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Chlorpromazine: a potential anticancer agent?

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SUMMARY: The antipsychotic drug chlorpromazine causes scission of the DNA in PY815 mouse mastocytoma cells or isolated PY815 cell nuclei and the broken DNA reseals when chlorpromazine is removed from nuclei. These properties suggest that chlorpromazine interferes with topoisomerase action as do several other DNA-intercalating anti-cancer drugs. However, protein is not associated with the broken DNA after chlorpromazine treatment suggesting a different mode of action on the topoisomerase. Reasons why chlorpromazine may have potential as an anti-cancer agent are considered.

INTRODUCTION: Several polycyclic heterocyclic compounds are now in clinical use as anti-cancer agents. These include various anthracycline and acridine derivatives such as adriamycin or amsacrine (Fig 1B and 1C) which intercalate into DNA (1,2). The cytotoxic effects of some of these drugs have been suggested to result from distortion of the DNA double-helix, which induces a type II topoisomerase to break and relax DNA (1-5) and this is supported by the formation of 5'-covalently linked DNA-protein adducts (2). However, during topoisomerase action on DNA inhibition of the resealing process by direct interaction of the drugs with topoisomerase itself may cause DNA breakage (6,7,8). Although other effects of the individual drugs have been reported, for example effects on membranes (9) or enzymes (10) their cytotoxicity is generally believed to result from scission of nuclear DNA(1,2).

Different DNA intercalators have many undesirable side effects such as cardiotoxicity, myelosuppression, hair loss, skin irritation, nausea and vomiting and this limits the dose of drug that can be given, and hence their efficacy. Some of these side effects may be a consequence of biotransformation of the drugs in vivo (9,11,), consequently there is still considerable scope for the development of improved anticancer drugs by increasing the activity or specificity of new or existing drugs.

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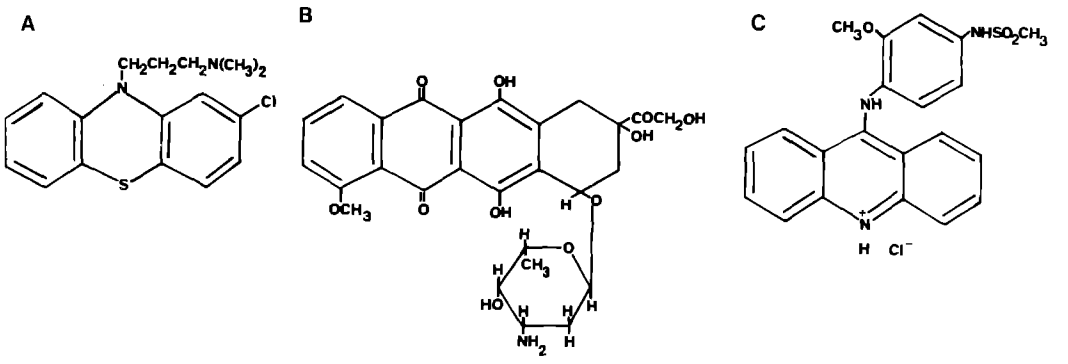

Figure 1. The structure of chlorpromazine (1A), adriamycin (1B) and amsacrine (1C).

In view of the conclusion that topoisomerase II may be the intracellular target for DNA intercalating anticancer drugs, we screened a variety of other heterocyclic compounds for their ability to produce breaks in the DNA of PY815 mastocytoma cells. Among these was the drug chlorpromazine, a substituted phenothiazine (Fig 1A) currently used clinically, as an anti-emetic, antipsychotic agent but which also has sedative, anti-pain and tranquilizing properties (12,13). The structure of chlorpromazine is vaguely reminiscent of several active substituted acridines which suggests that it might bind to DNA and, or inactivate topoisomerase II. Furthermore, phenothiazines such as chlorpromazine inhibit cyclic AMP phosphodiesterase (19,22) and calmodulin-dependent processes generally (14-16) and they can stimulate adenylate cyclase (17) which might elevate cyclic AMP in cells thereby inhibiting growth (18). In addition, chlorpromazine inhibits Na^+/K^+ and Ca^{2+} transport (19,22), oncovirus protein kinases (23), and protein kinase C (24), all of which appear to be necessary for, or involved in, events leading to cell growth (25-28). Finally, chlorpromazine appears to be relatively well tolerated in man (13). Therefore in toto the biochemical and pharmacological properties of chlorpomazine might be especially beneficial for the treatment of cancers.

