RNA SYNTHESIS AND RNA POLYMERASE ACTIVITY IN HEPATIC NUCLEI ISOLATED FROM RATS FED THE CARCINOGEN 2-ACETYLAMINOFLUORENE

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SUMMARY

An assessment was made of the activity of RNA polymerase I and the capacity for RNA synthesis, under conditions optimized for RNA polymerase I activity, in hepatic nuclei isolated from rats fed a diet containing the hepatic carcinogen AAF. Animals were maintained on the carcinogenic diet for either 4, 7 or 14 days. RNA polymerase activity progressively increased with time on the carcinogenic diet. However, the capacity for RNA synthesis remained quite constant. These results are suggestive of a progressive inhibition of DNA template activity during the early stages of AAF-induced hepatocarcinogenesis. The "permanance" of the increase in polymerase activity was examined by switching carcinogen fed animals to a control diet for either 2 or 5 days prior to making an assessment of the above parameters.

It is well established that the members of many classes of chemical carcinogens become covalently bound to DNA, RNA and proteins of the cells in target tissues (1,2,3), and it has become axiomatic that they induce cancer as a result of such binding to one or more of these macromolecules. However, at the present time, the critical macromolecular target(s) has (have) not been unequivocally identified (2). In an effort to elucidate the potential mechanism(s) of action of the hepatic carcinogen AAF[1] and its N-hydroxy derivative, N-OH-AAF, several investigators have examined their effect on various aspects of hepatic RNA synthesis. These studies have been primarily directed toward the question of whether carcinogen administration results in an inhibition of RNA polymerase activity or an inhibition of the capacity of DNA to serve as a template for RNA synthesis. Recently, Herzog et al. (4) reported that both RNA polymerase I and RNA polymerase II activities, in liver nuclei, are inhibited 2 hours after a single injec-

[1]Abbreviations: AAF, 2-acetylaminofluorene; N-OH-AAF, N-hydroxy-2-acetylaminofluorene; A-AAF, N-acetoxy-2-acetylaminofluorene; ^{3}H-ATP, [2,8-^{3}H] adenosine 5'-triphosphate; TCA, trichloracetic acid; Poly (dA-dT), a high molecular weight, double stranded, copolymer composed of alternating dA and dT units.

tion of N-OH-AAF. No inhibition of the template activity of chromatin isolated from the livers of the carcinogen-treated animals could be detected (4). These results essentially confirm earlier reports by Glazer et al. (5,6), and by Zieve (7) who indicated that the nucleolar RNA polymerase was more susceptable to inhibition than the nucleoplasmic form of the enzyme. However, Grunberger et al. (8) have reported that there is preferential impairment of the nucleolar DNA template following a single injection of N-OH-AAF rather than an affect on RNA polymerase. Inhibition of the capacity of hepatic DNA isolated from rats fed AAF for several weeks to serve as a template for RNA synthesis has been reported by Troll et al. (9).

This report describes studies in which a determination was made of the capacity for RNA synthesis and RNA polymerase activity in hepatic nuclei isolated from rats which had been fed a diet containing the hepatic carcinogen AAF. In view of the apparently conflicting reports as to whether acute administration of a carcinogen results in an inhibition of the nucleolar RNA polymerase (4,7) or the template activity of nucleolar DNA (8) our experimental conditions were optimized for an assessment of nucleolar RNA synthesis and the nucleolar RNA polymerase, RNA polymerase I (10). Studies involving exposure to carcinogens through feeding as opposed to a single injection of the compound, offer the potential for assessing progressive biochemical alterations which are likely to be involved in the carcinogenic process.

MATERIALS AND METHODS

Animals and carcinogenic diet: Male Sprague-Dawley rats weighing 175 ± 20 g, obtained from Spartan Research Animals, Inc., Haslett, MI, were used for these studies. A control basal diet (11) (carcinogenic basal diet) containing U. S. Pharmacopiea XIV salt mix and supplemented, for long term feeding, with p-aminobenzoic acid, 0.11 g/kg of diet; inositol, 0.11 g/kg of diet; and dry vitamin E acetate 0.24 g (121 international units) /kg of diet, was purchased from Teklad Mills, Madison Wisconsin. This control diet was supplemented with 0.05% (w/w) AAF, purchased from Eastman Kodak, Rochester, New York. Food inges-

tion was regulated by maintaining the animals in a windowless room in which the lights are on from 7 p.m. until 7 a.m. and off the other 12 hours of the day. Animals were maintained on the control diet for 3 to 5 days prior to being placed on the AAF-containing diet. The animals were always started on the carcinogenic diet at approximately 8 a.m. and all animals were killed at 9 a.m. ± 1 hour.

