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EFFECTS OF THE HEPATOCARCINOGEN 2-AMINOFLUORENE ON RNA SYNTHESIS IN RAT LIVER*

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2-Acetylaminofluorene (2-AAF) and its metabolites are known to influence RNA synthesis in target cells. *N*-Hydroxy-2-acetylaminofluorene (N-OH-AAF) was shown to inhibit rapidly (1-2 h after injection) rat liver nuclear and, more efficiently, nucleolar RNA synthesis [1, 2]. Rats injected with a single dose of N-OH-AAF showed the maximal level of adducts in hepatic DNA after 1.5 h [3] and it seemed probable that the observed inhibition of RNA synthesis was due to changes in template properties of the adducted DNA.

In other experiments however, it was demonstrated that N-OH-AAF when injected in a single dose, did not influence the template properties of isolated DNA [4] but affected the activities of RNA polymerases I and II [5]. On the other hand, the high level of DNA adducts in liver cells of rats fed 2-AAF caused inhibition of DNA template activity and this was probably the reason of hepatic RNA synthesis inhibition in these animals [6 - 8].

We tried to reexamine the time course of DNA adduct formation and RNA transcriptional activity in the nuclei isolated from livers of animals killed at different time intervals after a single injection of 2-aminofluorene (2-AF). Similarly as in the case of the N-OH-AAF, we observed rapid inhibition of RNA synthesis 1 - 2 h after treatment. 2-AF DNA adducts

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appeared much later suggesting that they did not cause the inhibition of RNA synthesis.

We used 3 month old male Wistar rats (3 - 4 in a group). Animals were treated with a single intraperitoneal injection of 2-AF suspended in maize oil (50 mg of 2-AF/kg). The effect of administration of 2-AF on the formation of DNA adducts, on transcriptional activity in isolated liver cell nuclei and on formation of tight DNA-protein complexes in these cells were studied.

DNA adducts. Administration of 2-AF *in vivo* induces formation of DNA adducts in liver cells. Figure 1 presents the amounts of adducts found in liver DNA at different time intervals after 2-AF treatment. The maximal level of DNA adducts was observed between 24 and 48 h after 2-AF

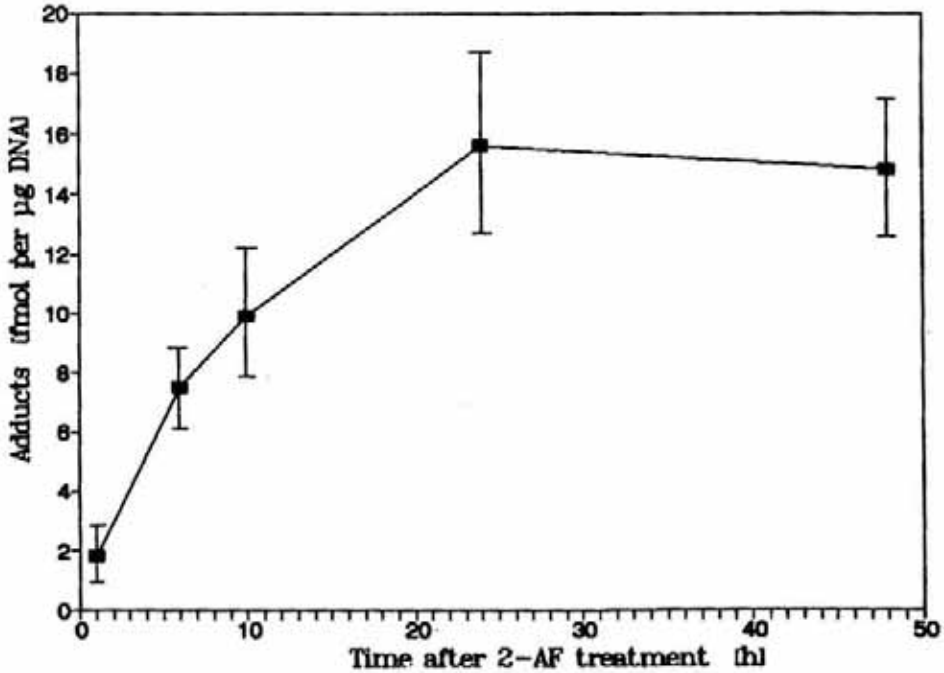

Fig. 1. The formation of adducts in DNA from liver of rats treated with a single dose of 2-aminofluorene. The values are the mean ± S.D. for 3 - 4 animals. The assay was performed according to Gupta *et al.* [11]

injection; 1- 2 h after treatment the level of adducts was only slightly augmented as compared with that of control DNA.

Transcription in isolated nuclei. A single dose of 2-AF caused rapid inhibition of total transcriptional activity measured in isolated liver nuclei of the animals killed 1 h after treatment with the carcinogen (Fig. 2). The

