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Anti-cancer activity studies of indolalthiohydantoin (PIT) on certain cancer cell lines

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Abstract

5-(2-Phenyl-3′-indolal)-2-thiohydantoin (PIT) has been evaluated as an anti-cancer compound on several cancer lines organised in to subpanels representing leukemia, melanoma, and cancer of lung, colon, kidney, ovary, breast, prostate and central nervous system by the National Cancer Institute (NCI) anti-cancer drug screen programme. The compound showed inhibitory activity on several cancer cell lines. No information is available on anti-cancer potency of this compound with normal cell lines. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Indole; Hydantoin; Thiohydantoin; Anti-cancer activity

1. Introduction

The discovery and development of novel therapeutic products for the treatment of malignancy is vitally important for the management of cancer patients. The tremendous potential advantages and challenges associated with the use of a molecular approach to cancer drugs have been reviewed [1]. The recent emphasis on the use of molecular modeling for the design of cancer drugs has substantial intellectual appeal. In fact, the major examples of rationally synthesised anti-tumour drugs, in use for the treatment of cancer in humans are restricted to the antimetabolites and the hormonally active agents [2]. Perhaps a more realistic objective is selective delivery of a cytotoxic agent to cancer cells, to maximise the concentration of the cytotoxic agent at the tumour while minimising its concentration around healthy tissues [3].

Indole and hydantoin derivatives constitute an important class of therapeutic agents in medicinal chemistry [4,5]. Indole-3-carbinol (I3C), a natural component from cruciferous vegetables, has been documented as acting as a modulator of carcinogenesis in various animal models [6]. Long term administration of I3C in the diet inhibits diethylnitrosamine-initiated hepatocarcinogenesis in the infant mouse model. Indeed, this naturally occurring compound is a promising anticancer agent that has been shown previously to induce a G1 cell cycle arrest of human breast cancer cell lines [7].

Some new 2-phenylindole derivatives with sulfur containing side chain showed significant in vivo antineoplastic activity [8]. Ge et al. found that a dimer of I3C induced apoptosis in human cancer cells and that the induction of apoptosis was independent from the P_{53} pathway [9]. Isoindole derivatives having a substitutent with strongly electron donating properties showed enhanced anti-tumour activity [10]. Spirohydantoin mustard (spiromustin) is a combination of nitrogen mustard and a derivative of phenytoin, an anticonvulsant drug that rapidly penetrates the blood–brain barrier and localises drug delivery to brain tumours [11].

This paper reports the anti cancer activity of 5-(2-phenyl-3′-indolal)-2-thiohydantoin (PIT) which was first synthesised and investigated as an aldose reductase inhibitor and for anti-HIV activity [12,13].

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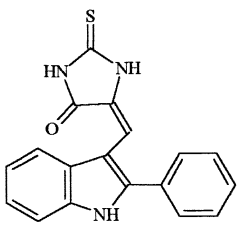
2. Experimental

Cytotoxicity tests were performed at National Cancer Institute (USA). The development and implementation of a pilot scale, in vitro, anticancer drug screen utilising a panel of 54 human tumour cell lines organised in to subpanels representing leukemia, melanoma, and cancer of lung, colon, kidney, ovary, breast, prostate and central nervous system was described. Each cell line was inoculated onto microtiter plates, then preincubated for 24–28 h. Subsequently, test agents were added in five tenfold dilutions and the culture was incubated for an additional 48 h. For each test agent, a dose–response profile was generated. End-point determinations of the cell viability or cell growth were performed by in situ fixation of cells, followed by staining with a protein-binding dye, sulforhadamine B (SRB) [14]. The SRB binds to the basic amino acids of cellular macromolecules; the solubilised stain was measured spectrophotometrically to determine relative cell growth or viability in treated cells.

The dose–response curves in Fig. 1 were created by plotting the percentage growths against the $\log_{10}$ of the corresponding concentration for every cell line. The cell line curves were grouped by subpanels. Horizontal lines were provided at the PG values of $+50$ and -50. The concentrations corresponding to points where the curves cross these lines are the GI_{50} (50% growth inhibition), TGI (total growth inhibition), LC_{50} (50% cell kill), respectively. Each concentration was expressed as the $\log_{10}$ (molar or μ/ml). Currently, the cell panel consists of the cell lines against PIT at a minimum of five concentrations at tenfold dilutions.



