2F1 female mice bearing i.p. transplants of 10^5 L1210 cells each were treated with ψ-OX at 100 mg/kg once daily on days 1, 4, and 7, increases in life span over controls of about 90% were obtained (%T/C = 190%). Moreover, like inox, ψ-OX-treated L1210 cells are blocked in G_2 + M [7]. Figure 1 shows the accumulation of L1210 cells into G_2 + M induced by ψ-OX at 100 μM in suspension culture. Vinblastine, 0.25 μM, was used here as the control drug. Based on this finding, possible inhibitory effects by ψ-OX on tubulin dimer (α + β tubulin) levels were investigated. Colchicine binding to tubulin dimers provided a convenient assay [9,10] especially since this method gives a characteristic and identifiable decay of binding with time. When L1210 cells in suspension culture were preincubated with ψ-OX at 100 μM for 18 hr, there was a decrease in tubulin dimer levels in the 100,000 g L1210 supernatant extracts as measured by colchicine binding (Table 1).

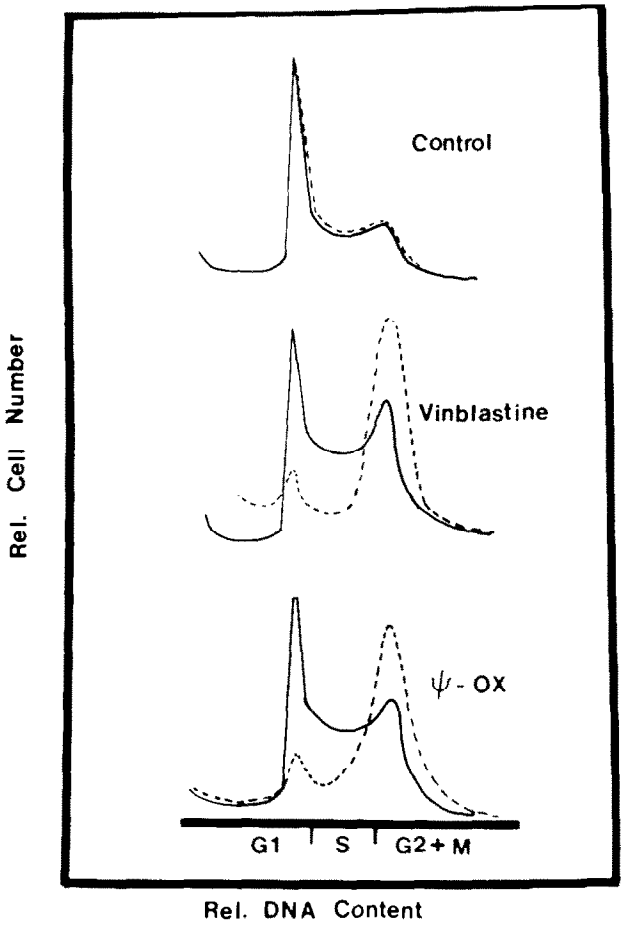

Fig. 1. Flow microfluorometric analysis of L1210 cells treated in suspension culture with either ψ-OX, 100 μM, or vinblastine, 0.25 μM, showing accumulation of cells into G_2 + M. The solid curves denote cells at 5 hours and dashed line curves denote cells at 20 hours.

At zero time, supernatant fractions from control cells (250 μg protein/ml) gave 175 cpm/μg protein of bound [^{3}H]colchicine while supernatant fractions from ψ-OX-treated cells (250 μg protein/ ml) only gave 36 cpm/μg protein or 21% of control value. Time decay of colchicine-binding occurred in the supernatant fractions from control and drug-treated L1210 cells. The drug preincubation time of 18 hr was selected to amply demonstrate the above effect on tubulin metabolism in a cell population largely viable (85% at 18 hr) since ψ-OX is very toxic to L1210 cells and cell viability decreases precipitously after 24 hr (approximately 2 cell cycles). Even so, the total protein (μg/100,000 cells) in control and drug-treated L1210 cells was not significantly different at 18 hr (66 for control cells and 64 for ψ-OX-treated cells). It seemed possible that ψ-OX was competing with colchicine in binding. To test this possibility, 100,000 g supernatant fractions from untreated L1210 cells were pre-incubated with 100 μM ψ-OX for 1 hr at 37° and then incubated with [^{3}H]colchicine for 2 hr. There was no competition by ψ-OX for colchicine binding to tubulin dimers. Alternatively, a

Table 1. Effect of ψ-OX on tuberlin dimers*

Drug (100 μM)	Specific activity of bound colchicine (cpm/μg protein) Binding time (hr) 0	2	4	6
Controls	175	130	80	48
ψ-OX	36	29	20	16

*Drug solutions sterilized by filtration were added at a final concentration of 100 μM to 25 ml culture flasks of L1210 cells at an initial concentration of 10^5 cells/ml in Fisher's growth medium. The cells at 18 hr were collected by centrifugation and washed three times with Dulbecco's phosphate buffered saline. Cell pellets were swollen with 2 vol. of ice-cold double-distilled water for 10 min. An equal volume of 2 x 0.067 M phosphate buffer (pH 6.8), containing 0.1 M KCl and 0.1 mM GTP was added to the swollen cells and the cells were disrupted with 40 strokes of a teflon pestle in an Elvehjem motor-driven tissue homogenizer. Cellular debris was removed in a clinical centrifuge at 1470 g. Subsequent centrifugation in a Beckman model LS-65 ultracentrifuge at 100,000 g for 1 hr at 4° gave the supernatant extracts used for protein determinations by the method of Lowry et al. [11] and for the colchicine binding assay as described by Borisy [9]. The colchicine-binding assay was carried out on 1 ml aliquots of supernatant fractions (250 μg protein/ml), to which [^{3}H]colchicine (2 μM, 10 μCi/μmole) was bound for the indicated times in a water bath at 37°. The reaction mixtures were poured onto pre-wetted 2.5 cm Whatman DE81 filter discs stacked four deep in a Millipore 122S sampling manifold. Each stack was washed five times, each time with 10 ml of phosphate buffer. Washed stacks were removed, placed in scintillation vials with 10 ml of Packard Instagel, held overnight, and counted in a Beckman LS 9000 scintillation counter. The 18 hr viabilities were determined in separate but parallel experiments by Trypan Blue dye exclusion. The viabilities of control and ψ-OX-treated cells were 98 and 85% respectively.

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metabolite of ψ-OX produced in treated cells might have competed with colchicine. When 100,000 g supernatant fractions from ψ-OX-treated and control cells were mixed in equal volumes and then assayed, the cpm/μg protein at time zero for the mixture was close to the theoretical average (310 cpm/μg protein for the mixture and 286 cpm/μg protein in theory). This indicated that a ψ-OX metabolite was not competing with colchicine in binding. It was also possible that L1210 cells blocked in G_2 + M might have lower levels of tubulin dimers

than control cells growing asynchronously. However, when L1210 cells were synchronized with thymidine and then released, drug-treated and control cells in G_2 gave the same results noted above. These data taken together imply that ψ-OX decreases in some fashion the absolute amount of tubulin dimers in L1210 cells to produce, in part, its antileukemic effect. However, other modes of action have not been excluded and should be investigated.

Acknowledgements - This research was supported by NCI Grant CA-12327, and Welch Grant E-321.

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