Isolation of hepatic nuclei: Hepatic nuclei were isolated, in hypertonic sucrose, as described by Yu and Feigelson (12). This procedure results in the retention of both the free and engaged forms of RNA polymerase (13).

Assay of the capacity for RNA synthesis: The assay conditions employed in these experiments were optimum for the nucleolar, α-amanitin resistant, RNA polymerase I. The reaction mixture employed was a modification of that described by Grunberger et al. (8) and contained Tris HCl (pH 8.2), 100 mM; $MgCl_2$, 6.0 mM; dithiothreitol, 2 mM; GTP, CTP, UTP, 0.4 mM each; ^{3}H-ATP (New England Nuclear), 0.4 mM, 20 μCi/umole; and 50 μl of nuclei (70 ± 20 μg DNA) in a final volume of 0.2 ml. Reactions were initiated by the addition of nuclei and incubated at 30°. The reaction was stopped by the addition of 2.0 ml of ice-cold 5% TCA. Bovine serum albumin, 0.5 ml of a 0.5% solution, was added and the percipitate collected by centrifuging at 1000 x g for 5 min. The percipitate was washed twice with 2.0 ml portions of ice-cold 5% TCA and once with ethanol-ether (4:1 v/v) prior to being dissolved in 0.5 ml of 88% formic acid (14). Radioactivity was measured with a Packard model 3380 liquid scintillation spectrometer, multisol (isolab Inc., Akron, Ohio) was employed as the scintillation fluor. All reactions were run for 3 min. during which time the rate was linear and directly proportional to the amount of nuclei added.

RNA polymerase I activity: The activity of RNA polymerase I in isolated nuclei was measured employing an exogenous template, poly (dA-dT). The template activity of the endongenous DNA was blocked by the addition of actinomycin-D. This procedure has been successfully employed by Grunberger et al. (8) and its validity has recently been verified by Yu (13). RNA polymerase I activity was assayed using the basic reaction mixture described above containing 20 μl of

nuclei (28 ± 6 μg DNA), 8 μg poly (dA-dT) (Miles), 4 μg actinomycin-D (Sigma) and omitting GTP and CTP. All reactions will run for 10 minutes during which time the rate was linear and directly proportional to the amount of nuclei added.

RESULTS AND DISCUSSION

A single injection of N-OH-AAF has been reported to inhibit hepatic RNA synthesis in vivo (5). Acute administration of AAF (40 mg/kg) to normal rats did not affect this process (5). However, at very high doses (160 mg/kg) injection of AAF did cause a transient inhibition of hepatic RNA synthesis in male

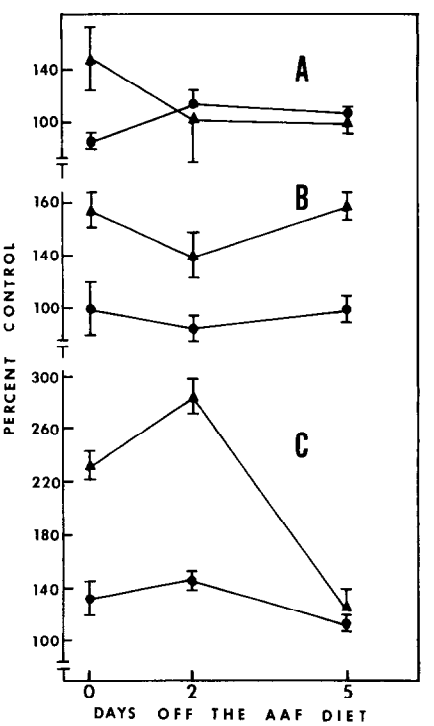

Fig. 1.: Effect of AAF ingestion on RNA synthesis and RNA polymerase I activity in isolated hepatic nuclei. RNA synthesis (●——●) and RNA polymerase activity (▲——▲) were determined, as described under Materials and Methods, in nuclei isolated from animals fed a diet containing 0.05% (w/w) AAF for 4 (A), 7 (B), or 14 (C) days. Groups of animals were killed at either the end of the period of carcinogen feeding (zero time on the chart) or at 2 and 5 days after being returned to the control diet. Each point represents the mean ± S.E. of the data obtained from 3 animals. The control values (mean ± S.E. of the data obtained from 12 animals) were 2,2028 ± 151 DPM/mg DNA/min and 488 ± 67 DPM/mg DNA/min for RNA synthesis and RNA polymerase I activity, respectively.

