Free Rad. Res. Comms., Vols. 12-13, pp. 751-757
Reprints available directly from the publisher
Photocopying permitted by license only

PREVENTION OF CANCER BY AGENTS THAT SUPPRESS OXYGEN RADICAL FORMATION

WALTER TROLL

Department of Environmental Medicine and Kaplan Cancer Center, New York University Medical Center, New York, NY 10016 USA.

The prevention of cancer by agents in our diet has led to the concept that oxygen radicals are a necessary component of a variety of human cancers including breast, colon and prostatic cancer. These cancers are putatively promoted by estradiol, bile acids and androgens. Epidemiological studies have shown that these cancers are suppressed in vegetarian populations. Vegetable components that may be responsible for this cancer prevention are Vitamin A, retinoids and protease inhibitors (PIs). These agents have been shown to suppress the formation of hydrogen peroxide in promoter-induced neutrophils. They also have been shown to block two-stage carcinogenesis and breast cancer when fed to animals. PIs also suppress experimentally-induced colon cancer and spontaneous liver cancer. Moreover, a new series of cancer-preventive agents, Sarcophytols (isolated by Fujiki and co-workers), are capable of suppressing two-stage carcinogenesis, breast and colon cancers in rodents when given in low concentrations. Sarcophytols were also active suppressors of H_2O_2 formation of 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced neutrophils. These observations point to an essential role of oxygen radicals in carcinogenesis. Suppression of the oxygen radical response of neutrophils in relation to cancer preventive agents is a facile assay of these important substances. The mechanism of action of oxygen radicals in promoting carcinogenesis is a multiple one, including: (1) activation of oncogenes, (2) modification of DNA bases, and (3) formation of single-strand breaks leading to poly(ADP)ribose polymerase activation.

KEY WORDS: Oxygen radicals, carcinogenesis, PADPR polymerase, cancer prevention.

INTRODUCTION

Carcinogenesis has been extensively investigated and significant progress has led to methods for identifying carcinogens in our environment. These carcinogenic agents were so prevalent that it appeared difficult to explain the relatively low cancer occurrence in certain populations without contemplating the presence of major anticarinogenic components in our environment. Epidemiological data on the occurrence of cancer throughout the world identified vegetarians as a having lower incidence of many cancers in comparison to meat (fat)-consuming populations. The highest rates of breast, colon and prostatic cancers were noted in countries where the people consumed a characteristically "western" diet high in meat content (*e.g.* the United States). The lowest levels were observed in populations that excluded meat and dairy products from their diet for economic reasons (*e.g.*, Japan and Thailand) or religious dictates (*e.g.*, Seventh-Day Adventists). Since such diets consist mainly of plants and their products, these findings suggest that plants contain anticarinogenic agents.[1-3]

Cellular DNA can be modified by a variety of agents, a process that may result in the creation of a new cell species. The first demonstration of the multistage nature of carcinogenesis was shown by Berenblum in skin cancer.[4] Inflammatory croton oil, when painted on the backs of mice, caused tumors only when the mice were pretreated

Correspondence: Walter Troll, Ph.D. Environmental Medicine – PHL 802, New York University Medical Center, 550 First Avenue, New York, NY 10016 USA.

with a minute dose of a carcinogen that by itself would be insufficient to cause cancer. This carcinogen putatively gives rise to a cell type that is indistinguishable from normal cells until exposure to a promoting agent converts it to a tumor cell. Many promoters, *e.g.*, phorbol esters which are the active compounds in croton oil, were identified as derivatives of TPA.[5,6] Fujiki *et al.* identified other types of promoting agents including teleocidin, aplysiatoxin and okadaic acid.[7]

