MECHANISMS OF ACTION INVOLVING REACTIVE METABOLITES AND OXIDATIVE STRESS: A THEORETICAL AND EMPIRICAL REVIEW.

Tom Muir, Environment Canada, Ontario Region, 867 Lakeshore Road, Burlington, Ontario, Canada.

Introduction

Until recently, regulatory science in North America has been dominated by the research program on chlorinated dioxins and related compounds. The initial focus appeared to be on grossly observable or macroscopic effects on reproduction and development in wildlife, particularly in the Great Lakes region (1). Later, as scientific knowledge accumulated, the focus shifted to a suite of effects or syndromes, and a variety of biomarkers of exposure, effects or impairment, and adverse effects or disability (2). The observable toxicological effects attributed to these "dioxin" compounds are numerous and varied, and mediated through a variety of mechanisms, however, the dioxin research program obscured the role of reactive metabolites, oxidative stress, and related mechanisms. It is only relatively recently that these mechanisms, and "dioxin" mediated aspects, are being rediscovered (4).Currently, the "endocrine disruption" issue has moved to the forefront, particularly in regulatory science. In addition to the concerns about disruptions to normal hormone activity, the metabolic by-products of estrogen and xenoestrogen cycling have also been identified as a risk factor. Of particular interest here are the interactions and relationships that might exist between the "dioxins", the endocrine disruption metabolism, and exposures to complex mixtures of chemical compounds and metals. The aim here is to review a selection of literature relevant to the hypothesis that reactive metabolites, oxidative stress, and related mechanisms are causally involved in environmental and human disease syndromes, including cancer.

Materials and Methods

The hypothesis will be investigated by examining several lines of evidence, including theoretical, experimental, empirical, and epidemiological. This examination will be an adaptation of that used by Aust, et al, 1993 (3). First, evidence will be reviewed on the capabilities of chemical and physical agents to generate free radical or other reactive species. Second, the extent to which certain specific and predictable interactions, such as lipid peroxidation and covalent binding, occur subsequent to the formation of the free radical species will be examined. Third, evidence regarding how compounds known to modulate free-radical trapping or scavenging defence systems alter the production of free radicals and toxic effects will be considered. Fourth, empirical and experimental data from molecular epidemiology studies will be examined. Fifth, conventional epidemiological data on the incidence and mortality rates of certain diseases will be examined in a relevant fashion. Finally, theoretical and conceptual considerations will be formulated to synthesise and unify the weight of evidence in order to see how it supports the hypothesis.

Results and Discussion

The role of reactive metabolites (notably free radicals), and oxidative stress in the toxicity of chemical and physical agents is long recognised in biochemical studies of disease syndromes and ageing (5, 6, 7, 8, 10, 42, 43). Until recently, the reasoning behind this role was largely in the theoretical realm due to a lack of knowledge of the molecular and cellular events involved. For example, the analytical or experimental methods to detect and quantitate free radicals did not exist until recently, and even so, free radicals are still difficult to identify, detect, quantitate and study (4). Further, it has only been recently that techniques have been developed that are capable of showing the presence of small lesions in the DNA, cell membranes, and other important macromolecules. These reactive species interactions, particularly involving a continous and progressive degeneration of DNA, or other key macromolecules, including antioxidant defences, can lead to a variety of lesions and disease states (12, 9, 11, 13, 7, 42, 43).

