PREFERENTIAL BINDING OF 2-ACETYLAMINOFLUORENE TO RAT LIVER rRNA DURING EARLY STAGES OF HEPATOCARCINOGENESIS

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Summary:

During continuous feeding of 2-acetylaminofluorene-9-^{14}C (AAF-9-^{14}C) under conditions known to induce a high incidence of liver tumors in male rats, there is preferential binding of the carcinogen to liver rRNA. Furthermore, simultaneous administration of 3-methylcholanthrene, which inhibits liver tumor induction by AAF, resulted in a 35-40% decrease in binding to liver rRNA as opposed to a 50% increase in the binding of AAF-9-^{14}C to liver tRNA. These data support the concept that binding of AAF to liver rRNA might be involved in hepatocarcinogenesis.

Administration of the carcinogen 2-acetylaminofluorene (AAF) or its N-hydroxy metabolite to rats leads to base substitution in liver nucleic acids by covalent attachment of 2-acetylaminofluorenyl and 2-aminofluorenyl residues, primarily at the C-8 position of guanine (1-3). The specific radioactivity of liver tRNA is several-fold greater than that of rRNA or DNA following a single injection of AAF-9-^{14}C or N-hydroxy-AAF-9-^{14}C (1,2,4). The preferential binding to rat liver tRNA has led to speculation regarding the involvement of binding to tRNA in the mechanism of action of these hepatocarcinogens (4,5). One fact that must be considered in the interpretation of the significance of these data is that rats do not develop liver tumors following a single injection of AAF or N-hydroxy-AAF. Induction of hepatocellular carcinoma requires the continuous feeding of these compounds for 8-12 weeks. As a result of additional experiments (6), we found that there was no correlation of binding of AAF to liver tRNA and hepatocarcinogenicity but that there was preferential binding of AAF to rat liver rRNA during initial stages of hepatocarcinogenesis.

Methods:

2-Acetylaminofluorene-9-^{14}C was fed to Holtzman rats or injected i.p. as a solution in dimethylsulfoxide/corn oil solution (1/6, vol/vol). Nucleic acids were isolated as follows. The liver was homogenized in cold 0.9% NaCl and the homogenate was centrifuged for 30 minutes at 10,000 rpm (Sorvall SS-34 rotor), yielding a pellet (P_1) and supernatant (S_1). P_1 was stirred with 25 ml of cold 6% sodium p-aminosalicylate for 45 minutes and DNA was isolated (7). S_1 was centrifuged for 3 hours at 38,000 rpm (Spinco No. 40 rotor) to give a pellet (P_2) and supernatant (S_2). Ribosomal RNA was obtained from P_2 (7) and tRNA was isolated from S_2, then purified by conversion to the cetyltrimethylammonium salt and re-conversion to the potassium salt (8). The specific radioactivities (dpm carbon-14 bound per mg nucleic acid, converted to equivalent picomoles of fluorenyl residues bound per mg) were determined by liquid scintillation counting (2).

Results and Discussion:

Upon continuous administration of AAF-9-^{14}C, binding of the carcinogen to liver DNA of male rats increased for 2 weeks, then leveled off at 300-400 pmoles/mg DNA from 2-8 weeks. In female rats, which are resistant to liver tumor induction by AAF, binding to liver DNA increased more slowly but reached the value obtained in male rats (300-400 pmoles/mg) at 4-8 weeks. Binding to liver tRNA was much less in both sexes. The maximum binding of AAF-9-^{14}C to liver tRNA of male rats occurred at 1-3 days (200-250 pmoles/mg tRNA) but the amount of bound carcinogen declined rapidly, and remained at 100-150 pmoles/mg from 2-8 weeks. Binding of AAF-9-^{14}C to tRNA of female rat liver was constant (100-150 pmoles/mg) between 1 day and 8 weeks. The most significant sex difference was observed in the amount of the carcinogen bound to liver rRNA. In male rats, binding to liver rRNA increased rapidly to 350 pmoles/mg rRNA by 2 weeks, then fell gradually to 100-150 pmoles/mg by 8 weeks. The binding of AAF-9-^{14}C to liver rRNA of female rats increased very

