Environmental Toxicants in Developing Countries

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Health effects from environmental toxicants may be a more serious problem in developing countries compared with developed countries because the problem is potentiated by other factors: a) the lack of or failure to enforce regulations, which allows human exposures to genotoxic agents; b) undernourishment of the lower economic and social classes that comprise the most exposed populations from industrial and agricultural activities; and c) parasitic infections that affect a wide range of populations in both urban and rural areas. Data on the genotoxic effects of different types of exposures, including environmental exposures (natural and industrial), occupational exposures, and infections and medical treatments, are presented and discussed with the point of view that all these factors must be taken into account with respect to regulation and the protection of human health. Occupational exposures in developing countries are higher than in developed countries due to lack of stringent regulations, lack of knowledge of the risks involved, and the negligence of workers. General pollution is another important issue since developed countries have established strict regulations and risky industrial processes are being exported to developing countries, along with banned substances and dangerous industrial wastes. It should be emphasized that stringent regulations in developed countries will not prevent exposures in the long term because toxic substances that are released into the environment will ultimately reach all our future generations. — Environ Health Perspect 104(Suppl 3):599–602 (1996)

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Introduction

The scientific community is, or should be, engaged in very serious considerations of strategies to urge conservation of natural resources and protection of the environment. There is a need for better education of individuals, communities, and businesses to change the mind-set for resource conservation and waste reduction technologies. Although we can separate good from bad, rich from poor, and developed from developing countries, the task to isolate polluted from unpolluted oceans or to stop a radioactive cloud that crosses my backyard is almost impossible.

Unlike much of basic research, environmental toxicology has often been instigated by public policy. Many laws have been established in the United States, Canada, Europe, and Japan to mandate toxicity testing or to require an assessment of toxicity of environmental pollutants. While environmental contamination of air, water, and soil in developing nations is recognized as a source of health problems, pollution controls are ignored. In addition, the extensive agricultural base is associated with widespread pesticide use and subsequent contamination beyond points of applications. Waste disposal practice typically consists of dumping into rivers, water supplies, and landfills without proper waste containment and treatment (1). The problem is potentiated by other factors such as undernourishment of the low-income workers who, at the same time, are the most exposed population due to industrial and agricultural activities. Also, parasitic infections afflict a wide range of populations in urban and rural areas. Individuals with parasitic infections might be more susceptible to toxicants. Moreover, since some of these infections are recurrent, medical treatment is prescribed (sometimes autoprescribed) more often in the lifetime of these individuals. The effect between medical treatment and exposure to contaminants is unknown.

Data from previous and ongoing studies investigating the genotoxic and cytotoxic effects of different types of exposures, including environmental and occupational exposures, infections, and medical treatments, are presented and discussed.

Cytotoxicity and Genotoxicity in Parasitic Infections

Parasitic infections affect more than 200 million people worldwide. Chronic infection and inflammation have been implicated in the development of human cancers (2). We have evaluated the frequency of mutations at the HPRT locus and the lymphocyte proliferation kinetics (LPK) in individuals infected with Taenia solium cysts in the brain (neurocysticercosis). These patients showed higher frequencies of mutations together with LPK retardation compared with non-infected controls. After a 15-day treatment with praziquantel (PZQ), the drug of choice, both parameters returned to control values (3). A reduction in chromosomal damage was observed in schistosomiasis patients after treatment with PZQ (W Anwar, personal communication), indicating that the infections by themselves were promoting genetic damage in hosts (3). In agreement with these observations, lymphocyte cultures from infected pigs showed higher proportions of polyploid cells before the PZQ treatment (4). A time-dependent increase of sister chromatid exchanges after inoculation with T. solium eggs and an increased lymphocyte proliferation for 6 to 8 weeks post inoculation, followed by an impaired proliferation after this period, were also observed (5).

There is also evidence for lymphocyte-function impairment caused by cestodes: a depression of cellular response in mice infected by T. taeniaeformis could be due to factors produced by the parasite (6). A factor secreted by T. solium cysticercus was isolated; this factor depressed 3H-thymidine uptake by T lymphocytes in vitro (7). These results are in agreement with the immunosuppression induced in mice and pigs (8–10) and in humans (11) infected with T. solium metacestodes.
In Brazil, Cetrón et al. (12) studied the humoral and cell-mediated immune response in 70 individuals (symptomatic and non-symptomatic) infected with Trypanosoma cruzi and 30 noninfected individuals. Peripheral blood mononuclear cells from infected individuals showed lower proliferative response to phytohemagglutinin (PHA), although the difference was not statistically significant. Definitive conclusions regarding the magnitude of the PHA responses among the groups were not possible, given the 5-day harvest time point. Patients with symptomatic Chagas' disease, which is caused by this parasite, had significantly decreased cellular response to T. cruzi lysate compared with those of the undetermined group (12).