It is known that there is endogenous production of free radicals in biological systems, and aerobic organisms are continuously exposed to oxidant stress. However, these organisms have evolved a number of significant and complex antioxidant and repair mechanisms to defend against these stresses. Although these defences are neither unlimited nor perfect, it is reported that oxidative deterioration of cell membranes (lipid peroxidation) does not occur to any significant extent in normal circumstances, attesting to their effectiveness (14).
There is evidence that many xenobiotics, including synthetic organic chemicals, inorganics, metals, complex industrial waste streams, and physical agents, also involve the formation of free radicals, oxidative stress, and related mechanisms, either in their metabolism by the cytochrome P450 enzymes, or as a result of other interactions with the organism. Included in this set are virtually the whole range of known environmental pollution and contamination agents, many of which are known or suspected carcinogens and cytotoxins. As well, it is known that the transition metal iron is a potent catalyst of oxidative stress, possibly through the generation of hydroxyl radical, and is a risk factor in atherosclerosis, cancer, arthritis, and general disease syndromes and ageing (15). This also supports the hypothesis that reactive metabolites and oxidative stress are causally involved in disease syndromes. Moreover, the relationship to the “dioxins” enters here since TEQs augment the induction of the cytochromes P450, and accelerate the production of radical species and OS from the metabolism of the complex mixtures of real world pollutant exposures, including halogenated hydrocarbons (11). Other P450 inducing compounds, such as PAH, that are confounded with the “dioxins” are also capable of prolonged MFO induction and the production of oxidative stress (16). As well, it is significant that certain of the P450 enzymes are also inducible by ethanol (alcohol), high fat diets, and obesity, all of which are known to enhance the metabolism of some halogenated hydrocarbons (11). It is known that certain of these agents also involve specific and predictable toxic interactions, such as lipid peroxidation or covalent binding, which are consistent with a free-radical related mechanism (4). Further, it is known that a number of compounds, such as the chemical and enzyme antioxidants or reducing potential, have the ability to modulate free-radical scavenging defence systems.

Some of these compounds (superoxide dismutase, catalase, dimethyl sulfoxide) have been shown to inhibit the production of reactive oxygen metabolites (ROS) induced by crocidolite asbestos (17). Alpha-tocopheral, an additional antioxidant, and a reducing agent, protect against oxidation of cellular lipids in hepatocytes induced by diquat, a redox cycler (18). Vitamin E protects against membrane lipid peroxidation (19). The addition of various nucleophiles, including glutathione, reduced the mutagenicity in the Ames Salmonella assay, of softwood kraft chlorination effluent

(20). This suggests that the effluent contains an electrophilic fraction and is consistent with a free radical or reactive species mechanism of the toxicity (21) Selenium treatment decreased the percentage incidence and tumour burden of skin carcinogenesis induced by 3-methylcholanthrene, and significantly increased the glutathione S-transferase activity (22). The antioxidants, butylated hydroxyanisole, vitamin E, vitamin C and cysteine significantly inhibited hepatic glutathione depletion and lipid peroxidation induced by endrin (23). Hodson, et al (16) also reports that the symptoms of dioxin toxicity in trout larvae can be reduced if the larvae are treated with antioxidants and inhibitors of MFO enzyme activity. Their current model of toxicity is based on increased oxyradical formation resulting from prolonged MFO induction. It has also been shown that some of these compounds are diminished or depleted by the administration of synthetic chemicals in animal models, again suggesting a free radical mechanism at work, for example, DDT decreased hepatic vitamin A storage in rats (24)) . There is evidence that dietary deficiency of the antioxidant selenium is associated with disease syndromes, elevated mortality, and accelerated ageing in human populations (25). There is evidence that dietary fat, primarily derived from animal fat and meat and dairy products consumption, is linked to cancer of the breast , prostate, and colon, and is also linked to coronary heart disease (CHD) (26) There is also evidence that the majority of general human exposure to fat soluble contaminants, such as dioxins, is derived from meat, fish, poultry, and dairy products, and the dietary fat contained therein (41). There is strong evidence that vegetables and fruits protect against cancer and heart disease, and that these foods contain a variety of active constituents that have potential anticancer activity, including antioxidants (26,27, 6)). For example, intake of vitamin E, and blood antioxidant levels are associated with lower rates of CHD (26), and vitamin C intake is reported to reduce breast cancer incidence in humans (28).