We report that low concentrations of chlorpromazine are cytotoxic to PY815 mouse mastocytoma cells and that the drug induces reversible DNA breakage in PY815 cells or isolated PY815 cell nuclei, as do amsacrine or adriamycin (1,2). These observations (19,22) suggest that chlorpromazine may affect topoisomerase action and that it and other phenothiazines could have potential as anticancer agents.

METHODS: PY815 Mouse mastocytoma cells were grown in suspension cultures in RPMI 1640 medium. Nuclei were prepared as previously described (4).DNA breakage by drugs was measured using a simple viscosity method (30). The procedures to measure DNA breakage and resealing with isolated nuclei have been described (4). To detect and quantitate DNA-bound proteins we employed a filter-binding assay which is sensitive and specific enough to detect a single protein molecule bound to adenovirus DNA (31). The viscosity procedure of Waring and Henley (32) was used to compare possible intercalative interactions between chlorpromazine or ethidium bromide and closed-circular supercoiled plasmid PNZ116 DNA. Radioactive DNA was prepared by phenol-sodium dodecylsulphate extraction from PY815 cells grown for 6h with [^{3}H]thymidine (2μCi/ml; 46Ci/mmole). Extreme care was taken not to shear the extracted DNA.

RESULTS: The inhibitory effect of increasing concentrations of chlorpromazine, trifluoperazine or amsacrine on the growth of PY815 mouse mastocytoma cells over 20h is illustrated in Fig 2. Chlorpromazine was more effective than trifluoperazine with a 90% reduction in growth by 10μM chlorpromazine. However both drugs were less effective inhibitors of growth and viability than amsacrine. Because chlorpromazine might have inhibited growth by affecting calcium-calmodulin action (16,27,33), while phenothiazines shift the cyclic AMP dose response in murine lymphoid cells (34) and PY815 cell growth is inhibited by 10^{-4} M N_6, $0^{2'}$- dibutyryl cyclic AMP + 1mM theophylline probably through effects on

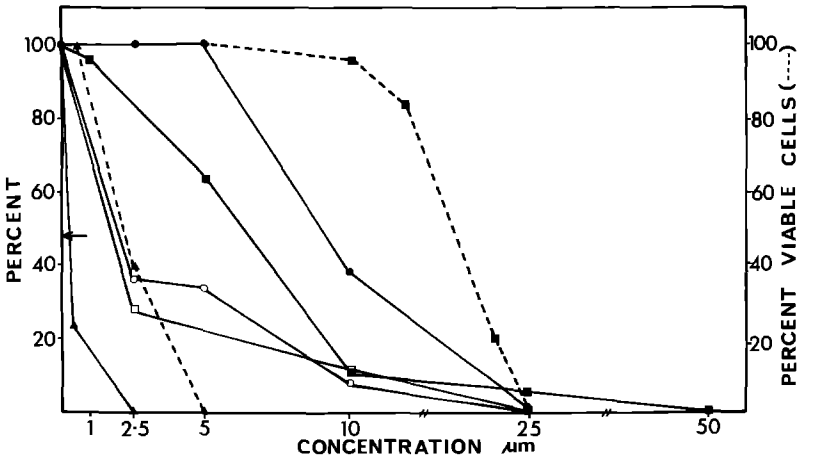

Figure 2. Effects of drugs on the growth and viability of PY815 cells. Cells were grown with the drugs for 20 h. ●—●, trifluoperazine; ■----■chlorpromazine; 0—0, trifluoperazine + 10^{-4}M N_6, $0^{2'}$-dibutyryl cyclic AMP + 1mM theophylline; □—□, chlorpromazine + 10^{-4}M N_6,$0^{2'}$-dibutyryl cyclic AMP + 1mM theophylline;▲----▲, amsacrine. The arrow indicates the growth inhibition by 10^{-4}M dibutyryl cyclic AMP + 1mM theophylline.

