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

by James E. Trosko,* B. Dawson,* and C.-C. Chang*

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 $9 \times 10^5$ 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
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](image1.png)

**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 100μl of PBB was added to each plate.

![Figure 2](image2.png)

**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.
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-fluorenylacetonitrile-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, pheno-barbital, etc.) (19), forms the basis for additional experiments, in animals, to verify both the promotion and teratogen potential of PBB.

This research was supported by a NCI grant (CA 21104), a MSU All-University Grant (80-37), a MSU Agriculture Experiment Station Grant, a MSU Toxicology Center grant, and a MSU College of Human Medicine grant to J.E.T. (CA 21104) and a NIEHS Young Environmental Scientists Award to C.-C. Chang (ES 01809).

REFERENCES

1. Slaga, T. J., Sivak, A., and Boutwell, R. K., Eds. Carcinogenesis, Vol. 2. Raven Press, New York, 1978.
2. Berenblum, I. A speculative review: The probable nature of promoting action and its significance in the understanding of the mechanism of carcinogenesis. Cancer Res. 14: 471 (1954).
3. Boutwell, R. K. The biochemistry of preneoplasia in mouse skin. Cancer Res. 35: 2631 (1976).
4. Mondal, S., Brankow, D. W., and Heidelberger, C. Two-stage chemical oncogenesis in cultures of C3H/10T ½ cells. Cancer Res. 36: 2254 (1976).
5. Mondal, S., and Heidelberger, C. Transformation of C3H/10T ½ CL8 mouse embryo fibroblasts by ultraviolet irradiation and a phorbol ester. Nature 260: 710 (1976).
6. Kennedy, A. R., Mondal, S., Heidelberger, C., and Little, J. B. Enhancement of x-radiation transformation by a phorbol ester using C3H/10T1/2 CL8 mouse embryo fibroblast. Cancer Res. 38: 438 (1978).
7. Reddy, B. S., Weisburger, J. H., and Wynder, E. L. Colon cancer: bile salts as tumor promoters, In: Carcinogenesis, Vol. 2, T. J. Slaga, A. Sivak, and R. K. Boutwell, Eds., Raven Press, New York, 1978, p. 453.
8. Weber, J., and Hecker, E. Experimental 34: 679 (1978).
9. Weiss, W. Changing incidence of thyroid cancer. J. Natl. Cancer Inst. 62: 1137 (1979).
10. Domellof, L. Gastric carcinoma promoted by alkaline reflux gastritis with special reference to bile and other surfactants as promoters of post operative gastric cancer. Med. Hypoth. 5: 463 (1979).
11. Oscarson, J. E. A., Veen, H. G., Ross, J. S., and Malt, R. A. Ileal resection potentiates 1,2-dimethylhydrazine-induced colonic carcinogenesis. Ann. Surgery 189: 503 (1979).
12. Trosko, J. E., and Chang, C.-C. The role of radiation and chemicals in the induction of mutations and epigenetic changes during carcinogenesis. In: Advances in Radiation Biology, Vol. 9, J. Lett and H. Adler, Eds., Academic Press, New York, in press.
13. Sivak, A., and Van Durren, B. L. Cellular interactions of phorbol myristate acetate in tumor promotion. Chem. Biol. Interact. 3: 401 (1971).
14. Wenner, C. E., Hackney, J., Kimelberg, H. K., and Mayhew, E. Membrane effects of phorbol esters. Cancer Res. 34: 1731 (1974).
15. Blumberg, P. M., Driedger, P. E., and Rossow, P. W. Effect of a phorbol ester on a transformation sensitive surface protein of chick fibroblasts. Nature 264: 446 (1976).
16. Horton, A. W., Eshlemon, D. N., Schuff, A. R., and
Perman, W. H. Correlation of carcinogenic activity among n-alkanes with their physical effects on phospholipid micelles. J. Natl. Cancer Inst. 56: 387 (1979).

