The Effect of Cigarette-Smoke Condensate on the in vitro Fixation of Benzo(a)Pyrene on DNA[1]

The existence of bonds between carcinogens and macromolecules (especially nucleic acids), which serves as a basis for modern theories of chemical carcinogenesis, has already received numerous experimental confirmations[2,3].

Cigarette smoke has been implicated directly in the etiology of lung cancer. Since it is not possible to show directly how substances as complex as tobacco tars attach to DNA, it appeared advantageous to us to demonstrate an interference between these substances and the fixation of DNA with benzo(a)pyrene metabolized by microsomal systems.

Male Wistar rats from the Commentry strain were used in these experiments. They received i.p. injections for 2 consecutive days in doses of either 20 mg/kg of MC dissolved in corn oil or 20 mg/kg of cigarette smoke condensate (SEITA, Paris) dissolved in propylene-glycol. The microsomes were isolated according to SCHNEIDER[4] and resuspended so that microsomes from 1 g of liver are contained in 1 ml of buffer. Preparation of the metabolites of B(a)P-^{3}H was according to SIMS[5].

A) *The induction of microsomal enzymes in vivo by cigarette smoke condensate.* As MC is the substance most often used to induce these enzyme systems, we used it as a reference. The activity of the enzymes is evaluated by the importance of the B(a)P-DNA bonds obtained. When the animals are injected with cigarette smoke condensate, the ability of their microsomes to provoke bonds between B(a)P-^{3}H and DNA increases in a similar fashion. In Table I, one can see that with MC treatment, the number of molecules of B(a)P-^{3}H fixed to DNA increases from 3.2. μMoles/nucleotide to 23; and with the condensate, the number of μMoles fixed also increase to 22.

Table I. The stimulating effect on microsomes of rats treated with MC and the condensate on the fixation of B(a)P-^{3}H on DNA in vitro

Origin of the microsomes	μM B(a)P-^{3}H/Mol. P-DNA
Control	3.2
Treatment with 3-MC	23.0
Treatment with the smoke condensate	22.0

The incubation medium contains: 5 mg of DNA dissolved in 5 ml of 0.05 *M* phosphate buffer (pH: 7.4) to which is added: 2.75 μ*M* of dry NADPH, 500 μ*M* of dry EDTA, 25 μg of B(a)P-^{3}H in 0.05 ml ethanol and 0.2 ml of the microsomal suspension. The incubations are for 30 min at 37°. The microsomes are removed by a 40-minute centrifugation at 100,000 *g*. DNA is precipitated with 2 volume of cold ethanol in the presence of 0.5 ml of 2 *M* $MgCl_2$ then dissolved in 3 ml of 5% PAS containing 1% of SDS and extracted in water-saturated phenol. The quantity of fixed B(a)P is estimated by radioactivity measurements and expressed in μ*M* of B(a)P-^{3}H/Mol. P-DNA (moles of nucleotides). The quantity of DNA recovered is determined by optical density measurements at 260 nm.

B) *Interference of the smoke condensate with the fixation B(a) P-^{3}H-DNA in vitro.* The addition of smoke condensate in the incubation medium inhibits the fixation of B(a) P to DNA. The inhibition noted is from 30% to 60% respectively for the 2 concentrations of 1 and 10 mg of condensate and for 25 μg of B(a) P (Table II).

C) *Interference of condensate with the fixation of metabolites of B(a) P-^{3}H on DNA.* In the preceding series, the incubations were made at one time, the production of metabolites of B(a) P-^{3}H and their fixation to DNA were made in the presence of microsomes with the condensate eventually added directly to the medium.

It is possible to proceed in several stages. During the first incubation, the substrate is hydroxylated by microsomal enzymes. The hydroxylated metabolites are then isolated and a study made of their fixation to DNA in the absence of microsomes during the second incubation. The advantages of using this procedure include a better definition of the metabolites effectively fixed and a means to obtain the intermediate complexes (DNA-condensate). As is shown in Table III, the incubation of DNA with the B(a) P-^{3}H metabolites previously formed gives a rate of fixation comparable to that obtained by incubation of B(a) P-^{3}H in the presence of microsomes: 24 μMoles B(a) P-^{3}H/nucleotide.

If DNA is replaced by the incubation product (DNA with condensate formed in the absence of microsomes), the rate of fixation of the metabolites of B(a) P-^{3}H is not modified: 24.6 and 26.6 μMoles. For an inhibition to occur, it is necessary to treat the condensate with the microsomes. The incubations are conducted as those of B(a) P-^{3}H, but the metabolites are not fractionated on TLC; we use the whole incubation product. It is therefore possible to prepare the complex DNA-metabolite of condensate. In Table IV, one can see that the metabolites of B(a) P-^{3}H are fixed less readily to the complexes between DNA and condensate metabolites than on DNA itself.

[1] Abreviations: B(a)P, benzo(a)pyrene; CI, initial condensate of cigarette smoke; NADPH, reduced nicotinamide adenine dinucleotide; MC, 3-methylcholanthrene.

