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PBB Inhibits Metabolic Cooperation in Chinese Hamster Cells *in Vitro:* Its Potential as a Tumor Promoter

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Using an *in vitro* assay system, polybrominated biphenyl (PBB) was assessed for its ability to inhibit metabolic cooperation between 6-thioguanine sensitive and resistant Chinese hamster V79 cells. Using a nonlethal range of the chemical, PBB was shown to inhibit metabolic cooperation (a form of cell-cell communication) in a manner similar to other known tumor promoters. Results suggest that PBB could act, epigenetically, as a teratogen and a carcinogenic promoter.

Introduction

Carcinogenesis in many animal test systems has been shown to be a complex and multistaged process (*1*), consisting of initiation and promotion phases (*2*, *3*). Not only has this initiation/promotion concept of carcinogenesis been found *in vivo* in animals, but also it appears to explain the multistep processes of *in vitro* transformation (*4-6*). Moreover, there are several reports which can lead one to believe that initiation and promotion phases exist in human carcinogenesis (*7-11*).

Initiation is now thought to involve the induction of DNA damage and the error-prone repair or replication of that damage in mutation fixation (*12*), whereas promotion seems to be the selective proliferation of the initiated cells by agents that affect cell membranes (i.e., tumor promoters) (*13-17*)

Yotti et al. (*18*), Trosko et al. (*19*), and Murray and Fitzgerald (*20*) have shown that known tumor promoters blocked metabolic cooperation between cells *in vitro*. Although the mechanism by which tumor promoters block this form of cell-cell communication is not known, it is suspected that interference with gap-junction function (*21*) might be involved (*18*, *20*). Once cells escape the contact-inhibiting (*22*) or chalone-inhibiting function of other neighboring cells (*23-28*), they can start proliferating.

The widespread exposure of the human population of Michigan to polybrominated biphenyl (PBB) by an accidental contamination of animal feed (*29*) has promoted a widespread number of studies on the potential biological consequences on human health. We report the results of our studies to test if PBB acts as other known tumor promoters in our *in vitro* assay using Chinese hamster cells.

Materials and Method

All cells used in the assay were originally derived from V79 Chinese hamster lung cells. The detailed protocol for the *in vitro* promoter assay has been reported (*19*). Wild-type cells [6-thioguanine-sensitive, presumptive hypoxanthine guanine-phosphoribosyltransferase (HG-PRT$^+$)] are seeded into 100 mm tissue culture dishes at a density of 9×10^5 cells per dish. Immediately after plating of wild-type cells, 100 6-thioguanine-resistant cells (HG-PRT$^-$) are seeded in the same dishes. Both cell lines are given 4 hr for attachment, at which time the PBB (Velsicol Corp.; dissolved in acetone) is added directly into each individual dish. 6-Thioguanine (final concentration, 10 μg/ml) was added immediately after treatment with PBB. The cells were incubated for two days without interruption, at which time the medium was changed. PBB-containing medium was removed and growth continued on the

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selective medium. After another two days of growth, the medium was again replaced with fresh selective medium. Three more days of growth resulted in colonies of a size sufficient to score visually. The medium is decanted, each dish was rinsed with 0.85% saline; the plates were air-dried, fixed with 95% ethanol and stained with Giemsa. The resulting colonies were scored visually.

Results and Discussion

In order to determine the cytotoxicity of PBB, the experiment in Figure 1 was performed. The colony-forming ability of V79 cells was measured after continuous exposure, during their growth, to increasing concentrations of PBB. We, then, performed a preliminary experiment using the highest concentration of PBB which had little lethal effect on colony-forming ability, to determine if PBB would inhibit metabolic cooperation between 6-thioguanine sensitive and resistant cells (Table 1). The results clearly indicate that PBB, although not as effective as 12-*o*-tetradecanoyl phorbol-13-acetate (TPA; an internal control promoter), did inhibit metabolic cooperation.

As an extension of these results, we designed a dose-response experiment. Figure 2 demonstrates that there does seem to be a definite dose response curve from 2.5 μg/ml to 10 μg/ml. The data also suggest a threshold level, below which no inhibition of metabolic cooperation is detectable. It must also be stressed that the inhibition of metabolic cooperation that was observed was at levels which were nontoxic to the cells.

If we assume that carcinogenesis in human beings consists of initiation and promotion phases, that this *in vitro* assay to detect tumor promoters, using