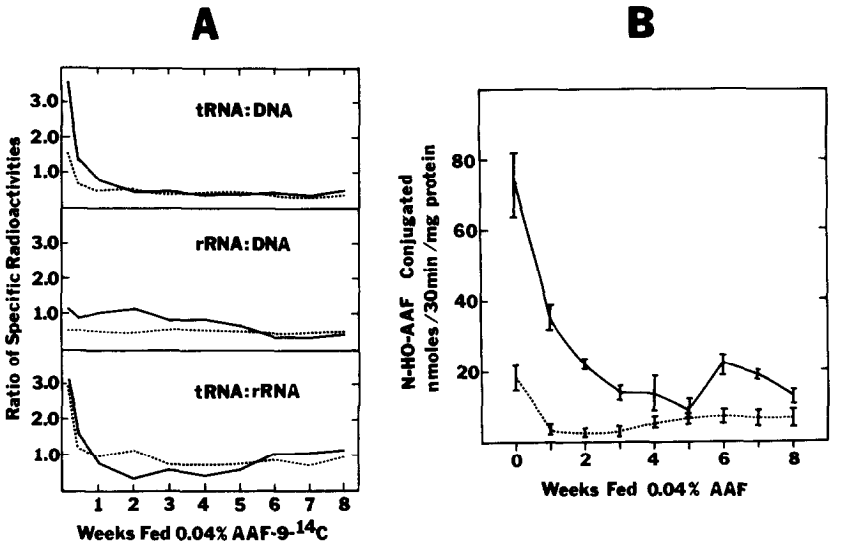

Figure 1. (A) Male (———) and female (------) rats were fed a grain diet (14) containing 0.04% AAF-9-^{14}C (1.91 dpm/pmole). At 1 and 3 days, then at weekly intervals for 8 weeks, 2 animals were sacrificed and tRNA, rRNA and DNA were isolated from the liver. The specific radioactivity (dpm/mg) of each nucleic acid was determined and the data expressed as the ratio of specific radioactivities indicated.

(B) Male (———) and female (------) rats were fed a grain diet containing 0.04% AAF. At weekly intervals, 4 animals were sacrificed and hepatic N-hydroxy-AAF sulfotransferase was determined (9).

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slowly and reached a maximum value of 100-150 pmoles/mg at 4-8 weeks. The data presented in Fig. 1A illustrate the dramatic change from an initial (1 day) preferential binding of AAF-9-^{14}C to rat liver tRNA to preferential binding to rRNA. The specific radioactivity of liver tRNA was greater than that of rRNA or DNA after 1 day of feeding AAF-9-^{14}C to rats. In male rats, at 1 day the tRNA/DNA and tRNA/rRNA ratios were 3.6 and 3.1, respectively. For female rats, these ratios were 1.6 (tRNA/DNA) and 3.0 (tRNA/rRNA). However, after one week of feeding AAF-9-^{14}C, the apparent preferential binding of the carcinogen to liver tRNA disappeared. The specific radioactivity of tRNA relative to that of DNA was about 0.4-0.5 from 2-8 weeks while that relative to rRNA was 0.4-1.0 from 2-8 weeks (Fig. 1A).

AAF-N-sulfate, an extremely reactive metabolite of AAF, is formed from N-hydroxy-AAF by the action of a sulfotransferase in the soluble fraction of rat liver homogenates (9). We believe that AAF-N-sulfate is responsible for most of the early binding of AAF-9-^{14}C to liver tRNA. The dramatic drop in the specific radioactivity of tRNA (Fig. 1A) relative to DNA and rRNA precisely parallels the decrease in hepatic N-hydroxy-AAF sulfotransferase observed during continuous feeding of AAF to rats (Fig. 1B). AAF-N-sulfate is not involved to a significant extent in the binding of AAF or N-hydroxy-AAF to rat liver DNA (10) and the results presented here (Fig. 1) suggest that AAF-N-sulfate may not be involved in binding of these carcinogens to liver rRNA.

