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COMMENTARY

Effects of Psychotropic Drugs on Cell Proliferation and Differentiation

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ABSTRACT. Some of the psychotropic agents widely used for the amelioration of anxiety, depression, and psychosis also show an effect at the cellular proliferation level. Surprisingly little research, however, has been directed to the antitumoral potential of these drugs, alone or in combination with established cancer treatments. Our review of the literature to date has yielded some promising early findings. Ligands active at the benzodiazepine (BZ) receptors have been studied the most extensively and were found to have differential, concentration-dependent effects on the growth and proliferation of both normal and cancer cells. Of the phenothiazines tested, chlorpromazine (CPZ) and perphenazine (PPZ) had the most potent cytotoxic action on fibroblasts and glioma cells. Antiproliferative effects also were noted by these and other agents in leukemic and breast cancer cell lines. Additional psychotropic drugs studied include the atypical antipsychotics, antidepressants, and mood stabilizers, especially lithium. Most of the reported activities were observed in *in vitro* studies and were achieved at high pharmacological concentrations. Further *in vivo* studies in well-designed animal models are warranted to determine whether these well-tolerated, relatively inexpensive, and widely available drugs or their derivatives may be added in the future to the armamentarium of cancer pharmacotherapy. BIOCHEM PHARMACOL **58**;8:1229–1236, 1999. © 1999 Elsevier Science Inc.

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The interaction of psychotropic drugs with the neural system has prompted their widespread use for the treatment of such mental disorders as anxiety, depression, and psychosis. However, some of these agents also possess non-neural activity at the cellular proliferation and differentiation level. Their direct effects on normal and cancer cell mitotic activity have been well demonstrated *in vitro*, although data on the impact of *in vivo* treatment with psychotropic agents on the progression of malignant tumors remain scarce. Further research is essential to determine whether these relatively inexpensive and widely available drugs or their derivatives could serve as an important addition to the armamentarium of cancer therapies.

The aim of the present commentary is to organize and clarify the information published to date in this new pharmaco-oncologic field.

ROLE OF PSYCHOTROPIC AGENTS IN PSYCHIATRIC DISORDERS IN CANCER PATIENTS

Cancer is a stressful life event, and people differ in their ability to cope with it. Some patients may demonstrate psychological disturbances, such as anxiety, depression with suicidal ideation, agitation, psychosis, and acute confusional state [1, 2]. Sometimes psychotropic drugs are indicated to treat these behavioral changes or to control the major side-effects of chemotherapy. The following families of drugs are the most frequently used:

- BZs†† (especially diazepam, lorazepam, alprazolam, and clonazepam) are highly effective in treating anxiety disorders in cancer patients, including those with brain tumors, and in controlling delayed emesis following anticancer chemotherapy [3, 4].
- Phenothiazines and butyrophenones are used for psychotic symptoms and, in low doses, for nausea and

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†† *Abbreviations*: BZ, benzodiazepine; PBR, peripheral-type benzodiazepine receptor; CBR, central type benzodiazepine receptor; TFP, trifluoroperazine; PPZ, perphenazine; CPZ, chlorpromazine; LAK, lymphocytic activated killer; IL-2, interleukin-2; MDR, multidrug resistant; NGF, nerve growth factor; and Con A, concanavalin A.

intractable hiccups [5]. The novel atypical neuroleptics (e.g. risperidone and olanzapine), which have fewer extrapyramidal side-effects, are expected to be beneficial in psychotic cancer patients, who also may benefit from their stimulatory effect on appetite.
- Some antiepileptic drugs, which are also mood stabilizers (e.g. carbamazepine, valproic acid, gabapentin, and lamotrigine), are used to control neuropathic pain syndromes in cancer patients [6].

In contrast to the broad data available on the beneficial effects of these agents on the psychiatric complications of cancer, little is known of their effect on the tumorigenicity and progression of the cancer itself. The following sections describe the *in vitro* and *in vivo* findings to date on their effects on normal and cancer cell proliferation and differentiation.

BENZODIAZEPINES

Normal Cells

A variety of ligands active at the BZ receptors are known to affect the proliferation of normal cells. Studies in V79 Chinese hamster lung cells have shown that the selective PBR ligands Ro5–4864 and PK 11195 cause mitotic arrest in the G_2/M stage of the cell cycle but do not affect DNA synthesis. This action is apparently unrelated to a specific interaction with the PBR itself [7]. The CBR does not seem to play a role in the impact of BZs on cell proliferation. Exposure of rat thymus cells to diazepam (a mixed CBR and PBR ligand) resulted in a significant increase in mitotic activity, whereas the PBR ligand Ro5–4864 significantly suppressed it; flumazenil, a CBR antagonist, was ineffective [8]. Another group reported that diazepam and clonazepam (a selective CBR ligand) markedly inhibited the proliferation of clonal cell lines derived from cultured brain cortex neurons at concentrations 2- to 3-fold higher than the blood concentration achieved in patients with clinical therapeutic doses. The effect was cytostatic rather than cytotoxic [9]. Diazepam and medazepam also inhibited mitosis of dividing flagellate cells [10]. No correlation was found between the effect of these BZs on the central nervous system and their antimitotic activity [10]. One study of the effect of the PBR agonist Ro5–4864 and the putative antagonist PK 11195 on primary astrocyte cultures showed that at concentrations of 50 nM to 10 μM these ligands inhibited cell proliferation in a concentration-dependent manner. Some antiproliferative effect also was obtained when Ro5–4864 and PK 11195 were combined, suggesting that the PBR may be specifically involved in mediating antiproliferative effects in astrocyte cultures [11]. Furthermore, the observation that diazepam, Ro5–4864, and PK 11195 all inhibited growth factors (basic fibroblast growth factor, epidermal growth factor, and platelet-derived growth factor) activity and induced DNA synthesis in rat astrocytes [12] may indicate a possible antagonistic activity of the BZ receptor ligands on growth factor–cell interaction. Finally, Lafi and Parry [13] treated cultured Chinese hamster cells with diazepam, midazolam, medazepam, and bromazepam. A concentration-dependent reduction in the number of diploid cells was obtained with medazepam and midazolam; both agents induced significant levels of hyperdiploidy. Bromazepam was the most potent in inducing chromosomal aberrations (low levels only). The authors concluded that these structurally related BZs could be regarded as potentially genotoxic agents.