In contrast to initiation, which requires only one dose, many applications of a promoting agent are needed to produce tumors. Thus, promotion requires multiple or prolonged exposure to the agent before tumor growth becomes inevitable. Tumor development has been divided into three phases: stage I – conversion, stage II – promotion, and stage III – progression to malignant neoplasm.[4,8-11] The concept of multistage carcinogenesis in mouse skin led to identification of promoting agents that contribute to the development of other cancers as well. The determination of the site where the tumor occurs facilitated identification of ovarian hormones as tumor promoters. The hormones estradiol and testosterone are considered possible causative agents in breast and prostatic cancers, respectively. Since the initiators of these common human cancers are not known, the identification of agents as tumor promoters depends on their ability to produce cancer at specific sites where receptors that bind them are present.[12,13] Such cancer in humans appears to be preventable by nutritional components that inhibit tumor promotion. Similar considerations have led to the tentative identification of bile acids as contributors to colon cancer.[14]

Mechanism of Anticarcinogenesis

Studies on the mechanism of tumor promotion have revealed a number of common characteristics that are shared by the seemingly unrelated agents that contribute to cancer. These include protein kinase C activation, proteases, as well as induction of oxygen radicals and poly(ADP)ribose polymerase (PADPR polymerase).[15-18] The most interesting common aspect of tumor promotion at certain sites has come from the observations that the same anticarcinogenic compounds, capable of counteracting experimental tumor promotion by TPA in the mouse model, counteract breast and colon cancers in animals and also, as shown by epidemiological studies, in humans.[19] After it was noted that croton oil or its purified principle TPA induced proteases in mouse skin it was hypothesized that proteases may be involved in tumor promotion. To test this possibility, synthetic protease inhibitors were applied to mouse skin and found to block the promoting action of TPA. Low doses (1–10 μg) of tosyl-L-lysine chloromethyl ketone, tosyl-phenylalanine-chloromethyl ketone and the competitive substrate tosyl-l-arginine methyl ester specifically counteracted promotion. The number and incidence of tumors decreased and latent periods increased. The inhibition of tumor promotion by protease inhibitors was confirmed by applying leupeptin to mouse skin.[20] Feeding a raw soybean diet rich in protease inhibitors not only suppressed tumors in mouse skin initiated with 4-nitroquinoline-N-oxide and promoted by TPA, but also breast tumors induced by X-ray irradiation in Sprague-Dawley rats and spontaneous liver cancer in C_3H mice.[18] Feeding animals leupeptin suppressed rat mammary tumors induced by 7,12-dimethylbenz(a)anthracene, whereas ε-aminocaproic acid or Bowman-Birk soybean inhibitor inhibited colon cancer induced by 1-,2-dimethylhydrazine.[18] The epidemiological studies of Correa showed decreased breast, prostatic and colon cancers in populations consuming seeds rich in protease inhibitors (e.g., rice, soybeans, chick-peas).[21]

A similar crossover of anticarinogenic agents that were active in the mouse skin system was evidenced with retinoids which also suppressed breast cancer.[22] Bryos-

tatin, a modifier of protein kinase C that had been shown to inhibit tumor promotion by phorbol esters in SENCAR mice, was found to inhibit leukemia in humans.[23,24] These apparent common anticarinogenic actions also have recently been observed with Sarcophytols A and B, cembrane type diterpenes isolated from the soft coral *Sarcophyton glaucum*. These compounds were shown to inhibit teleocidin-mediated tumor promotion in mouse skin. When fed to mice, Sarcophytol A inhibited tumor promotion by TPA, aplysiatoxin and okadaic acid in large bowel carcinogenesis, as well as spontaneous mammary and liver tumors.[25,26] These compounds are reminiscent of retinoids in structure but appear more effective in suppressing a variety of tumors and exhibit only negligible toxicity.

Role of Oxyradicals

Recent developments have stimulated a growing interest in the role of oxygen radicals and particularly of hydrogen peroxide in tumor promotion. Hydrogen peroxide and organic peroxides have been shown to be tumor promoters in SENCAR mice.[27] Tumor promoters including phorbol esters and indole alkaloids induce a respiratory burst in polymorphonuclear leukocytes (PMNs) resulting in the formation of superoxide anion radicals ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($\cdot OH$) and singlet oxygen.[7,28-32] Phorbol derivatives that are inactive as tumor promoters (*i.e.*, phorbol, phorbol diacetate or 4-O-methyl-TPA) also fail to elicit production of $\cdot O_2^-$ and H_2O_2 by PMNs.[33,34] Interestingly, chemopreventive protease inhibitors and retinoids suppress formation of $\cdot O_2^-$ as well as H_2O_2 by TPA-activated PMNs.[35,36] Thus, the ability of the above agents to inhibit the production of active oxygen species may be responsible for their chemopreventive properties in respect to suppression of tumor promotion and cancer development.

