Antimutagenic activity of fluphenazine in short-term tests

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Fluphenazine, an antipsychotic drug that belongs to the phenothiazine family, reduced the genotoxicity of standard direct- and indirect-acting mutagens in the Ames test, both in the presence and in the absence of promutagen-activating S9 fraction. In short-term tests on human lymphocytes, the inhibitory effect of fluphenazine on the genotoxicity of standard mutagens was strongest in the cytokinesis-blocked micronucleus assay and in the thioguanine-resistance test, and weakest in the sister chromatid exchange test. Fluphenazine also considerably reduced the level of free radicals estimated in in vitro samples of human granulocytes. The results suggest that, in the range of the tested concentrations, fluphenazine could be considered for use to prevent the genotoxicity of daunorubicin, methyl methanesulfonate, benzo[a]pyrene and mitomycin C. Reduction in the level of free radicals appears to be an important mechanism of the antimutagenic action of fluphenazine.

Introduction

Analysis of clinical data, in which unexpected beneficial side-effects of commonly used drugs were discerned, has led to the identification of several antimutagenic and cancer-chemopreventive compounds among old drugs. Similarly, it has been shown that phenothiazines significantly reduce the incidence of cancer among schizophrenic patients treated with them (Katz et al., 1967; Jones, 1985). This suggested that phenothiazines could have antimutagenic and cancer-chemopreventive activity in humans. The phenothiazine family includes major tranquilizers used to treat psychotic disorders, such as schizophrenia, mania, paranoid syndromes and alcoholic hallucinosis. The finding that phenothiazines reduce the incidence of cancer in humans generated a broad range of experimental studies, which confirmed their antitumor, cancer-chemopreventive and antimutagenic activities (e.g. Jones, 1985; Leszek et al., 1988; Motohashi et al., 1991; Tanaka et al., 1997).

Fluphenazine is a member of the phenothiazine drug family and is often used in the treatment of various psychoses, mainly schizophrenia and paranoid syndromes. However, the fact that it is a member of this family does not mean that it will also have antimutagenic and cancer-chemopreventive action. Published studies on fluphenazine’s genotoxicity in short-term tests and on its effects on the genotoxicity of standard mutagens have not provided an unequivocal answer. In the standard bacterial Ames test, fluphenazine, like other tested phenothiazines, reduces the mutagenicity of benzo[a]pyrene (Calle and Sullivan, 1982; Kittle et al., 1981). On the other hand, fluphenazine induces micronuclei in mouse bone marrow cells (Rao and Rao, 1981) and increases the frequency of sister chromatid exchanges and chromosomal aberrations in human peripheral lymphocytes in vitro (Shafer et al., 1987). It also suppresses unscheduled DNA synthesis (UDS) in hypothalamic cells of rats chronically treated with the drug (Šram et al., 1990). Since fluphenazine is used in humans in the treatment of various psychoses, these doubts should be dispelled and the drug’s action on mutagenesis and carcinogenesis clarified.

Fluphenazine is a piperazine phenothiazine that is structurally similar to perphenazine and prochlorperazine (Budavari, 1996). Its general chemical formula is given in Figure 1. The chemical structure of fluphenazine suggests that it could form complexes with and/or reduce chemical mutagens and also scavenge free radicals.

The aim of this study was to evaluate in vitro the effect of fluphenazine on the genotoxicity of standard mutagens in a battery of short-term tests, including the bacterial Ames test and three short-term assays on human lymphocytes. The effect of fluphenazine on the generation of free radicals in human granulocytes was also estimated, because of the importance of free radicals in mutagenesis and carcinogenesis (e.g. see Troll and Weisner, 1985; Marnett, 1987; Cavaliere and Rogan, 1992; Frenkel, 1992) and because the chemical structure of fluphenazine suggested it might have radical-scavenging activity.

The lymphocyte short-term assays were carried out with cells separated from the venous blood of psychiatric clinic patients to whom fluphenazine had been prescribed as the main drug for treatment of their mental disorder. Since these patients were also heavy smokers, and thus would have been exposed to higher concentrations of genotoxic agents, we intended to check whether fluphenazine would be effective in decreasing the genotoxicity of standard mutagens in their blood cells in vitro.