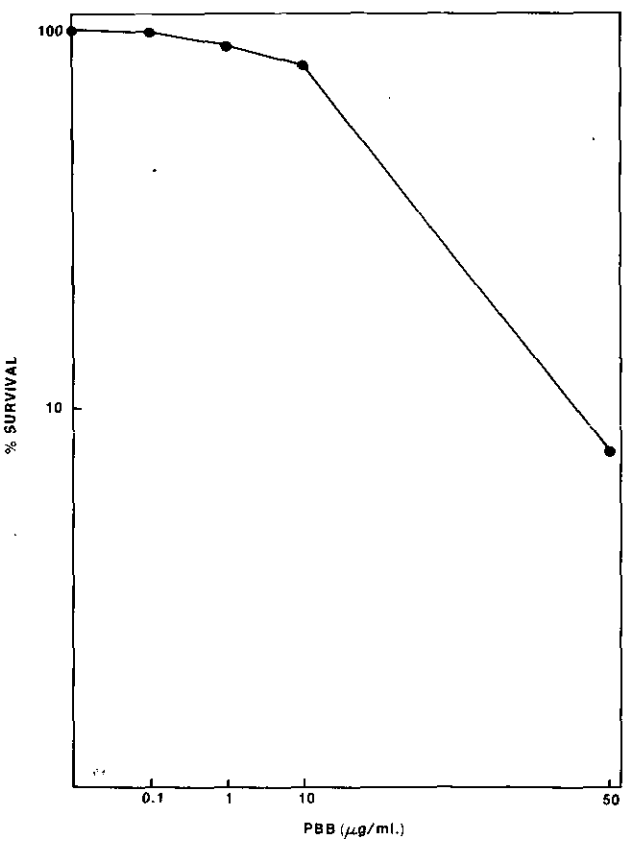

FIGURE 1. Colony-forming ability of Chinese hamster (V79) cells in increasing concentration of PBB. The data are expressed as the percentage of plated cells which formed colonies when 200 cells were plated in four plates for each group. PBB was prepared by serial dilution in concentration such that 100λ of PBB was added to each plate.

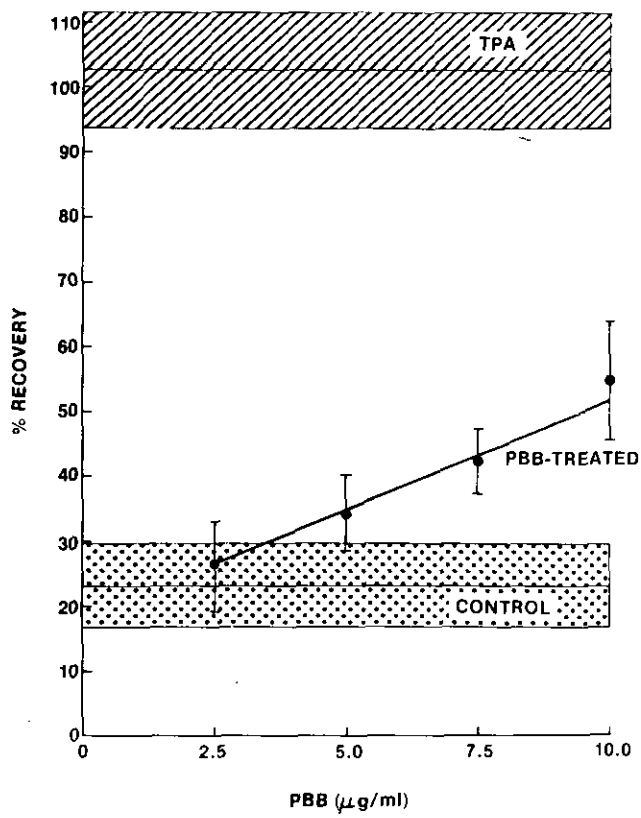

FIGURE 2. Dose-response curve for the effect of PBB on the inhibition of metabolic cooperation between HG-PRT+ and HG-PRT- Chinese hamster V79 cells. The shaded areas correspond to the mean recovery of HG-PRT- colonies for the control and TPA-treated groups ± SD. The data are expressed as the percentage of HG-PRT- cells which formed colonies in the presence of HG-PRT+ cells using 21 plates for each group.

Table 1. Recovery of $6TG^R$ Cells in the presence of TPA and PBB.[a]

V79 (HG-PRT$^+$), cells/plate	$6TG^R$ (HG-PRT$^-$), cells/plate	Drug	Concentration, μg/ml	Recovery, %
9×10^5	100	—	-	24.0 ± 0.8
9×10^5	100	TPA	0.1 μg/ml	90.2 ± 1.8[b]
9×10^5	100	PBB	7.5 μg/ml	38.7 ± 1.5[b]

[a]TPA = 12-σ-tetradecanoylphorbol-13-acetate (dissolved in 100% ethanol); PBB = Firemaster Bp-6 (dissolved in acetone).
[b]Treatment significantly increased the % recovery above control according to the Student-Newman-Keuls' (SNK) Test ($p < 0.01$)

Chinese hamster lung cells, is relevant to the human situation, and that blocking of metabolic cooperation (as a marker for cell-cell communication) is a property of all tumor promoters, then we feel that PBB has the properties of a potential tumor promoter. Since all three of the former assumptions have yet to be rigorously verified, we cannot conclude that PBB is a definite tumor promoter for human beings. However, because of the general similarity of carcinogenesis in all mammals and the general (but by no means) concurrence of *in vitro* and *in vivo* tests for carcinogenicity, one would predict PBB could be a tumor promoter.

PBB has been shown to reduce, significantly, the incidence of *N*-2-fluorenylacetamide-induced mammary gland and ear duct tumors in female rats (*30*). Of importance is the observation that PBB has many properties similar to phenobarbital (*31*, *32*) and butylated hydroxytoluene (*31-34*), in that these chemicals can inhibit chemically induced carcinogenesis if administered prior to the carcinogen, but they act as promoters when given after carcinogen exposure. These observations in animals, plus our *in vitro* results, lead us to believe that, *in vivo*, PBB could be a promoter of certain types of tumors under conditions where the organism has been previously initiated.

Furthermore, because of a recent *in vitro* assay for teratogens which is based on the ability of a chemical to affect cell membrane surface adhesion (*35*), one might predict PBB could also be a teratogen. Since both our assay and the teratogen assay are dependent on specific membrane components, not all cells in all organs will be negatively affected by PBB (or any other chemical). However, this demonstration that PBB does affect a cell type, in a manner similar to a wide number of other known promoters (i.e., saccharin, DDT, Tween 60, phenobarbital, etc.) (*19*), forms the basis for additional experiments, in animals, to verify both the promotion and teratogen potential of PBB.

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