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Selective inhibitor (PB-100) of human glioblastoma cell multiplication

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PB-100, a selective anticancer agent, suppresses the mitogenic effect of several steroid hormones, catecholamines and cytokines on human BCNU-resistant glioblastoma cells.

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SUMMARY

Numerous biological substances stimulate cancer cell proliferation in vitro and in vivo. We show here that the steroid hormones progesterone and testosterone, the catecholamines dopamine and epinephrine, the interleukins IL4, IL-6 and IL-10, and the iron transport protein ferritin, dose-dependently enhance in vitro the proliferation of a human BCNU-resistant glioblastoma cell line (U 251) and, to a lesser extent, that of a normal (non malignant) astrocyte line (CRL 1656).

We then demonstrate that PB-100, a potent, selective anticancer agent, abolishes the mitogenic effect of each of the tested substances both on cancer cells and on non malignant cells. PB-100 by itself inhibits multiplication of cancer cells and leads to their destruction. In contrast, it does not inhibit multiplication and activities of normal cells.

PB-100, which crosses the blood-brain barrier, and can act in synergy with conventional therapies, may be considered as a model for treatments aiming at the selective destruction of brain tumor cells.

OBJECTIVES

Cancer cell multiplication is enhanced by many biological substances involved in normal physiological processes, such as cytokines or steroid hormones, and especially so when these molecules are produced in excessive amounts. The rationale of our reseach was to look for a chemotherapeutic drug capable of suppressing stimulation of cancer cell proliferation by such mitogens and of killing malignant cells without affecting normal cell multiplication and activities.

There is a particularly urgent need for such substances in brain tumor therapy: the few known anticancer drugs which can cross the blood-brain barrier are toxic for normal cells.

The anticancer agent PB-100, which crosses the blood-brain barrier, is closely targeted to malignant cells and does not harm normal cells. In vivo assays showed that it can be used in monotherapy or in combination with conventional anticancer therapies. The aim of the present research was to study in vitro the interaction between PB-100 and several mitogens of prime physiological importance.

METHODS

Cell lines and culture technique

The established human glioblastoma cell line (U 251) and normal astrocyte cell line (CRL 1656 Mpf cell repository line) were grown at 37°C in an incubator. Culture medium (RPMI 1640) was renewed 3 days after seeding and subsequently twice a week. Absence of bacteria and mycoplasma was checked every 3 months. No antibiotic was used. Each cell type was subcultured in 6-well tissue culture plates (9.8 cm^2/well) starting from a $4x10^4$ inoculum. Cell viability was determined using trypan blue dye. Viability was 99 % to 100 % at start of experiments. Cells were counted with a Coulter counter.

Inhibition and stimulation of cell multiplication

After a 5 minute cell incubation, the filtration-sterilized substance under test was added to the culture medium. Increasing concentrations were assayed. Cells were detached from wells by trypsinization, which was then stopped by addition of 4 ml RPMI 1640 per well. Results are means of three experiments, each performed in triplicate.

Isolation of DNA and UV absorbance measurements

DNAs from glioblastoma (U 251) and astrocyte (CRL 1656) cell lines were isolated and purified using phenol and chloroform. After dialysis, purity and amount of DNA were determined from its UV absorbance at 260 nm (260/280=2.05).

In the presence of 0.1 KOH, DNA exhibited a 45 % - 52 % hyperchromicity. To evaluate in vitro binding of PB-100, DNA was dissolved in Tris-HCl buffer at pH 7.3 (10 µg/ml). PB-100 was dissolved in 1 ml buffer and added to the blank (distilled water) and to the DNA solution. Mixture was gently agitated at room temperature. Its UV spectrum was used to detect formation of a PB-100 / DNA complex.

To investigate the effect of the mitogens and of PB-100 on DNA secondary structure, percent increase or decrease of UV absorbance at 260 nm were determined. Three separate determinations were performed.

