Effect of chlorpromazine on intact and irradiated aliquot ctdsDNA samples

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Summary:

Bakground:*Chlorpromazine is widely used in human medicine in the therapy of schizophrenia, organic psychosis and the manic phase of manic depressive illness. It expressed a selective cytotoxicity and the results of genotoxicity were positive.*

Objectives: *This study is designed to explore the effect of chlorpromazine on irradiated and non irradiated calf thymus double strands DNA (ctdsDNA) molecule.*

Methods: *Aliquots of irradiated (subjected to UVB light) and non-radiated ctdsDNA samples were incubatyed with different concentrations of chlorpromazine. Further series of experiments studied the simultaneous effects of chlorpromazine and UVB light on aliquots of ctdsDNA, The changes in optic densities of ctdsDNA aliquots were mointered and recorded bu UV-spectrophotometer at 260 nm.*

Results: *Chlorpromazine exerts dual effects on non-radiated ctdsDNA aliquots represented by hyperchromasia and hypochromasia in regard to its concentration. It potentiates the effect of UVB radiation on ctdsDNA molecules. Its effect is differed in respect to the radiation status.*

Conclusion: *chlorpromazine exerts several effects on aliquot ctdsDNA samples which are related to the nature of DNA molecule as well as to the concentration of chlorpromazine.Also chlorpromazine potentiates the hyperchromasic effect of UVB radiation on aliquot ctdsDNA samples but it produces completely damage of DNA molecule when the aliquot ctdsDNA samples irradiated in presence of chlorpromazine.*

Introduction:

Introduction of chlorpromazine was the breakthrough in psychiatric practice.

Pharmacotherapy with chlorpromazine started in Polish hospitals in the autumn and winter in 1954 and the first papers covering clinical experiences started to be published in 1955.

It belongs to the group of phenothiazine derivatives [Fig. 1]. It is widely used in human medicine in the therapy of schizophrenia, organic psychoses and the manic phase of manic-depressive illness. In veterinary medicine it is used as a tranquillizer and antiemetic agent.

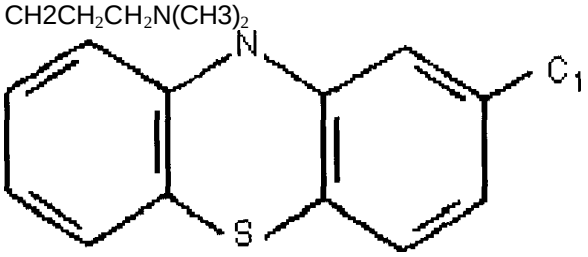

Fig. I. Chemical structure of chloropromazine

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Although the results of chloropromazine genotoxicity were positive (Jin-fu *et al* 1988; Obaseiki-Ebor & Akerele 1988), its effect on DNA molecule is varied in respect to the experimental model. In vitro, de-Mol and Maanders (1983) demonstrated non-covalent binding of chlorpromazine to calf thymus DNA by using equilibrium dialysis technique. Chlorpromazine and related phenothiazines stimulate luciferase DNA uptake expression at 10^{-5} M in Hela cell model as well as it interacts with plasmid DNA and DNA-polylysine complexes (Hawtrey *et al* 2002). Partial dissociation of intercalated calf thymus (ctDNA) complexes with ethidium bromide (EB) is observed with chlorpromazine (Hawtrey *et al* 2002).

Phenothiazines including chlorpromazine in clinically relevant doses (up to 20 µM) expressed a selective cytotoxicity and antiproliferative activity. It induced apoptosis in leukemic cells lines without any influence on the viability of normal lymphocytes (Zhelev *et al* 2004). It induced DNA fragmentation in almost all leukemic cell lines during a 48-h incubation. Chlorpromazine mediates apoptosis and DNA breaks when it incubated with human lymphoblasts through specific activation of intracellular proapoptotic signaling cascades (Hieronymus *et al* 2000). Other studies showed that, *in vivo*, chlorpromazine showed no effects on DNA (i.e not induced DNA fragmentation) after a single dose at half the LD_{50} in mice (Carlo *et al* 1986). Tsutsui *et al* 2003 demonstrated that

chlorpromazine as a calmodulin inhibitor, inhibits DNA fragmentation of hepatocytes induced by galactosamine.