Genotoxicity of Antiparasitic Drugs

Several chemical agents currently in use to treat parasitic infections are known or suspected mutagens/carcinogens (13). The metabolic activation of some of these drugs generates genotoxic metabolites. Since metabolic capacities vary among individuals, genotoxic effects may also vary among treated individuals.

Niclosamide is a drug used to treat tapeworm intestinal infections. Experiments in our laboratory using the Salmonella typhimurium microsomal test system showed that niclosamide induced frameshift mutations following metabolic activation. Mutagenic activity was also detected in the urine of mice after oral administration of the drug (14). Clastogenic damage was observed after S9 metabolic activation in vitro, and a significant increase of chromosomal aberrations was found in lymphocytes from three of the five patients treated with niclosamide (15).

In the case of metronidazole (MTZ), an imidazole derivative which is the drug of choice for the treatment of Trichomonas vaginalis, Entamoeba histolytica, and Giardia lamblia, several studies were conducted in animal models and in humans. Treated Pelibuey sheep showed a significant increase in the HPRT variant frequency (V6) (16). The highest Vf were found in the animals with the highest steady-state MTZ in plasma. An inverse relationship between the Vf and drug elimination was found. The fact that the animals with the lowest elimination rates of MTZ had increased gene mutations indicated that differences in pharmacokinetics constitute one potential mechanism of genotoxic susceptibility (16).

We studied the effects of therapeutic doses of MTZ in 12 healthy nonsmoker male volunteers, who were 18 to 25 years old and weighed 55 to 65 kg. The frequency of chromosome aberrations and the LPK in lymphocyte cultures were compared before and after treatment. Increased frequencies of chromosome breaks with interindividual variation were observed. Also, grouped data revealed a marginal effect on mitotic index (MI), while this increment was highly significant in 3 of the 12 volunteers. At the same time, a faster LPK was observed in 10 individuals after treatment. In agreement with the different MTZ pharmacokinetics observed in sheep, different concentrations of MTZ in the plasma of these volunteers were found. Since MTZ is metabolized by the P450 enzymes, particularly by that encoded in the CYPIA1 locus which is polymorphic (G Elizondo, manuscript in preparation: (17)), individual differences observed could be due to differences at this level.

Genotoxicity and Malnutrition

Malnourishment and undernourishment constitute important factors for consideration when monitoring studies are performed. It has been demonstrated that malnourished children showed more genetic damage in their lymphocytes than well-nourished ones (18–20). In rats maintained under an isocaloric and hypoproteic diet, an induction of P450 liver enzymes involved in the metabolism of several mutagens was observed (J Espinosa, personal communication).

Environmental Exposure

Xenobiotic exposure generates concern in developing countries, especially because the levels of exposure are often several times higher than in developed nations. Lung concentrations of heavy metals such as Cd, Cu, Co, Ni, and Pb found in autopsy cases in Mexico City during the 1980s were higher than levels found in cases from the 1950s. A possible explanation for this increase is the growing number of vehicles and industries operating in Mexico City (T Fortoul, personal communication).

Arsenic, a human carcinogen, is mobilized from its natural sources by many activities such as contamination of water, smelting of metals, combustion of fossil fuels, the use of pesticides or herbicides containing arsenic, and more recently, by the semiconductor industry. Although earlier studies indicated occupational risk in smelter workers (21), the genotoxicity of inorganic arsenic in exposed groups has been a matter of debate (22). However, a recent study in individuals with lifetime exposure to approximately 300 mg/l of arsenic in their drinking water showed elevated frequencies of chromatid-type aberrations and of micronuclei in buccal and urothelial cells. Genetic damage in males appears to be related to their methylation capacities (ME Gonsbatt, manuscript in preparation). Also, increased single-strand DNA breaks were detected in blood lymphocytes using the single cell electrophoresis assay (23).

Personnel working at landfills are at risk, especially when they handle toxic materials with little protection. Although blood lead, urinary mercury, hair cadmium, and blood and urinary phenol levels were at control levels, a significantly increased level of chromosomal damage was detected among a group of high-risk workers. Moreover, this damage was found to increase with exposure time (24).

Lymphocyte Proliferation Studies

The methods of cellular replication, e.g., labeling, mitotic, and replication indexes, are used as indicators of toxicity; these methods have given valuable information on the mechanisms of action of certain drugs (25,26) and have been shown to be affected by medical treatments and environmental exposures. While parasitic infections impair lymphocyte proliferation, MTZ treatment enhanced it. Chronic exposure to arsenic via drinking water inhibited lymphocyte stimulation and proliferation (27,28), especially in those individuals showing arsenic skin lesions; squamous cell carcinoma is one type of skin cancer frequently observed in these individuals and is the only type of cancer with a nonviral origin that is associated with immunosuppression (29). Although these indexes are not specific parameters of immune impairment, they have been shown to be sensitive parameters in our human monitoring studies as a first approach to evaluate immunotoxic effects (Table 1).

