OAT and 3'MeDAB Azo Compounds Similarly Cause Liver Tumors in GR Mice, but Differently Modify Activities of FoxA Transcription Factors

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Transcription factors of the FoxA family (forkhead box A) regulate cell metabolism and differentiation and maintain specificity of liver cell proteome and phenotype of mature hepatocytes. The relationship between hepatocarcinogenicity of azo compounds *o*-aminoazotoluene (OAT) and 3'-methyl-4-dimethylaminobenzene (3'MeDAB) for GR mice and one of the early events, modulation of the DNA-binding activity of FoxA transcription factor, was studied. Single injection of 3'MeDAB to 12-day-old mice caused liver tumors in 100% males and females similarly as OAT, a well-known mouse hepatocarcinogene. The DNA-binding activity of FoxA in the liver decreased 2.5-3 times by OAT, this resulting in a 40% reduction of glucocorticoid induction of tyrosine aminotransferase (liver-specific gene). In contrast to these, 3'MeDAB did not modify FoxA protein activities or the degree of glucocorticoid induction of tyrosine aminotransferase.

Key Words: *azo compounds; tyrosine aminotransferase; mice; FoxA transcription factors*

Carcinogenesis is characterized by unbalanced proliferation, apoptosis, and differentiation processes [8]. This balance is maintained by regulatory proteins, transcription factors providing proteome specificity of certain cells, among them FoxA (forkhead box A) transcription factor family proteins. Proteins of this family (FoxA1, FoxA2, FoxA3) are expressed in the liver, pancreas, and lungs; they promote chromatin decompactization and regulate the expression of numerous target genes. More than 100 FoxA target genes were found in the liver. Their products regulate cell differentiation and metabolism and maintain cell phenotype [3,5,8,9]. We studied the involvement of FoxA factors in tumor induction on the model of various hepatocarcinogenic substances, primarily azo compounds [6,7,11] characterized by pronounced tissue, species, and strain specificity. It was found that the decrease in activities of FoxA proteins and attenuation of glucocorticoid induction of tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase; TAT) gene expressed exclusively in the liver take place only if the substance is hepatocarcinogenic for this rodent species, strain, or gender, but not in resistant animals. For example, 2'3-dimethyl-4-aminoazobenzene (*o*-aminotoluene; OAT; CAS: 97-56-3) reduces DNA-binding activity of FoxA and attenuates glucocorticoid induction of TAT in sensitive mice more than 2-fold. Another azo compound, 3'-methyl-4-dimethylaminoazobenzene (3'MeDAB; CAS: 55-80-1), causes tumors in rats but not in adult mice [6,7]. This compound 2-fold reduces FoxA activity and level of glucocorticoid induction of TAT in rats, but does not change it in mice [6,7]. Other substances (acetylaminofluorene, benzidene, diethylnitrosoamine, estragol, *etc.*) exhibit similar effects and also only if the substance causes liver tumors [2]. It should be noted that the hepato-

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carcinogene effects on FoxA activities were primarily studied on adult animals. On the other hand, it is known that many compounds exhibit more pronounced carcinogenic effects in 12-day-old mice.

For better understanding of the role of FoxA proteins in the mechanisms of tumor development in the liver, we studied the effects of OAT and 3'MeDAB on tumor induction in 12-day-old GR mice, DNA-binding activity of FoxA transcription factors in the liver, and the level of glucocorticoid induction of TAT in these animals.