Role of PADPR Polymerase

The induction of PADPR polymerase is of specific interest among the many possible contributions of oxygen radicals to tumor promotion. For example, it has been observed that inhibitors of PADPR polymerase cause deletion of oncogenes from transfected NIH 3T3 cells.[15] Benzamide, an inhibitor of PADPR polymerase, has been

TABLE I
Effect of various inhibitors on hydrolysis of [^{3}H]casein by chymotrypsin and trypsin

Inhibitor	Concentration of inhibitor (mM)	Inhibition (%)	
		Chymotrypsin[a]	Trypsin[a]
Nicotinamide	10	7	6
	20	22	12
	40	53	14
	60	52	40
Benzamide	10	57	24
	20	64	37
	40	76	44
	60	88	64
3-Aminobenzamide	10	34	6
	20	50	27
	40	69	31
	60	72	40

[a]Twenty nanograms chymotrypsin or trypsin per assay.

TABLE I
Inhibition of superoxide anion formation in human neutrophils

Concentration of inhibitor	Inhibition (%)		
	Nicotinamide	Benzamide	3-Aminobenzamide
2 mM	18	23	15
5 mM	23	34	48
10 mM	35	84	73

shown to cause a loss of exogenously-supplied H-*ras* genes from NIH 3T3 cells, which results in morphologically-normal flat cells. Benzamide, 3-aminobenzamide and nicotinamide were all found to be protease inhibitors with a preference for chymotrypsin. They also inhibited TPA-induced formation of oxygen radicals by human neutrophils[37] (Tables I and II).

Naturally-occurring chymotrypsin-inhibiting protease inhibitors, e.g. potato inhibitor I, have been shown to effectively block H_2O_2 formation by TPA-activated human neutrophils and perhaps they also are inhibitors of poly(ADP)ribosylation.[35] Antipain, another protease inhibitor, has been shown to be a PADPR polymerase inhibitor as well.[8] The contribution of PADPR polymerase to promotion becomes of particular interest when the actions of protease inhibitors, retinoids and antioxidants are examined in their role of interfering with PADPR formation.

Expression of Oncogenes

Garte *et al.*[39] have demonstrated that H-*ras* oncogene-induced transformation can be inhibited by leupeptin, antipain, ε-aminocaproic acid and α_1-antitrypsin. Inhibition of cell transformation occurred only when antipain was added to NIH 3T3 cells three to nine days after transfection with *ras*, which suggests cell proliferation as the sensitive zone for suppression of *ras* expression. Similar time requirements for suppressing transormation of NIH 3T3 cells after transfecting *ras*, v-*raf* and v-*mos* oncogenes have been noted with the PADPR inhibitor 3-amino-benzamide.[40] Such results suggest a central role for poly(ADP)ribosylation in oncogene expression – a possible step in tumor promotion. The demonstration that PADPR inhibitors cause deletion of exogeneously-supplied *ras* with concomitant reversion of cells from transformed to morphologically-normal flat cells also points to poly-(ADP)ribosylation as being involved in the transformation process. Luminol, a potent inhibitor of PADPR polymerase, also induced loss of exogeneous *ras* sequence and formation of the flat cells.[15] It is of interest to speculate if other chemopreventive anticarinogens are active through repressing PADPR polymerase. For example, it was shown that all-*trans*-retinoic acid is effective in suppressing *ras*-induced transformation.[41] It may be that retinoids inhibit formation of PADPR by interfering with activation of PMNs to form oxygen radicals, which by causing DNA damage are responsible for inducing poly(ADP)ribosylation. This could be the connection of antioxidants to PADPR polymerase inhibition. The inhibition of PADPR polymerase may not be the only mechanism of action by anticarcinogenic agents, since ε-aminocaproic acid and α_1-trypsin inhibitor have been shown to suppress the induction of DNA polymerase α, a necessary enzyme for carcinogen-mediated DNA amplification.[42,43] The suppression of *ras*-oncogene expression demonstrated by these particular protease inhibitors is more likely due to this latter effect since they are unlikely prospects for being inhibitors of PADPR polymerase.[35,38,39] On the other hand, free radical scavenger modulation of oncogenic action of X-rays, bleomycin and TPA in hamster cells is probably due to the inhibition of PADPR formation.[44]