Conclusions

Due to its rapid industrialization, Latin America is undergoing socioeconomic changes and an increase in diseases related to development. Human monitoring is therefore especially relevant in developing countries. While monitoring studies in developed countries showed that workers tend to take protective measures, workers
in Latin America still are, in many cases, not aware of health problems related to chemical and radiation exposures. Developing countries are also becoming disposal sites for industrialized nations whose regulations and laws prohibit the disposal of some types of wastes. Here is where our findings must impact most. We must continue our efforts in working with government, business, and people to develop legislation that will protect natural resources, diminish exposure to toxicants (environmental or occupational), and improve education in the importance of prevention of infectious and exposure-related diseases. Many changes are required, not just in the developed nations but globally. "Think globally, act locally" applies very well to the spirit of this presentation. If we want to protect the future of our world and our species, international policies, as well as local ones, should be established and experts in the field should be prepared, especially in developing countries.

The acquisition of new technologies, industries, or products should have the same safety standards in developed and developing countries because the genotoxic or immunotoxic effects we observed in highly exposed individuals are examples of what can be expected when regulations are avoided or ignored. International collaborative studies should be encouraged to monitor high-risk groups using the approaches that have proven to be successful and developing new ways to identify different levels of exposure and their effects. In addition, these studies could help create awareness in the exposed groups and in their communities. Good examples are the arsenic study mentioned previously, which was a joint project between the United States and Mexico (unpublished data), and the biomonitoring study on occupational exposure to ethylene oxide performed between Brazilian and European scientists (30).

Finally, we should keep in mind a statement made by Malling and Valkovic indicating that "the present generation is only a caretaker of the human genome of future generations" (31).

**REFERENCES**

1. Sullivan JB, Krieger, GR. Introduction to hazardous materials toxicology. In: Hazardous Materials Toxicology (Sullivan JB, Krieger GR, eds). Baltimore, MD: Williams & Wilkins, 1992;2–8.

2. IARC. IARC Monograph on the Evaluation of Carcinogenic Risks to Humans. Vol 61: Schistosomes, Liver Flukes and Helicobacter pylori. Lyon: International Agency for Research on Cancer, 1994;39–42.

3. Montero R, Fliesser A, Madrazo I, Cuevas C, Ostrosky-Wegman P. Mutation at the Hprt locus in patients with neurocysticercosis treated with praziquantel. Mutat Res 305:181–188 (1994).

4. Fliesser A, Gonzalez D, Plancarte P, Ostrosky-Wegman P, Montero R, Stephano A, Correa D. Praziquantel treatment of brain and muscle Taenia solium cysticercosis II. Parasitol Res 76:640–642 (1990).

5. Herrera LA, Santiago P, Rojas G, Salazar PM, Tato P, Molinari JL, Schiffman D, Ostrosky-Wegman P. Immune response impairment, genotoxicity and morphological transformation induced by *Taenia solium* metacestode. Mutat Res 305:223–228 (1994).

6. Letonja T, Hambergger C, Schuring G. Evaluation of spleen lymphocyte responsiveness to a T-cell mitogen during early infection with larval *Taenia taeniaformis*. Parasitol Res 73:265–270 (1987).

7. Molinari JL, Tato P, Reynoso OA, Cazares JML. Depressive effect of *Taenia solium* cysticercus factor on cultured human lymphocytes stimulated with phytohaemagglutinin. Ann Trop Med Parasitol 84:205–208 (1990).

8. Williams K, Merchant M, Arcos L, Salas M, Díaz S, Díaz de León L. Immunopathology of cysticercosis. In: Molecules, Cells and Parasites in Immunology (Larralde C, Williams K, Ortiz L, Seal M, eds). New York: Academic Press, 1980;145–162.

9. Tato P, Valles Y, Rolon R, Molinari JL. Effects of immunization in immunodepressed hogs naturally parasitized with *Cysticercus cellulosae*. Rev Latinoam Microbiol 29:67–71 (1987).

10. Molinari JL, Tato P, Valles Y. Helper T lymphocyte depressed by *Cysticercus cellulosae* in immunized and control hogs. Rev Latinoam Microbiol 29:293–300 (1987).

11. Fliesser A, Woodhouse E, Larralde C. Human cysticercosis: antigens, antibodies and nonresponders. Clin Exp Immunol 39:27–37 (1980).