MATERIALS AND METHODS

The study was carried out on 12-day-old male and female GR mice from vivarium of Institute of Cytology and Genetics. The animals were kept in plastic boxes 6-8 per box at natural light and free access to water and fodder (standard PK 120-1 fodder, Laboratorsnab). All manipulations with animals were carried out in accordance with the European Communities Council Directive 86/609/EEC). Azo compounds were dissolved in olive oil and injected to mice intraperitoneally in single doses of 250 mg/kg for 3'MeDAB (Koch-Light) and 225 mg/kg for OAT (ICB). Controls were injected with the solvent. In order to measure FoxA activity and level of TAT induction, the mice were sacrificed after 1 day or were observed over 18 months. Tumors were diagnosed in males [2] and females [1] as described previously. Dexamethasone phosphate (Sigma) was injected intraperitoneally in a dose of 5 mg/kg 19 h after injection of azo compounds. Tyrosine aminotransferase activity was evaluated as described previously [2] and expressed in μmol p-hydroxyphenylpyruvate/100 mg protein/h.

Protein extracts from liver cell nuclei were isolated by the method of Gorsky–Shapiro [7] in our modification. DNA-binding activities of proteins were evaluated by the gel retardation assay [2]. DNA-binding activity of ETS not changing under the effects of OAT, 3'MeDAB, and other compounds [2,6,7] served as the negative control. Double-stranded oligonucleotides (Biosset) served as DNA probes and corresponded to the known FoxA binding sites: 5'-CAGTCGAGTTGACTAAGTCAATAATCAGAATCAGTCG-3'; ETS: 5'-CAGTTCGAACTTCCTGCTCGA-3' (second strand is not shown). After annealing, the oligonucleotides were labeled with *E. coli* DNA polymerase I Klenov fragment (Sibenzyme) in the presence of [α-^{32}P]dATP (6000 Ci/mmol; Amersham). Protein concentration was measured after Bradford.

The data were statistically processed using Excel 6.0 software.

RESULTS

One year after injection of OAT to 12-day-old GR mice, tumors were found in 100% females and 80% males (in 12 of 15). Administration of 3'MeDAB induced liver tumors in 100% males and females (Table 1). In females, the number of tumor nodes in the liver in response to 3'MeDAB was higher to OAT. Hence, 3'MeDAB, noncarcinogenic for adult mice [7] in comparison with the known hepatocarcinogene OAT, exhibited a pronounced tumor-inducing effect in 12-day-old mice.

We showed (Fig. 1) that OAT injected to 12-day-old mice significantly reduced DNA-binding activity of FoxA (by 2.8 times according to densitometry data). These data were in line with the previous results obtained on several strains of adult mice sensitive to OAT [2,6,7]. However, 3'MeDAB, hepatocarcinogenic for these mice, virtually did not reduce DNA-binding activity of FoxA. Changes in the glucocorticoid induction of TAT (Table 2) directly reflected changes in FoxA protein activities, which was in line with the data according to which FoxA proteins determined the amplitude of glucocorticoid induction of TAT [12]. After OAT injection the glucocorticoid induction of TAT decreased by 40-42%, while 3'MeDAB did not modify the level of glucocorticoid induction of TAT in females at all and just slightly inhibited it in males (Table 2).

The transcription factors have attracted special attention of scientists searching for causes of some

TABLE 1. Incidence of Liver Tumors after Single Injection of OAT and 3'MeDAB to 12-Day-Old GR Mice

Parameter	Oil		3'MeDAB		OAT	
	males	females	males	females	males	females
Incidence of liver tumor development	-	-	100% (*n*=6)	100% (*n*=5)	80% (*n*=15)	100% (*n*=6)
Number of nodes in the liver per mouse	-	-	4.7±2.4	13.6±4.8	3.6±0.8	5.7±1.4
larger than 2 mm	-	-	2.2±0.7	2.4±1.2	1.7±0.7	-

