CHLORPROMAZINE ENHANCEMENT OF EPIRUBICIN CYTOTOXITY *IN VITRO*: EFFECTS ON PLASMA MEMBRANE AND DNA DAMAGE

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ABSTRACT

The frequently used antiemetic drug chlorpromazine has previously been shown to augment anthracycline-induced toxicity to cultured Chinese hamster fibroblasts measured by cloning /1/. We therefore tested the ability of chlorpromazine to affect the induction of plasma membrane and DNA damage by the anthracycline epirubicin. Plasma membrane damage was determined by the cells' ability to accumulate ^{86}Rb after incubation with 10 mg/l epirubicin, and DNA damage was determined by measuring the amount of DNA precipitation after incubation with 25 mg/l epirubicin. The epirubicin-induced inhibition of ^{86}Rb-accumulation as well as the enhancement of epirubicin-induced DNA damage were markedly enhanced in the presence of chlorpromazine. Chlorpromazine augmentation of epirubicin cytotoxicity, including plasma membrane and DNA damage, may be due to its calmodulin antagonistic action and related to the maintenance integrity. Further studies are justified to evaluate the effects of chlorpromazine influence on antineoplastic drug action *in vitro* and *in vivo*.

KEY WORDS

chlorpromazine, epirubicin, Rb accumulation, strand break

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INTRODUCTION

The potent and selective antiemetic drug chlorpromazine is frequently used during cancer treatment to counteract the emesis caused by antineoplastic drugs. Recently, chlorpromazine has been suggested to interact with the cytotoxicity of antineoplastic agents. An enhancement of nitrosourea /2/ and epirubicin /1/ toxicity has been demonstrated in different experimental models. In the present study the ability of chlorpromazine to affect the induction of plasma membrane and DNA damage caused by epirubicin was evaluated.

METHODS

DNA strand break assay

DNA strand breakage was measured with the DNA precipitation assay /3/ modified by the use of double radiolabelling according to the principles, outlined by Rydberg /4/ and Sandström and Johansson /5/, for detection of DNA strand breaks with improved accuracy. Exponentially growing V79 cells were prelabelled with either 3.7 kBq (0.1 μCi) [^{3}H]thymidine/ml (specific activity 74 GBq/mmol) or 0.8 kBq (0.02 μCi)[^{14}C]thymidine/ml (2.2 GBq/mmol). After approximately 18 h of labelling, the radioactive medium was discarded and the cells were washed twice with Hepes-buffered (20 mmol/l) medium. The cells were then exposed to the drug or used as controls. When chlorpromazine was used to affect epirubicin-induced DNA strand breakage it was added 30 min before the drug. The cells were exposed to 25 mg/l epirubicin for 1 h, and then immediately loosened by trypsinization. Drug-treated, ^{3}H-labelled cells were mixed with ^{14}C-labelled control cells (in ice-cold Hepes-buffered medium to prevent DNA rejoining), and *vice versa*, to improve DNA strand break measurement accuracy.

^{86}Rb-accumulation

Plasma membrane damage was determined by the cultured Chinese hamster fibroblasts' ability to accumulate ^{86}Rb for 2 h after 30 min preincubation with/without chlorpromazine followed by epirubicin 10 mg/l for 60 min. They were then washed twice with Eagle's medium and the incubation continued for 120 min in the presence of 28 μmol/l ^{86}RbCl. The cells were briefly rinsed,

trypsinized (0.1%) and transferred to scintillation vials to which scintillation fluid was added. Radioactivity was determined in a liquid scintillation counter and the number of counts of treated cells was compared to that of untreated cells.

Materials

Epirubicin (4'-epidoxorubicin) as a crystalline powder was obtained as a kind gift from Farmitalia Carlo Erba, Milan, Italy. Methyl-^{14}C-thymidine and methyl-^{3}H-thymidine, and ^{86}RbCl was from Amersham International, Amersham, Buckinghamshire, UK. Chlorpromazine was from Sigma Chemical Co., St Louis, MO, USA. Eagle's minimal essential medium (Eagle's MEM) was from GIBCO Ltd, Paisley, Scotland, UK. Fetal calf serum was obtained from Biochrom KG, Berlin, Germany. All other chemicals were of analytical grade.