12. Cetron MS, Basilio FP, Moraes A P, Sousa AQ, Paes JN, Kain SJ, Wener MH, Van Voorhis WC. Humoral and cellular immune response of adults from northeastern Brazil with chronic *Trypanosoma cruzi* infection: depressed cellular immune response to *T. cruzi* antigen among Chagas' disease patients with symptomatic versus indeterminate infection. Am J Trop Med Hyg 49:370–382 (1993).

13. Gentile JM, Ostrosky-Wegman P. Preface. Mutat Res 305:117 (1994).

14. Cortinas de Nava C, Espinosa J, García L, Zapata AM, Martínez E. Mutagenicity of antiamebic and antihelmintic drugs in the *Salmonella typhimurium* microsomal test system. Mutat Res 117:79–91 (1980).

15. Ostrosky-Wegman P, García G, Montero R, Pérez Romero B, Alvarez Chacón R, Cortinas de Nava C. Susceptibility to genotoxic effects of nicosamide in human peripheral lymphocytes exposed in *vitro* and in *vivo*. Mutat Res 173:81–87 (1986).

16. Ostrosky-Wegman P, Lares Asseff I, Santiago P, Elizondo G, Montero R. Metronidazole hprt mutation induction in sheep and the relationship with its elimination rate. Mutat Res 307:253–259 (1994).

17. Elizondo G, Montero R, Herrera JE, Hong E, Ostrosky-Wegman P. Lymphocyte proliferation kinetics and sister-chromatid exchanges in individuals treated with metronidazole. Mutat Res 305:133–137 (1994).

18. Armendares S, Salamanca F, Fran S. Chromosome abnormalities in severe protein calorie malnutrition. Nature 232:271–273 (1971).
19. Ortiz R, Campos C, Gómez JL, Espinoza M, Ramos-Motilla M, Betancourt M. Sister-chromatid exchange (SCE) and cell proliferation in lymphocytes from infected and non-infected children with severe protein calorie malnutrition (PCM). Mutat Res 312:33–37 (1994).

20. Betancourt M, Ortiz R, González C, Pérez P, Cortés L, Rodríguez L, Villaseñor L. Assessment of DNA damage in leukocytes from infected and malnourished children by single cell gel electrophoresis/comet assay. Mutat Res 331:65–77 (1995).

21. Nordenson I, Beckman G, Beckman L, Nordström 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 (1978).

22. U.S. EPA. Health Assessment Document for Inorganic Arsenic. Final Report. EPA 600/8-83-021F. Research Triangle Park, NC: U.S. Environmental Protection Agency, 1984.

23. Valverde M. Study of a population exposed to chronic hydroarsenicism using the single cell electrophoresis assay (SCGE) [Graduate Thesis]. Faculty of Sciences, UNAM. México 1995.

24. Gonsebatt ME, Salazar AM, Montero R, Díaz Barriga F, Yáñez L, Gómez H, Ostrosky-Wegman P. Genotoxic monitoring of workers at a hazardous waste disposal site in Mexico. Environ Health Perspect 103(Suppl 1):111–113 (1995).

25. Rojas E, Herrera LA, Sordo M, Gonsebatt ME, Rodríguez R, Ostrosky-Wegman P. Mitotic index and cell proliferation kinetics for the identification of antineoplastic activity. Anticancer Drugs 4:637–640 (1993).

26. Montero R, Gonsebatt ME, Gerson R, Rojas E, Herrera LA, Ostrosky-Wegman P. AS-101: a modulator of in vitro T-cell proliferation. Anticancer Drugs 4:351–354 (1993).

27. Ostrosky-Wegman P, Gonsebatt ME, Montero R, Vega L, Barba H, Espinosa J, Palao A, Cortinas C, García-Vargas G, del Razo LM, Cebrián M. Lymphocyte proliferation kinetics and genotoxic findings in a pilot study on individuals chronically exposed to arsenic in Mexico. Mutat Res 250:477–482 (1991).

28. Gonsebatt ME, Vega L, Montero R, García Vargas G, Del Razo LM, Albores A, Cebrián ME, Ostrosky-Wegman P. Lymphocyte replicating ability in individuals exposed to arsenic via drinking water. Mutat Res 313:293–299 (1994).

29. IARC. Consensus report. In: Mechanisms of Carcinogenesis in Risk Identification. IARC Sci Publ 116 (Vainio H, Magee P, McGregor D, Michael AJ, eds). Lyon:International Agency for Research on Cancer 1992:9–54.

30. Ribeiro LR, Salvadori DMF, Rios ACC, Costa SL, Tates AD, Törnyqvist M, Natarajan AT. Biological monitoring of workers occupationally exposed to ethylene oxide. Mutat Res 313:81–87 (1994).

31. Brusick D. Principles of Genetic Toxicology. New York:Plenum Press. 1984.ix.