Left abscissa: growth as a percentage of the growth of untreated cultures. Right asbcissa: viability 20h after amsacrine (▲....▲) or chlorpromazine (■....■) treatment.

calcium availability (35,36), we also tested whether a synergistic effect of chlorpromazine and the cyclic AMP analogue on PY815 cell growth might occur. However the drugs appeared to be more additive than synergistic at low chlorpromazine or trifluoperazine concentrations.

To assess whether chlorpromazine or trifluoperazine caused scission of the DNA in PY815 cells, cultures were grown for 10 or 30 min with or without 4μM or 10μM chlorpromazine, then the harvested cells were washed, lysed and the viscosity of the lysates was determined as previously described (30). There was a progressive decrease in viscosity with increasing time of treatment or drug concentration consistent with DNA breakage by either drug (Table 1a).

To ensure that the DNA breakage was a result of the direct action of chlorpromazine or trifluoperazine rather than an indirect consequence of inhibition of growth, isolated nuclei were also treated with 4μM or 10μM chlorpromazine, trifluoperazine, or 4μM amsacrine, then lysed, and the viscosities measured. Treatment of nuclei with either drug, for 10 or 30 min caused the viscosity of nuclear lysates to decrease indicating scission of nuclear DNA and the drugs appeared to be almost equally effective (Table 1b).

Table 1 Effects of chlorpromazine (CP), trifluoperazine (TFP) or amsacrine (mAMSA) treatment of PY815 cells or isolated nuclei on the viscosity of cell or nuclei lysates

Treatment		FLOW RATE (min)[1]	
		1A Whole Cells[2]	1B Isolated nuclei[3]
Untreated	10 min	6:56±30	7:46±14
	30 min	6:41±18	7:37± 8
4μM CP	10 min	3:34± 9	4:21±30
	30 min	3:24± 6	3:03± 8
10μM CP	10 min	3:18±11	2:48±12
	30 min	2:41±18	1:57± 1
4μM TFP	10 min	4:37± 1	4:24±18
	30 min	2:37± 6	2:32±11
10μM TFP	10 min	3:19± 4	3:57± 4
	30 min	2:34±14	2:25± 2
4μM mAMSA	10 min	3:00± 4	3:13±20
	30 min	2:40± 4	2:11± 4

1. mean of 3 measurements .
2. 3.0×10^6 cells per assay.
3. 5.0×10^6 nuclei per assay.

Table 2: Repair of Chlorpromazine or Amsacrine-induced breaks in Nuclear DNA

Treatment [a]	Flow Rate (min)[b]
Untreated nuclei	7:53 ± 30
+4μM mAMSA	3:38 ± 20
+ 4μM CP	3:43 ± 11
+4μM mAMSA, then resealed	7:26 ± 30
+4μM CP, then resealed	7:01 ± 10
+ 4μM mAMSA then 4μM CP during resealing.	5:54 ± 30
+4μM CP then 4μM CP during resealing	3:44 ± 4

[a] $5x10^6$ nuclei per assay.
[b] mean of 3 measurements.

To assess whether chlorpromazine interfered with the resealing of DNA during topoisomerase action, nuclei treated for 10 min with 4μM chlorpromazine or 4μM amsacrine, were recovered, washed and reincubated with or without chlorpromazine (4). Nuclei treated with amsacrine resealed their DNA during subsequent incubation without drugs, whereas the DNA largely remained broken in the presence of 4μM chlorpromazine (Table 2).