Cancer Cells

PROLIFERATION. In addition to their action on normal cells, BZ receptor ligands have been reported to affect the proliferation of a variety of cancer cell types, including neuroblastoma, glioma, pituitary tumor, and melanoma. In one study, diazepam, clonazepam, Ro5–4864, and PK 11195, at micromolar concentrations, inhibited the proliferation of rat C6 glioma and mouse neuroblastoma (neuro-2A) cells [14]. The researchers suggested that the antiproliferative action of these ligands was not mediated by the PBR, as evidenced by the lack of correlation between the potency and specificity of the compounds for antiproliferative activity and for binding affinities to the PBR. Moreover, these ligands also inhibited the proliferation of mouse SP2/O-Ag14 hybridoma and rat NCTC epithelial cells, which have no detectable high-affinity PBR [14].

Maguire *et al.* [15] investigated the water-soluble BZ clorazepate and found that it inhibited the proliferation of rat C6 glioma cells in a concentration-dependent manner, with an IC_{50} value of 280 μM. Specifically, it synchronized cultured C6 cells in the early G_1 phase of the cell cycle [15]. Since G_1 arrest is characteristic of the action of differentiating agents on tumor cells [16, 17], clorazepate may possess differentiating properties. In another study, PK 11195 and Ro5–4864, in serum-free medium at nanomolar concentrations, enhanced DNA synthesis and increased the proliferation of C6 glioma cells by 20–30%. However, the use of higher concentrations led to growth inhibition [18]. These results indicate possible bidirectional effects of PBR ligands on cell proliferation, depending on the concentration of the ligand.

In an estrogen-induced rat pituitary tumor model, Ro5–4864, PK 11195, clonazepam, Ro15–788, Ro15–4513, and diazepam inhibited cell proliferation at concentrations of 10^{-4} to 10^{-8} M; this effect was Ca^{2+} dependent. Agents active at the PBR and mixed-type BZs were more potent inhibitors of cell growth than those active at the CBR [19]. Thus, in this cell type, the PBR may mediate the antiproliferative effect of BZ receptor active ligands.

The effects of agents active at the BZ receptors have also been tested on human melanoma M6 cells. Diazepam and Ro5–4864 inhibited the proliferation of M6 melanoma cells, with IC_{50} values of 139 and 107 μM, respectively. None of the BZ receptor-active agents tested (i.e. clonazepam, flunitrazepam, PK 11195, and flumazenil) altered the

antiproliferative effect of diazepam and Ro5–4864, whereas the protein kinase C activator phorbol-12-myristate 13-acetate partially antagonized it [20]. Based on these observations, the authors suggested that the inhibitory effect of the ligands on cell growth is not mediated by BZ receptors, but it may involve the inhibition of a calcium/protein kinase C-related pathway.

Finally, one study examined the effects on human HO-1 melanoma cells of Ro7–3351 and Ro5–4608, alone or in combination with interferons α, β, and γ [21]. Ro7–3351 alone inhibited the proliferation of HO-1 cells. Inhibition of cell growth required cell exposure to the ligand for at least 72 hr, and the effect did not correlate with the binding affinities of these BZs to PBR. A marked synergistic growth inhibitory effect was obtained by combining Ro7–3351 with interferon β. Ro5–4608 alone had only a marginal effect on HO-1 melanoma cell growth.

DIFFERENTIATION. In a study on rat pheochromocytoma cells (PC12), Curran and Morgan [22] found that ligands active at the PBR augmented the induction of c-fos by NGF by 100-fold; this action was stereoselective. In another study with the same model, NGF increased the binding of PBR ligands to their receptor; neither Ro5–4864 nor PK 11195 altered PC12 cell proliferation or catecholamine secretion [23]. In contrast, in rat pituitary GH3 tumor cells, agents active at the PBR induced both growth hormone cell secretion and an increase in mitochondrial volume and mitochondrial DNA synthesis [24].

Mak *et al.* [25] showed that midazolam can induce monocytic and granulocytic differentiation of WEHI 3B (JCS) M1 murine myeloid leukemia cells, as manifested by the morphological differentiation and enhanced expression of the differentiation antigens Mac-1, F4/80, and Gr-1, and increased phagocytic activity towards opsonized yeast cells [25]. Midazolam also induced an up-regulation of tumor necrosis factor α mRNA and the neutrophil-specific J11d differentiation marker. In a B16 melanoma cell line, our group recently showed that diazepam, clonazepam, Ro5–4864, and PK 11195 all inhibit proliferation at micromolar concentrations and induce phenotypic alterations compatible with a more differentiated phenotype, such as dendrite-like structures, enhanced γ-glutamyltranspeptidase activity, lipid droplets, and melanogenesis. The alterations induced were distinct for each compound. The mechanism of action may involve BZ receptor ligand reduction of the intracellular pyrimidine nucleotides UTP or CTP, or the purine nucleotide GTP [26].

