IRCS Medical Science: Biochemistry; Cancer; Cell and Membrane Biology; Drug Metabolism and Toxicology; Pharmacology, 11, 363-364 (1983)

TUMOR PROMOTER (TPA), DNA CHAIN OPENING AND UNSCHEDULED DNA SYNTHESIS

M. Beljanski and L. Le Goff

Laboratoire de Pharmacodynamie Faculté de Pharmacie, 92290 Châtenay-Malabry, France

Paper received: 31st January, 1983; amended 11th April, 1983

Tumor promoters accelerate the proliferative capacity of tumor cells (1), although at high concentrations they may induce carcinogenesis in animals (2). Recently it was shown that TPA, (12-0-tetradecanoyl-phorbol-13-acetate), the tumor promoting phorbol ester, appears to act in a similar fashion on normal embryonal diploid and cancer cells (3). TPA induces differentiation of leukemic cells (4), activates silent genes for specific r-RNA synthesis in hybrid cells (5) and promotes cell proliferative capacity or phenotypic cellular changes (6). Here we report the *in vitro* effect of TPA on DNA secondary structure and DNA *in vitro* synthesis.

Materials and methods: DNAs from tissues or cells and bacteria were isolated and purified by the phenol method (8, 9). Buffalo rat liver and grafted hepatoma tissues (Morris 7777) from three animals were excised and separately used for DNA isolation. Breast cancer and healthy tissues (4 cases) as well as neurocarcinoma DNA (2 cases) originated from patients treated by chemotherapy before exeresis. Total leukocytes were taken from three patients with acute myeloblastic leukemia (60 000–100 000 cells/mm³), whilst normal leukocytes came from healthy human beings (6000–8000 cells/mm³). Wild strain of *Salmonella typhimurium* LT_2 and His^- mutants (TA 1538, 1537, 1535) were cultured in shaken nutrient medium described elsewhere (8). Exponentially growing cells were used for DNA isolation.

Conditions for DNA chain opening (hyperchromicity) were as follows. UV absorbance at 260 nm of cancer and control tissue DNA, as well as of His^- mutant DNA and wild type DNA (His^+) (20 μg in 1 ml of Tris-HCl buffer 10^{-2}M pH 7.65), was measured at room temperature before and after addition of TPA or α-phorbol. Blank cuvettes contained the equivalent amount of the same compounds. Contact between DNA and TPA or α-phorbol was 1 min with gentle shaking. The results are expressed as UV absorbance increase (see also ref.9).

The incubation conditions for *in vitro* DNA synthesis have been described elsewhere (7, 8). Incubation time was 10 min at 36 °C. The amount of acid precipitable ³H-labelled DNA (TCA, 5% solution) was determined in the absence and presence of TPA or α-phorbol. The acid precipitable product was filtered on a millipore (GF/C glass filter, washed with TCA solution and dried. Its radioactivity was then measured with Packard liquid spectrometer.

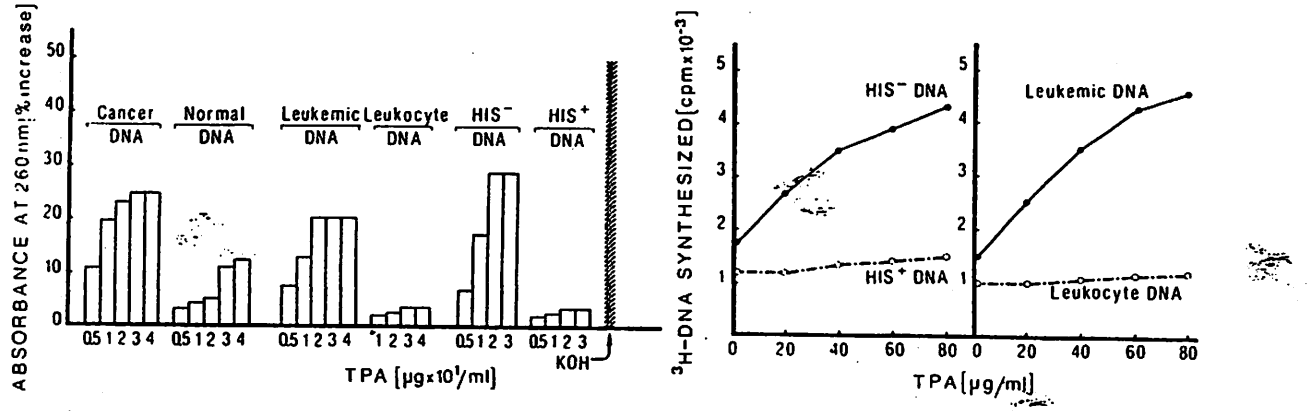

Figure 1 (left): Effect of TPA on DNA strand separation. Cancer DNA (rat hepatoma); normal DNA (rat liver); leukemic DNA (total leukocytes from acute myeloid leukemic patients); leukocyte DNA (healthy humans); His^- DNA (mutant TA1538); His^+ DNA (wild type of S. typhimurium LT2). Figure 2 (right): in vitro *DNA synthesis in the absence and presence of TPA. His^- DNA (mutant TA 1538); His^+ DNA (wild type of S.* typhimurium *LT2). Leukemic DNA (total leuykocytes from acute myeloid leukemic patients). Leukocyte DNA (healthy humans).*