Field evidence on herring gulls nesting in an industrialised urban site revealed a two-fold or greater rate of heritable genetic mutation (minisatellite DNA) compared to rural controls. The exposure route was thought to be via the air (29). Exposure to thermal power plant emissions was associated with increases in blood cholesterol and low density lipoproteins, decreased high density lipoproteins, and higher prevalence of ischemic heart disease in the exposed group as compared to controls (30). The examination of molecular and genetic damage in humans due to environmental pollution in Poland revealed significant increases in PAH-DNA and aromatic adducts, in sister chromatid exchange, chromosomal aberrations, and a doubling on the frequency of ras oncogene overexpression. It was also found that the aromatic adducts on DNA were significantly correlated with chromosomal mutation in a population with increased risk of cancer and reproductive outcomes (31). There are a number of studies consistently reporting an association between exposure to chlorination by-products in household water supplies and bladder cancer (32, 40). Recall that these by-products, usually represented by trihalomethane, involve reactive metabolites in their metabolism. A survey of US cancer mortality from 1950-69 revealed that cancers of the lung and the nasal cavity and sinuses were significantly elevated in males in counties where the petroleum industry was most concentrated, compared to controls. Further, when the petroleum industry areas that also had heavy involvement in the chemical industry were examined, the nasal cancer mortality rate was reported as “exceptionally high”, and the lung cancer rate was also the highest (33). More recently, it is reported that exposure to a polluted urban atmosphere induces single strand breaks in human nasal respiratory epithelium (34). It is relevant that P450 enzymes were present and inducible by ethanol treatment in rat nasal epithelial microsomes (11).

Some of the most powerful and supportive evidence is emerging from a new paradigm called "radical induced DNA disorder" (RIDD), and results from the redox cycling of estrogens and xenoestrogens, and the generation of hydroxyl radical damage to DNA bases, and to mutagenesis and carcinogenesis, with direct and strong evidence of specific links to DNA damage in wild fish, and to breast and prostate cancer in humans (35, 28, 36). Moreover, the models had a sensitivity and specificity of 100% for classifying normal prostate versus cancer and normal versus benign prostatic hyperplasis (BPH), and close to 100% for BPH versus cancer. The use of spectral models to discriminate the DNA of cancerous, metastatic, BPH, and normal tissue provides a physical and theoretical perspective on chemical and biological phenomena, and can be used more generally. Indeed, there is evidence that mutational spectroscopy may provide a means to establish cause and effect relationships between environmental agents and genetic change (37).

It can be deduced from first principles that the interactions of reactive metabolites (e.g., electrophiles or free radicals) with nucleophiles, involve the existence and motion of atomic charge (e.g., electrons), or an electromagnetic field or force. It is this field that Malins et al (36) is measuring and provides an almost exact discriminatory power between different levels of hydroxyl radical interactions, DNA damage, and cancer or disease progression. In this way, the microscopic mechanisms of action, or mechanics, can be linked to macroscopic dynamics and conceived of, and represented by fields (a "field" being any physical quantity which takes on different values at different points in space or time). At this molecular level, chemistry, biology, and physics come together, and the physical description is sufficient. Newton's Second Law, f=ma, where the electromagnetic force is substituted for f, can be used to relate observed risk fields and complex pollutant fields. This is based on the physical equivalence of an accelerated point and an electric current (and field) of increasing intensity. This approach can be used in analysing GIS-based data on mortality and morbidity risks, and on pollutant release and integrated exposure source strengths. The object is to relate disease rates (which can be seen as a matrix of probabilities) and their variations or relative accelerations from point to point (which accelerations mean the existence of some force), to the material-dependent interactions of that force. These interactions are the integrated source/exposure densities, reactive metabolites and sequelae, and other possible risk factors (all of which can also be seen as matrices of probabilities) and their relative accelerations from point to point in relation to the actual risk field. The "mass" or "resistance" part "m" reflects the defence and repair mechanisms of the life form.

This macroscopic approach looks at a large aggregate of individuals and /or over time, and is based on a probabilistic or statistical interpretation of causal laws. Theoretically, a meaningful macroscopic description involves an averaging that washes out detailed information of the microworld. This means that we don't need a precise knowledge of the individuals in the large aggregate population because that is always subject to contingencies and chance. Instead, we utilise statistical properties or laws indicating that variations in a particular cause, or class of causes, produce regular and predictable trends in the effects, at the large scale or macroscopic level alone (38, 39). Empirically, there is substantial unexplained variation in several risk fields that may be accounted for by the approach.