Interestingly, an increased density of PBR has been demonstrated in ovarian cancer cells as compared with benign tumor or normal ovarian cells [27]. This observation suggests the need to evaluate the possible role of PBR ligands as selective growth inhibitors or differentiating agents in ovarian cancer cells.

Immunocompetent Cells

Low micromolar concentrations of BZ receptor ligands inhibited T- and B-cell stimulation by Con A and by lipopolysaccharides, and reduced alloantigen recognition. At the secretory level, high concentrations of diazepam and clonazepam stimulated IL-2 production by Con A-stimulated spleen cells [28]. In another study, Ro24–7429 inhibited apoptosis of chimpanzee T cells and reduced antigen-induced cell proliferation [29]. The impact of BZ receptor ligands on malignancy of the immune system merits further investigation.

PHENOTHIAZINES

Normal Cells

The phenothiazines investigated thus far for their effects on normal cell proliferation include TFP, PPZ, and CPZ. TFP, at a concentration of 7.5 μM, inhibited DNA polymerase α activity in normal rat kidney cells, thereby arresting the cells in the S phase of the cell cycle [30]. Like PPZ, it also inhibited the proliferation of osteoblasts and affected their alkaline phosphatase activity and collagen synthesis *in vitro* and *in vivo*. This action was not cytotoxic, but cytostatic [31]. In normal keratinocytes, TFP induced an irreversible arrest of the cell cycle, an early event in normal keratinocyte terminal differentiation [32]. Likewise, in the epidermis of guinea pigs, topical application of TFP and CPZ affected the cell cycle and the keratinization process; this finding may have clinical relevance for the treatment of psoriasis [33]. Finally, oral administration of PPZ (0.1% in drinking water) to rats led to a sustained elevation of prolactin levels and consequent growth of breast cell components. However, cell growth was stimulated to a lesser extent than expected, suggesting a possible enhancement of apoptotic cell death [34].

Cancer Cells

PROLIFERATION. Phenothiazines have been shown to exert antiproliferative effects on many tumor cells. In studies on leukemic cells, fluphenazine (1–10 μM) inhibited the proliferation of the human leukemic cell line HL-60 and the T-cell line H33-HJ JA1, an IL-2 producing cell line derived from Jurkat cells and stimulated by IL-2 autocrine mechanisms [35]. CPZ and TFP also inhibited the proliferation and clonogenicity of murine L1210 leukemic cells. This effect was concentration dependent and was influenced by the duration of exposure to the drugs. The main effect was observed during the logarithmic phase of cell growth. The antiproliferative activity of the phenothiazines correlated with their antagonistic effect on calmodulin activity, suggesting that calmodulin might be the intracellular target of phenothiazine-induced cell growth inhibition [36, 37]. Support for this assumption was provided by Glass-Marmor *et al.* [38, 39], who found that the antipsychotic agent thioridazine, a calmodulin antagonist, inhibits

B16 melanoma cell proliferation and the glycolytic supply of ATP, which is required for cell growth. The elevated level of calmodulin in malignant keratinocytes also may explain their resistance to the growth arrest induced by TFP in normal keratinocytes [32]. In other studies, CPZ and TFP inhibited the proliferation of IRSC-10M small cell lung cancer cells, most probably due to inhibition of protein kinase C activity [40], and PPZ and fluphenazine arrested human MCF-7 breast cancer cells in the G_1 phase of the cell cycle, leading to a reduced percentage of cells in the S phase [41].

Of thirteen phenothiazines screened for cytotoxic activity on fibroblasts and glioma cells, the most potent were PPZ and CPZ [42]. CPZ and CPZ sulfate inhibited the proliferation of C6 astrocytoma cells [43], and CPZ arrested the growth of T9 anaplastic glioma cells [44]. In one study, a battery of calmodulin antagonists, including CPZ, TFP, fluphenazine, and penfluridol (which penetrates the blood–brain barrier), were found to inhibit cell proliferation and to sensitize C6 rat glioma cells towards the cytotoxic drug bleomycin [45]. This antiproliferative effect correlated with their ability to inhibit the activation of calmodulin-sensitive phosphodiesterase. Penfluridol, which was highly potent in glioma cells, also enhanced bleomycin sensitivity by 90-fold in L1210 leukemia cells and by 4-fold in MCF-7 human breast cancer cells on clonogenic assays. *In vivo*, CPZ, when combined with the antineoplastic drug 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), significantly inhibited the growth of experimental rat glioma tumors [46].

DIFFERENTIATION. A study of the action of phenothiazines and eight benzo[*a*]phenothiazine derivatives in the leukemic cell lines ML-1, U-937, and THP-1 indicated that 12*H*-benzo[*a*]phenothiazine and 5-oxo-5*H*-benzo[*a*]phenothiazines were the most potent inducers of monocytic/macrophage differentiation [43]. Phenothiazines lacking the benzyl group were ineffective. CPZ induced cytoplasmic spread in T9 glioma cells and the development of lamellipodium-like appendages, which resembled the differentiating effects of NGF [44].

MDR Cancer Cells

Molnár *et al.* [47] showed that 5*H*-benzo[*a*]phenothiazine-5–1 inhibits the proliferation of mouse and human tumor cell lines. The effect on MDR cell lines was more potent than on parental cells. Its impact on the accumulation of rhodamine 123 (a substrate of P-glycoprotein) suggested a direct action of the agent on the efflux pump P-glycoprotein, which is overexpressed in MDR cells and is responsible for the MDR phenotype of tumor cells [47].