DNA molecules absorb photon energy directly for wavelengths <320 nm, and lead to well-characterized mutagenic DNA damage. A few light-absorbing pharmaceuticals have long been known to cause photo(geno)toxic effects. Drugs that contain chlorine substituents in their chemical structure, such as chlorpromazine, exhibit photochemical activity that is traced to the UV-induced dissociation of the chlorine substituent leading to free radical reactions with lipids, proteins and DNA (Moore 2002). Notably, chlorpromazine derivatives has been established as photomutagen (Gocke 2001).

As early as 1974, Blumendrantz & Asboe-Hansen showed the possible correlation between the ultraviolet degradation of chlorpromazine and complexation with DNA and/or RNA. Irradiation of supercoiled plasmid DNA in the presence of phenothiazine derivatives(fluphenazine HCI, thioridazine HCl, and perphenazine) leads to single strand breaks, and the highest photocleavage activity was observed with perphenazine and thioridazine HCI (Viola *et al* 2003).

Others had an opposite opinion that said the properity of irreversible photobinding of chlorpromazine make it a useful compound as it absorbs both ultraviolet A and B lights but this effect is not extended to DNA molecule (Van-Henegouwen et al 1990)

This study is aimed to explore the effect of chlorpromazine on calf thymus double strand DNA molecule as well as on the irradiated DNA molcule by ultravioletB rays in in vitro expeimental model.

Materials and Methods:

Preparation of ctds-DNA samples

The experimental work was performed with mammalian macromolecular DNA isolated from calf thymus (BHD, England) (Box.1). The DNA samples were prepared by dissolving the DNA in sodium chloride-sodium citrate buffer (0.0015M NaCl , 0.00015 M trisodium citrate). UV-spectrophotometric measurements of DNA samples (concentrations ranged between 1 to 6 pg/mL) at 260nm were performed in order to established the standard curve.

Effect of chlorpromazine on intact ctdsDNA

Aliquots of the DNA samples (3 µg/mL) were incubated with different concentrations of chloropromazine (4, 8, 12, 16 and 20 gg/mL) then the UV-spectrophotometric measurements of DNA samples at 260nm were obtained.

Effect of ultraviolet - B radiation on ctdsDNA

Aliquots of the DNA samples (3 pg/mL) were irradiated by UVB-lamp (closed box system of 5x15x10 cm dimensions) for 30, 60,90,120 minutes.

Effect of chlorpromazine on irradiated ctdsDNA

At the end of 30 minutes interval period, chlorpromazine of different concentrations (4, 8, 12, 16 and 20 µg/mL) were added to each sample and then the UV-spectrophotometric measurements of DNA samples at 260nm were immediately obtained.

In another series of experiment, aliquots containing both chlorpromazine (4,8, 12, 16 and 20 µg/mL) and ctdsDNA (3 µg/mL) samples were irradiated by UVB-lamp for 30 minutes. Then the UV-spectrophotometric measurements of DNA samples at 260nm were obtained at the end of exposure.

Statistical analysis:

The data are analysed by Student's "t" test (paired, two tailed) and simple correlation test taking p - 0.05 as the lowest limit of significance.

Results:

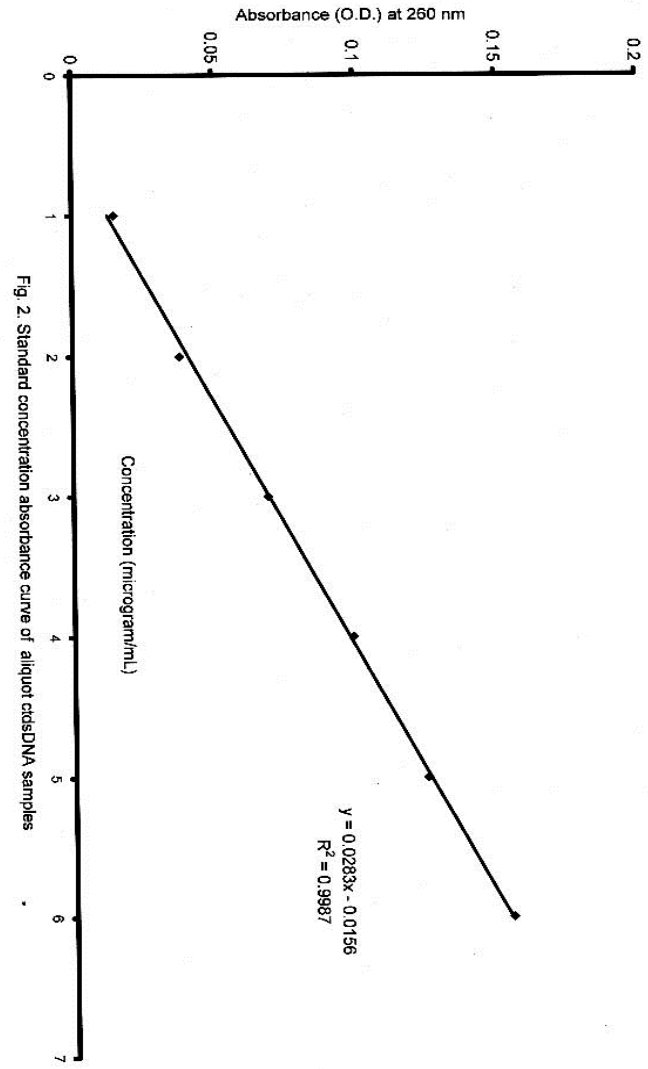

Fig. 2. Standard concentration absorbance curve of aliquot ctdsDNA samples

Fig.2 shows standard absorbance-concentration curve of aliquot ctdsDNA samples. The calculated absorbance for each I µg/mL ctdsDNA is 0.028 absorbance O.D. at 260 nm.

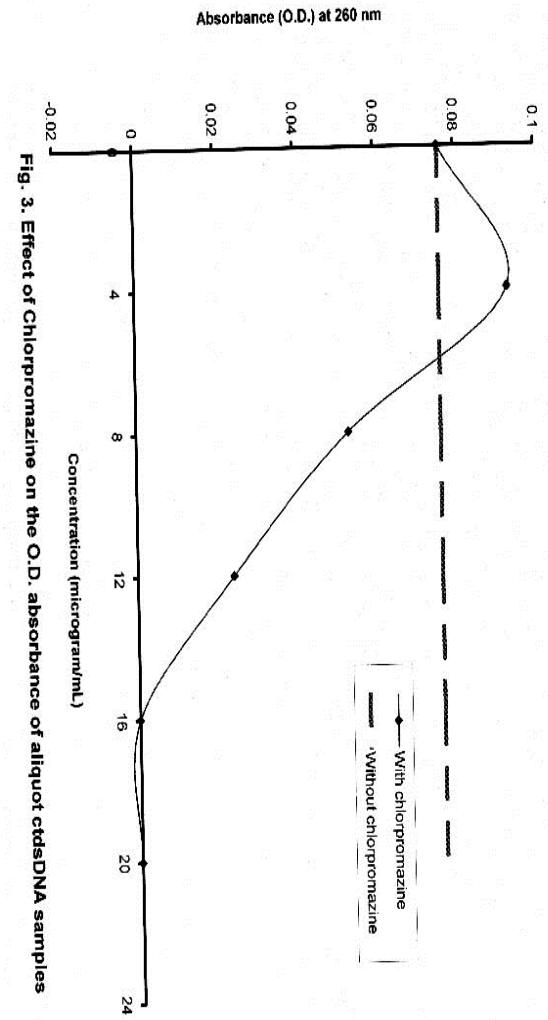

Fig. 3. Effect of Chlorpromazine on the O.D. absorbance of aliquot ctdsDNA samples