Materials and methods

Chemicals
Fluphenazine–HCl, analytical grade, was kindly supplied by Jelfa (Jelenia Góra, Poland). B(α)P, daunorubicin (DRC), methyl methanesulfonate (MMS), cytochalasin B, mitomycin C (MMC), 5-bromo-2’-deoxyuridine (BrdUrd), phorbol 12-myristate 13-acetate (PMA) and dimethyl sulfoxide (DMSO) were purchased from Sigma (St Louis, MO). Aroclor 1254 was from Analabs (New Haven, CT). In the preparation of bacterial growth media, Oxoid Nutrient Broth no. 2 (Oxoid, Basingstoke, UK). Difco Bacto Nutrient Broth and Difco Agar (Difco, Detroit, MI), glucose 6-phosphate and NADP (Sigma) were used. Blood-cell separation solutions (Histopaque-1077 and Histopaque-1119) and components of cell culture media [RPMI 1640, fetal calf serum (FCS) and l-glutamine] were obtained from Sigma. Phytohemagglutinin (PHA-M) was obtained from Gibco (Gaithersburg, MD). Acridine Orange, ethidium bromide, Azur II, eosin B, Trypan Blue, Giemsa solution and nitroblue tetrazolium (NBT) were purchased from Sigma. Other reagents used for preparing buffers and culture media were from POCH (Gliwice, Poland).

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The effect of fluphenazine on the mutagenic activity of standard mutagens was estimated using the *Salmonella typhimurium* tester strains recommended for particular mutagens (Ames et al., 1975; Maron and Ames, 1983). The *S. typhimurium* strains TA 98 and TA 100 were kindly supplied by Professor B.N.Ames. The mutagenic activities of mixtures containing the tested mutagen and fluphenazine were assayed following the routine Ames protocol (Maron and Ames, 1983) in the presence or absence of promutagen-activating fraction S9. The fraction was prepared according to Ames et al. (1975) from the liver of Aroclor 1254-treated male Wistar rats. The fluphenazine was dissolved in distilled water and added at 5, 10, 25 or 50 µg/plate, in consecutive plates. B[a]P and MMS were dissolved in DMSO, while DRC was dissolved in distilled water; these standard mutagens were added to tester plates at the concentrations recommended by Maron and Ames (1983): B[a]P, 2.5 µg/plate; MMS, 0.5 µg/plate; DRC, 0.5 µg/plate. The tested mixtures contained 0.1 µl of mutagen solution, 0.1 µl of fluphenazine solution, 0.1 µl of overnight cultures of the *S. typhimurium* tester strain and 0.5 µl of the S9 mix (10% S9, v/v) or 0.5 µl of phosphate buffer (pH 7.4), and 2 µl of top agar. The mixtures were poured on to minimal glucose agar plates. To determine the number of surviving bacterial cells, 0.1 µl of each tested mixture was diluted 10-fold and then poured on to nutrient agar plates. The numbers of revertants and viable cells were counted after incubation for 2 days at 37°C. The number of surviving bacterial cells was calculated as the number of viable cells/plate multiplied by 10² (Maron and Ames, 1983). The results obtained in the Ames test (mean ± SD; n = 6) are presented in histograms as the net number of his⁺ revertants, i.e. the number after subtraction of spontaneous revertant numbers.

Separation of blood cells

Heparinized blood (20 ml) was obtained by venipuncture from five males aged 43–50 years, each of whom smoked 20–30 cigarettes/day. The blood donors were all from the psychiatric clinic (none had been admitted previously); two of them were diagnosed as suffering from schizophrenic psychosis, and the others from non-specific paranoid syndrome suffering from a psychiatric disorder, the patients were generally healthy. The blood cells were separated by a single-step discontinuous density-gradient centrifugation technique with Histopaque-1119 and Histopaque-1077 layers (English and Anderson, 1974). Isolated lymphocytes and granulocytes were washed in phosphate-buffered saline (PBS), pH 7.2.