Phenothiazine derivatives have also been tested for their ability to inhibit proliferation and overcome MDR in human sensitive and Adriamycin©-resistant MCF-7 breast cancer cell lines. *trans*-Flupenthixol was found to be the most active reverser of MDR, with no evidence of acute clinical toxicity [48]. The investigators suggested that the ideal phenothiazine structure for reversing MDR is a hydrophobic nucleus with a —CF_3 ring substitution at position 2, connected by a four-carbon alkyl bridge to a *para*-methyl-substituted piperazinyl amine.

Immunocompetent Cells

CPZ and fluphenazine at pharmacological doses inhibited mitogen-induced activation [49] and proliferation [50] of human T cells; they also reduced the accumulation of lymphokine-specific mRNA and suppressed human thymocyte production of interferon-γ [49]. Both agents, however, increased natural killer (NK) activity [50]. In another study, CPZ and TFP were found to induce immunosuppression, an activity unrelated to their dopamine antagonistic properties [51]. These observations prompted the suggestion that phenothiazines might be helpful in immune-suppressive regimens. Unfortunately, there are no data on the impact of phenothiazines on the proliferation of malignant immune cells.

OTHER ANTIPSYCHOTIC AGENTS

Haloperidol, like estrogen, induced the rapid and transient expression of the oncogenes c-myc and c-fos in rat anterior pituitary gland [52]. This action may be involved in prolactin secretion and stimulation of pituitary cell proliferation. In addition, the atypical antipsychotic agent clozapine can induce agranulocytosis in about 1% of patients. *In vitro* studies with liquid culture systems and immunofluorocytometry have revealed that clozapine and its major metabolite *N*-desmethyl clozapine exhibit toxic effects against myeloid maturation and myeloid mitotic components [53].

Antidepressants

Antidepressants possess both stimulatory and inhibitory effects on cell proliferation, depending on the cell type. The serotonin reuptake inhibitors fluoxetine and zimelidine inhibited the proliferation of prostate carcinoma cell lines PC-3, DU-145, and LN CaP *in vitro* and of xenografts in nude mice. This effect was attributed to the inhibition of serotonin uptake by prostate cells, for which serotonin is mitogenic [54]. In a related work, acute treatment with a single intraventricular injection of 10 mg/kg of fluoxetine into rat brains reduced the cytolytic activity of NK cells and inhibited mitogen-induced lymphocyte proliferation [55]; this was not true for chronic treatment. By contrast, *in vivo* administration of fluoxetine and amitriptyline to mice increased the development of fibrosarcoma, melanoma, and breast tumors. This effect correlated with the *in vitro* enhancement of DNA synthesis by these drugs [56].

MOOD STABILIZERS: LITHIUM

Normal Cells

Davies and Garrod [57] reported that lithium induced early stages of kidney tubule differentiation. The renal mesenchyme-epithelial transition showed more DNA synthesis than control cells, although less than that elicited in the spinal cord, which is the known inducer of nephrogenic differentiation. Treatment of mammary epithelial cells obtained from virgin mice resulted in predominant proliferation of luminal-type epithelial cells [58].

Cancer Cells

Lithium chloride at millimolar concentrations inhibited the proliferation of HL-60 promyelocytic and WEHI-3B D^+ myelomonocytic leukemia cells. Growth inhibition was accompanied by induction of differentiation, expressed by an increase in nitroblue tetrazolium reduction and by increased binding of myeloid-specific antibodies. Low levels of retinoic acid markedly enhanced the antiproliferative effects of lithium, whereas other differentiating agents (dimethyl sulfoxide or selenazofurine) failed to do so. These observations suggest that the combination of lithium and retinoic acid may be of clinical relevance in the treatment of white blood cell malignancy [59].

Lithium carbonate at 2.5 to 5 mM inhibited anchorage-independent growth of short-term culture of a neural precursor cell line (NT) developed from murine teratocarcinomas. Lithium arrested the cells in the G phase. Higher concentrations (10 mM) were toxic and led to 33% cell death [60].

Our team has shown that millimolar concentrations of lithium chloride markedly inhibit the proliferation and reduce the clonogenicity of B16 mouse and HT-144 human melanoma cell growth [61].

Pretreatment of B16 melanoma cells with lithium chloride in syngeneic mice delayed the appearance and growth of melanoma tumors. The antiproliferative effects were accompanied by morphological alterations and enhancement of the activity of the endoplasmic reticulum marker NADH cytochrome *c* reductase. These effects were partially reversed by the addition of *myo*-inositol. The results suggest that the effects of lithium on melanoma cell growth and differentiation are related to its effect on phosphatidylinositol metabolism [61, 62]. Along the same lines, lithium was reported to enhance the cytotoxicity of the chemotherapeutic agents bleomycin and vincristine against melanoma tumors in mice [63].

Administration of the lithium salt of γ-linolenic acid to patients with advanced pancreatic cancer resulted in plasma levels of lithium not exceeding 0.8 mmol/L. The highest doses were associated with longer survival. The authors suggested that this compound be investigated in a randomized prospective clinical trial [64].