Results and discussion: Incubation of DNA from rat hepatoma tissues and leukemic cells with increasing concentrations of TPA resulted in a high increase of UV absorbance measured at 260 nm (figure 1). Hyperchromic increase of the same order of magnitude was also observed with DNA from human breast cancer and neurocarcinoma (results not shown here). Rat liver DNA responded to TPA but to a small degree (figure 1). The same was true for healthy monkey brain and spleen DNAs (not shown here). DNA from normal leukocytes appeared to resist TPA action (figure 1). Since His^- mutants of *S. typhimurium*, used for testing the mutagenic effect of carcinogens (10), undergo *in vitro* strand separation in the presence of these compounds (9), we tested the *in vitro* effect of TPA on DNAs isolated from His^- mutants and DNA of wild type. Figure 1 shows that TPA indeed induced strand separation of His^- DNA (DNA from three mutants) but not of His^+ DNA. TPA-induced chain opening in DNAs from His^- mutants as well as from cancer cells correlated with increased *in vitro* DNA synthesis (figure 2). DNAs from cancer tissues and from His^- mutants of *S. typhimurium* have in common a destabilized physicochemical structure which may react with TPA and different carcinogenic compounds depending on the pH and nature of the implicated molecules (8, 9). In contrast α-phorbol, which is inactive in cell proliferation and/or differentiation, has no effect either on DNA *in vitro* strand separation or in DNA *in vitro* synthesis (figure 3).

Different authors have reported that various drugs induce the appearance of single strand regions in the nuclear DNA of

animal cells (11, 12), in DNA maintained in *in vitro* conditions (13) and increase the number of initiation sites on DNA of cancer cells cultures *in vitro* (14). We have recently demonstrated that croton oil induces strand separation in DNAs isolated from mammalian cancer and healthy tissues (9) and that carcinogens destabilize His⁻ DNA from *S. typhimurium* (8). DNA *in vitro* synthesis occuring in the presence of TPA is the consequence of *in vitro* strand separation in local areas of DNAs isolated from cancer tissues and from some healthy tissues among those tested so far. These results may explain for skin and embryonal cell increased proliferative capacity in nude mice treated with TPA (2) or *in vitro* accelerated growth of tumor cells in the presence of this agent (1, 2). TPA may induce the synthesis of a set of proteins in normal fibroblasts and increases their amount in fibroblasts from patients with Bloom syndrome (15). The correlation between DNA strand separation, DNA *in vitro* synthesis and cell proliferation, already shown with carcinogens (9) is observed also with TPA. TPA induces the expression of silent ribosomal genes in hybrid cells (5), an event which requires DNA strand separation, necessary for transcription.

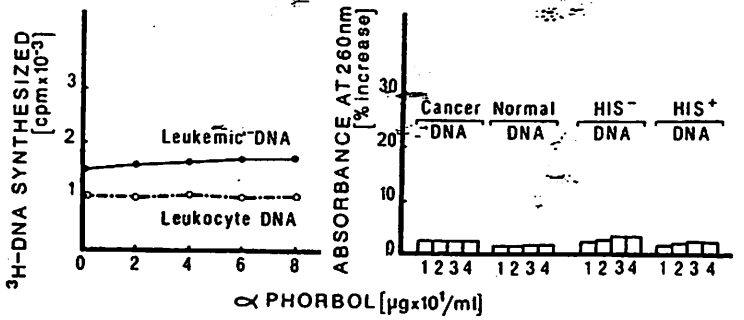

Figure 3: Absence of effect of α-phorbol on DNA in vitro *synthesis and DNA strand separation. For the origin of DNAs used, see legend to figure 1.*

The *in vitro* response of DNA from His⁻ mutants of *S. typhimurium* to TPA might at first sight appear surprising. It should be recalled that these mutants respond to many carcinogenic compounds whilst wild strain of *S.typhimurium* does not (10). Our data described for carcinogens (8) and those reported here suggest that mutations in Salmonella strains have locally led to destabilized segments of DNA chains. TPA used in *in vitro* conditions further separates His⁻ DNA chains but not in His⁺ DNA. α-Phorbol was inactive with all DNAs used here.

1. Friedman, E.A. (1981) *Cancer Res.*, **41**, 4588-4591
2. Hecker, E. (1968) *Cancer Res.*, **28**, 2338-2349
3. Kopelovich, L. (1982) *Exp. Cell Biol.*, **50**, 266-270
4. Huberman, L. and Callaham, M.F. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 1293-1297
5. Soprano, K.J. and Baserga, R. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 1566-1569
6. Wigler, M. and Weinstein, I.B. (1976) *Nature*, **259**, 232-259
7. Beljanski, M. (1979) *IRCS Med. Sci.*, **7**, 476
8. Beljanski, M. *et al.* (1982) *Exp. Cell Biol.*, **50**, 271-280
9. Beljanski, M. *et al.* (1981) *Exp. Cell Biol.*, **49**, 220-231
10. Ames, B.N. *et al.* (1973) *Proc. Natl. Acad. Sci. USA*, **70**, 2281-2285
11. Center, M.S. (1979) *Biochem. Biophys. Res. Commun.*, **89**, 1231-1238
12. Neubort, S. *et al.* (1982) *Mol. Pharmacol.*, **21**, 739-743
13. Mong, S. *et al.* (1981) *Cancer Res.*, **41**, 4020-4026
14. Walters, R.A. *et al.* (1976) *Biochem. Biophys. Res. Commun.*, **69**, 212-217
15. Mallick, U. *et al.* (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 7886-7890