PIT

3. Results and discussion

We describe here the development of a pilot scale, in vitro, anti cancer drug screen of anti cancer drug candidate PIT. A panel of human tumour cell lines was

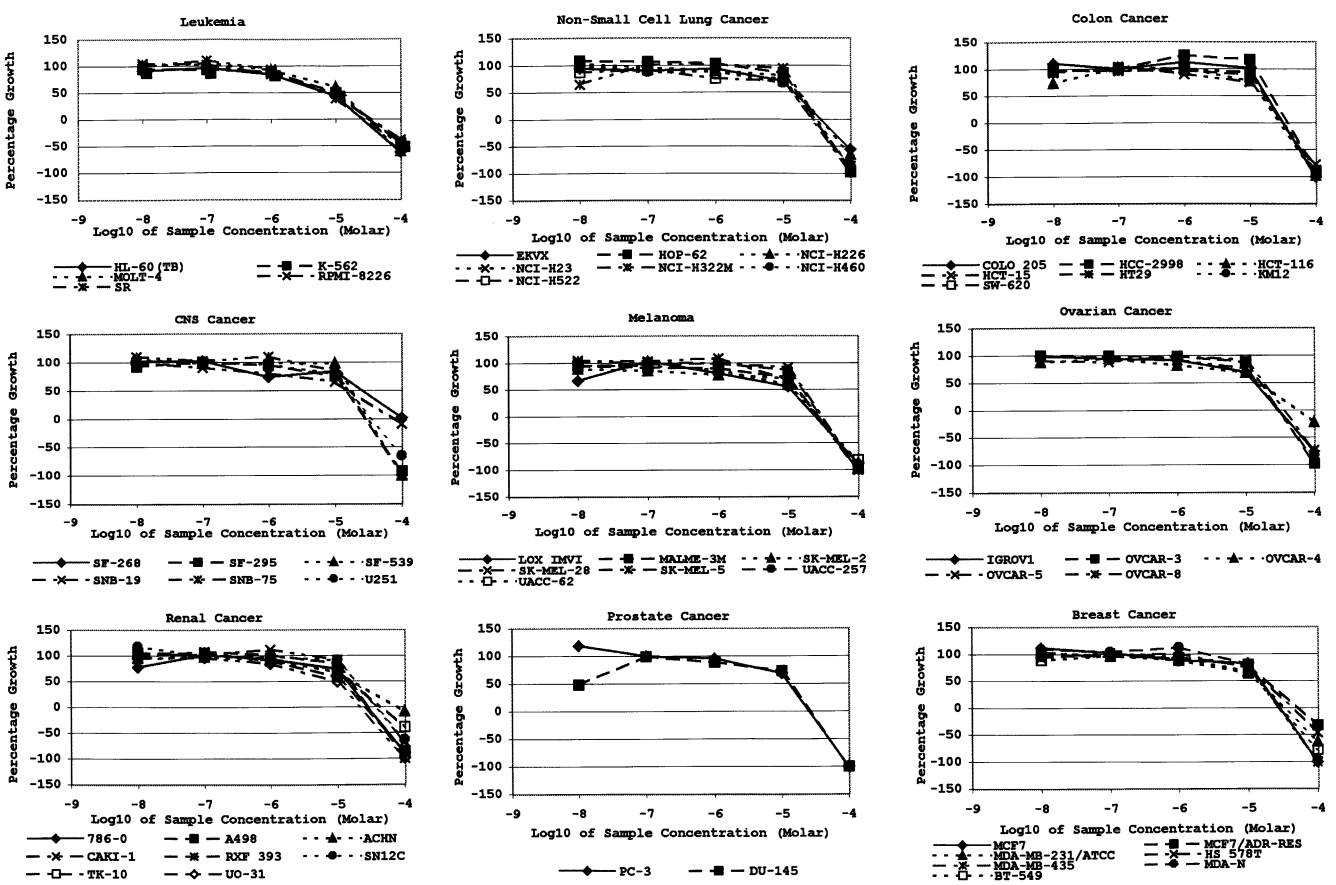


Fig. 1. Dose–response curves for nine cell lines incubated with PIT.

used for the tests. Most significant activity value was obtained against leukemia cell lines. Within these cell lines, a fairly dramatic difference was observed between leukemia cells and the other cancer cell lines. In leukemia cells, except MOLT-4 cell line, low log GI_{50} values (− 5.14 for HL-60; − 5.00 for K-562; − 5.06 for RPMI-8226; and − 5.20 for SR) were observed when compared with the other cell lines. Interestingly, PIT showed slightly better log GI_{50} value (− 5.03) for UO-31 cell line in renal cancer panel.

Fig. 1 shows dose response curves of PIT. The results of this screening showed a certain cellular subpanel selectivity around 10^{-4} molar concentrations. The most diluted concentration (10^{-9} M) was not considered because of its scarce significance. Unfortunately, the high doses required for the best activity prevented any screening in vivo.

A study with a series of sulphonylurea derivatives reported to possess a broad spectrum of activity in several solid tumour models [15,16]; therefore new 2-phenylindole derivatives with sulfur containing side chain have considerable interest. Sulfonyl urea derivatives have been found to accumulate in the cell mitochondria, the mitochondria may be the target site for anti tumour activity of these compounds [17,18]. Modes of action of these compounds differ from traditional anti cancer drugs which typically inhibit DNA, RNA, or protein synthesis.