In contrast to its inhibitory effects, lithium chloride at 1–5 mM stimulated cell proliferation of breast cancer cells, specifically, the estradiol-dependent cell lines MCF-7, T47-D, and ZR-75–1, but not the hormone-independent MDA-MB-231 cells or an estrogen-independent clone of MCF-7 cells. Lithium, estradiol, and epidermal growth factor were also shown to increase the rate of uptake of *myo*-inositol into MCF-7 cells and to increase steady-state levels of inositol phosphates. Apparently, lithium can stimulate the proliferation of human breast cancer cells by a mechanism that involves the phosphoinositide signaling pathway [65, 66].

Immune Cells

Lithium chloride stimulates human monocytes to secrete tumor necrosis factor α, which, in turn, stimulates endothelial cells to produce granulocyte macrophage colony stimulating factor (GM-CSF) in order to enhance granulocytosis. This effect may be relevant to the leukocytosis described in lithium-treated patients [67, 68].

Lithium (1 mM) and interleukin are effective in modulating the toxic action of zidovudine, a drug used for the treatment of acquired immunodeficiency syndrome, on hematopoietic progenitors [69, 70]. Lithium was also effective in reversing the myelosuppression and thrombocytopenia induced by 3′-azido-3′-deoxythymidine (AZT), but less effective in ameliorating AZT-induced anemia [70].

Based on observations of a decrease in chemotherapy-induced myelosuppression in ovarian cancer patients by lithium carbonate, some authors suggested that lithium co-administration may enable escalation of chemotherapy doses in selected patients [68]. In this study, lithium increased LAK cell activity by decreasing cyclic AMP levels. In a complementary work, lithium alone, or IL-2/LAK alone, inhibited the growth of mouse melanoma tumors [71]. The combination of lithium and IL-2/LAK resulted in the strongest inhibitory effect on tumor growth, as reflected by tumor size and prolongation of survival [68, 71].

Based on studies on mice after irradiation and bone marrow transplantation, Gallicchio *et al.* [72] suggested that lithium treatment of the donor may effectively enhance hematopoietic recovery and engraftment in the transplant recipient. Thanks to its wide-ranging effects, lithium may even be superior to the combination of several growth factors [72].

MOOD STABILIZERS: ANTIEPILEPTIC DRUGS

Valproic acid analogues have been shown to induce antiproliferative and differentiating activities in C6 glioma cells [73], and G_1 arrest at 6 to 6.5 hr prior to the S phase [74]. At nontoxic concentrations (0.5 to 2 mM), valproic acid arrested cell growth, induced differentiation, and increased immunogenicity in the human neuroblastoma cell lines OKF-NB-2 and UKF-NB-3. The differentiated characteristics included morphological and ultrastructural alterations compatible with a more differentiated phenotype, a de-

crease in the expression of the N-myc oncoprotein, and an increase in the expression of the membrane neural cell adhesion molecule. Increased immunogenicity was reflected by higher sensitivity to lysis by LAK cells [75]. In another study, valproate, carbamazepine, and phenytoin inhibited the proliferation of B-myeloma and T-lymphoma cell proliferation [76].

CONCLUDING REMARKS

A growing body of evidence, mainly from *in vitro* studies, indicates that a variety of psychotropic agents have an inhibitory effect on normal and cancer cell growth and differentiation. However, this activity usually is achieved at high pharmacological concentrations, and the relevance to clinically relevant therapeutic doses remains unclear. Moreover, the cellular and molecular mechanisms responsible for the cytostatic activity of these agents are puzzling. Most researchers have not yet extended the promising *in vitro* results to complementary *in vivo* studies. Well-designed animal models are essential to confirm the possible role of psychotropic agents, in original form or their non-psychoactive derivatives, in the treatment of malignant tumors as well as benign proliferative diseases. Despite the current lack of definitive data on their clinical application in human cancer, the general safety and tolerability of the psychotropic drugs, as opposed to the currently available antiproliferative drugs, justify further investigation into their use alone or in combination with established treatments.

