Chemical Interaction: Enhancement and Inhibition of Clastogenicity
by Wagida A. Anwar

Most environmental exposures involve concurrent or sequential exposure to multiple chemicals in air, water, and food. Interactive effects in carcinogenesis have been described for certain combinations of agents. They are described in terms of enhancement or inhibition of carcinogenesis. Enhancement effects have been documented for cigarette smoking in combination with exposure to asbestos, radon, alcohol, or other exposures. A variety of inhibitors of carcinogenesis have also been described. They are classified into agents preventing formation of carcinogens; blocking agents; and suppressing agents. Assessment of risk from exposure to multiple agents can be derived either from epidemiological studies in relation to actual exposure or from laboratory studies after controlled exposure to different agents. Prediction of how toxic components of mixtures will interact should be based on an understanding of the mechanisms of such interactions. Compounds may interact chemically, yielding new toxic components or causing a change in the biological availability of the existing components or metabolites. In humans, great individual variability in response is to be expected because of genetic heterogeneity or acquired host susceptibility factors. Interaction is thus a key component in the risk assessment process. In this paper, the definition of interaction and the theoretical basis for different types of interaction in cancer causation are reviewed. Epidemiological and experimental studies showing interactive effects of two chemical carcinogens are also presented.

Introduction

In considering the health risk of exposed populations, it must be borne in mind that people are not exposed to only one potentially hazardous agent. Interactive effects in induction of cancer have been documented from exposure to multiple agents (1,2). The term “interaction” describes the way in which the joint effect of two or more agents differs from the simple additive effects. The effects are further classified according to their enhancement or inhibition of carcinogenesis. Such effects constitute an important consideration in assessment of the risk.

Knowledge about interaction in human carcinogenesis is based entirely on epidemiologic studies of effects from cigarette smoking, which is a complete mixture, in conjunction with exposure to other agents. An extensive review of this topic is provided by Saracci (3). The report shows enhancement of lung cancer in cigarette smokers by asbestos. Both agents are individually associated with increased risk for development of lung cancer, while combined exposure results in an enhanced risk. Although chronic exposure to asbestos slightly increases the risk of bronchogenic carcinoma in nonsmokers, asbestos workers who smoke are at an 8-fold greater risk than nonexposed smokers and at a 92-fold greater risk than nonsmoking asbestos workers (4,5). Enhancement of cancer of the upper alimentary tract was observed in cigarette smokers who consume excessive alcohol (7). High incidence of liver cancer was seen in populations with endemic hepatitis and with simultaneous exposure to mycotoxins (8). The interaction between alcohol consumption and tobacco smoking has been studied for several sites by Esteve and Tuyns (6). They found that the two agents may interact in different ways, depending on the site.

Obviously, the complex mixture of greatest importance in these interactions is cigarette smoke. Another complex mixture that may contribute significantly to the cause of cancer is pyrolyzed products in cooked food (7).

Interactions observed in epidemiologic studies are also substantiated in experimental investigations using tobacco smoke condensate, coal combustion fumes, automobile exhausts, and used engine oil (1). Enhancement of lung cancer risk from exposure to arsenic and cigarette smoke in smelter workers is supported by the observation of increased chromosomal damage (8,9).

Because it is not feasible to test all substances through epidemiological and long-term animal studies, in vivo and in vitro short-term tests for DNA damage, gene mutation, aneuploidy, chromosomal damage, and cell transformation are viable alternative procedures. The main advantage of such short-term tests is that they are rapid and inexpensive by comparison with other studies (10). Data from these tests can be used alone or in groups, as in the genetic activity profile (GAP) database (11,12), which provides a computer-generated graphic representation of genetic bioassay data (13).

---

1Department of Community, Environmental and Occupational Medicine, Faculty of Medicine, Ain Shams University, Cairo, Egypt.
My laboratory has reported the induction of chromosomal damage among traffic policemen in Cairo (14). The frequencies of chromosomal aberrations and sister chromatid exchanges are significantly higher among the traffic policemen than in the control group. The increase in chromosomal damage among the traffic policemen is enhanced further by smoking. Another study was conducted to evaluate the cytogenetic effects in male workers exposed to mercury fulminate (15). The frequencies of chromosomal aberrations and micronucleated lymphocytes are significantly higher in the exposed group compared to the control group. Smokers in the exposed group showed the highest frequency of chromosomal damage.