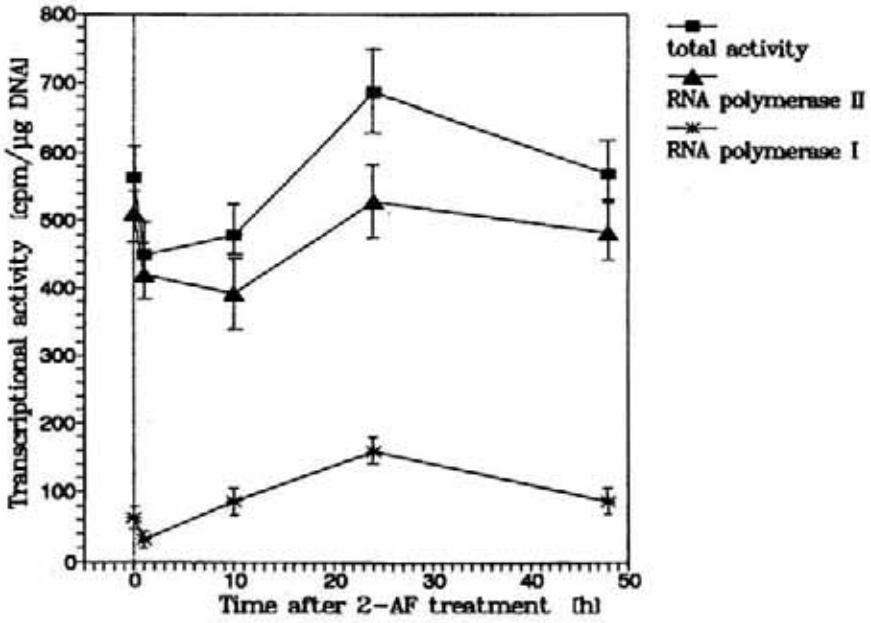

Fig. 2. The effects of 2-aminofluorene administration *in vivo* on the transcriptional activity in isolated rat liver nuclei (measured as the ratio of incorporation of RNA precursors). The values are the mean ± S.D. for 3 - 4 animals. The assay was performed according to Widnell & Tata [12] with modifications [13]

inhibitory effect was the strongest on the RNA polymerase I activity whereas RNA polymerase II was less affected. In the nuclei isolated from livers of animals killed 24 h after the injection we observed a stimulation of the RNA polymerase I dependent transcriptional activity.

Our results concerning the changes in RNA transcription induced by 2-AF are similar to those presented by others for N-OH-AAF [1, 2]. We also observed a decline in RNA synthesis shortly after treatment with the carcinogen. However, the 2-AF induced adducts appear in DNA much later than those induced by N-OH-AAF. Rats injected with N-OH-AAF showed the maximal level of adducts 1-2 h after the injection [3] simultaneously with the inhibition of transcriptional activity [1, 2], whereas 2-AF gave the

maximal number of adducts in DNA after 24 h. Using 2-AF which required a longer path of activation in the cell than N-OH-AAF did, we were able to separate the inhibitory effect on transcription from adduct formation in liver cells. Our results suggest that early inhibition of liver RNA synthesis was not related to the number of AF-DNA adducts. They also confirm the results of early works of Zieve [4] and Herzog *et al.* [5], who reported that template properties of rat liver DNA were not affected by a single dose of N-OH-AAF.

The stimulation of RNA polymerase I at longer time intervals after treatment with 2-AF seems to be also in good agreement with the results of Adams & Goodman [6], who observed an increase in hepatic RNA polymerase I activity on feeding rats with 2-AAF.

However, the possibility that in some cases DNA adducts are able to inhibit RNA synthesis can not be excluded. Nath and coworkers showed that AF and AAF adducts inhibited the RNA chain elongation in reaction on modified plasmid pDR100 [9]. We found that one of the animals in the group examined 24 h after treatment, had higher level of DNA adducts than other animals. In liver cells of this animal the lowest transcriptional activity and segregation of nucleolar components typical for inhibition of nucleolar RNA synthesis were detected.

Tight DNA-protein complexes. The conformation of the transcriptionally active chromatin regions is different from that of inactive parts. The interactions of active genes with nuclear skeletal structures seem to be of some importance for the transcription process [10]. The interactions between DNA and nuclear matrix proteins include formation of tight complexes probably with covalent bonds between DNA and protein. The effect of administration of 2-AF on the protein-DNA interactions in rat liver nuclei was studied using filtration through nitrocellulose filters which retain tight DNA-protein complexes (Table 1). The percentage of DNA retained on filters was similar in most of the DNA probes with the exception of DNA probes obtained from animals killed 10 and 24 h after 2-AF treatment. In those animals the amounts of tightly bound proteins seemed to be lower.

To check whether the effect observed was due to the presence of adducts in DNA of the treated animals or to the changes occuring in nuclear proteins we complexed labelled control DNA with matrix proteins originating from liver cells of animals injected with 2-AF and killed 1, 10 and 24 h after the injection. Table 2 shows the amounts of stable complexes formed by DNA of non-treated rats with proteins of animals injected with 2-AF. Stable

Table 1

The effect of 2-AF administration on the level of tight DNA-polypeptide complexes in rat liver nuclei

The values are the mean ± S.D. for 3 - 4 animals. The complexes were assayed according to Neuer-Nitsche & Werner [14], and the results presented as percentage of total nuclear DNA

Time after 2-AF treatment						
Control	1 h	6 h	10 h	24 h	48 h	72 h
DNA in tight complexes (%)						
6.1 ± 0.4	6.4 ± 0.3	6.2 ± 0.3	5.5 ± 0.2	5.2 ± 0.3	6.1 ± 0.5	6.5± 0.4

Table 2

The influence of 2-AF administration on the binding of rat liver nuclear matrix proteins to total DNA isolated from control animals

The values are the mean ± S.D. for 2 - 3 animals. The assay was performed according to Zenk *et al.* [15]. The amount of DNA in SDS/K^+ stable complexes was estimated as percentage of total bound DNA

Time after 2-AF treatment	Control	1 h	10 h	24 h
DNA in stable complexes (%)	4.2 ± 0.2	4.3 ± 0.2	3.3 ± 0.3	4.1 ± 0.2

protein-DNA complexes were formed with lower efficiency when the proteins from nuclear matrix of animals killed 10 h after 2-AF treatment were used. The result suggests that changes in formation of tight DNA -protein complexes could be related to some properties of nuclear proteins which probably can also interact with carcinogen molecules.

The changes in DNA-protein interactions might be involved in the stress-response mechanism. However, the elucidation of the possible role played by tight complexes needs further experimental work.

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