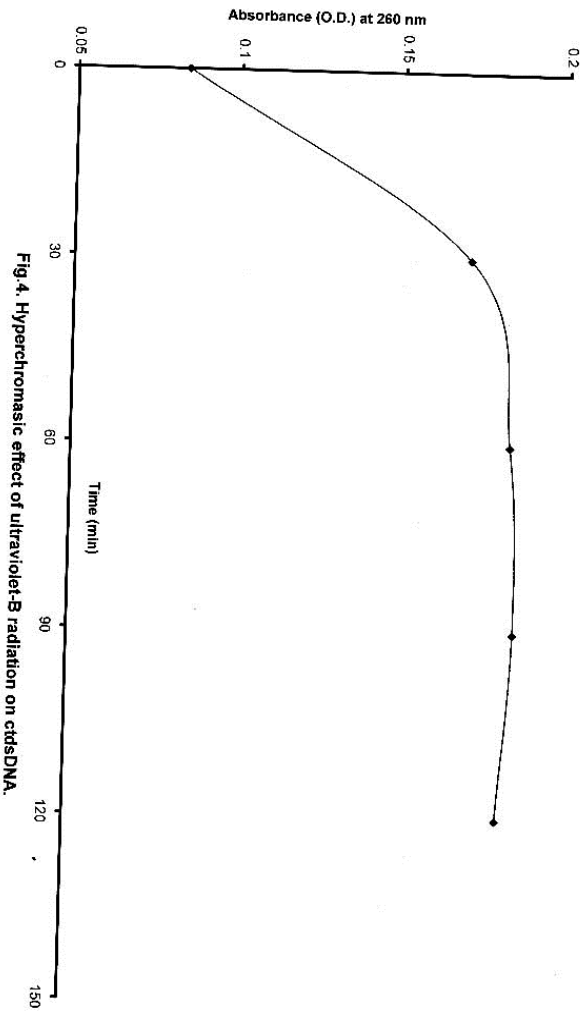

Fig.4. Hyperchromasic effect of ultraviolet-B radiation on ctdsDNA.

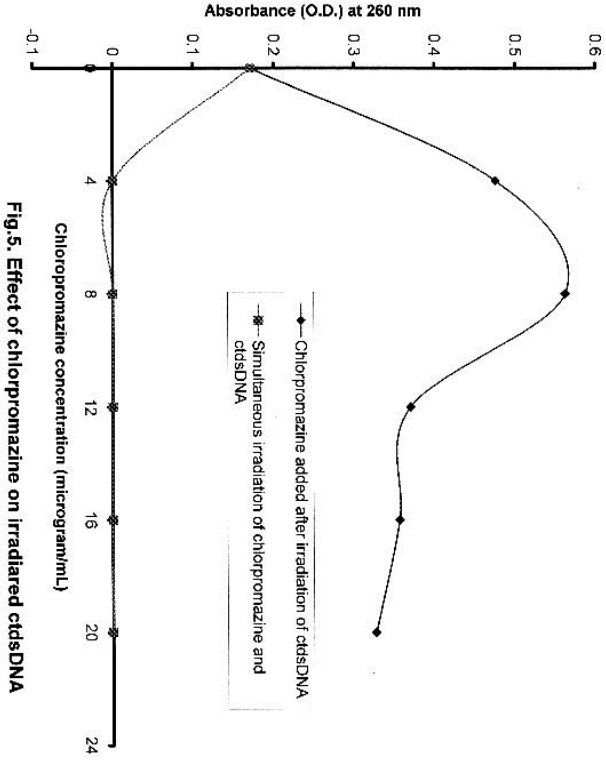

Fig.5. Effect of chlorpromazine on irradiared ctdsDNA

Fig. 3 shows that chlorpromazine shows hyperchromasic effect at concentrations 4 and 8 µg/mL(an increase in O.D. absorbance) while concentrations higher than 8 µg/mL produces significant ($p < 0.001$) hypochromasic effect (i.e. DNA fragmentation).