CONCLUSION

The chemopreventive agents[45] capable of suppressing tumor promotion (protease inhibitors, retinoids, Sarcophytols, nicotinamide) have two characteristic properties, which are that they suppress oxyradical induction of tumor promoter-induced neutrophils and they prevent oncogene transformation in *ras*-induced NIH 3T3 cells. PADPR induction may be the connecting link between oxyradical induction and oncogene expression. Oxyradicals induce PADPR by DNA strand breaks. Inhibitors of PADPR (*e.g.*, aminobenzamide) cause oncogene deletion, preventing the transformation of NIH 3T3 cells. This may present the major mechanism of chemoprevention.

Acknowledgements

Special thanks are expressed to Drs. Takashi Sugimura and Minako Nagao for discussing their recent findings relating PADPR to oncogene expression, and to Dr. Hirota Fujiki for making available his recent data on chemopreventive agents.

Supported in part by the Foundation for Promotion of Cancer Research, Tokyo, Japan, by NIEHS Grants ES 00260 and P42 ES 048995, and by PHS Grants CA 37858 and P30CA 16087, awarded by the National Cancer Institute, DHHS.

References

1. B. Armstrong and R. Doll (1975) Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary factors. *International Journal of Cancer*, **15**, 617–631.
2. K.K. Carroll (1975) Experimental evidence of dietary factors and hormone-dependent cancers. *Cancer Research*, **35**, 3374–3383.
3. R.L. Phillips (1975) Role of life style and their habits in risk of cancer among Seventh-Day Adventists. *Cancer Research*, **35**, 3513–3522.
4. I. Berenblum (1941) The cocarinogenic action of croton resin. *Cancer Research*, **1**, 44–48.
5. E. Hecker (1968) Cocarcinogenic principles from the seed oil of *Croton tiglium* and from other euphorbiaceae. *Cancer Research*, **28**, 2338–2349.
6. B.L. Van Duuren and L. Orris (1965) The tumor-enhancing principles of *Croton tiglium*. *Cancer Research*, **25**, 1871–1875.
7. H. Fujiki, M. Mori, M. Nakayasu, T. Terda, T. Sugimura and R.E. Moore (1981) Indole alkaloids: Dihydroteleocidin B, teleocidin, and lyngbyatoxin A as members of a new class of tumor promoters. *Proceedings of the National Academy of Sciences USA*, **78**, 3872–3876.
8. R.K. Boutwell (1964) Some biological aspects of skin carcinogenesis. *Progress in Experimental Tumor Research*, **4**, 207–250.
9. R.K. Boutwell (1983) Diet and anticarcinogenesis in the mouse skin two-stage model. *Cancer Research*, **43**, (Suppl.), 2465s–2468s.
10. V. Kinzel, G. Fürstenberger, H, Loehrke and F. Marks (1986) Three-stage tumorigenesis in mouse skin: DNA synthesis as a prerequisite for the conversion stage induced by TPA prior to initiation. *Carcinogenesis*, 7(5), 779–782.
11. T.J. Slaga, S.M. Fischer, K. Nelson and G.L. Gleason (1980) Studies on mechanism of action of anti-tumor-promoting agents: Evidence for several stages in promotion. *Proceedings of the National Academy of Sciences USA*, **77**, 3659–3663.
12. W.A. Knight, R.B. Livingstone, E.J. Gregory and W.L. McGuire (1977) Estrogen receptors as an independent prognostic factor for early recurrence in breast cancer. *Cancer Research*, **37**, 4669–4671.
13. M. Pollard and P.H. Luckert (1986) Promotional effects of testosterone and dietary fat on the development of autochronous prostate cancer in rats. *Cancer Letters*, **32**, 223–227.
14. I.B. Weinstein (1989) Multistage carcinogenesis: A progressive disorder in signal transduction. *Proceedings of the American Association for Cancer Research*, **30**, 683.
15. M. Nakayasu, H. Shima, S. Aonuma, H. Nakagama, M. Nagao and T. Sugimura (1988) Deletion of transfected oncogenes from NIH 3T3 transformants by inhibitors of poly(ADP-ribose)polymerase. *Proceedings of the National Academy of Sciences USA*, **85**, 9066–9070.
16. Y. Nishizuka (1984) The role of protein kinase C in cell surface signal transduction and tumor promotion. *Nature*, **308**, 693.
17. W. Troll and R. Wiesner (1985) The role of oxygen radicals as a possible mechanism of tumor promotion. *Annual Review of Pharmacology and Toxicology*, **25**, 509–528.

