BINDING OF 2-ACETYLAMINOFLUORENE-9-^{14}C WITH NUCLEIC ACIDS OF RAT LIVER TISSUE DURING HEPATOCARCINOGENESIS AND IN PRIMARY HEPATOMAS

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In the course of hepatocarcinogenesis induced by 2-acetylaminofluorene (2-AAF) residual binding of 2-AAF-9-^{14}C with rat liver nucleic acids was studied 2 weeks after a single administration of the labeled compound. Until the sixth month of administration of unlabeled 2-AAF with the diet, rat liver RNA bound an increased amount of the radioactive carcinogen, but in primary hepatomas induced by 2-AAF increased binding of the label with DNA was observed by comparison with the corresponding polynucleotides of normal liver. Prolonged binding of the carcinogen with total RNA of primary hepatomas was due mainly to its heterodisperse components with a maximum in the 18S RNA region, and also to fractions of the biopolymer with sedimentation constants of 10S and 5S, which have a high content of polyadenylate fragments.

KEY WORDS: *hepatocarcinogenesis; RNA; DNA; 2-acetylaminofluorene; binding of carcinogen.*

It has been suggested that disturbance of repair of intracellular biopolymers is an inducing mechanism for tumor growth [9]. In turn, the repair system of macromolecules mainly determines the residual binding of the carcinogen with nucleic acids (NA). It is thus possible that only the carcinogen not removed from biopolymers by repair systems of the cell is essential for tumor production.

The object of this investigation was to study residual binding of radioactive 2-acetylaminofluorene (2-AAF) with rat liver NA in the course of chemical hepatocarcinogenesis and in primary hepatomas.

EXPERIMENTAL METHOD

2-AAF-9-^{14}C (France) had a specific radioactivity of 26.7 mCi/mmole. Tumors of the liver were induced in noninbred male albino rats weighing initially 120-150 g with the aid of 2-AAF [6] or diethylnitrosamine (DENA) [3]. The radioactive carcinogen was given to the rats 2 and 6 months after the beginning of feeding with unlabeled carcinogen and to animals with primary hepatomas induced by 2-AAF and DENA. The rats were killed 14 days after intraperitoneal injection of 2-AAF-9-^{14}C in a dose of 0.13 mCi/kg body weight.

The content of total RNA and DNA in the liver tissue was determined spectrophotometrically [4] after extracting them from the acid-insoluble residue. Native NA were obtained by Georgiev's method [1]. Electrophoresis of RNA in 2.5% polyacrylamide gel, staining of the gels, and isolation of RNA fractions enriched with polyadenylate sequences were carried out as described earlier [5]. The gels were washed to remove excess of dye and cut longitudinally into two equal parts, dried on slides at 55-60°C for 3-4 h, and exposed with a photographic plate (photosensitivity 90 GOST 10691-63 units; emulsion No. 2245 "Mikro," 9 × 12 cm) at 20°C for 28 days. The gels dried on the slides, and the developed photographic plates were

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TABLE 1. Binding of 2-AAF-9-^{14}C with Liver Tissue NA at Various Times during Hepatocarcinogenesis and in Primary Hepatomas (M ± m)

Tissue	Specific radioactivity, counts/min/mg NA		Content in liver tissue, mg %	
	DNA	RNA	DNA	RNA
Control (intact liver)	276±49	304±95	324±34	627±48
Liver after feeding with 2-AAF for 2 months	274±26	648±50*	327±22	664±58
Liver after feeding with 2-AAF for 6 months	377±39	671±31*	376±9	726±21
Primary hepatoma induced by 2-AAF (12 months after beginning of administration of agent)	538±43*	236±9	214±24	464±59
Primary hepatoma induced by DENA (6 months after beginning of administration of agent)	390±47*	266±21	533±107	985±363

*P < 0.05 compared with control.

subjected to densitometry on the MF-4 microphotometer with automatic recording of absorption. The radioactivity profile (from the plates) was compared with the profile of RNA content in gels. The radioactivity of the tissues and of NA was measured in a Mark II liquid scintillation counter in dioxan scintillator. The sedimentation constants of the individual RNA fractions were calculated on the basis of the results of electrophoretic fractionation of the biopolymer in polyacrylamide gel [8]. The results were subjected to statistical analysis by means of the Mann–Wilcoxon–Whitney nonparametric criterion [2].