Approximately 10% of the labeled carcinogen remained firmly bound to liver DNA after administration of a single dose of AAF-9-^{14}C, whereas the label completely disappears from liver rRNA by 4 weeks (2). Bound carcinogen (single dose)

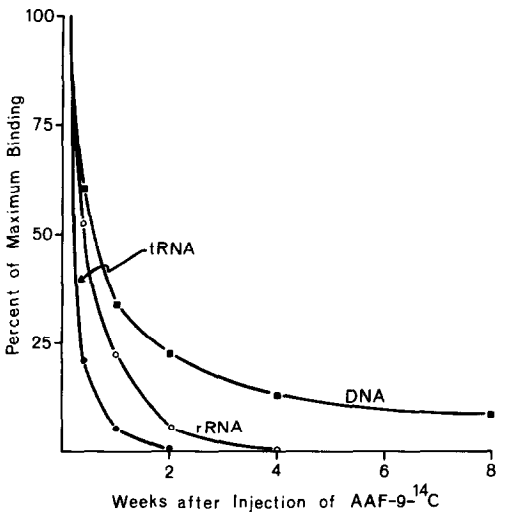

Figure 2. Twelve male rats (180-200 g) were each given a single i.p. injection of 2-acetylaminofluorene-9-^{14}C (dose, 30 mg/kg; specific radioactivity, 8.90 dpm/pmole). At 1, 3, 7, 14, 28 and 56 days, 2 animals were sacrificed and liver tRNA, rRNA and DNA were isolated and the specific radioactivities of the nucleic acids were determined. The ordinate is expressed as percent of the maximum binding (attained at day 1) for each nucleic acid. Values for the amount of carcinogen bound at day 1 were: tRNA, 116 pmoles/mg; rRNA, 49; and DNA, 26.

disappears from liver tRNA at a much faster rate than from rRNA or DNA as seen in Fig. 2, and is completely removed (< 1% of maximum) by 2 weeks after administration of AAF-9-^{14}C. The biological half-life of the tRNA-bound radioactivity was 1.5 days (calculated from data between 3 and 14 days), in contrast to 3 days for rRNA found here (Fig. 2) and previously reported (2). Mechanisms involved in the removal of the carcinogen bound derivatives from tRNA and rRNA are unknown, but it is interesting to note the greatly accelerated removal from (or turnover of) the tRNA. Normal liver rRNA and tRNA each have half-lives of approximately 5 days (11-13) so that disappearance of bound carcinogen is not due simply to normal turnover of the rRNA and tRNA.

3-Methylcholanthrene (MC) inhibits liver tumor induction in male rats by AAF (see ref. 14) and also inhibits the binding of AAF-9-^{14}C to rat liver rRNA and DNA

TABLE I

INFLUENCE OF 3-METHYLCHOLANTHRENE (MC) ON THE BINDING OF AAF-9-^{14}C TO RAT LIVER NUCLEIC ACIDS[a]

Nucleic acid	pmoles bound/mg nucleic acid		Influence of MC (%)
	AAF-9-^{14}C diet	AAF-9-^{14}C + MC diet	
DNA	358 ± 30	232 ± 29	- 36
rRNA	328 ± 24	200 ± 6	- 39
tRNA	148 ± 10	224 ± 9	+158

[a]Male rats were fed a grain diet containing 0.04% AAF-9-^{14}C (1.91 dpm/pmole) without or with MC (0.005%) for 2 weeks before sacrifice. Mean ± S.E. (3 rats). The average amounts of AAF-9-^{14}C ingested during the 2-week period were: AAF-9-^{14}C diet, 0.32 mmoles; and AAF-9-^{14}C + MC diet, 0.40 mmoles.

following a single injection of the carcinogen (14). When 3-methylcholanthrene (0.005%) was fed simultaneously with AAF-9-^{14}C for 2 weeks, binding of AAF-9-^{14}C to liver rRNA and DNA was inhibited almost 40% (Table 1). However, the binding to liver tRNA was increased 50% under these conditions (Table 1).

Thus, several lines of evidence suggest that the extent of covalent binding of AAF-9-^{14}C to liver tRNA is not correlated with hepatocarcinogenicity of AAF in the rat. The preferential binding of AAF-9-^{14}C to liver rRNA in the male rat during the initial 2-5 weeks of carcinogen feeding, the time at which the early proliferative phase of hepatocarcinogenesis by AAF begins (15,16), may be of significance.

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