References

1. Government of Canada. 1991. Toxic Chemicals in the Great Lakes and Associated Effects. Vol. II - Effects. Ottawa, Ontario.
2. Fox, Glen A. 1993. What have biomarkers told us about the effects of contaminants on the health of fish-eating birds in the Great Lakes? The theory and a literature review. J. Great Lakes Res. 19(4): 722-736.
3. Aust, S.D., et al. 1993. Free Radicals in Toxicology. Toxicology and Applied Pharmacology 120, 168-178.
4. McLean, M. Et al. 1998. A new mechanism of toxicity for polychlorinated biphenyls (PCBs): redox cycling and superoxide generation. Organohalogen Compounds, Vol. 37, pp. 59-62.
5. Rothstein, M. 1986. Biochemical studies of ageing. Chemical and Engineering News, Aug.11, pp26-39.
6. Ames, B.N. 1983. Dietary carcinogens and anticarcinogens. Science 221: 1254-1264.
7. Hartman, P.E. 1983. Mutagens: some possible health impacts beyond carcinogenesis. Environmental Mutagenesis 5:139-152.
8. Bhatnagar, R.S., Ed. 1980. Molecular Basis of Environmental Toxicity. Ann Arbor Science Publishers Inc., 589 p.
9. Burch, P.R.J. 1969. An Inquiry Concerning Growth, Disease, and Ageing. University of Toronto Press. 213 pages.
10. Legator, M., and Epstein, S. Eds. 1971. The Mutagenicity of Pesticides. MIT Press. Cambridge, Mass.
11. Raucy, J. L. et al. 1993. Bioactivation of halogenated hydrocarbons by cytochrome P4502E1. Critical Reviews in Toxicology, 23(1): 1-20.
12. Parke D.V. 1994. The cytochromes P450 and mechanisms of chemical carcinogenesis. Environmental Health Perspectives, Vol. 102, No. 10. Pp. 852-853.
13. Kehrer, James P. 1993. Free radicals as mediators of tissue injury and disease. Critical Reviews in Toxicology, 23(1): 21-48.
14. Horton, A.A., and Fairhurst, S. 1987. Lipid peroxidation and mechanisms of toxicity. Critical Reviews in Toxicology, Vol. 18. Issue 1:27-28.
15. Ryan, Timothy P, and Aust, Steven D. 1992. The role of iron in oxygen-mediated toxicities. Critical Reviews in Toxicology, 22(1): 119-141.
16. Hodson, P. and Billiard S. 1998. What's causing recruitment failure of fish? Canadian Network of Toxicology Centres News. Spring 1998. P.6.
17. Ishizaki, T. Et al. 1994. Crocidolite-induced reactive oxygen metabolites generation from human polymorphonuclear leukocytes. Environmental Research 66, 208-216.
18. Sandy, M. S. et al. 1988. Relationships between intracellular vitamin E, lipid peroxidation, and chemical toxicity in hepatocytes. Toxicology and Applied Pharmacology 93, 288-297.
19. Patel, J. M. and Edwards, D. A. 1988. Vitamin E, membrane order, and antioxidant behaviour in lung microsomes and reconstituted lipid vesicles. Toxicology and Applied Pharmacology 96, 101-114.
20. Tachibana et al. 1988. The effect of various nucleophiles on the mutagenicity of softwood kraft chlorination effluent. Chemosphere, Vol. 17, No. 7, pp. 1343-1354.
21. Suntio et al. 1988. A review of the nature and properties of chemicals present in pulp mill effluents. Chemosphere, Vol. 17, No. 7, pp. 1249-1290.
22.Bansal M.P., and Gupta, G. 1985. Influence of selenium on 3-methylcholanthrene induced skin carcinogenesis in mice.
23. Numan, I.T. et al. 1990. Protective effects of antioxidants against endrin-induced lipid peroxidation, glutathione depletion, and lethality in rats. Arch. Environ. Contam. Toxicol. 19, 302-306.
24. De Waziers, I, and Azais V. 1987. Drug-metabolising enzyme activities in the liver and intestine of rats exposed to DDT: effects of vitamin A status. Arch. Environ. Contam. Toxicol. 16, 343-348.
25. Foster, H. And Zhang, L. 1995. Longevity and selenium deficiency: evidence from the People's Republic of China. The Science of the Total Environment 170, 133-139.
26. Willett, Walter C. 1994. Diet and health: what should we eat? Science, Vol. 264, 532-537.
27. Davis, D.L. 1989. Natural anticarcinogens, carcinogens, and changing patterns in cancer: some speculation. Environmental Research, 50, 322-340.
28. Malins, D.C. et al. 1996. Progression of human breast cancers to the metastatic state is linked to hydroxyl radical-induced DNA damage. Proc. Natl. Acad. Sci. USA Vol. 93, pp. 2557-2563. Med. Sci.
29. Yauk, C.L., and Quinn, J. S. 1996. Multi-locus DNA fingerprinting reveals high rate of heritable genetic mutation in herring gulls nesting in an industrialised urban site. Proc. Natl. Acad. Sci. USA Vol. 93, pp. 12137-12141. Applied Biological Sciences.
30.Balaz, V and Micuda, J. 1985. Development of ischemic heart disease in relation to certain metabolic risk factors. In; QSAR in Toxicology and Xenobiochemistry, M. Tichy, ed. Elsevier Science.
31. Perera, F. P. Et al. 1992. Molecular and genetic damage in humans from environmental pollution in Poland. Nature, Vol. 360, November 19. Pp. 256-258.
32. King, W. D. And Marrett, L.D. 1996. Case Control study of bladder cancer and chlorination by-products in treated water (Ontario, Canada) Cancer causes and Control 7, pp. 596-604.