References

1. Bruera E and Neumann CM, The uses of psychotropics in symptom management in advanced cancer. *Psychooncology* **7**: 346–358, 1998.
2. Derogatis LR, Morrow GR, Fetting J, Penman D, Piasetsky S, Shmale AM, Henricks M and Carnicke CL Jr, The prevalence of psychiatric disorders among cancer patients. *JAMA* **249**: 751–757, 1983.
3. Greenberg DB, Strategic use of benzodiazepines in cancer patients. *Oncology (Huntingt)* **5**: 83–88, 1991.
4. Kris MG, Pisters KM and Hinkley L, Delayed emesis following anticancer chemotherapy. *Support Care Cancer* **2**: 297–300, 1994.
5. Goodman M, Risk factors and antiemetic management of chemotherapy-induced nausea and vomiting. *Oncol Nurs Forum* **24** (Suppl 7): 20–32, 1997.
6. Kloke M, Hoffken K, Olbrich H and Schmidt CG, Antidepressants and anti-convulsants for the treatment of neuropathic pain syndromes in cancer patients. *Onkologie* **14**: 40–43, 1991.
7. Camins A, Diez-Fernandez C, Pujadas E, Camarasa J and Escubedo E, A new aspect of the antiproliferative action of peripheral type benzodiazepine receptor ligands. *Eur J Pharmacol* **272**: 289–292, 1995.
8. Stepien H, Pawlikowska A and Pawlikowski M, Effects of benzodiazepines on thymus cell proliferation. *Thymus* **12**: 117–121, 1988–89.
9. Regan CM, Gorman AM, Larsson OM, Maguire C, Martin ML, Schousboe A and Williams DC, *In vitro* screening for anticonvulsant-induced teratogenesis in neuronal primary culture and cell lines. *Int J Dev Neurosci* **8**: 143–150, 1990.
10. Miernick A, Santa-Marcia A and Marano F, The antimitotic activities of some benzodiazepines. *Experientia* **42**: 956–958, 1986.
11. Bruce JH, Ramirez AM, Lin L, Oracion A, Agarwal RP and Norenberg MD, Peripheral-type benzodiazepines inhibit proliferation of astrocytes in culture. *Brain Res* **564**: 167–170, 1994.
12. Neary JT, Jorgensen SL, Oracion AM, Bruce JH and Norenberg MD, Inhibition of growth factor-induced DNA synthesis in astrocytes by ligands of peripheral-type benzodiazepine receptors. *Brain Res* **675**: 27–30, 1995.
13. Lafi A and Parry JM, A study of the induction of aneuploidy and chromosome aberrations after diazepam, medazepam, midazolam and bromazepam treatment. *Mutagenesis* **3**: 23–27, 1988.
14. Gorman AM, O'Beirne GN, Regan CM and Williams DC, Antiproliferative action of benzodiazepines in cultured brain cells is not mediated through the peripheral-type benzodiazepine acceptor. *J Neurochem* **53**: 849–855, 1989.
15. Maguire C, O'Connell C and Regan CM, Clorazepate synchronizes cultured rat C6 glioma in the early G_1 phase of the cell cycle. *Brain Res* **590**: 74–80, 1992.
16. Sidi Y, Beery E, Panet C, Wasserman L, Novogrodsky A and Nordenberg J, Growth inhibition and induction of phenotypic alterations by tiazofurin: Differential effects on breast cancer and HB-100 breast cell lines. *Eur J Cancer Clin Oncol* **25**: 883–889, 1989.
17. Vaziri C, Stice L and Faller DV, Butyrate-induced G_1 arrest results from p21-independent disruption of retinoblastoma protein-mediated signals. *Cell Growth Differ* **9**: 465–474, 1998.
18. Ikezaki K and Black KL, Stimulation of cell growth and DNA synthesis by peripheral benzodiazepine. *Cancer Lett* **49**: 115–120, 1990.
19. Kunert-Radek J, Stepien H and Pawlikowski H, Inhibition of rat pituitary cell proliferation by benzodiazepines *in vitro*. *Neuroendocrinology* **59**: 92–96, 1994.
20. Crocker CE and Niles LP, Benzodiazepine-induced inhibition of human malignant melanoma (M-6) cell growth. *Anticancer Res* **16**: 1259–1263, 1996.
21. Solowey WE, Pestka S, Spector S, Fryer RI and Fisher PB, Peripheral-acting benzodiazepines inhibit the growth of human melanoma cells and potentiate the antiproliferative activity of recombinant interferons. *J Interferon Res* **10**: 269–280, 1990.
22. Curran T and Morgan JI, Superinduction of c-*fos* by nerve growth factor in the presence of peripherally active benzodiazepines. *Science* **229**: 1265–1268, 1985.
23. Miller LG, Tischler AS, Jumblatt JE and Greenblatt DJ, Benzodiazepine binding sites on PC12 cells: Modulation by nerve growth factor and forskolin. *Neurosci Lett* **89**: 342–348, 1988.
24. Black KL, Shiraishi T, Ikezak K, Tabuchi K and Becker DF, Peripheral benzodiazepine stimulates secretion of growth hormone and mitochondrial proliferation in pituitary tumor GH3 cells. *Neurol Res* **16**: 74–80, 1994.
25. Mak NK, Szeto YY, Fung MC, Leung KN and Kwan SK, Effects of midazolam on the differentiation of murine myeloid leukemia cells. *Chemotherapy* **43**: 272–281, 1997.
26. Landau M, Weizman A, Zoref-Shani E, Beery E, Wasserman L, Landau O, Gavish M, Brenner S and Nordenberg J, Antiproliferative and differentiating effects of benzodiazepine receptor ligands on B16 melanoma cells. *Biochem Pharmacol* **56**: 1029–1034, 1998.
27. Katz Y, Ben-Baruch G, Kloog Y, Menczer J and Gavish M, Increased density of peripheral benzodiazepine-binding sites