In addition to using cells from exposed humans, mice were used in our interaction studies. Praziquantel (PQ) is a commonly used drug to treat patients with schistosomiasis. We have conducted a cytogenetic-urine metabolite study in mice to determine the in vivo clastogenic and coclastogenic potential of PQ with a ubiquitous environmental contaminant, benzene. Our study showed that PQ is not clastogenic but can enhance the clastogenic activity of benzene in mice by shifting the metabolic pathway of benzene toward formation of muconaldehyde, which may be responsible for the enhancement effect (16). In another study, similar enhancement interaction was observed between benzene and cremophore (17). Cremophore E1 (CR), a frequently used solubilizer and emulsifier in the pharmaceutical and cosmetic industry, is made up of ethylene oxide and caster oil.

Using cells in culture, Hermann (18) noticed that several nonmutagenic hydrocarbons with 2- and 3-unsubstituted rings enhanced the mutagenicity of benzo[a]pyrene (BaP). Combined treatment of Chinese hamster cells with an extract from automobile exhaust and a mutagen yielded higher mutant frequency than expected (the sum of their individual effects (19)). Dubins and La Velle (20) examined the ability of nickel to act as a comutagen with simple alkylating agents in bacterial system. Nickel chloride potentiated the mutagenicity of methyl methanesulfonate in polymerase-prolific strains. Similar results have also been reported for the comutagenic actions of arsenite (21), chromate (22), and cadmium (23) using bacterial assays. Other metal salts such as CuCl₂, MnCl₂, and NaMoO₄ were found to enhance UV mutagenesis in E. coli WP2, which has wild-type DNA repair capability (24).

Cytoxan interacts with either benzamide, 3-aminobenzamide, or theophylline to induce sister chromatid exchanges (SCEs). The SCE frequencies in the presence of the poly(ADP-ribose)polymerase inhibitors are significantly higher than those in the absence of inhibitor over the cytoxan control. In the same study, the inhibitors in combination with melphanal, thiopeta, or cytoxan act synergistically in causing cell division delay (25). Reiss et al. (26) observed that simultaneous exposure of adult, male rat liver (ARL-18) epithelial cells to chrysotile asbestos and BaP is more mutagenic than an additive effect of these substances. The clastogenicity of cis-diaminedichloro- platinum (II) and 8-methoxypsoralen plus ultraviolet light (UVA) are enhanced by post-treatment with sodium arsenite in Chinese hamster ovary (CHO) cells and in human skin fibroblasts (27).

On the other hand, Beckman and Nordenson (28) studied the rates of chromosomal aberrations and sister chromatid exchanges in human lymphocytes exposed to combinations of arsenic, lead, and sulfur dioxide, which are the major toxic emissions from copper smelters. No synergistic effects were found. Selenium in combination with the three other agents showed antagonistic effect.

**Inhibition of Carcinogenesis**

A variety of inhibitors of carcinogenesis have been described. They are classified into agents preventing formation of carcinogens, blocking agents, and suppressing agents (1). Selenium, for instance, has been found to have a protective effect against chromosomal damage induced by arsenic (29). It showed an antagonistic (protective) effect against sodium arsenite, lead acetate and sodium sulfite (28).

A number of dietary components have been identified as inhibitors of mutagenesis induced by various chemical mutagens. These include vitamins, trace metals, fatty acids, protease inhibitors, polyphenols, porphyrins, sulfydryl compounds, essence of vanilla and cinnamon oil, and several other agents (30). Among vitamins, retinoids, tocopherol, ascorbic acid, and riboflavin were shown to inhibit mutagenicity of a number of chemical mutagens (Table 1).

In one of our studies (31), we demonstrated that the mutagenic and potentially carcinogenic activity of benzene can be blocked by a free-radical scavenger, dimethyl sulfoxide (DMSO). Therefore, the potential use of free-radical scavenger to protect workers exposed to benzene may be considered.

