Short Communication

SACCHARIN DOES NOT BIND TO DNA OF LIVER OR BLADDER IN THE RAT

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Introduction

Saccharin was found to induce bladder tumours in male rats of the first and the second generation after life-long feeding of a diet containing 5% saccharin [1]. On the other hand, with lower doses, and in epidemiological studies on the human consumption as sweetening agent there is no clear evidence for a carcinogenic activity despite the long and widespread use [2]. This controversy prompted us to investigate the interaction of saccharin with DNA in an intact mammalian organism.

Most of the known chemical carcinogens undergo a covalent binding to DNA [3], and the resulting damage can lead to a mutation. Saccharin has not been found to be mutagenic [4,5] and the present study shows that it does not undergo a covalent binding to DNA of the liver or the bladder of male rats either. The tumor induction is therefore probably due to a secondary damage to the bladder.

Materials and Methods

^{35}S-labelled saccharin was obtained from the Radiochemical Centre, Amersham, with a specific activity of 12.4 mCi/mmol. 137.1 mg were suspended in 4 ml 0.01 M Na_2HPO_4 and dissolved after the addition of one aequivalent 1 N NaOH. 1 μl of this solution was chromatographed on a thin layer silica gel (Merck 60 F 254) together with inactive saccharin as described by Kennedy et al. [6]. The radiochemical purity was found to be better than 99.5%. Repeated recrystallization of a trace amount of radioactive saccharin with inactive material did not alter the specific activity.

Two male rats (SIV 50, Sprague—Dawley derived, 180 and 173 g) were gavaged with 2.37 and 2.39 ml of the saccharin solution at 09 : 00 after starving overnight. The radioactivity administered per animal was 4.53 and 4.57 mCi and the corresponding dose was 372 and 390 mg/kg.

The animals were held in open metabolism cages with water ad libitum and were gavaged daily with 4 ml of a concentrated saccharose solution (2.5 g) so providing for about a third of the daily need of calories. This procedure was chosen for maximizing the absorption of saccharin and reducing the excretion in the faeces [6,7]. 48 h after the administration the animals were given 6 ml tap water and killed at 50 h. Urine and faeces were collected in separate portions for the periods of 0—24, 24—48 and 48—50 h. 66 and 84% of the radioactivity administered appeared in the first urine fraction. The total recovery of radioactivity from urine, faeces and cage rinse was 93 and 102%. Urine was chromatographed with the same thin layer system as used for saccharin. At least 99.6% of the radioactivity was found at the saccharin retention. This confirms that saccharin is excreted unchanged [6,7].

The animals were bled by open heart puncture under ether anaesthesia, the livers (5.39 and 5.57 g) were excised and frozen over liquid nitrogen. The bladders were rinsed with 0.9% NaCl, excised and kept on ice until 4 bladders, 2 from the treated animals and 2 from untreated animals were ready. The 4 bladders were cut open, blotted dry on filter paper (0.61 g) and homogenized for the isolation of DNA. The two additional bladders were added in order to reduce the loss of DNA always encountered when working with very small amounts during the isolation procedure.

DNA was isolated according to Markov and Ivanov [8] by a method which yields a DNA with maximal contaminations of 1% RNA and 0.1% protein. The dried DNA was redissolved in 4 ml 0.014 M sodium phosphate buffer, pH 6.8, and counted after the addition of 10 ml Insta-Gel (Packard) in a narrow ^{35}S-channel in a liquid scintillation counter. The amount of DNA was determined after diluting an aliquot of the DNA solution by assuming an optical density at 260 nm of 19 for a neutral DNA solution of 1 mg/ml.

Yield of DNA and counting results are compiled in Table I.

TABLE I

INCORPORATION OF RADIOACTIVITY INTO DNA OF LIVER AND BLADDER OF MALE RATS 50 H AFTER A GAVAGE OF ^{35}S-LABELLED SACCHARIN

The background from 3.9 mg inactive DNA is 18.6 cpm. The variability of the glass vials is bigger than the statistical counting error and gives rise to ± 0.65 cpm as 1 standard deviation. The net radioactivity in our samples is expressed with ± 2 standard deviations of the difference.