Aliquot ctdsDNA samples exposed to ultraviolet-B radiation showed significant (p < 001) hyperchromasic effect up to two hours of exposure (Fig.4). The pronounced effect of radiation is observed after 30 minutes of exposure to radiation and then a slight increment in hyperchromasic effect is observerd in the following periods of exposure.

Hyperchromasia of aliquot ctdsDNA samples induced by radiation is significantly ($P < 0.001$) increased when chlorpromazine is immediately added after irradiation (Fig.5). Such effect is observed with concentrations of 4 and 8 µg/ml- . Higher concentrations seemed to fragment the DNA molecule since the increment in optic density did not obviously differ from the effect of irradiation alone (Figs 4&5). Simultaneous incubation of chlorpromazine of whatever concentration and aliquot ctdsDNA samples resulted in complete fragmentation of DNA molecule ($P <0.001$) (Fig. 5).

Discussion:

The results obtained in this work showed that chlorpromazine exerts significant effect on aliquot ctdsDNA samples which is related to its concentration as well as to the nature of ctdsDNA whether it is intact or irradiated. Chlorpromazine at 4 µg/ml, produces hyperchromasic effect upon aliquot ctdsDNA samples mainfested by 22.4% increment in optic density absorbance above the baseline level. On the other side, chlorpromazine at concentrations ranged from 8 to 20 µg/mL produces significant hypochromasic effect which is inversely correlated with chlorpromazine concentration (r = -0.938) Therefore we predict from the equation of regression (0.083 - 0.0045x concentration) that for each I µg/mL of chlropromazine there is 0.0045 decline in optic density absorbance.

The hyperchromasic effect of chlorpromazine is due to its interaction with ct-DNA double helix causing conformational changes of ctdsDNA and leading to cleave the phosphodiester bond of DNA (Biver *et al* 2004) . This effect is not restricted to phenothiazines but other drugs like polymyxins or quercetin can produce such effect (Jiang *et al* 2002; Kang *et al* 2004). Viola *et al* 2003 studied three phenothiazine derivatives; fluphenazine hydrochloride, thioridazine hydrochloride, and perphenazine and suggested that phenothiazines bind to the DNA at least in two ways: intercalation and external stacking on the DNA helix, depending on their relative concentrations. This study adds the effect of chlorpromazine, depending on its concentration, to the above phenothiazine derivatives.

There are a plenty of evidences pointed out to the photomutagenicity / photogenotoxicity of ultraviolet light (Brendler-Schwaab *et al* 2004; Caricchio *et al* 2004). Our results were in agreement with Yu and Ku 2000 who found Raman hyperchromicity in UV-induced ctdsDNA only with aqueous solution. UV-induced DNA hyperchromasi may be attenuated by using several chemicals like sanguinarine and berberine (Das *et al* 2003), and vitamin A (Antille *et al* 2003). On the other side there are several chemicals able to induce DNA damage like phenothiazines (Zhelev *et al* 2004). Chlorpromazine is the lowest cytotoxic compound among phenothiazine in this respect. We observed that the effect of chlorpromazine on intact ctdsDNA is completely differ on irradiated DNA. Its effet is similar to caffiene which able to enhance genotoxicity on damaged DNA (Kaufmann *et al* 2003). In this work chlorpromazine potentiated the DNA hyperchromasia -induced by UVB when it added on irradiated DNA. Simultaneous exposure of aliquot ctdsDNA samples and chlorpromazine to UVB radiation resulted in complete DNA damage. This finding is in agreement with Viola *et al* 2003 who demonstrate photocleavage of irradiated plasmid DNA in presence of one of the following phenothiazine derivatives; fluphenazine hydrochloride, thioridazine hydrochloride and perphenazine.

We conclude that chlorpromazine exerts several effects on aliquot ctdsDNA samples which are related to the nature of DNA molecule as well as to the concentration of chlorpromazine.Also chlorpromazine potentiates the hyperchromasic effect of UVB radiation on aliquot ctdsDNA samples but it produces completely damage of DNA molecule when the aliquot ctdsDNA samples irradiated in presence of chlorpromazine.

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