in ovarian carcinoma as compared with benign ovarian tumours and normal ovaries. *Clin Sci (Colch)* **78**: 155–159, 1990.
28. Ramseier H, Lichtensteiger W and Schlumpf M, *In vitro* inhibition of cellular immune responses by benzodiazepines and PK 11195. Effects on mitogen- and alloantigen-driven lymphocyte proliferation and on IL-1, IL-2 synthesis and IL-2 receptor expression. *Immunopharmacol Immunotoxicol* **15**: 557–582, 1993.
29. Ehret A, Westendorp MO, Herr I, Debatin KM, Heeney JL, Frank R and Krammer PH, Resistance of chimpanzee T cells to human immunodeficiency virus type I Tat-enhanced oxidative stress and apoptosis. *J Virol* **70**: 6502–6507, 1996.
30. L-pez-Girona A, Colomer J, Pujol MJ, Bachs O and Agell N, Calmodulin regulates DNA polymerase α activity during proliferative activation of NRK cells. *Biochem Biophys Res Commun* **184**: 1517–1523, 1992.
31. Komoda T, Ikeda E, Nakatani Y, Sakagisyi Y, Mead N, Kato T and Kumegawa M, Inhibitory effect of phenothiazine derivatives on bone *in vivo* and osteoblastic cells *in vitro*. *Biochem Pharmacol* **34**: 3885–3889, 1985.
32. Grief F, Soroff HS, Albers KM and Taichman LB, The effect of trifluoperazine, a calmodulin antagonist, on the growth of normal and malignant epidermal keratinocytes in culture. *Eur J Cancer Clin Oncol* **25**: 19–26, 1989.
33. Niczyporuk W, Krajewska-Kulak E and Zimnoch L, Preliminary study on the effect of the selected calmodulin antagonists on the skin. *Rocz Akad Med Bialymst* **41**: 515–524, 1996.
34. Stringer BM, Rowson J, Greer W, Wynford-Thomas D and Williams ED, Effect of sustained serum prolactin elevation on breast epithelial and myoepithelial proliferation. *Cell Tissue Kinet* **23**: 17–30, 1990.
35. Schleuning M, Brumme V and Wilmanns W, Growth inhibition of human leukemic cell lines by the phenothiazine derivate fluphenazine. *Anticancer Res* **13**: 599–602, 1993.
36. Hait WN and Lee AL, Characteristics of the cytotoxic effects of the phenothiazine class of calmodulin antagonists. *Biochem Pharmacol* **34**: 3973–3978, 1985.
37. Hait WN, Grais L, Benz C and Cadman EC, Inhibition of growth of leukemic cells by inhibitors of calmodulin: Phenothiazines and melittin. *Cancer Chemother Pharmacol* **14**: 202–205, 1985.
38. Glass-Marmor L, Morgenstern H and Beitner R, Calmodulin antagonists decrease glucose 1,6-bisphosphate, fructose 1,6-bisphosphate, ATP and viability of melanoma cells. *Eur J Pharmacol* **313**: 265–271, 1996.
39. Glass-Marmor L and Beitner R, Detachment of glycolytic enzymes from cytoskeleton of melanoma cells induced by calmodulin antagonists. *Eur J Pharmacol* **328**: 241–248, 1997.
40. Zhu HG, Tayeh I, Israel L and Castagna M, Different susceptibility of lung cell lines to inhibitors of tumor promotion and inducers of differentiation. *J Biol Regul Homeost Agents* **5**: 52–58, 1991.
41. Sutherland RL, Watts CK, Hall RE and Ruenitz PC, Mechanisms of growth inhibition by nonsteroidal antiestrogens in human breast cancer cells. *J Steroid Biochem* **27**: 891–897, 1987.
42. Motohashi N, Sakagami H, Kamata K and Yamamoto Y, Cytotoxicity and differentiation-inducing activity of phenothiazine and benzo[*a*]phenothiazine derivatives. *Anticancer Res* **11**: 1933–1937, 1991.
43. Lee GL and Hait WM, Inhibition of growth of C6 astrocytoma cells by inhibitors of calmodulin. *Life Sci* **36**: 347–354, 1985.
44. Marushige Y, Marushige K and Koestner A, Chemical control of growth and morphological characteristics of anaplastic glioma cells. *Anticancer Res* **9**: 1729–1735, 1989.
45. Hait WN, Gesmonde JF and Lazo JS, Effect of anti-calmodulin drugs on the growth and sensitivity of C6 rat glioma cells to bleomycin. *Anticancer Res* **14**: 1711–1721, 1994.
46. Aas AT, Brun A, Pero RW and Salford LG, Chlorpromazine in combination with nitrosourea inhibits experimental glioma growth. *Br J Neurosurg* **8**: 187–192, 1994.
47. Molnár J, Pusztai R, Hevér A, Nagy S and Motohashi N, Effect of two benzo[*a*]phenothiazines on multi-drug resistance (mdr) and tumor antigen expression. *Anticancer Res* **15**: 2013–2016, 1995.
48. Ford JM, Prozialeck WC and Hait WN, Structural features determining activity of phenothiazines and related drugs for inhibition of cell growth and reversal of multidrug resistance. *Mol Pharmacol* **35**: 105–115, 1989.
49. Schleuning M, Brumme V and Wilmanns W, Inhibition of cyclosporine on A/FK506 resistant, lymphokine-induced T-cell activation by phenothiazine derivatives. *Naunyn Schmiedebergs Arch Pharmacol* **350**: 100–103, 1994.
50. Petri IB, Szekeres E, Varga E, Berek I, Molnár J, Berek L, Kawase M and Motohashi N, Immunomodulating activities on cellular cytotoxicity and the blast transformation of human lymphocytes by 10-[*n*-(phthalimido)alkyl]-2-substituted-10*H*-phenothiazines and 1-(2-chloroethyl)-3-(2-substituted-10*H*-phenothiazin-10-yl)alkyl-1-ureas. *Anticancer Res* **16**: 1247–1250, 1996.
51. Roudebush RE, Berry PL, Layman NK, Butler LD and Bryant HU, Dissociation of immunosuppression by chlorpromazine and trifluoperazine from pharmacologic activities as dopamine antagonists. *Int J Immunopharmacol* **13**: 961–968, 1991.
52. Chernavsky AC, Valerani AV and Burdman JA, Haloperidol and oestrogens induce c-myc and c-fos expression in the anterior pituitary gland of the rat. *Neurol Res* **15**: 339–343, 1993.
53. Veys PA, Wilkes S, Shah S, Noyelle R and Hoffbrand AV, Clinical experience of clozapine-induced neutropenia in the UK. Laboratory investigation using liquid culture systems and immunofluorocytometry. *Drug Saf* **7** (Suppl 1): 26–32, 1992.
54. Abdul M, Logothetis CJ and Hoosein NM, Growth inhibitory effects of serotonin uptake inhibitors on human prostate carcinoma cell lines. *J Urol* **154**: 247–250, 1995.
55. Pellegrino TC and Bayer BM, Modulations of immune cell function following fluoxetine administration in rats. *Pharmacol Biochem Behav* **59**: 151–157, 1998.
56. Brandes LJ, Arron RJ, Boydanovica RP, Tony J, Zabioniayh CL and Hogg GR, Stimulation of malignant growth in rodents by antidepressant drugs at clinically relevant doses. *Cancer Res* **52**: 3796–3800, 1992.
57. Davies JA and Garrod DR, Induction of early stages of kidney tubule differentiation by lithium ions. *Dev Biol* **167**: 50–60, 1995.
58. Levay-Young BK, Amamoto S, Imagawa W and Nandi S, Casein accumulation in mouse mammary epithelial cells after growth stimulated by different hormonal and nonhormonal agents. *Endocrinology* **1256**: 1173–1182, 1990.
59. Sokoloski JA, Li J, Nigam A and Sartorelli AC, Induction of the differentiation of HL-60 and WEHI-3B D^+ leukemia cells by lithium chloride. *Leuk Res* **17**: 403–410, 1993.
60. Hasgekar NN, Gohkhale PP, Amin MK, Shesadri R and Lalitha VS, Lithium inhibits growth in a murine neural precursor cell line. *Cell Biol Int* **20**: 781–786, 1996.
61. Nordenberg J, Panet C, Wasserman L, Malik Z, Fux A, Stenzel KH and Novogrodsky A, The anti-proliferative effect of lithium chloride on melanoma cells and its reversion by *myo*-inositol. *Br J Cancer* **55**: 41–46, 1987.
62. Becchetti A and Whitaker M, Lithium blocks cell cycle transitions in the first cell cycles of sea urchin embryos, an effect rescued by *myo*-inositol. *Development* **124**: 1099–1107, 1997.
63. Ballin A, Aladjem M, Banyash M, Biochis H, Barzilay Z, Gal