Since the 2-phenylindole and some hydantoin derivatives are important in cancer research, in our continuing search for anti tumour agents, we are planning to design and synthesised more indolalthiohydantoin derivatives to investigate. Considering this group of therapeutic agents that has not been studied previously for their anti cancer activity, it is promising to search new derivatives.

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References

[1] M.R. Grever, S.A. Schepartz, B.A. Chabner, Cancer drug discovery and development program, Semin. Oncol. 19 (1992) 622–638.

[2] B.I. Sikic, Anti cancer drug discovery, J. Nat. Cancer Inst. 83 (1991) 738–740.

[3] L.N. Junghim, T.A. Shepherd, Design of anti tumor products, Chem. Rev. 94 (1994) 1553–1566.

[4] I. Chen, S. Safe, L. Bjeldanes, Indole-3-carbinol and diindolylmethane as aryl hydrocarbon (Ah) receptor agonists and antagonists in T47D human breast cancer cells, Biochem. Pharmacol. 51 (1996) 1069–1076.

[5] B.A. Diwan, J.M Rice, R.A Lubet, H. Hu, J.M. Ward, P-450 enzyme induction by 5-ethyl-5-phenylhydantoin and 5,5-diethylhydantoin, analogues of barbiturate tumor promoters phenobarbital and barbital, and promotion of liver and thyroid carcinogenesis initiated by *N*-nitrosodiethylamine in rats, Cancer Res. 48 (1998) 2492–2498.

[6] A. Oganasian, J.D. Henrdicks, D.E. Williams, Long term dietary indole-3-carbinol inhibits diethylnitrosamine-initiated hepatocarcinogenesis in the infant mouse model, Cancer Lett. 118 (1997) 87–94.

[7] C.M. Cover, S.J. Hsieh, E.J. Cram, C. Hong, J.E. Riby, L.F. Bjeldanes, G.L. Firestone, Indole-3-carbinol and tamoxifen cooperate to arrest the cell cycle of MCF-7 human breast cancer cells, Cancer Res. 59 (1999) 1244–1251.

[8] C. Biberger, E. Von Angerer, 2-Phenylindoles with sulfur containing side chains, J. Steroid Biochem. Mol. Biol. 58 (1996) 31–43.

[9] X. Ge, S. Yannai, G. Rennert, N. Gruener, F.A. Fares, 3,3′-Diindolylmethane induces apoptosis in human cancer cells, Biochim. Biophys. Res. Commun. 228 (1996) 153–158.

[10] C.L. Chan, E.J. Lien, Z.A. Tokes, Synthesis, biological evaluation, and quantitative structure–activity relationship analysis of 2-hydroxy-1H-isoindolediones as new cytostatic agents, J. Med. Chem. 30 (1987) 509–514.

[11] M.J. Resio, H.L. De Vroom, Spiromustine and intracarotid artery cisplatin in the treatment of glioblastoma multiform, J. Neurosci. Nurs. 18 (1986) 13–16.

[12] E. Buyukbingol, S. Suzen, G. Klopman, Studies on the synthesis and structure-activity relationships of 5-(3′-indolal)-2-thiohydantoin derivatives as aldose reductase enzyme inhibitors, Farmaco 49 (1994) 443–447.

[13] S. Suzen, E. Buyukbingol, Evaluation of anti-HIV activity of 5-(2-phenyl-3′-indolal)-2-thiohydantoin, Farmaco 53 (1998) 525–527.

[14] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, New colorimetric cytotoxicity assay for anticancer-drugscreening, J. Natl. Cancer. Inst. 82 (1990) 1107–1112.

[15] P.J. Houghton, F.C. Bailey, G.S. Germain, G.B. Grinday, J.J. Howbert, J.A. Houghton, Studies on the cellular pharmacology of *N*-(-4-methylphenylsulfonyl)-*N*′-(4-chlorophenyl)urea, Biochem. Pharmacol. 39 (1990) 1187–1192.

[16] P.J. Houghton, F.C. Bailey, G.S. Germain, G.B. Grinday, B.C. Witt, J.A. Houghton, *N*-(5-Indanylsulfonyl)-*N*′-(4-chlorophenyl)urea, a novel agent equally cytotoxic to nonproliferating human colon adenocarcinoma cells, Cancer Res. 50 (1990) 318–322.

[17] J.W. Chern, Y.L. Leu, S.S. Wang, C.F. Lee, P.S. Tsou, S.C. Hsu, Y.C. Liaw, H.M. Lin, Synthesis and cytotoxic evaluation of substituted sulfonyl-*N*-hydroxyguanidine derivatives as potential anti tumor agents, J. Med. Chem. 40 (1997) 2276–2286.

[18] J.H. Thaker, C. Chapin, R.H. Berg, R.A. Ashmun, P.J. Houghton, Effect of anti tumor diarylsulfonylurea on in vivo and in vitro mitochondrial structure and functions, Cancer Res. 51 (1991) 6286–6289.