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DETECTION AND QUANTITATION OF BENZO[a]PYRENE-DNA ADDUCTS IN BRAIN AND LIVER TISSUES OF BELUGA WHALES (Delphinapterus leucas) FROM THE ST. LAWRENCE AND MACKENZIE ESTUARIES.

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Introduction

The rationale underlying the strategy of measuring levels of chemicals which become covalently attached to cellular macromolecules as a bioassay for genotoxicity is based on the current understanding of the mode of action of chemicals once they become bioavailable (1).

The metabolism of organic chemicals is a relatively complex phenomenon and detoxification by cellular mechanisms is not always complete, sometimes resulting in highly reactive electrophilic metabolites that react with nucleophilic centers in macromolecules such as lipids, proteins, DNA and RNA. Binding with DNA can cause formation of altered bases that can be repaired, be innocuous, or result in alterations which become fixed and are transmitted to daughter cells. Current research suggest that damage to DNA that goes uncorrected is probably one of the initial stages in chemical carcinogenesis (2-5).

Given that genotoxic agents exert their activity through irreversible reactions with nucleophilic atoms to form adducts, the amount of metabolite bound to cellular DNA could provide a basis upon which to estimate exposure and possibly, to assess the risk connected with that exposure (1,6-8).

It should be noted that there are few analytical techniques available for the detection and quantitation of chemical adducts in the DNA of living organisms. The reasons for this are: (a) the analytical technique often has to accommodate the unique chemical and/or physical properties of the individual chemical or its metabolite; (b) the percentage of total chemical that becomes attached to the DNA in the target tissue is quite small because most of the parent compound is usually detoxified and excreted; (c) not all adducts that form between the genotoxic agent and DNA are stable or are involved in the development of subsequent deleterious events in the organism; and (d) the amount of DNA available for analysis is often quite limited.

Recently (9), I demonstrated a sensitive and specific analytical method for the detection of benzo[a]pyrene (BaP) bound to cellular DNA. This technique was used to analyze DNA for adducts to BaP of several stranded beluga whales from the St. Lawrence Estuary (10) as well as the DNA of beluga whales from the Mackenzie estuary.

Methodology

Whale tissue:

Whale brain tissue from three stranded beluga whales from the St. Lawrence Estuary was provided by Dr. P. Beland, Fisheries Ecology Research Center, Kimouski, Quebec. Whale brain and liver tissue from four beluga whales from the Mackenzie Estuary was provided by Patt Weaver, Freshwater Institute, Winnipeg, Manitoba.

DNA prepration:

DNA isolation was accomplished by: (a) homogenizing the tissue in 1 N NH_4OH/0.2% Triton X100; (b) partitioning of nucleic acids with chloroform/isoamyl alcohol/phenol (24/1/25-v/v), and; (c) desalting by passage through a molecular sieve column (Sephadex G50). Further purification was accomplished by enzymatic depolymerization of RNA and spermine precipitation of DNA (9).

DNA concentration was determined with bisbenzamide (Hoechst dye 33258), which forms a stable, fluorescent product with DNA (11).

Benzo[a]pyrene binding to DNA:

A sensitive HPLC method coupled with fluorescence detection was used (9). Essentially the technique consists of the acid-induced removal of the benzo[a]pyrene-adduct from the DNA as the strongly fluorescent free tetrols which are then separated and quantitated. The technique not only allows femtomole quantiaties of BaP adducts to be detected, but also measures only the adducts that are formed between the DNA and the "ultimate" carcinogenic form of BaP (2-5), the diolepoxide (BaPDE).

Results and Discussion

Analyses for BaP adducts in the DNA of brain tissue from three stranded beluga whales from the St. Lawrence estuary and in the DNA of brain and liver tissues from four whales from the Mackenzie estuary were performed using the fluorescence/HPLC assay (Table I). Values obtained for the St. Lawrence belugas were 2.15, 0.98, and 0.73 adducts of BaPDE per 10^7 nucleotides of DNA. No detectable adducts were found in the DNA of the belugas from the Mackenzie estuary.

The data obtained are important for several reasons. First, they provide evidence that the whales from the St. Lawrence estuary had been exposed to BaP, and had metabolized it to BaPDE, which subsequently became covalently bound to the DNA of the brain tissue. Over the past several years a strong and convincing argument has developed for a causal relationship between the carcinogenic potency of BaP and the amount bound to an organism's DNA as a result of cellular metabolism (2-5). This is reinforced by the premise that the integrity of DNA is essential for survival. Alterations, if left uncorrected, could trigger a sequence of events that culminates in the appearance of an overt maligancy. Second, the level of adducts detected approaches that found in animals, both terrestrial and aquatic, exposed under controlled laboratory conditions to

Table I. Detection of Benzo[a]pyrene Adducts in DNA of Beluga Whales (Delphinapterus leucas)

Sample		Tissue	BaP Adduct binding*	Formation level**
St. Lawrence Estuary				
	#1	Brain	206	2.15
	#2	Brain	94	0.98
	#3	Brain	69	0.73
Mackenzie Estuary				
	#1 - #4	Brain	none detected***	
	#1 - #4	Liver	none detected***	

* BaP adducts to DNA expressed as nanograms of tetrol I-1 per gram of DNA. St. Lawrence data taken from (10).
** Level of adduct formation expressed as BaP adducts per 10^7 DNA nucleotides.
*** Detection limit is 10 picograms of tetrol (9).

carcinogenic doses of BaP (12-15). Third, cells in brain tissue are known to have slow turnover rates and lack or have very low capacity for excission repair of DNA damage. Therefore, significant accumulation of DNA adducts could occur in this tissue from long-term chronic exposure to environmental levels of BaP too low to induce neoplasia (16).

Several notes of caution are advisable in assessing and interpreting the data presented. First, the sparseness of samples examined precludes a definitive statement about the status of the DNA of the whales in the population from which they were taken. Second, the data provide neither an insight as to the source of BaP exposure (water, food, air, etc.) of these animals, nor information as to the type of exposure (acute vs chronic). Third, although it would seem reasonable to relate BaPDE adduct levels in the DNA of the whales from the two different estuaries to the level of BaP in their respective environments, it should be noted that the health of an animal may influence the formation of adducts. In this regard, the whales from the Mackenzie estuary were hunted animals and not known to be diseased at the time of their capture, while the whales from the St. Lawrence estuary were diseased animals that had beached.

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