Treatment of PY815 cells or nuclei with amsacrine causes attachment of topoisomerase to the 5'-termini of broken nuclear DNA (2). Therefore, the filter-binding procedure of Coombs et.al. (31) was used to detect the association of protein with DNA prepared from parallel PY815 cell cultures grown with [^{3}H]thymidine for 6h prior to 4μM drug treatment for 30 min. Only 13% of the radioactive DNA from untreated cells bound to filters, whereas 65% of the DNA from amsacrine-treated cells and 15% of the DNA from chlorpromazine--treated cells was bound by the filters. From this data it appeared that the mechanism of action of chlorpromazine differed in some subtle way from that of amsacrine and other intercalating drugs since protein was not associated with broken DNA after chlorpromazine action. Because this difference could have been due to the non-planar nature of the phenothiazine ring system and inability of the drug to intercalate into DNA, the interaction of chlorpromazine with covalently-closed supercoiled plasmid PNZ116 DNA was examined by viscometry and compared with that of ethidium bromide.

As observed with PM_2 DNA by Waring (32) increasing ethidium bromide concentrations initially increased, then decreased the viscosity of PNZ116 DNA

solutions consistent with unwinding and rewinding of the DNA into positive supercoils. The equivalence point was at approximately 1 ethidium molecule bound/24 nucleotides. In contrast, chlorpromazine caused little change in the viscosity of PNZ116 DNA over a wide concentration range giving no evidence of intercalation into DNA.

DISCUSSION: The preceeding results showed that chlorpromazine and trifluoperazine break the DNA in PY815 cells or isolated PY815 cell nuclei and the effects of chlorpromazine on breakage and resealing of DNA in isolated nuclei were comparable with effects of similar concentrations of amsacrine suggesting that chlorpromazine affects topoisomerase action as does amsacrine (2,3,7). However, chlorpromazine was a less effective inhibitor of PY815 cell growth and viability than amsacrine, possibly because it does not enter cells as readily as amsacrine (3), or it does not interact with DNA or topoisomerase II in exactly the same way as amsacrine. Ohnishi and McConnell (37) proposed that the radical ion of chlorpromazine intercalates into DNA. In contrast, Waring (38) obtained no evidence that chlorpromazine intercalated into phage ϕX174 supercoiled Rf DNA at pH6 or pH 7.9 and we could detect no evidence of intercalation of chlorpromazine into PNZ116 DNA. Consequently, it is possible that the effects of chlorpromazine on PY815 cell DNA arise from the direct interaction of the drug with topoisomerase rather than with DNA. Similar suggestions have been made by Filipski (6) and Nelson et.al. (7) to explain the effects of other intercalative and non- intercalative drugs that break DNA.

We believe chlorpromazine could be an effective anti-cancer agent because it appears to be reasonably well tolerated in man allowing high and prolonged doses to be employed (13) and because several of its properties, for example its anti-emetic, anti-pain and tranquilizing effects could be, and have been useful adjuncts during chemotherapy. Furthermore, the known inhibitory effects of phenothiazines on calmodulin-dependent and other reactions and the requirement for calmodulin in the cell cycle (24,27,33) could give chlorpromazine a multi-faceted action against cancer cells. In addition, chlorpromazine may be more stable

than amsacrine, which is rapidly inactivated by sulphydryl reagents (39) and chlorpromazine also acts on the central nervous system and the brain (13), locations poorly accessible to many other anti-cancer drugs. It remains to be determined whether chlorpromazine will be an effective inhibitor of growth of other tumour cells in vitro or in vivo although spasmodic reports of inhibition of growth of different cancer cells by chlorpromazine or trifluoperazine have appeared (40-44) and it has been proposed that the use of chlorpromazine might explain the lower incidence of cancer in mental institutions (45). In most of these studies little effort was made to determine optimal drug doses or dose regimes, therefore a thorough re-evaluation of the anti-cancer potential of this class of drugs seems warranted.

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