Description	Units	Animal 1 Liver	Animal 2 Liver	Animals 1 + 2 Bladders
DNA in scintillation vial	mg	2.75	3.33	0.68
Radioactivity measurement (4 × 40 min counting time)	cpm	19.1	18.3	19.8
Net radioactivity on DNA	cpm	0.5 ± 1.8	—0.3 ± 1.8	1.2 ± 1.8

Results

The radioactivity measurements on the DNA isolated from the liver and the bladders show that no incorporation of saccharin can be detected (Table I). This negative result can also mean that a binding of saccharin to DNA is below our limit of detection. A radioactivity measurement of 20.6 cpm, i.e. 3 standard deviations above background (2 cpm or 2.5 dpm net radioactivity) would be significant on a 0.001 confidence level. Based upon the specific activity of our starting material of 12.4 mCi/mmol or 150 dpm/ng, we therefore would have detected about 17 pg or $1 \cdot 10^{-13}$ mol of saccharin in a scintillation vial. The 3 mg of DNA from the liver represent about 10^{-5} mol DNA phosphate (= nucleotides). Our limit of detection of saccharin bound to liver DNA is therefore $1 \cdot 10^{-8}$ mol saccharin/mol DNA phosphate.

For the bladders, the limit of detection is about 10 times higher since the yield of DNA from the bladders of the treated rats was only 0.34 mg. The corresponding value can be calculated $1 \cdot 10^{-7}$ mol saccharin/mol DNA phosphate.

Our results therefore show that the binding of saccharin to DNA, if any, is less than one molecule saccharin per 10^8 nucleotides in the liver and less than one per 10^7 nucleotides in the bladder.

Discussion

This lowest possible binding of saccharin to DNA can be put into relation to the one found with other carcinogens: For such a comparison, the binding must be expressed as a function of the dose administered:

We define a "Covalent Binding Index"* as damage on DNA per dose, i.e.

$$\frac{\mu \text{mol bound molecules/mol DNA phosphate}}{\text{mmol/kg}}$$

In these units, the lowest detectable binding of saccharin to DNA in our experiments can be calculated to be 0.005 for liver and 0.05 for bladder.

These figures are compared to the ones found with typical carcinogens in Table II. In the first two examples, with *N,N*-dimethylnitrosamine in rat liver and benzo(a)pyrene on mouse skin, the high covalent binding index correlates well with the significant carcinogenicity in long-term studies. The binding of benzo(a)pyrene and benzene to rat liver is much smaller (examples 3 and 4 of Table II) and correspondingly, no tumours have been detected in long-term studies with these systems.

The values found with saccharin are another two orders of magnitude lower so that it is highly probable that the carcinogenicity of saccharin is not due to covalent binding to DNA but to an indirect mechanism: with high dosage and the corresponding high concentration in the urine, saccharin could lead to concrements which are suspected to induce bladder tumours by stimulating the mitotic activity of the bladder epithelium [16].

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* We prefer the term "Covalent Binding Index" to the word "alkylation", since the latter describes the addition of an aliphatic residue only, and should not be used for aromatic molecules.

TABLE II

COVALENT BINDING INDEX OF SELECTED CHEMICAL CARCINOGENS TO DNA EXPRESSED AS "DAMAGE TO DNA PER DOSE"

The unit chosen is $\dfrac{\mu\text{mol bound chemical/mol DNA phosphate}}{\text{mmol/kg}}$