mice (15). The capacity for RNA synthesis was essentially unchanged in nuclei prepared from rats maintained on the carcinogenic diet for either 4 days (Fig. 1A) or 7 days (Fig. 1B). It was elevated slightly, approximately 40%, in nuclei prepared from animals fed the AAF diet for 14 days, and returned to the control level 5 days after the animals were taken off the carcinogenic diet (Fig. 1C).

Some (4,5,6,7) but not all (8) of the studies dealing with the acute effect of N-OH-AAF on RNA polymerase activity in isolated hepatic nuclei have concluded that polymerase activity was inhibited. Recent evidence has been presented which indicates the presence, in hepatic nuclei, of two distinct populations of RNA polymerase, free and engaged (13,16). The free polymerase population can account for as much as 50% of the total nuclear RNA polymerase activity and is easily lost when the nuclear isolation procedure involves the use of an isotonic buffer (13). In view of these findings those studies (4,5,6,7) in which the nuclei isolation procedure favored the loss of free RNA polymerase may have to be reevaluated.

The synthesis of RNA in isolated nuclei is a function of the level of RNA polymerase activity and the capacity of chromatin to serve as a template for RNA synthesis. The primary *in vitro* manifestation of gene repression in whole chromatin relative to DNA is a reduction of 10-fold in the number of RNA polymerase binding sites and 3-fold decrease in the rate of RNA chain elongation (17). Alkylation of DNA results in a decrease in the ability of the polymer to serve as a template for RNA polymerase (18,19). Arylamidation of DNA, as a result of reacting it *in vitro* with A-AAF, markedly decreases its capacity to act as a template for the synthesis of RNA and this appears to be primarily due to blocking chain elongation rather than interfering with initiation (20, 21). Therefore, those situations in which RNA polymerase activity is increased while the capacity for RNA synthesis remains unchanged may be indicative of an inhibition of template activity.

Increasing the duration of carcinogen exposure resulted in a progressive increase in RNA polymerase I activity (Fig. 1, zero time values). At the end

of 4, 7 and 14 days of carcinogen feeding, RNA polymerase activity was increased to 145%, 155% and 230% of control values, respectively. The capacity for RNA synthesis, under conditions optimized for RNA polymerase I activity, remained quite constant (Fig. 1). Taken together, these results indicate that ingestion of the hepatic carcinogen AAF results in a progressive inhibition of the capacity of DNA, in the target organ, to serve as a template for RNA synthesis. A possible alternative explanation is that in our experiments there was an excess amount of RNA polymerase I (i.e., all initiation sites for the enzyme on nucleolar chromatin were saturated) and consequently it would not play a limiting role in gene expression. However, this may not have been the case. There are reports in the literature of a direct correlation between increased levels of RNA polymerase I and increased ribosomal RNA synthesis. This has been shown to occur, in rat liver, following cortisone administration (12) and after partial hepatectomy (22).

The stability of the carcinogen-induced alteration in polymerase activity was variable depending upon the duration of carcinogen exposure. Five days after being switched to the control diet, RNA polymerase activity returned to the control value in those animals which had been fed the AAF diet for either 4 or 14 days (Fig. 1, A & C). However, polymerase activity remained elevated in those animals which had been fed the carcinogenic diet for 7 days (Fig. 1B).

The evoking of DNA repair synthesis (excision repair) is one of the early consequences of exposure to chemical carcinogens (23). This process has been extensively examined in mammalian cells in culture (24) and it has also been shown that chemical carcinogens can evoke DNA repair, in target organs, in the intact animal (25,26,27). The fidelity of the DNA excision repair process in mammalian cells, has been accessed and this appears to be an error free process (28,29). However, there are some reaction products formed between chemical carcinogens and DNA bases which are not readily corrected by the DNA excision repair process (30,31,32). These appear to be potentially damaging (32), i.e. may lead to cell death, mutation and possibly carcinogenesis.