**Mechanisms of Interaction**

Predicting how toxic components of mixtures will interact should be based on an understanding of the mechanisms of such interactions. The interaction may occur

| Table 1. Examples of dietary inhibitors of mutagenesis (30). |
|-------------------------------------------------------------|
| **Dietary agent** | **Test system** | **Inhibit the mutagenic activity of** |
| Vitamin A | *S. typhimurium* | Cyclophosphamide (CP), protein pyrolysates, aflatoxin B₁, benzo[a]pyrene (BaP) |
| | V79 CHO cells | CP, aflatoxin B₁ |
| Vitamin E | Fibroblast of golden hamster | Methyl mercury |
| | Drosophila | Radiation |
| | CHO cells | BaP |
| Ascorbic acid | *S. typhimurium* | Dimethyl nitrosamine |
| | Cells in exposed workers | Polycyclic aromatic hydrocarbons, benzene |
| Selenium | Bacteria, human cells | Nitrosamines, BaP |
| Cobalt | *E. coli* | UV, γ rays |

| CHO, Chinese hamster ovary cells. |
during any stage of the toxicological process: absorption, distribution, metabolism, and excretion. Compounds may also interact chemically, yielding new toxic components or causing a change in the biological availability of the existing components or metabolites. In humans, great individual variability in response is to be expected because of genetic heterogeneity or acquired host susceptibility factors.

Hermann (18) stated that the higher the number of cycles of the polycyclic aromatic hydrocarbons (PAH), the lower the amount of PAH necessary to enhance and/or inhibit the mutagenicity of BaP. Metabolic activation of BaP was evidently involved in synergistic phenomenon because no effect was observed on BaP-4,5 oxide, a direct-acting metabolite of BaP. In the presence of exogenous activation, enhancement may also be due to competition for deactivating enzymes, as suggested by Ashby and Styles (22). After activation of mutagen, the expression of genotoxicity, such as chromosomal aberrations, can be modified by DNA repair processes (33).

Reiss et al. (26) discussed several possible means by which asbestos fibers could cause enhancement of BaP mutagenesis. The fibers may adsorb BaP and/or affect cellular membrane structure. These activities would facilitate transport of BaP across the plasma membrane. Asbestos fibers may also increase the binding of BaP to isolated microsomes. This property of asbestos could increase the availability of the carcinogen for metabolic activation. Nickel could affect DNA repair by decreasing the fidelity of DNA synthesis via some action on DNA polymerases (20). Moreover, Rossman and Molina (24) suggest that the comutagenic effects of metals might occur either via metal induced infidelity of repair replication or (in case of CuCl2) via metal-induced depurination.

Lee et al. (27) observed that the cogenotoxic activity of sodium arsenite is confined to interaction with S-dependent agents. Such activities may be due to inhibition of repair of the induced lesions, induction of error prone repair or interference with DNA replication.

The mechanisms of desmutagenesis have been proposed to involve chemical inactivation of mutagens, enzymatic inactivation of mutagens, inhibition of metabolic activation of promutagens, and inactivation of activated mutagens, including scavenging processes (34).

Risk Assessment of Interaction

Interaction between different agents in a mixture can produce effects either less than (antagonistic) or greater than (synergistic) the sum of the effects of individual components. This will lead to underestimation or over-estimation of the actual risks (L28). Therefore, in estimation of risk, potential interaction among different agents should be considered (2).

The first step of a risk assessment process is probably the documentation of mutagen burden, which is referred to as the average amount of any given mutagenic chemical that was unavoidably ingested, absorbed, or inhaled from the environment and/or produced and carried endogenously within the body (35). The net total burden would take into account the amount of antimutagens that also might enter the body. Unfortunately, at the present time there is no practical way to measure such net or total burden. The problem is complicated further by the fact that mutagen doses vary at different stages along the pathway from exposure to biological response. However, it is possible in the future that surveys of the mutagenicities of various body fluids, coupled with monitoring, could provide some useful insight into the magnitudes of these quantities (36).

A mathematical approach to understanding interaction was applied to the interaction between two or more toxic agents (37). The results indicate the existence of a strong synergistic interaction between ethyl methanesulfonate and ultraviolet light for cell killing in the diploid yeast. Application of such mathematical models may help in risk assessment of combined exposure.

Information generated by using mathematical models may complement data generated by epidemiological studies and from laboratory studies after exposure to different agents. Prediction of how toxic components of mixtures will interact should be based on an understanding of the mechanisms of such interactions.