Example No.	Chemical	Dose (single)	Route of administration	Species	Organ	Covalent binding index	Ref.	Carcinogenicity of similar dose by continued administration	Ref.
1	N,N-dimethylnitrosamine	27 μmol/kg	i.p.	Rat	Liver	5600	9	Yes	13
2	Benzo(a)pyrene	4 μmol/kg	Painting	Mouse	Skin	430	10	Yes	14
3	Benzo(a)pyrene	164 nmol/kg	i.p.	Rat	Liver	10	11	No	14
4	Benzene	900 μmol/kg	Inhalation	Rat	Liver	1.7	12	No	15
5	Saccharin	2.1 mmol/kg	Gavage	Rat	Liver	< 0.005		No	2
6	Saccharin	2.1 mmol/kg	Gavage	Rat	Bladder	< 0.05		No	2

REFERENCES

1 D.L. Arnold, S.M. Charbonneau, C.A. Moodie and I.C. Munro, Long-term toxicity study with orthotoluenesulfonamide and saccharin, Toxicol. Appl. Pharmacol., 41 (1977) 164.
2 W.C. Lepkowski, Saccharin ban goes beyond issue of cancer, Chem. Eng. News, 55, 15 (1977) 17.
3 J.A. Miller, Carcinogenesis by Chemicals: An Overview, G.H.A. Clowes Memorial Lecture, Cancer Res., 30 (1970) 559.
4 P.G.N. Kramers, The mutagenicity of saccharin, Mutat. Res., 32 (1975) 81.
5 T. Sugimura, T. Yahagi, M. Nagao, M. Takeuchi, T. Kawachi, K. Hara, E. Yamasaki, T. Matsushima, Y. Hashimoto and M. Okada, Validity of Mutagenicity Tests Using Microbes as a Rapid Screening Method for Environmental Carcinogens, in R. Montesano, H. Bartsch and L. Tomatis (Eds.), Screening Tests in Chemical Carcino- genesis, IARC Scientific Publications No. 12, International Agency for Research on Cancer, Lyon, 1976, p. 81.
6 G. Kennedy, O.E. Fancher, J.C. Calandra and R.E. Keller, Metabolic fate of saccharin in the albino rat, Food Cosmet. Toxicol., 10 (1972) 143.
7 H.B. Matthews, M. Fields and L. Fishbein, Saccharin: distribution and excretion of a limited dose in the rat, J. Agr. Food Chem., 21 (1973) 916.
8 G.G. Markov and I.G. Ivanov, Hydroxyapatite column chromatography in procedures for isolation of purified DNA, Analyt. Biochem., 59 (1974) 555.
9 P.J. O'Connor, M.J. Capps and A.W. Craig, Comparative studies of the hepatocar- cinogen N,N-dimethylnitrosamine in vivo: reaction sites in rat liver DNA and the significance of their relative stabilities, Br. J. Cancer, 27 (1973) 153.
10 P. Brookes and P.D. Lawley, Evidence for the binding of polynuclear aromatic hydrocarbons to the nucleic acids of mouse skin: relation between carcinogenic power of hydrocarbons and their binding to deoxyribonucleic acid, Nature (Lond.), 202 (1964) 781.
11 W.K. Lutz and Ch. Schlatter, Excretion of carcinogenicity data to low doses with a dose-response study of the binding of benzo(a)pyrene to rat liver DNA, Arch. Toxicol., in press.
12 W.K. Lutz and Ch. Schlatter, Mechanism of the carcinogenic action of benzene: irreversible binding to rat liver DNA, Chem.-Biol. Interactions 18 (1977) 241.
13 P.N. Magee and J.M. Barnes, The production of malignant primary hepatic tumors in the rat by feeding dimethylnitrosamine, Br. J. Cancer, 10 (1956) 114.
14 IARC Monographs on the Evaluation of Carcinogenic Risk of the Chemical to Man, Vol. 3, International Agency for Research on Cancer, Lyon, 1973, p. 91.
15 IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 7, International Agency for Research on Cancer, Lyon, 1974, p. 203.
16 D.B. Clayson, Experimental studies in urothelial cancer, in E.H. Cooper and R.E. Williams (Eds.), The Biology and Clinical Management of Bladder Cancer, Blackwell Scientific Publications, Oxford, 1975, p.1.