Estimation of fluphenazine doses with lymphocyte survival test

The effect of fluphenazine on the viability of human lymphocytes in 4 and 18 h cultures was assessed with the standard Trypan Blue exclusion test and PHA-M (1% v/v) in the presence of 2 µg/ml ethidium bromide. Briefly, 0.1 ml of mutagen solution, 0.1 ml of fluphenazine and 0.1 ml of lymphocyte suspension (0.8 × 10⁶ cells/ml) were added to the culture in a volume of 50 µl to obtain the desired final concentration. We did not apply an external promutagen activating fraction, because freshly prepared human lymphocytes have a microsomal enzymatic system which activates polyaromatic hydrocarbons to form genotoxic derivatives (Guengerich, 1992; Goldstein and Faletto, 1993). Fluphenazine, dissolved in distilled water, was added simultaneously to the culture in a volume of 50 µl to obtain the final concentrations in the culture medium. The lymphocytes were incubated for the last 48 h of the culture time at a final concentration of 30 µM. The cultures were harvested according to the standard cytogenetic method. Cell smears on glass slides were air-dried for 3 days. Afterwards, the slides were immersed in 2× SSC (0.03 M sodium citrate in 0.3 M sodium chloride) at 62°C for 30 min and, simultaneously, they were illuminated under a UV lamp (Perry and Wolff, 1974). The slides were then stained with a mixture of dyes (azur II/ eosin) to reveal the differential chromatic staining (Antoshina and Poriadkova, 1978) and examined under a microscope. The mean number of sister chromatids per metaphase among 25 metaphases was calculated for each slide analysed.

Cytogenetic assessment of lymphocyte proliferation

The replication indices (RIs) of lymphocyte cultures were estimated by counting the number of metaphases that were in the first (M₁), second (M₂) or third (M₃) division in the presence of BrdUrd and calculated according to the formula RI = (M₁ + 2M₂ + 3M₃)/100. The mitotic indices (MIs) were determined in each culture’s slide within 1000 cells found randomly by microscopy examination. The proliferation potential was calculated as the product of MI and RI for each lymphocyte culture.

Cytokinesis-blocked micronucleus assay

The lymphocyte micronuclear assay was performed by cytokinesis-blocked assay in accordance with standard procedures (Fenech, 1993; Lee et al., 1994). Briefly, lymphocyte cultures were stimulated with the lectin PHA-M (1% v/v) and the standard micronucleus-inducing clastogenic agent MMC, was added to the culture medium to give a final concentration of 0.03 µg/ml. Fluphenazine dissolved in water was added to the cultures in a volume of 50 µl to give the required final concentration. Control cultures contained the same volume of water instead of MMC and/or fluphenazine. MMC and fluphenazine were present in the culture medium for the whole period of culture, i.e. for 72 h. Lymphocytes were exposed to a cytokinesis-blocking agent, cytochalasin B, added to give a final concentration of 0.8 µg/ml in culture medium, for the last 48 h of this culture period. The cultures were harvested according to Lee et al. (1994) with mild hypotonic treatment (0.075 M KCl for 10 min) followed by fixation with cold methanol:acetic acid (3:1). The cell suspension was gently spread on microscope slides, dried and stained with 10% Giemsa solution (in PBS, pH 6.8) for 5 min. Slides were then air-dried under a microscope and the number of micronuclei in 1000 binucleated lymphocytes was counted.

Thioguanine-resistance assay

Lymphocytes were suspended in culture medium and stored in a plastic culture bottle for 18 h at 4°C to prevent phenocopies. They were then suspended in fresh medium and cultured for 48 h according to published protocols (Ostrosky-Wegman et al., 1987; Montero et al., 1993), with minor modifications. Briefly, 0.8 × 10⁶ cells/ml were cultured in 24-well plastic dishes and stimulated with PHA-M (1% v/v) in the presence of 2 × 10⁻³ M thioguanine. The tested mutagen, B[a]P (16 µM), and supposed antimitagen, fluphenazine (concentration range: 0.625–5 µM), were added at the start of culture. After 24 h, BrdUrd (30 µM) was added to the cultures for 24 h. The harvesting procedure comprised centrifugation of the cultures, fixation of pellets with 75% ethanol for 30 min, treatment with lysis solution (0.2 mg/ml RNase; 0.5 mM disodium EDTA and 0.5% Nonidet P-40) for 30 min at 10°C. For partial denaturation of DNA, the cell pellets were resuspended in 0.5 N HCl, kept at room temperature for 30 min and washed twice with PBS; smears were prepared on microscope slides. Cells in which BrdUrd had been incorporated into the DNA were detected by an immunocytochemical procedure using a monoclonal mouse antibody able to recognize BrdUrd in single-stranded DNA (clone Bu20a, DAKO, Glostrup, Denmark). Subsequent visualization and staining were performed using the alkaline phosphatase-anti-alkaline phosphatase (APAAP) technique (Cordell et al., 1984; Vanderlaan and Thomas, 1985; Magaud et al., 1988). Thioguanine-resistant lymphocytes (which become intensely stained red) were counted among 5000 cells randomly found under the microscope.
Estimation of granulocyte superoxide radicals