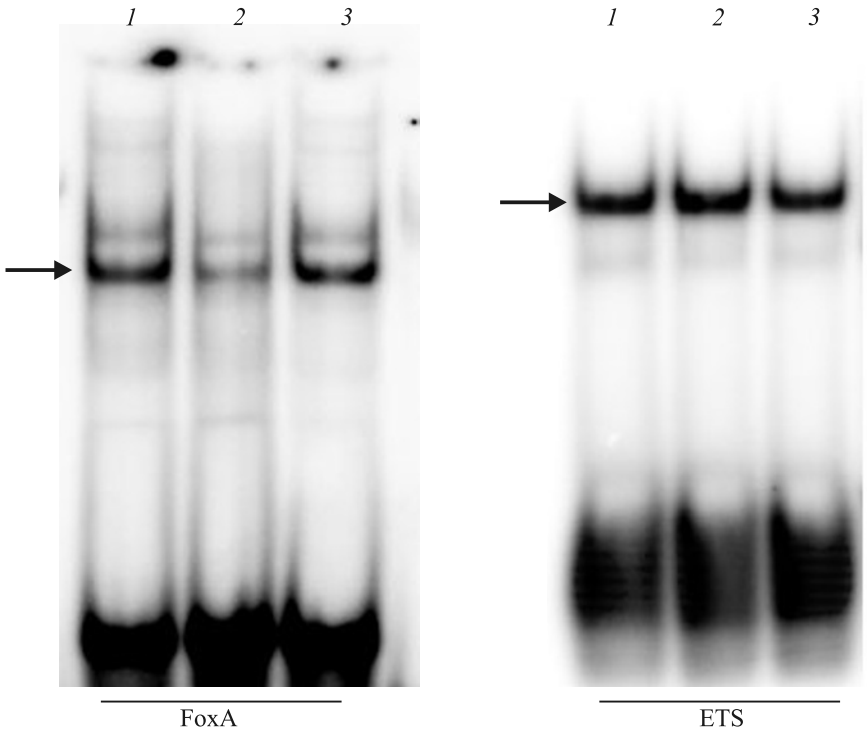

Fig. 1. Effects of OAT and 3'MeDAB on FoxA and ETS transcription factor DNA-binding activity in the liver of 12-day-old GR mice. DNA-binding activity of ETS in the liver served as the negative control. *1*) liver cell nuclei extract from animals receiving no carcinogens; *2*) OAT injection; *3*) 3'MeDAB injection. Typical autoradiography (1 of 3 independent experiments) is presented. For each point, 3-4 mice were examined.

TABLE 2. Effects of OAT and 3'MeDAB on Glucocorticoid Induction of TAT in the Liver of 12-Day-Old GR Females and Males

Parameter	Males	Females
Basal level	15.8±5.0 (*n*=3)	15.4±3.4 (*n*=3)
Glucocorticoid induction	137.5±2.9 (*n*=6)	127.0±5.3 (*n*=4)
Induction+OAT	80.7±11.2** (*n*=5)	77.0±6.6** (*n*=4)
Induction+3'MeDAB	121.5±3.9 (*n*=6)	127.0±5.3 (*n*=4)

Note. Activity of TAT is expressed in μmol p-hydroxyphenylpyruvate/100 mg protein/h. *p<0.01: significant reduction of the level of glucocorticoid induction (Student's t test).

human diseases in recent years. About 270 diseases and syndromes directly linked with dysfunctions of 164 transcription factors are known [14]. The key role of some of these factors in the carcinogenesis has been shown [4,9,10,13]. For example, the presence of an extra C/EBPa allele is responsible for a 2-fold reduction of sensitivity to carcinogens [13]. Transfection of malignant cells by the plasmid providing a high level of C/EBPa expression leads to proliferation reduction and triggering of apoptosis processes [4]. The expression of another factor, HNF4a, gradually reduces throughout hepatocarcinogenesis [10]; recovery of expression leads to antioncogenic effect. The contribution of FoxA factors to the carcinogenic process remains not quite clear and deserves further research. It seems that FoxA proteins are not the key transcription factors triggering the cell degeneration process, but this fact does not preclude the presence of antioncogen targets among them [3,5,9]. For example, it is known that disorders in FoxA expression leads to a significant reduction of the production of many other liver-specific transcription factors with tumor-suppressive effects, for example, HNF4a [5].

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