33. Blot, W. J et al. 1977. Cancer Mortality in U.S. Counties with petroleum industries. Science 198 51-53.
34. Calderon-Garciduenas, L., et al. 1996, DNA strand breaks in human nasal respiratory epithelium are induced upon exposure to urban pollution. Environmental health Perspectives, Vol.104, No.2, pp. 160-168.
35. Malins, D.C., and Gunselman, S. J. 1994. Fourier-transform infrared spectroscopy and gas chromotography-mass spectrometry reveal and remarkable degree of structural damage in the DNA of wild fish exposed to toxic chemicals. Proc. Natl. Acad. Sci. USA Vol. 91, pp. 13038-13041. Biochemistry.
36. Malins D.C., et al. 1997. Models of DNA structure achieve almost perfect discrimination between normal prostate, benign prostatic hyperplasia (BPH), and adenocarcinoma and have a high potential for predicting BPH and prostate cancer. Proc. Natl. Acad. Sci. USA Vol. 94, pp. 259-264. Medical Sciences.
37. Coller, H.A. and Thilly, W. G. 1994. Development and applications of mutational spectra technology. Environ. Sci. Technol. Vol. 28, No. 11, pp. 478A-487A.
38. Bohm, David. 1957. Causality and Chance in Modern Physics. Harper Torchbooks. New York.
39 Horz, H. 1979. Philosophical concepts of space and time. In: Einstein, a Centenary Volume. Harvard University Press, 229-242.
40. Morris, Robert D., et al. 1992. Chlorination, chlorination by-products, and cancer: a meta-analysis. American Journal of Public Health. Vol. 82, No. 7, July. pp. 955-963.
41 Schecter, A., et al. 1994. Congener-specific levels of dioxins and dibenzofurans in U.S. food and estimated daily dioxin toxic equivalent intake. Environmental Health Perspectives, Vol. 102, No.11, 962-966.
42. Simic, M.G. 1991. DNA damage, environmental toxicants, and rate of aging. Environ. Carcino. & Ecotox. Revs., C9(1), 113-153.
43. Sahu, Saura C. 1991. Role of oxygen free radicals in the molecular mechanisms of carcinogenesis: a review. Environ. Carcino. & Ecotox. Revs., C9(1), 83-112.