RESULTS AND DISCUSSION

In the course of previous in vitro research, we had shown that the selective anticancer agent PB-100, overcoming the drug resistance (BCNU) of the U 251 human cell line derived from the highly malignant and frequent glioblastoma brain tumor, arrests multiplication of these cells in a dose-dependent manner ; moreover, 100 µg PB-100/ml kill 100 % of the glioblastoma cells. In contrast, even at this concentration, this agent does not affect multiplication of the CRL 1656 normal astrocyte cell line chosen as control.

In the present in vitro study, we demonstrated the mitogenic effect of several important physiological molecules on glioblastoma cells and astrocytes and the ability of PB-100 to inhibit this mitogen-induced stimulation of cell proliferation.

Used at ng/ml concentrations, progesterone, testosterone, dopamine, epinephrine, the interleukins IL-4, IL-6 and IL-10, and ferritin all significantly enhance (by 50 to 220 %) glioblastoma cell proliferation and also, to a lesser extent (by 5 to 20 %), that of non-malignant astrocytes.

PB-100 (µg/ml) added to the culture medium containing any one of these mitogens drastically inhibits their stimulatory effect on cell multiplication, which may experience a 75 % decrease. In addition, even in the presence of the mitogens, chosen PB-100 concentrations may kill over 98 % of the cancer cells. PB-100 also dose-dependently decreases the effect of the mitogens on astrocyte multiplication until it returns to physiological levels.

Though all the tested mitogens bind to cell surface receptors, mediation of their activity differs afterwards: some are translocated to the nucleus (steroid hormones), while others trigger a second messenger (catecholamines). PB-100 might compete with the mitogens at any one of these levels and this is being investigated.

However, when assayed in vitro on isolated DNA, all the tested substances prove able to bind directly to this molecule. DNA absorbance at 260 nm is seen to increase (hyperchromicity) under the influence of the mitogens for both glioblastoma and astrocyte DNA, proportionately to the effect of these agents on cell multiplication. On the contrary, PB-100 decreases glioblastoma DNA hyperchromicity, lowers that of astrocyte DNA to the extent it had been increased by the mitogens, but does not modify control UV absorbance values of normal astrocyte DNA.

Increase of 260 nm UV absorbance is due to H-bond breakage inside the DNA molecule. Some fifteen years ago, after comparing numerous DNAs from cancer and normal cells, we demonstrated that 260 nm UV absorbance is at all times higher for malignant than for non-malignant DNA. This means that cancer DNA permanently contains large areas where H-bonds holding the strands together are broken, whereas normal DNA strands only separate locally and temporarily for closely regulated replication and gene expression. The characteristic property of cancer DNA is thus its relaxed secondary structure, which leads to molecular destabilization. On the separated strand portions, normally unused initiation sites become available to enzymes in charge of replication and transcription, accounting for uncontrolled replication and abnormal protein synthesis. UV absorption values at 260 nm, in vitro DNA replication and cell multiplication closely correlate.

It also becomes easy for numerous endogenous and exogenous substances to bind to the relaxed strands of cancer DNA, and many of them further increase chain relaxation and DNA destabilization. In contrast, we found a number of restabilizing compounds able to bring the separated cancer DNA strands back together into their proper position. An example of this is PB-100: it binds to the abnormal cancer DNA initiation sites, shutting off the supply of abnormal metabolites on which the malignant cell had come to depend, and increasing concentrations of the anticancer agent finally kill the cancer cell. Access to non relaxed normal DNA strands is much more difficult. PB-100 does not bind to them; for this reason it is not toxic for normal cells.

CONCLUSIONS

The selective anticancer agent PB-100, when assayed in vitro on human BCNU-resistant glioblastoma cells, efficiently arrests the mitogenic effect of eight different molecules of physiological importance. PB-100 is also able to completely destroy the glioblastoma cells.

Even at the highest concentrations used, PB-100 does not impair non-malignant astrocyte multiplication; it only suppresses mitogen-induced enhancement and returns proliferation of these cells to normal levels. PB-100 shows no toxicity for normal (non malignant) cells.

Results obtained in this study, added to those of prior investigations, indicate that PB-100, which crosses the bloodbrain barrier, is a potential candidate for brain cancer therapy, where it may be used in synergy with conventional anticancer treatments.