REFERENCES

1. Williams, G. M. Chemical mixtures and interactive carcinogenesis: in vitro studies. In: Complex Mixtures and Cancer Risk (H. Vainio, M. Sorsa and A. J. McMichael, Eds.), International Agency for Research on Cancer, Lyon, 1990, pp. 107–112.
2. Kaldor, J. M., and L’Abbe, K. A. Interaction between Human Carcinogens. In: Complex Mixtures and Cancer Risk (H. Vainio, M. Sorsa, and A. J. McMichael, Eds.), International Agency for Research on Cancer, Lyon, 1990, 1990.
3. Saracci, R. The interaction of tobacco smoking and other agents in cancer etiology. Epidemiol. Rev. 9: 175–193 (1987).
4. Selikoff, I. J., Hammond, E. C., and Chung, J. Asbestos exposure, smoking and neoplasia. J. Am. Med. Assoc. 204: 104–110 (1968).
5. Hammond, E. C., and Selikoff, I. J. Relation of cigarette smoking to risk of death of asbestos-associated disease among insulation workers in the United States. In: Biological Effects of Asbestos (P. Bogovski, V. Timberell, J. C. Gilson, and J. C. Wagner, Eds.), International Agency for Research on Cancer, Lyon, 1973, pp. 213–317.
6. Esteve, J., and Tuyns, A. J. Models for combined action of alcohol and tobacco on risk of cancer: what do we really know from epidemiological studies? In: Chemical Carcinogenesis (F. Feo, P. Pani, A. Columbano, and R. Garcea, Eds.), Plenum, New York, 1988, pp. 649–655.
7. Sugimura, T., Sato, S., Ohgaki, H., Takayama, S., Nagao, M., and Wakabayashi, K. Mutagens and Carcinogens in cooked food. In: Genetic Toxicology of the Diet (I. B. Knudsen, Ed.), Alan R. Liss, New York, 1986, pp. 86–107.
8. Porshagen, G., Wall, S., Taube, A., and Linnmann, L. On the interaction between occupational arsenic exposure and smoking and its relationship to lung cancer. Scand. J. Work Environ. Health 7: 302–309 (1981).
9. Nordenson, I., Beckman, G., Beckman, L., and Nordstrom, S. Occupational and environmental risks in and around a smelter in northern Sweden. II. Chromosomal aberrations in workers exposed to arsenic. Hereditas 88: 47–50 (1977).
10. Anderson, D. The use of short-term tests in detecting carcinogenesis of complex mixtures. In: Complex Mixtures and Cancer Risk (H. Vainio, M. Sorsa, and A. J. McMichael, Eds.), International Agency for Research on Cancer, Lyon, 1990, pp. 89–100.
11. IARC. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement 6, Genetic and Related Effects: An Updating of Selected IARC Monographs from Volumes 1–42. International Agency for Research on Cancer, Lyon, 1987.
12. Waters, M. D., Stack, H. F., Brady, A. L., Lohman, P. H. M., Haroun, L., and Vainio, H. Use of computerized data listings and activity
profiles of genetic and related effects in the review of 195 compounds. Mutat. Res. 205: 285–312 (1988).

12. Waters, M. D., Claxton, L. D., Stack, H. F., and Graedel, T. E. Genetic activity profiles-application in assessing potential carcinogenicity of complex environmental mixtures. In: Complex Mixtures and Cancer Risk (H. Vainio, M. Sorsa, and A. J. McMichael, Eds.), International Agency for Research on Cancer, Lyon, 1990, pp. 75–88.

13. Anwar, W. A., and Kamal, A. M. Cytogenetic effects in a group of traffic policemen in Cairo. Mutat. Res. 208: 225–231 (1988).

14. Anwar, W. A., and Gabal, M. S. Cytogenetic study in workers occupationally exposed to mercury fulminate. Mutagenesis 6(3): 189–192 (1991).

15. Anwar, W. A., Au, W. W., Sadagopan Ramanujam, V. M., and Legator, M. S. Enhancement of benzene clastogenicity by praziquantel in mice. Mutat. Res. 222: 283–289 (1989).

16. Au, W. W., Anwar, W. A., Paolini, M., Ramanujam, S., Cantilli-Forti, G. Mechanism of clastogenic and co-clastogenic activity of cremophore with benzene in mice. Carcinogenesis 12(1): 53–57 (1991).

17. Hermann, M. Synergistic effects of individual polycyclic aromatic hydrocarbons on the mutagenicity of their mixtures. Mutat. Res. 90: 399–409 (1981).