There is a need to determine the consequences of carcinogen-induced DNA lesions, both those which are and those which are not readily repaired by the excision process. These experiments represent a step in that direction, and indicate that an assessment of DNA template activity may be an important parameter to examine in those studies aimed at elucidating the functional consequences of carcinogen-DNA interactions.

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REFERENCES

1. Miller, J.A., Cancer Res., 30: 559-576 (1970).
2. Miller, E.C. and Miller, J.A., in The Molecular Biology of Cancer, ed. H. Busch, Academic Press, New York, 377-402 (1974).
3. Heidelberger, C., Eur. J. Cancer, 6: 161-172 (1970).
4. Herzog, J., Serroni, A., Briesmeister, A. and Farber, J. L., Cancer Res., 35: 2138-2144 (1975).
5. Glazer, R.I., Nutter, R.C., Glass, L.E. and Menger, F.M., Cancer Res., 34: 2451-2458 (1974).
6. Glazer, R.I., Glass, L.E. and Menger, F.M., Mol. Pharmacol, 11: 36-43 (1975)
7. Zieve, F.J., J. Biol. Chem., 247: 5987-5995 (1972).
8. Grunberger, G., Yu, F-L., Grunberger, D. and Feigelson, P., J. Biol. Chem., 248: 6278-6281 (1972).
9. Troll, W., Belman, S., Berkowitz, E., Chmielewicz, Z.F., Ambrus, J.L. and Bardos, T.J., Biochim. Biophys. Acta, 157: 16-24 (1968).
10. Chambon, P., in The Enzymes, 3rd edition, ed. P.D. Boyer, Academic Press, New York, 10: 261-331 (1974).
11. Farber, E., Cancer Res., 16: 142-153 (1956).
12. Yu, F-L. and Feigelson, P., Proc. Nat. Acad. Sci. U.S.A., 68: 2177-2180 (1971).
13. Yu, F-L., Biochim. Biophys. Acta, 395: 329-336 (1975).
14. Novello, F. and Stripe, F., Biochem. J., 112: 721-727 (1969).
15. Dawson, K.M., Chem.-Biol. Interactions, 5: 153-165 (1972).
16. Yu, F-L., Nature, 251: 344-346 (1974).
17. Cedar, H. and Felsenfeld, G., J. Mol. Biol., 77: 237-254 (1973).
18. Mamet-Bratley, M.C., Biochim. Biophys. Acta, 247: 233-242 (1971).
19. Roberts, J.J., Int. J. Cancer, 16: 91-102 (1975).
20. Zieve, F.J., Mol. Pharmacol., 9: 658-669 (1973).
21. Millette, R.L. and Fink, L.M., Biochemistry, 14: 1426-1432 (1975).
22. Yu, F-L., Biochem. Biophys. Res. Comm., 64: 1107-1115 (1975).
23. Strauss, B.S., Life Sciences, 15: 1685-1693 (1975).
24. Lieberman, M.W., Baney, R.N., Lee, R.E., Sell, S. and Farber, E., Cancer Res., 31: 1297-1306 (1971).
25. Goodman, J.I. and Potter, V.R., Cancer Res., 32: 766-775 (1972).
26. Goodman, J.I., Biochem. Biophys. Res. Comm., 56: 52-59 (1974).
27. Damjanov, I., Cox, R., Sarma, D.S.R. and Farber, E., Cancer Res., 33: 2122-2128 (1973).

28. Lieberman, M.W. and Poirier, M.C., Biochemistry, 13: 5384-5388 (1974).
29. Lieberman, M.W. and Poirier, M.C., Proc. Nat. Acad. Sci. U.S.A., 71: 2461-2465 (1974).
30. Kriek, E., Cancer Res., 32: 2042-2048 (1972).
31. Goth, R. and Rajewsky, M.F., Proc. Nat. Acad. Sci. U.S.A., 71: 639-643 (1974)
32. Frei, J.V. and Lawley, P.D., Chem.-Biol. Interactions, 10: 413-427 (1975).
33. Lehmann, A.R., Life Sciences, 15: 2005-2016 (1975).