17. Shoyab, M., Delarco, J. E., and Todaro, G. H. Biologically active phorbol esters specifically alter affinity of epidermal growth factor membrane receptors. Nature 279: 387 (1979).

18. Yotti, L. P., Chang, C.-C., and Trosko, J. E. Elimination of metabolic cooperation in Chinese hamster cells by a tumor promoter. Science 206: 1089 (1979).

19. Trosko, J. E., Yotti, L. P., Dawson, B., and Chang, C.-C. In Vitro assay for tumor promoters. In: Short Term Tests for Chemical Carcinogens, H. Stich and R. H. C. San, Eds., Springer-Verlag, New York, in press.

20. Murray, A. W., and Fitzgerald, D. H. Tumor promoters inhibit metabolic cooperation in cocultures of epidermal and 3T3 cells. Biochem. Biophys. Res. Commun. 91: 395 (1979).

21. Loewenstein, W. R. Junctional intercellular communication and the control of growth. Biochim. Biophys. Acta 580: 1 (1979).

22. Levine, E. M., Becker, Y., Boone, C. W., and Eagle, H. Contact inhibition macromolecular synthesis and polyribosomes in cultured human diploid fibroblasts. Proc. Natl. Acad. Sci. (U.S.) 53: 350 (1965).

23. Bullough, W. S. Mitotic and functional homeostasis: a speculative review. Cancer Res. 25: 1683 (1965).

24. Krig, L., Kulhmann, I., and Marks, F. Effect of tumor-promoting phorbol esters and of acetic acid on mechanisms controlling DNA synthesis and mitosis (Chalones) and on the biosynthesis of histidine-rich protein in mouse epidermis. Cancer Res. 34: 3135 (1974).

25. Houck, J. C., Chalones. American Elsevier, New York, 1976.

26. Bell, G. I. Model of carcinogenesis as an escape from mitotic inhibitors. Science 192: 569 (1976).

27. Onda, H. A new hypothesis on mitotic control mechanisms in eukaryotic cells: Cell-specific mitosis-inhibiting protein excretion hypothesis. J. Theor. Biol. 77: 367 (1979).

28. Gilbert, D. A. The mechanism of action and interaction of regulators of cell replication. Biosystems 10: 227 (1978).

29. Carter, L. J. Michigan's PBB incident: Chemical mix-up leads to disaster. Science 192: 240 (1976).

30. Schwartz, E. L., Kluwe, W. M., Sleight, S. D., Hook, J. B., and Goodman, J. I. Inhibition of N-2-fluorenylaceticamide-induced mammary tumorigenesis in rats by dietary polybrominated biphenyls. J. Natl. Cancer Inst. 64: 63 (1980).

31. Peraino, C., Fry, R. J., Staffeldt, E. Reduction and enhancement by phenobarbital of hepatocarcinogenesis induced in the rat by 2-acetylaminofluorine. Cancer Res. 31: 1506 (1971).

32. Peraino, C., Fry, F. J., Staffeldt, E., Christopher, J. P. Enhancing effects of phenobarbital and butylated hydroxytoluene on 2-acetylaminofluorene-induced hepatic tumorogenesis in the rat. Food Cosmet. Toxicol. 15: 93 (1975).

33. Ulland, B. M., Weisburger, J. H., Yamamoto, R. S., and Weisburger, E. K. Antioxidants and carcinogenesis: Butylated hydroxytoluene, but not diphenyl-p-phenylenediamine, inhibits cancer induction by N-2-fluorenylaceticamide and by N-hydroxy-N-2-fluorenylaceticamide in rats. Food Cosmet. Toxicol. 11: 199 (1973).

34. Goodman, J. I., Trosko, J. E., Yager, J. D., Jr. Studies on the mechanism of inhibition of 2-acetylaminofluorine toxicity by butylated hydroxytoluene. Chem. Biol. Interact. 12: 171 (1976).

35. Braun, A. G., Emerson, D. J., and Nicholson, B. B. Teratogenic drugs inhibit tumor cell attachment to lectin-coated surfaces. Nature 282: 507 (1979).