EXPERIMENTAL RESULTS

The results of measurements of binding of 2-AAF-9-^{14}C with NA and liver tissue during hepatocarcinogenesis induced by 2-AAF are given in Table 1. Clearly during malignant change in the liver the intensity of binding of the labeled carcinogen by NA increased. In the first stages of the process of carcinogenesis, more of the bound labeled 2-AAF was retained in RNA, it will be noted, than in DNA (judging from residual binding 2 weeks after indicator labeling with 2-AAF-9-^{14}C). In primary hepatomas induced by 2-AAF the opposite distribution of radioactivity was observed; under these conditions the degree of binding of the carcinogen with DNA was significantly greater than in the control, whereas the specific radioactivity of RNA in the induced tumors was close to the control. In another primary hepatoma induced with DENA, taken for comparison, the change in binding of radioactivity of the carcinogen with NA showed a similar tendency (Table 1).

Binding of the labeled hepatocarcinogen with DNA (allowing for its content in liver tissue) increased during feeding with 2-AAF; it also exceeded the control values in the primary hepatoma. Radioactivity bound with total liver RNA exceeded that bound with total DNA in the first 6 months of carcinogenesis, but in the primary hepatoma it was below the control level and even below the radioactivity of total DNA.

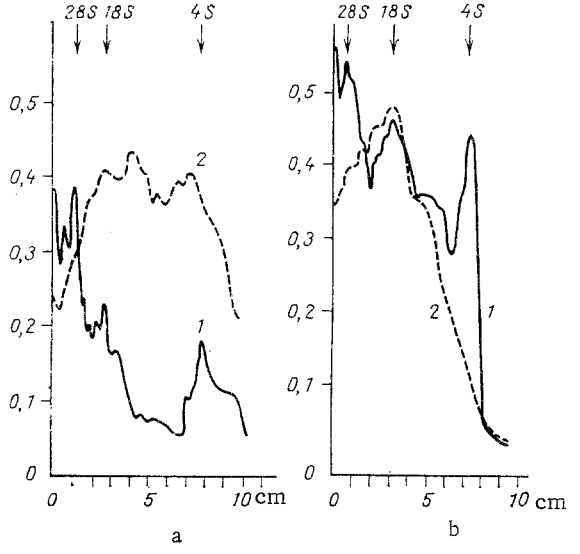

Fig. 1. Densitograms of RNA of primary hepatomas, stained with methylene blue, 2 weeks after administration of 2-AAF-9-^{14}C and densitograms of photographic plates after exposure for 28 days with dried gel: a) total RNA; b) fraction of total RNA not enriched with polyadenylate sequences. 1) Optical density of staining of gel; 2) optical density of photographic plate. Abscissa, distance from starting line of RNA in gel (in cm); ordinate, optical density in beam of incandescent lamp.

Similar results for binding of 2-AAF with DNA and RNA in the initial stages of hepatocarcinogenesis were obtained previously by other workers [7, 10]. The presence of residual incorporation into hepatoma DNA after administration of 2-AAF-9-^{14}C is evidence that changes in the genetic apparatus take place in tumors and cannot be corrected by the repair systems of the cell. It was therefore interesting to determine which RNA fractions could contribute to the realization of the tumor genetic information in hepatomas by virtue of their prolonged binding with the carcinogen.

Densitograms of dried gels, after fractionation of their hepatoma RNA, and also of autoradiographs obtained from the photographic plates are shown in Fig. 1. The radioactivity in the total RNA 2 weeks after administration of the labeled circinogen remains mainly in fractions with sedimentation constants of 18, 10, and 5S (Fig. 1a). The distribution of bound radioactivity of the labeled hepatocarcinogen in the RNA fraction unenriched with polyadenylate sequences is shown in Fig. 1b. Clearly most binding of the label was revealed within a wide range of molecular weights of the biopolymer with a maximum in the region of the 18S fraction of RNA. It can be concluded from differences between the two graphs that fractions with sedimentation constants of 10 and 5S in the total RNA of the primary hepatomas were enriched with polyadenylate sequences. These fractions were possibly messenger RNAs or their precursors, which have been shown to be capable of binding the carcinogen for a long time.

The distribution of residual radioactivity in the RNA spectrum of the primary hepatomas is thus evidence of prolonged interaction between the chemical carcinogen and the heterodispersed RNAs, 18S-ribosomal RNA, and certain messenger RNAs.

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