The influence of fluphenazine on superoxide radical generation by human granulocytes in vitro was evaluated using the routine NBT reduction test (Metcalf et al., 1986), both in the presence and in the absence of the standard granulocyte stimulator (PMA; 100 ng/ml) and, separately, in the presence of the promutagen B[a]P (16 µM). Granulocytes were suspended at a density of $2 \times 10^9$ cells/ml in PBS, pH 7.2, containing NBT (final concentration 0.2%) and the desired concentration of fluphenazine and/or the tested agent (PMA, B[a]P). After incubating samples for 45 min at 37°C in a shaking water bath, the reaction was terminated and the cells were centrifuged at 4°C. The pellets were then lysed with dimethylformamide at 56°C in a shaking bath and absorption ($A_{515nm}$) relative to that of cell-free blank samples was measured using a spectrophotometer.

Statistical analysis

The results obtained with the Ames test were examined by means of the standard r-test to determine their statistical significance. Regression equations were calculated to see whether the observed effects of fluphenazine on lymphocytes and granulocytes were significantly dose-related. For this purpose, we estimated if the slopes of the regression curves ($a_i$) were significantly different from zero. Multifactor analysis of variance (MANOVA) was performed in order to assess the significance of differences between various test systems and to confirm the significance of dose–response relations. All statistical estimations were computed using Systat software (Evanston, IL, USA).

Results

We evaluated the effect of fluphenazine on the genotoxicity of standard mutagens in the Ames test. When used alone or together with a mutagen, fluphenazine at concentrations of $\leq 50$ µmol/plate did not inhibit the growth of bacterial cells, whereas at higher concentrations it had a considerable inhibitory effect. Therefore, the highest dose of fluphenazine evaluated by us in the Ames test was 50 µmol/plate.

The effects of fluphenazine on the standard indirectly acting mutagen, B[a]P, and two directly acting mutagens, MMS and DRC, are shown in Figure 2. Fluphenazine moderately decreased the genotoxic effect of B[a]P in the Ames test. At the highest fluphenazine concentration tested (50 µmol/plate), there was a 20% decrease in B[a]P mutagenic activity (Figure 2A). In the case of directly acting mutagens, tested without the presence of S9 fraction (Figure 2C and D), fluphenazine had a weak effect on the genotoxicity of MMS (causing a 14% decrease at the highest concentration tested); in the case of DRC, the inhibitory effect of fluphenazine was stronger (by almost 50%) at the highest concentration tested. We also tested the genotoxicity of DRC in the presence of the S9 fraction and the effect of fluphenazine on the mutagenic activity of DRC in the TA 98 + S9 system. The results are shown in Figure 2B. As may be seen, fluphenazine decreased the mutagenic action of DRC in the presence of S9 fraction by ~25% at the highest concentration tested, and the inhibitory effect was concentration-dependent.

In human lymphocyte cultures we estimated the background genetic damage caused by 16 µM B[a]P and 0.03 µg/ml MMC by assaying four different end-points. The results obtained using lymphocytes from five blood donors were collected and are given in Table I as a mean ± SD. The results presented in Table I were taken as reference values ($E_{0i}$) with which we compared data on the effect of fluphenazine on the genotoxicity of standard mutagens ($E/E_{0i}$ ratio).

The effect of fluphenazine on the mutagenicity of 16 µM B[a]P or 0.03 µg/ml MMC to human lymphocytes in vitro is shown in Figure 3 in the form of linear regression lines describing the dependence of effects on the concentration of fluphenazine in a culture medium. As may be seen from this figure, the inhibitory effect of fluphenazine was greatest in the CBMN assay (~70% decrease at the highest fluphenazine concentration), moderate in the thioguanine resistance test (25% decrease) and weakest (~20% decrease) in the sister chromatid exchange test. For direct comparison, the influence of fluphenazine on the proliferation potential of the B[a]P-treated lymphocytes is also shown in Figure 3. Fluphenazine decreased the proliferation rate of B[a]P-treated lymphocyte cultures by 50% at the highest concentration tested (5 µM) as compared with the control cultures (containing only B[a]P). The slopes of the regression lines shown in Figure 3 were of negative direction and were significantly different from zero.