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Table II. Interaction of benzo(a) pyrene-^{3}H with DNA in the presence of the cigarette smoke condensate

N	DNA + substances	μM B(a) P-^{3}H/Mol. P-DNA	Inhibition μM B(a) P-^{3}H/Mol. P-DNA
3	B(a) P-^{3}H	21.1 (19.8–22.4)	0
3	B(a) P-^{3}H + CI 1 mg	13.4 (12.2–14.6)	7.7 (36.5%)
3	B(a) P-^{3}H + CI 10 mg	7.9 (7.2– 8.6)	13.2 (62.5%)
2	B(a) P-^{3}H without microsomes	not measurable < 1	

N, number of experiments; B(a) P-^{3}H — 25 μg; Microsomes — 0.2 ml containing 2 mg of proteins. The mode of operation is the same as for Table I.

Table III. Interaction of the metabolites of B(a) P-^{3}H on DNA pre-incubated with cigarette smoke condensate

N	Substances	Interaction of metabolites with B(a) P on DNA (in μM of B(a) P-^{3}H/Mol. P-DNA)	Interaction of the metabolites of B(a) P-^{3}H (in % of the controls)
3[a]	Metabolites of B(a) P-^{3}H	24.2 (23.5–25.8)	100
3[b]	Metabolites of B(a) P-^{3}H + DNA pre-incubated with 1 mg CI	24.6 (23.0–26.4)	100.2
3[b]	Metabolites of B(a) P-^{3}H + DNA pre-incubated with 10 mg CI	26.2 (24.0–32.2)	108.2

Initial condensate not incubated with microsomes. *N*, number of experiments. [a] 4 *M*n of metabolites of B(a) P-^{3}H are incubated 1 h at 37°C with 1 mg of DNA. At the end of the incubation, the DNA complexes are precipitated, washed several times with ethanol, suspended overnight in ethanol washed with acetone, dried, their radioactivity measured and the number of μMoles of B(a) P fixed is deduced. [b] DNA is replaced by the complex prepared in the following manner: 1 mg of DNA is incubated 30 min with 1 or 10 mg of condensate in 1 ml of phosphate buffer (pH 7.4) containing 0.55 μM of NADPH. DNA complex is precipitated, washed and dried. It is then utilized for the second incubation as indicated above[a].

Table IV. Interaction of the metabolites of B(a) P-^{3}H with the complexes between DNA and metabolites of condensate

N	Substances	Interaction of metabolites with B(a) P on DNA (in μM of B(a) P-^{3}H/Mol. P-DNA)	Interaction of the metabolites of B(a) P-^{3}H (in % of the controls)
3[a]	Metabolites of B(a) P-^{3}H + DNA (control)	25.7 (25 –27.4)	100
3[b]	Metabolites of B(a) P-^{3}H + complex (metabolites of 1 mg tar — DNA)	15.3 (14.2–17.4)	59.5
3[b]	Metabolites of B(a) P-^{3}H + complex (metabolites of 10 mg tar — DNA)	16.6 (15.8–18.2)	64.6

Condensate incubated with the microsomes. *N*, number of experiments. [a] 1 mg of DNA or [b] 1 mg of the DNA-condensate complex prepared according to Table III are incubated with 4 nM of metabolites of B(a) P-^{3}H for 1 h at 37°C. Some 2 M sodium acetate is added at the rate of 0.1 ml per ml in the incubation medium. The DNA precipitated by 2 volume of ethanol is washed and dried. The radio-activity is measured and the fixation of B(a) P is deduced.

Discussion. Bonds between the carcinogen B(a) P and the DNA molecule are possible in vitro with certain metabolites of carcinogen produced by aryl-hydrolase located in the microsomes[6,7]. In our experiments the cigarette smoke condensate is a very strong inducer of the hepatic microsomal enzymes.

Comparable results have been reported showing that the inhalation of cigarette smoke is capable of greatly stimulating the enzymatic oxydation of B(a) P in the lungs of rats[8]. Phenomena of a similar order have been demonstrated in the placenta of pregnant women who smoke.

The smoke condensate when incubated with the microsomes is also capable of forming complexes with DNA. A second incubation of the DNA-condensate complexes with the metabolites of B(a) P-^{3}H results in a much lower rate of fixation of B(a) P.

The cigarette smoke condensate contains benzopyrene but in quantities too weak to cause a direct intervention: 1 mg of condensate that inhibits fixation in the order of 60% does not contain more than 0.04% of B(a) P added in the middle of the incubation.

In addition, we must admit that in the cigarette smoke condensate there are substances different to B(a) P that can occupy the specific sites of fixation of this B(a) P on the DNA molecule[9].

Résumé. Le condensat de fumée de cigarette induit in vivo, l'aryl hydrolase microsomiale. Il est capable de former avec l'ADN des complexes qui diminuent le taux de fixation ultérieure du B(a) P. Ces résultats montrent qu'il contient des substances différentes du B(a) P et capable d'entrer en compétition avec lui.

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