Statistics

Statistical significance was tested with Wilcoxon's rank sum test. The level of significance for rejecting the null hypothesis of zero treatment effect was taken to be $P = 0.05$.

RESULTS

Epirubicin caused a dose-dependent decrease in ^{86}Rb accumulation during 2 h and also a dose-dependent decrease in the amount of precipitated DNA (Figs. 1 and 2).

Chlorpromazine (0.1 mg/l) augmented the inhibitory effect of 10 mg/l epirubicin on ^{86}Rb accumulation of the fibroblasts (Table 1) and also augmented the decrease of the amount of DNA precipitated in the presence of 25 mg/l epirubicin (Table 2). Chlorpromazine at a lower concentration (0.01 mg/l) was without an enhancing effect on epirubicin toxicity as measured with both methods.

DISCUSSION

Chlorpromazine, as do phenothiazines in general, has many diverse actions. Apart from effects of its own, chlorpromazine enhances the activity of a number of analgetic and central depressant drugs, and also markedly affects the actions of other

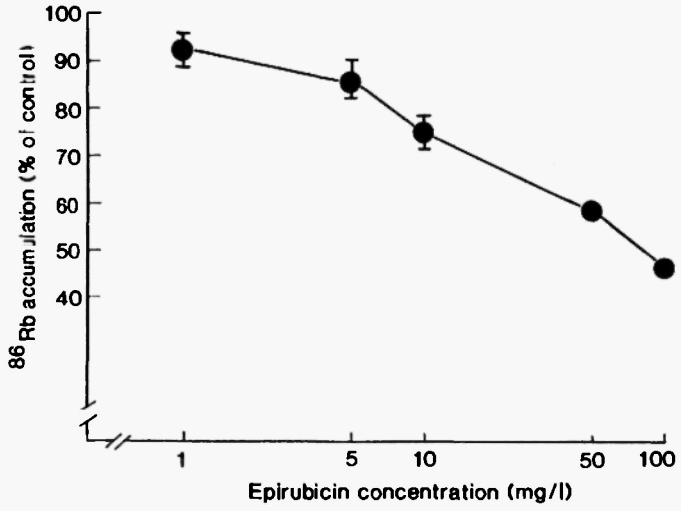

Fig. 1: Percentage of accumulated ^{86}Rb (% of control) after 1 h treatment with epirubicin.

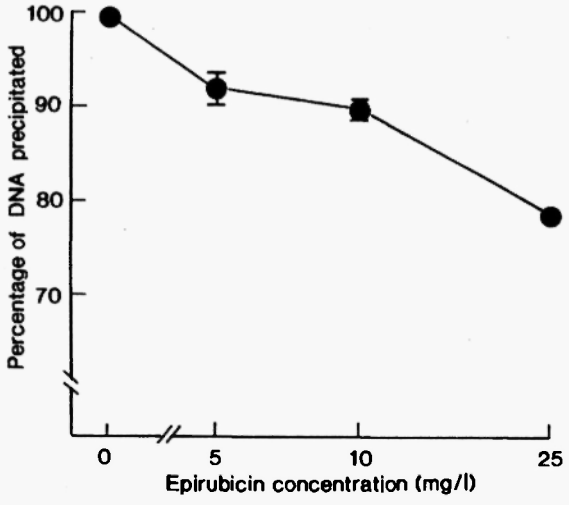

Fig. 2: Percentage of DNA precipitated after treatment with epirubicin for 1 h.