MANOVA showed that the effect of fluphenazine on lymphocyte cultures was significantly concentration-dependent ($F$ = 243.29; three degrees of freedom; $P < 10^{-6}$). It also estimated significant differences between the applied tests in their description of the fluphenazine effect ($F$ = 810.56; three degrees of freedom; $P < 10^{-6}$).

The level of free radicals in control granulocyte samples was estimated spectrophotometrically; the $A_{515nm}$ (mean ± SD; $n = 5$) was as follows: sample containing PMA (100 ng/ml), 0.117 ± 0.0111; sample containing 16 µM B[a]P, 0.08 ± 0.0094; sample containing solvent only (DMSO, 10 µl), 0.055 ± 0.0089. We took these results as reference levels ($E_{0i}$), with which the results of fluphenazine impact were compared, and presented as an $E/E_{0i}$ ratio.

The effect of fluphenazine on the level of free radicals in human granulocytes is shown in Figure 4. In samples where solely fluphenazine was present (spontaneous release, without PMA) the level of free radicals was 55% lower than in control samples. In the granulocyte samples in which the standard free radical-cascade activator (PMA, 100 ng/ml) was present, fluphenazine reduced the level of free radicals by 25% (at 10 µM) compared with the samples in which solely PMA was present. In the presence of the promutagen B[a]P (16 µM), fluphenazine reduced the level of free radicals in granulocytes by ~40% at the highest concentration tested. Regression equations showed that the inhibition by fluphenazine was strongly concentration-dependent; the slopes of the regression lines were significantly different from zero. Analysis of variance confirmed this concentration-dependence ($F$ = 200.02; four degrees of freedom; $P < 10^{-6}$).

Discussion

The question of the genotoxicity and/or anti-genotoxicity of fluphenazine still remains controversial, some reports suggesting that it is mutagenic while others suggest that it is anti-mutagenic. Extensive work with the Ames test showed that phenothiazines were not mutagenic in vitro either in the presence or in the absence of microsomal S9 fraction (Balbi et al., 1980). On the other hand, in short-term human lymphocyte tests, fluphenazine at concentrations of 2.5–10 µg/ml (i.e. 4.89–19.59 µM) significantly increased chromosome damage and the frequency of sister chromatid exchange (Shafer et al., 1987); however, these authors acknowledged that the tested doses were high and notched the need for low-dose studies.

In our initial experiments (data not shown), the genotoxicity of fluphenazine was evaluated by means of a sister chromatid exchange test in human lymphocyte cultures at concentrations ranging from 0.625 to 5.0 µM (i.e. 0.319–2.55 µg/ml). The calculated regression equation showed that changes in the frequency of sister chromatid exchange did not depend on the fluphenazine concentration in the culture medium ($F =$
Fig. 2. Effect of fluphenazine (FPh) on the mutagenicity of (A) B[a]P, (B and D) DRC and (C) MMS in the Ames test. The promutagen-activating fraction 59 was present (A and B) or absent (C and D) in the test. Results are means ± SD (n = 6). Statistical significance (t-test) of the differences between results obtained in plates with fluphenazine and without fluphenazine (relative controls):

***P < 0.001, **P < 0.01, *P < 0.05.

2.526; P = 0.1872; not significant). The discrepancy between our results and previously cited results may have been caused by the difference in the ranges of fluphenazine concentrations tested. We chose low concentrations (≤5 µM), since at 10 µM (i.e. 5.1 µg/ml) a considerable increase in the number of dead lymphocytes occurred.