R and Witz IP, The effect of lithium chloride on tumour appearance and survival of melanoma-bearing mice. *Br J Cancer* **48**: 83–87, 1983.
64. Fearon KCH, Falconer JS, Ross JA, Carter DC, Hunter JO, Reynolds PD and Tuffnell Q, An open-label phase I/II dose escalation study of the treatment of pancreatic cancer using lithium gammalinolenate. *Anticancer Res* **16**: 867–874, 1996.
65. Taylor JA, Grady LH, Engler KB and Welshons WV, Relationship of growth stimulated by lithium, estradiol, and EGF to phospholipase C activity in MCF-7 human breast cancer cells. *Breast Cancer Res Treat* **34**: 265–277, 1995.
66. Welshons WV, Engler KS, Taylor JA, Grady LH and Curran EM, Lithium-stimulated proliferation and alteration of phosphoinositide metabolites in MCF-7 human breast cancer cells. *J Cell Physiol* **165**: 134–144, 1995.
67. Kleinerman ES, Knowles RD, Blick MB and Zwelling LA, Lithium chloride stimulates human monocytes to secrete tumor necrosis factor/cachectin. *J Leukoc Biol* **46**: 484–492, 1989.
68. Richman CM, Makii MM, Weiser PA and Herbst AL, The effect of lithium carbonate on chemotherapy-induced neutropenia and thrombocytopenia. *Am J Hematol* **16**: 313–323, 1984.
69. Gallicchio VS, Hughes NK, Hulette BC and Noblitt L, Effect of interleukin-1, GM-CSF, erythropoietin and lithium on the toxicity associated with 3′-azido-3′-deoxythymidine (AZT) *in vitro* on hematopoietic progenitors (CFU-GM, CFU-MEG, and BFU-E) using murine retrovirus-infected hematopoietic cells. *J Leukoc Biol* **50**: 580–586, 1991.
70. Gallicchio VS, Hughes NK and Tse KF, Modulation of the haematopoietic toxicity associated with zidovudine *in vivo* with lithium carbonate. *J Intern Med* **233**: 259–268, 1993.
71. Wu Y and Cai D, Study of the effect of lithium on lymphokine-activated killer cell activity and its antitumor growth. *Proc Soc Exp Biol Med* **201**: 284–288, 1992.
72. Gallicchio VS, Messino MH, Hulette BC and Hughes NK, Lithium and hematopoiesis: Effective experimental use of lithium as an agent to improve bone marrow transplantation. *J Med* **23**: 195–216, 1992.
73. Courage-Maguire C, Bacon CL, Nau H and Regan CM, Correlation of *in vitro* anti-proliferative potential with *in vivo* teratogenicity in a series of valproate analogues. *Int J Dev Neurosci* **15**: 37–43, 1997.
74. Martin ML and Regan CM, The anticonvulsant valproate teratogen restricts the glial cell cycle at a defined point in the mid-G1 phase. *Brain Res* **554**: 223–228, 1991.
75. Cinatl J Jr., Cinatl J, Scholz M, Driever PH, Henrich D, Kabickova H, Vogel JU, Doerr HW and Kornhuber B, Antitumor activity of sodium valproate in cultures of human neuroblastoma cells. *Anticancer Drugs* **7**: 766–773, 1996.
76. Tittle TV and Schaumann BA, Effect of antiepileptic drugs on growth of murine lymphoid tumor cells in single-cell culture. *Epilepsia* **33**: 729–735, 1992.