TABLE 1

^{86}RbCl accumulated for 2 h (% of untreated controls) after 30 min pre-incubation with/without chlorpromazine followed by epirubicin (10 mg/l) for 60 min

	Controls	Epirubicin
--	--	86.4 ± 1.7 (15)
Chlorpromazine (0.1 mg/l)	97.4 ± 3.6 (8)	77.0 ± 0.8* (15)
Chlorpromazine (0.01 mg/l)	98.0 ± 3.1 (8)	83.8 ± 2.6 (15)

Mean ± S.E.M.
* $p < 0.02$ against epirubicin alone

TABLE 2

Percentage of DNA precipitated after 30 min pre-incubation with/without chlorpromazine followed by epirubicin (25 mg/l) for 60 min

	Controls	Epirubicin
--	99.9 ± 0.2 (30)	90.1 ± 1.1 (30)
Chlorpromazine (0.1 mg/l)	--	87.0 ± 1.1* (30)
--	99.6 ± 0.2 (25)	89.9 ± 0.6 (25)
Chlorpromazine (0.01 mg/l)	--	91.4 ± 0.6 (25)

Mean ± S.E.M.
* $p < 0.002$ against epirubicin alone

drugs /6/. In the present study, the complexity of chlorpromazine action was further shown by its enhancement of the effects of epirubicin in causing damage to DNA and the cell membrane.

The mechanisms of action of the interaction of chlorpromazine and the antineoplastic drugs are difficult to outline. The drugs known to be affected by chlorpromazine, e.g., nitrosoureas /2/, epirubicin and bleomycin /1/, are chemically and pharmacologically different. Apart from enhancing the cytotoxic effect of epirubicin, chlorpromazine has also been shown to increase by more than 5-fold the cytotoxicity of vinca alkaloids /7/. Calmodulin inhibitors, such as chlorpromazine, have also been suggested to affect the efflux of anthracyclines from mast cells, thereby affecting the intracellular anthracycline concentration /8/. Phenothiazines enhance the cellular retention and cytotoxicity of adriamycin in P338 cells /9/.

The generation of free radicals is believed to be of prime importance in the antitumoural and toxic actions of anthracyclines /10-12/. However, it has been suggested that oxygen radicals are not involved in the direct interaction between epirubicin and antiemetics /13/. The exclusion of free-oxygen radicals in the mechanism of interaction with chemotherapeutic toxicity by chlorpromazine is further supported by the fact that the action of nitrosoureas is not generally associated with oxygen radicals, and that bleomycin cytotoxicity, linked to induction of oxygen radicals, seemed not to be affected by chlorpromazine /1/. On the other hand, chlorpromazine, one of the most useful phenothiazine analogues, is known to be easily oxidized by either a metal ion (Fe^{3+}) or horseradish peroxidase or catalase, yielding a coloured intermediate, a cation radical /14/. Other plausible explanations, such as an elevated cellular accumulation by inhibition of outward transport, which has been seen following, e.g., calcium modifiers /15,16/, or an interaction with DNA repair as has been suggested for metoclopramide potentiation of cisplatin toxicity /17/, could partially explain the results. As chlorpromazine is also a calmodulin antagonist, it has been suggested that chlorpromazine action is related to the maintenance of cellular calcium homeostasis and membrane integrity /18,19/. Chlorpromazine has been shown to interact with various components of the cytoplasmic network, including calmodulin /20-22/, and to have profound effects on membrane structure and function /23,24/. At high concentrations, ca. 10^{-6} M, chlorpromazine is cytotoxic, causing cytoplasmic vacuolization and membrane blebbing and fragmentation. Chlorpromazine enhancement of epirubicin cytotoxicity, measured as cloning survival, ^{86}Rb accumulation, or DNA strand breakage,

may be due to its calmodulin antagonistic action and could indicate that clinical studies on chlorpromazine and other antiemetics during antineoplastic treatment are justified to evaluate the significance of the interaction results found *in vitro*.

In conclusion, the present results suggest that the enhancement by chlorpromazine of epirubicin cytotoxicity found earlier involves damage to both DNA and cell membranes. To determine whether one of these actions is primary will require further studies.

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