The antimutagenic action of fluphenazine is another issue. It has been documented in the literature that phenothiazines and its derivatives inhibit the mutagenicity of B[a]P in the Ames test (Kittle et al., 1981; Calle and Sullivan, 1982). Our results obtained with the Ames test showed that fluphenazine had a rather mild antimutagenic effect on the genotoxicity of
Antimutagenicity of fluphenazine

Table I. Background level of genotoxic damage induced by the standard mutagens B[α]P (16 μM) and MMC (0.03 μg/ml) in lymphocytes from five blood donors as estimated with four end-point assays

<table>
<thead>
<tr>
<th>Test</th>
<th>Standard mutagen</th>
<th>Estimated end-point</th>
<th>Genotoxic effect (mean ± SD; n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sister chromatid exchange</td>
<td>B[α]P</td>
<td>frequency of chromatid exchanges per metaphase</td>
<td>39.6 ± 4.36</td>
</tr>
<tr>
<td>Thioguanine resistance</td>
<td>B[α]P</td>
<td>frequency of resistant cells × 10⁶</td>
<td>78.4 ± 6.28</td>
</tr>
<tr>
<td>Cytokinesis-blocked micronuclei</td>
<td>MMC</td>
<td>percentage of binucleated lymphocytes containing micronuclei</td>
<td>5.6 ± 1.01</td>
</tr>
<tr>
<td>Proliferation potential</td>
<td>B[α]P</td>
<td>MI × RI</td>
<td>4.7 ± 1.08</td>
</tr>
</tbody>
</table>

![Image](image.png)

Fig. 3. Effect of fluphenazine (FPh) on the proliferation potential (PP), sister chromatid exchanges (SCEs), cytokinesis-blocked micronuclei (CBMN) and thioguanine resistance in human lymphocytes cultured in vitro in the presence of standard mutagens. In the proliferation potential, sister chromatid exchange and thioguanine resistance tests, 16 μM B[α]P was present in each culture, whereas in the CBMN assay 0.03 μg/ml MMC was the standard mutagen. The results obtained in the presence of fluphenazine and mutagen (E) are compared with the results of the relative positive control cultures (E₀), i.e. in the presence of mutagen only. The dose-dependent effect of fluphenazine was calculated with regression equations; statistical evaluation of regression coefficients is shown in the lower part of the figure.

three standard mutagens; the effect was statistically significant at higher drug concentrations (i.e. 25-50 μM).

In the case of promutagen, B[α]P, the decrease in mutagenicity found in the Ames test could be explained by fluphenazine inhibiting free radical pathways during mutagen processing by enzymes in the S9 fraction. The S9 microsomal fraction from rat livers contains mixed-function oxidases, including aromatic hydrocarbon hydroxylase, which can transform B[α]P and other aromatic hydrocarbons to genotoxic metabolites by two-electron oxidation processes (Gelboin, 1980; Frenkel, 1992). Also the family of cytochrome P450 oxidases can metabolize B[α]P and other aromatic hydrocarbons by one-electron oxidation, yielding genotoxic free radicals (Cavalieri and Rogan, 1992; Frenkel, 1992). Those oxidation pathways could be inhibited by fluphenazine, since it is able to decrease free radical levels.

At present it is difficult to explain the observed antimutagenic effects of fluphenazine against the directly acting mutagens DRC, MMC and MMS. Indeed, several papers have proved that directly acting mutagens, such as MMC and DRC, are modified inside the cell by intracellular oxido-reductases (Farmer, 1985; Parke, 1987). We noticed that the mutagenic activity of DRC was almost 30% higher in the presence of the S9 fraction. However, the effect of fluphenazine on the mutagenicity of DRC was weaker in the presence of the S9 fraction than in the absence of the S9 (decrease by 25% and 50%, respectively). This suggests that inhibition of the S9 free radical pathways by the drug may not be the only mechanism of fluphenazine’s antimutagenic action against the genotoxicity of DRC, since when no S9 fraction was added, the antimutagenic effect of fluphenazine was even stronger. However, inhibition of free radical pathways would not be the mechanism of the antimutagenic action exerted by fluphenazine against the simple alkylating agent, MMS. Thus, a search for mechanisms of fluphenazine’s antimutagenic activity, besides the anti-free radical action, has been undertaken.