18. W. Troll, K. Frenkel and R. Wiesner (1984) Protease inhibitors as anticarcinogens. *Journal of the National Cancer Institute*, **73**(6), 1245–1250.
19. W. Troll (1989) Cancer prevention by inhibitors of tumor promotion. *Proceedings of the American Association for Cancer Research*, **30**, 684–685.
20. M. Hozumi, M. Ogawa, T. Sugimura, T. Takeuchi and H. Umezawa (1972) Inhibition of tumorigenesis in mouse skin by leupeptin, a protease inhibitor from *Actinomycetes*. *Cancer Research*, **32**, 1725–1729.
21. P. Correa (1981) Epidemiological correlations between diet and cancer frequency. *Cancer Research*, **41**, 3685–3690.
22. A.K. Verma, B.G. Shapas, H.M. Rice and R.K. Boutwell (1979) Correlation of the inhibition by retinoids of tumor promoter-induced mouse epidermal ornithine decarboxylase activity and of skin tumor promotion. *Cancer Research*, **39**, 419–425.
23. H. Hennings, P.M. Blumberg, G.R. Pettit, C.L. Herrald, R. Shores and C.H. Yuspa (1981) Bryostatin 1 an activator of protein kinase C inhibits tumor promotion by phorbol esters in SENCAR mouse skin. *Carcinogenesis (Lond.)*, **8**, 1343–1346.
24. A.S. Kraft, F. William, G.R. Pettit and M.B. Lilly (1989 Varied differentiation responses of human leukemias to bryostatin 1. *Cancer Research*, **49**, 1287–1293.
25. H. Fujiki, M. Suganuma, H. Suguri, S. Yoshizawa, K Takagi and M. Kobayashi (1989) Sarcophytols A and B inhibit tumor promotion by teleocidin in two-stage carcinogenesis in mouse skin. *Journal of Cancer Research in Clinical Oncology*, **115**, 25–28.
26. T. Narisawa, M. Takahashi, M. Niwa, Y. Fukaura and H. Fujiki (1989) Inhibition of methylnitrosourea-induced large bowel cancer development in rats by Sarcophytol A, a product from a marine soft coral *Sarcophyton glaucum*. *Cancer Research*, **49**, 3287–3289.
27. T.J. Slaga, A.J.P. Klein-Szanto, L.L. Triplett, L.P. Yotti and J.E. Trosko (1981) Skin tumor promoting activity of benzoyl peroxide, a widely used free radical-generating compound. *Science*, **213**, 1023–1025.
28 J.A. Badwey and M.L. Karnovsky (1980) Active oxygen species and the functions of phagocytic leukocytes. *Annual Review of Biochemistry*, **49**, 695–726.
29. J.C. Fantone and P.A. Ward (1982) Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. *American Journal of Pathology*, **107**, 397–418.
30. J. Formisano, W. Troll and T. Sugimura (1983) Superoxide response induced by indole alkaloid tumor promoters. *Annals of the New York Academy of Science*, **407**, 429–431.
31. A. Janoff, A. Klassen and W. Troll (1970) Local vascular changes induced by the cocarcinogen, phorbol myristate acetate. *Cancer Research*, **30**, 2568–2571.
32. S.J. Klebanoff (1980) Oxygen metabolism and the toxic properties of phagocytes. *Annals of Internal Medicine*, **93**, 480–489.