18. Li, A. P., and Royer, R. E. Diesel-exhaust-particle extract enhancement of chemical-induced mutagenesis in cultured Chinese hamster ovary cells: Possible interaction of diesel exhaust with environmental carcinogens. Mutat. Res. 103: 349–355 (1982).

19. Dubins, J. S., and La Velle, J. M. Nickel(II) genotoxicity: potentiation of mutagenesis of simple alkylating agents. Mutat. Res. 162: 187–199 (1986).

20. Rossman, T. G. Effect of metals on mutagenesis and DNA repair. Environ. Health Perspect. 40: 189–195 (1981).

21. La Velle, J. M., and Wittmer, C. M. Chromium (VI) potentiates mutagenesis by sodium azide but not ethylmethane sulfonate. Environ. Mutagen. 6: 311–320 (1984).

22. Mandel, R., and Ryser, H. J. P. Mutagenicity of cadmium in Salmonella typhimurium and its synergism with two nitrosamines. Mutat. Res. 138: 9–16 (1984).

23. Rossman, T. G., and Molina, M. The genetic toxicity of metal compounds: II. Enhancement of ultraviolet light-induced mutagenesis in Escherichia coli WP2. Environ. Mutagen. 8: 263–271 (1986).

24. Mourredato, D., Kourakis, A., Kolloukas, D. E., Hatziheodoridou, P., and Dozi-Vassiliades, J. Enhancement of cytogenetic damage by inhibitors of poly (ADP-ribose) polymerase in human lymphocytes exposed to antineoplastics in vivo and in vitro. Teratog. Carcinog. Mutagen. 6: 485–492 (1986).

25. Reiss, B., Tong, C., Telang, S., and Williams, G. M. Enhancement of benzo(a)pyrene mutagenicity by chrysotile asbestos in rat liver epithelial cells. Environ. Res. 31: 100–104 (1983).

26. Lee, T. C., Lee, K. C., Tseng, Y. J., Huang, R. Y., and Jan, K. Y. Sodium arsenite potentiates the clastogenicity and mutagenicity of DNA crosslinking agents. Environ. Mutagen. 8: 119–128 (1986).

27. Beckman, L., and Nordenson, I. Interaction between common genotoxic agents. Hum. Hered. 36: 397–401 (1986).

28. Sweins, A. Protective effect of selenium against arsenic-induced chromosomal damage in cultured human lymphocytes. Hereditas 98: 249–252 (1983).

29. Chauhan, P. S., Bhilwade, H. N., Chaubey, R. C., and Aravindakshan, M. Diet as a source of inhibitors of mutagenesis. In: Mutation and The Environment. Part E: Environmental Genotoxicity, Risk, and Modulation (M. L. Mendelson and R. Albertini, Eds.), Wiley-Liss, 1990, pp. 339–349.

30. Anwar, W. A., Au, W. W., Legator, M. S., Sadagopan Ramanujam, V. M. Effect of dimethyl sulfoxide on the genotoxicity and metabolism of benzene in vivo. Carcinogenesis 10: 441–445 (1989).

31. Ashby, J., and Styles, J. A. Comutagenicity, comparative enzyme substrates, and in vitro carcinogenicity assay. Mutat. Res. 54: 105–112 (1978).

32. Natarajan, A. T., Csukas, L., Degraffi, F., van Zeeland, A. A. Influence of inhibition of repair enzymes on the induction of chromosomal aberrations by physical and chemical agents. Prog. in Mutat. Res. 4: 47–59 (1982).

33. Kuroda, Y. Antimutagenesis studies in Japan. In: Antimutagenesis and Anticarcinogenesis Mechanisms I. Basic Sciences (Y. Kuroda, D. M. Shankel, and M. D. Waters, Eds.), Plenum Press, New York, 1990, pp. 1–22.

34. Hollander, A. A history of attempts to quantify environmental mutagenesis. In: Environmental Mutagens and Carcinogens (T. Sugimura, S. Kondo and H. Takebe, Eds.), University of Tokyo Press, Tokyo, 1982, pp. 21–36.

35. JCP/EMC Committee 4 Report. Mutat. Res. 115: 255–291 (1983).

36. Agar, D. D., and Haynes, R. H. Analysis of interactions between mutagens. II. Ethyl methanesulfonate and ultraviolet light in Saccharomyces cerevisiae. Mutat. Res. 232: 327–336 (1990).