In human lymphocyte cultures we noticed that addition of fluphenazine to cells cultured in the presence of 16 μM B[α]P reduced the proliferation potential by 50% at the highest dose of the drug (5 μM). A detailed analysis of the proliferation potential showed a marked (~40%) decrease in mitotic index and a mild (15%) decrease in replication index. At the highest concentration of drug, an increase in M₁ metaphases (by 13%) and decrease in M₃ metaphases (by ~20%) occurred. A marked inhibition of proliferation by fluphenazine was also confirmed by the data from the CBMN assay, which showed that the fraction of binucleated cells decreased by almost 30% at the highest concentration of drug. It should be remembered that, in the range of tested concentrations (0.625–5 μM), fluphenazine was not cytotoxic to lymphocyte cultures, either when added separately or together with B[α]P or MMC. Thus, we excluded the possibility that fluphenazine was cytotoxic alone or when added together with standard mutagens.

When cells are genetically damaged, a decrease in proliferation rate may help detoxification and repair of damage caused by mutagens (Ames et al., 1993; Kaston and Kuerbitz, 1993; Tomatis, 1993). In response to DNA damage, the p53 gene becomes activated, and this triggers the transcription of genes involved in cell cycle arrest and/or apoptosis (e.g. Darnton, 1998; Taylor et al., 1999). If apoptosis is induced in genetically damaged cells by ‘genome guardians’ (the best-known of which is the p53 gene product), this will lead to selective elimination of those cells that are unable to repair their DNA damage (e.g. Smith and Fornace, 1996; Wang, 1998; Wylie...
Thus, apoptosis can be perceived as ‘apoptotic repair of genotoxicity of mutagens towards lymphocyte cultures. The applied tests measure different end-points of genotoxicity: point mutations (the thioantimutagenic action strongly depended on the concentration of the drug in lymphocyte cultures, and showed that the applied tests differed significantly in their description of the fluphenazine antimutagenic action. The differences between the tests suggested that various mechanisms unequally contributed to the final antimutagenic effect of fluphenazine. The detailed mechanisms of the fluphenazine antimutagenic action remain to be investigated in future.

We tried to confirm one possible mechanism of fluphenazine’s antimutagenic action, i.e. inhibition of free radical pathways. The results demonstrated that free radical generation by human granulocytes in vitro was considerably inhibited in the presence of fluphenazine. The regression equations and analysis of variance showed that fluphenazine’s inhibitory effect strongly depended on the concentration of the drug in the samples.

The activation of a granulocyte oxidative burst caused by PMA is a consequence of the phorbol ester binding and activating protein kinase C, a mediator of the major cellular signal transduction pathway (Blumberg, 1988; Tauber et al., 1989). PMA binding and overactivation of the kinase can explain a vast number of PMA biological activities, including its influence on cellular proliferation, differentiation, carcinogenesis and tumor promotion (Blumberg, 1988). Phenothiazines competitively inhibit the interaction between phorbol esters and their specific cellular receptors (Shoyab et al., 1982), which could be interpreted as their potentially antitumor-promoting action. Our results describing the inhibitory action of fluphenazine on the granulocyte free radical-generating system in the presence of PMA may also suggest an antitumor-promoting potency of the drug. Fluphenazine also decreased considerably the level of granulocyte oxygen free radicals in the presence of B[a]P. The B[a]P-derived free radicals appear to make up a significant part of the genotoxic by-products (Dix and Marnet, 1981; Cavalieri and Rogan, 1992) and may add their genotoxic action to DNA damage caused in vivo by B[a]P metabolites formed with a bay-region diol epoxide (Nesnow et al., 1995). The decrease caused by fluphenazine in the level of free radicals in the presence of B[a]P might
indicate that the drug inhibits the free radical activation of this promutagen.

Summing up the results presented in this paper, we conclude that in the range of the tested concentrations, fluphenazine exhibited antimutagenic activity in vitro, and undoubtedly should be studied further to elucidate the mechanisms of its effect on mutagenesis and carcinogenesis in vivo. The antimutagenic potency of fluphenazine in humans requires separate studies. The first step of such studies could be evaluation of the level of genotoxic damage by standard mutagens in vitro in lymphocytes isolated from schizophrenic patients treated with fluphenazine for various periods of time. However, at present the psychiatrists who prescribe fluphenazine as an anti-psychotic drug should also consider its additional, beneficial effect—a possible decrease in mutation frequency, which seems especially important for patients heavily exposed to mutagens.

References


Dix,T.A. and Marnet,L.J. (1981) Free radical epoxidation of 7,8- antipsychotic tricyclic compounds competitively inhibit the interaction 244 of phenothiazine as an anti-psychotic drug should also consider

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