33. K. Frenkel and K. Chrzan (1987) Hydrogen peroxide formation and DNA base modification by tumor promoter-activated polymorphonuclear leukocytes. *Carcinogenesis*, **8**(3), 455–460.
34. B.W. Goldstein, G. Witz, M. Amoruso, D.S. Stone and W. Troll (1981) Stimulation of human polymorphonuclear leukocyte superoxide anion radical production by tumor promoters. *Cancer Letters*, **11**, 257–262.
35. K. Frenkel, K. Chrzan, C. Ryan, R. Wiesner and W. Troll (1987) Chymotrypsin-specific protease inhibitors decrease H_2O_2 formation by activated human polymorphonuclear leukocytes. *Carcinogenesis*, **8**, 1207–1212.
36. W. Troll, K. Frenkel and G. Teebor, G. (1984) Free oxygen radicals: Necessary contributors to tumor promotion and cocarinogenesis. In *Cellular Interactions by Environmental Tumor Promoters* (eds. H. Fujiki, E. Hecker, R.E. Moore, T. Sugimura, and I.B. Weinstein), Japan Scientific Societies Press, Tokyo/VNU Science Press BV, Utrecht, pp. 207–218.
37. W. Troll, R. Wiesner and K. Frenkel (1987) Anticarcinogenic action of protease inhibitors. In *Advances in Cancer Res.*, Vol. 49 (eds. G. Klein and S. Weinhouse). Academic Press, Inc., New York, pp. 265–283.
38. J.E. Cleaver, M.J. Banda, W. Troll and C. Borek (1986) Some protease inhibitors are also inhibitors of poly(ADP-ribose) polymerase. *Carcinogenesis*, **7**(2), 323–325.
39. S.J. Garte, D. Currie, J. Motz and W. Troll (1988) Inhibition of H-*ras* oncogene transformation of NIH 3T3 cells by protease inhibitors. *Cancer Research*, **47**, 3159–3162.
40. A.M. Diamond, C.J. Der and J.L. Schwartz (1989) Alterations in transformation efficiency by the ADPRT-inhibitor 3-aminobenzamide are oncogene specific. *Carcinogenesis*, **10**(2), 383–385.
41. S.J. Garte, D.D. Currie and W. Troll (1987) Retinoic acid inhibits transformation of NIH 3T3 cells by the human H-*ras* oncogene. *Proceedings of the American Association of Cancer Research*, **29**, 140.

42. A. Bürkle, T. Meyer, H. Hilz and H. zur Hausen (1987) Enhancement of N-methyl-N'-nitro-N-nitrosoguanidine-induced DNA amplification in a simian virus 40-transformed Chinese hamster cell line by 3-aminobenzamide. *Cancer Research*, **47**, 3632–3636.
43. R. Heilbronn, J.R. Schleohofer, A.O. Yalkinoglu and H. zur Hausen (1985) Selective DNA amplification induced by carcinogens (initiators): Evidence for a role of proteases and DNA polymerase α. *International Journal of Cancer*, **36**, 85–91.
44. C. Borek and W. Troll (1983) Modifiers of free radicals inhibit *in vitro* the oncogenic actions of X-rays, bleomycin, and the tumor promoter 12-O-tetradecanoylphorobl 13-acetate. *Proceedings of the National Academy of Sciences USA*, **80**, 1304–1307.
45. L.W. Wattenberg (1985) Chemoprevention of cancer. *Cancer Research*, **45**, 18.

Accepted by Prof. G. Czapski