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MECHANISM OF THE CARCINOGENIC ACTION OF BENZENE: IRREVERSIBLE BINDING TO RAT LIVER DNA

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Introduction

It has been established that exposure of animals and men to benzene (see Ref. 1 for a recent review on benzene toxicity) can result in damage to the haematopoietic system and a relationship between such exposure and the development of leukemia is suggested by many case reports [2]. Since a common feature of chemical carcinogens is their interaction with biological macromolecules [3], most probably a covalent binding of a reactive metabolite to DNA, and since the carcinogenicity of benzene to animals has not yet been unequivocally proved, we thought it necessary to examine the interaction of benzene with DNA.

We have exposed adult male rats to radioactive benzene (tritium or carbon-14 label) in a closed inhalation chamber and were able to show for the first time a covalent binding of a benzene metabolite to DNA in vivo. The liver was chosen as a model system since it is the main organ for the metabolism of foreign compounds containing all the enzymes necessary for an activation and since this organ provides ample DNA for the determination of low specific radioactivity.

Materials and methods

Labeled benzene was obtained from ICN at a specific activity of 1.8 Ci/mmole (tritium) or 35.8 mCi/mmole (carbon-14) and was diluted, if necessary, to the total dose indicated on line 2 of the table. The total amount of radioactivity used for one animal is given on line 3.

Adult male rats (SIV 50, 270—340 g) were exposed for the period of time indicated on line 4.

The closed inhalation system, which will be described in detail elsewhere [4], consisted of a 2-l desiccator. The expired carbon dioxide was adsorbed with soda lime at the bottom of the desiccator and the resulting reduced pressure was balanced with oxygen. The radioactive benzene was transferred

TABLE I

BINDING OF RADIOACTIVE BENZENE AND TRITIATED WATER TO RAT LIVER DNA

The experimental details are described in Materials and Methods

Line No.	Description	Units	Tritiated benzene		Carbon-14 benzene		Control non-covalent binding	Control tritiated water	
1	Experiment	No.	1	2	3	4	5	6	7
2	Total benzene administered	mg	19.7	19.7	21.2	21.2	44		
3	Total radioactivity	mCi	12.2	12.2	1.0	1.0	0.53	3.4	3.7
4	Period of exposure	h	19	10.5	9.25	11.5	2	26	23
5	Radioactivity in whole liver	dpm/mg	8200	11 900	1400	820	$2.4 \cdot 10^5$	3100	3200
6	Radioactivity of isolated DNA	cpm	545	496	112	98	23.3	96	126
7	Background of equal volume buffer	cpm	29.4	22.5	29.0	29.0	21.6	26.6	29.4
8	Specific activity of DNA	dpm/mg	834	821	41.6	37.0	3.4	122	162
9	Radioactivity bound to DNA calculated as *μmole benzene / mole DNA phosphate*		2.40	2.36	*1.57*	*1.40*			

into the chamber in the oxygen supply stream. The exposure was stopped when the benzene concentration in the chamber had fallen below 0.1 mg/l.

The liver was excised under ether anaesthesia, frozen over liquid nitrogen and stored at -20°C until further use. DNA was isolated from the big liver lobe (3—4 g) according to Markov and Ivanov [5], by a method yielding a DNA (2.1—2.6 mg) with maximal contaminations of 1% RNA and 0.1% protein.

The radioactivity of whole liver (line 5 of the table) was determined after digestion of about 50 mg wet tissue in Soluene 100 (Packard). Purified DNA was dissolved in 3—4 ml 0.014 M sodium phosphate buffer, pH 6.8. The radioactivity was counted in 10 ml Insta-Gel (Packard).

The binding of benzene to DNA is expressed on a molar ratio on line 9.

Results

The first two experiments (column 1 and 2) were performed with tritiated benzene and revealed a considerable amount of radioactivity on the DNA (line 6). Since part of the tritium label is lost from benzene during its oxidation and could be incorporated into DNA during its biosynthesis the apparent binding of tritiated benzene to DNA (line 9) must be interpreted with caution.

Therefore, carbon-14 labeled benzene had to be used. The results of these experiments are given in column 3 and 4 of the table clearly showing that about 1.5 μmole benzene is bound per mole DNA phosphate. To prove a covalent binding, the following control experiment was performed (column 5): 44 mg tritiated benzene (0.52 mCi) were added to the homogenate of an inactive big liver lobe in 25 ml lysing medium. The minute amount of radioactivity found on the DNA after this incubation shows that non-covalently bound benzene is removed from DNA during the isolation procedure.

As has been pointed out above, the apparent difference of about 60% in the binding of tritium or carbon-14 label to DNA could be attributed to the incorporation of tritium from water into DNA, which should account for about 300 dpm/mg DNA of the specific activity in experiments 1 and 2 (line 8). The control experiments 6 and 7 show that the incorporation of tritium from an external dose of tritiated water is about 40 dpm/mg DNA per mCi gavaged water after one day. One would therefore expect the release of more than 8 mCi tritiated water from benzene for an explanation of the difference of 300 dpm/mg DNA. This is much more than is actually formed since only a very small percentage of the radioactivity in the urine collected from Exp. 2 was found to be tritiated water. The discrepancy is most probably due to the fact that with tritiated benzene the release of tritium occurs at the site of metabolism only. The intracellular specific activity and the corresponding incorporation into DNA will therefore be higher than from the external dose in our control experiments which is diluted into the total water pool of the animal.

Discussion

Our experiments have revealed a covalent interaction of a benzene metabolite with DNA in vivo, but do not give any information about the chemical nature of this metabolite. A likely intermediate in benzene metabolism [1] is benzene oxide. In neutral aqueous media it rearranges only slowly to the phenol [6] so that its lifetime could be long enough for a diffusion from the site of activation to the DNA. Alternatively, the metabolic appearance of polyhydroxy derivatives suggests the formation of a phenol epoxide, so that the reactive molecule could be a secondary metabolite like in the case of benzo(a)pyrene where a diol-epoxide has been found as the ultimate carcinogen [7]. Only a chemical analysis of the nucleosides will answer this question.

To get a better idea of the alkylating and perhaps carcinogenic potency of benzene, we can compare it to the typical alkylating and hepatocarcinogenic agent *N,N*-dimethylnitrosamine (DMN): Chronic feeding of adult rats with 50 ppm DMN, which is equivalent to about 4 mg/kg per day leads to liver tumours in almost 100% of the animals [8]. The reaction of a single intraperitoneal dose of 2 mg/kg produces in the liver 0.5 μmole methylations per g DNA [9], which is about 150 μmole methylations per mole DNA phosphate. Our benzene dose of 60 mg/kg is 30 times higher but yields only 1.5 μmole alkylated nucleotides per mole DNA phosphate. The alkylating potency of benzene to liver DNA is therefore about 3000 times lower than that of DMN.

This comparison shows how potent an alkylation assay can be for the screening of weak carcinogens. With a radioactive material of high specific activity we can detect a reaction with DNA at a level which is ineffective in a long-term carcinogenicity study.

In the complete process leading to a chemically induced cancer many more parameters play an important role, such as the repair capacity of the cell or the mutagenicity of that specific type of damage. A positive answer in an alkylation assay will therefore only be a warning sign but a strong reason for further studies.

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