ONCOLOGY

FOXA Transcription Factors Determine the Amplitude of Glucocorticoid Induction of Tyrosine Aminotransferase in Mice

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o-Aminoazotoluene was more potent than 3'-methyl-4-dimethylaminoazobenzene in modulating glucocorticoid induction of tyrosine aminotransferase and DNA-binding activity of FOXA (HNF3) in 12-day-old ICR mice. In adult animals, induction of tyrosine aminotransferase and FOXA activity were modulated by *o*-aminoazotoluene, while 3'-methyl-4-dimethylaminoazobenzene was ineffective. Our results suggest that FOXA proteins determine glucocorticoid induction of tyrosine aminotransferase in mice (similarly to rats).

Key Words: *FOXA transcription factors; aminoazo dyes; tyrosine aminotransferase; glucocorticoid induction*

FOXA transcription factors (HNF3 according to old nomenclature) in combination with other transcription factors play an important role in tissue-specific gene expression. They determine basal and exogenous signal-induced gene expression (*e.g.*, carbamoyl phosphate synthase I in the liver [3], lipocalcin-5 in the epidermis [10], and glucagon in the pancreas [9]).

Tyrosine aminotransferase (TAT) gene is expressed and induced by glucocorticoid hormones only in liver parenchymal cells. Glucocorticoid regulation of rat TAT gene is provided by two enhancers localized at -5.5 and -2.5 kb from the transcription start. Both enhancers and glucocorticoid receptor binding sites have numerous binding sites for FOXA and C/EBP proteins [2]. Factors of the FOXA family determine the amplitude of glucocorticoid induction of TAT in these animals [8]. However, the mechanisms for glucocorticoid induction of TAT in other species remain unknown.

Our previous study of the early effects of tissue-specific hepatocarcinogenic aminoazo dyes revealed a correlation between the decrease in glucocorticoid induction of TAT and DNA-binding activity of FOXA proteins in the liver of rats and mice. Administration of specific hepatocarcinogen 3'-methyl-4-dimethylaminoazobenzene (3'-MeDAB) to rats reduced activity of FOXA proteins, which was followed by a decrease in glucocorticoid induction of TAT. However, *o*-aminoazotoluene (OAT) had no effect on TAT induction and FOXA protein activity. Other results were obtained in experiments on mice. As differentiated from 3'-MeDAB, specific hepatocarcinogen OAT decreased DNA-binding activity of FOXA and glucocorticoid induction of TAT in SWR mice. Activity of other transcription factors involved in glucocorticoid induction of rat TAT gene (*e.g.*, C/EBP and GME-binding proteins [2,7]) remained unchanged after administration of aminoazo dyes to mice or rats [4,6].

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Here we studied the sensitivity of ICR mice to hepatocarcinogenic activity of OAT.

MATERIALS AND METHODS

Experiments were performed on ICR mice aging 12 days and 3 months. OAT and 3'-MeDAB were dissolved in olive oil and injected intraperitoneally in doses of 22.5 and 25 mg per 100 g body weight, respectively. Control animals received the solvent in a similar dose. The animals were decapitated 5 h after induction with dexamethasone (5 mg/kg) and 6 h after administration of azo dyes. TAT activity was measured in cytosol fraction of the liver [1].

Double-stranded oligonucleotide 5'-cagtCGAG TTGACTAAGTCAATAATCAGAATCAGTCG-3' corresponding to the FOXA site and obtained from mouse transthyretin gene (strand 2 not shown) was used in the experiment [5]. Small letters designate the additional extended ends labeled after annealing with *E. coli* DNA polymerase I Klenow fragment in the presence of [α-^{32}P]-dATP. Oligonucleotides were synthesized on an ASM-102I automatic analyzer (Biosset). Nuclear extracts of liver cells were prepared as described elsewhere [1]. DNA-binding activity of nuclear proteins was estimated by gel retardation assay [1]. The gel was dried. The binding pattern was visualized on a Molecular Imager FX Pro Plus phosphoimager (Bio-Rad) or using X-ray film.

RESULTS

Liver tumor in 12-day-old ICR mice developed 1 year after single treatment with OAT. The degree of spontaneous carcinogenesis in control animals did not exceed 13%. Hence, ICR mice are sensitive to the hepatocarcinogenic effect of OAT. It was interesting to evaluate the effect of OAT and 3'-MeDAB on glucocorticoid induction of TAT and activity of FOXA proteins in these mice.

OAT significantly decreased DNA-binding activity of FOXA in 12-day-old mice. 3'-MeDAB was less potent in this respect (Fig. 1). The difference in the effect of azo dyes was more pronounced in 3-month-old mice. Similarly to 12-day-old mice, OAT decreased DNA-binding activity of FOXA in these animals. However, 3'-MeDAB had little effect on this parameter in 3-month-old mice.

Variations in the glucocorticoid induction of TAT after administration of OAT and 3'-MeDAB to 12-day-old and adult mice corresponded to changes in DNA-binding activity of FOXA. OAT completely inhibited glucocorticoid induction of TAT in 12-day-old mice, while the effect of 3'-MeDAB was less pronounced. Administration of OAT significantly decreased glucocorticoid induction of TAT in adult mice, while 3'-MeDAB had little effect on this parameter (Fig. 2).

A correlation was found between the decrease in DNA-binding activity of FOXA and glucocorticoid induction of TAT in ICR mice. After admini-

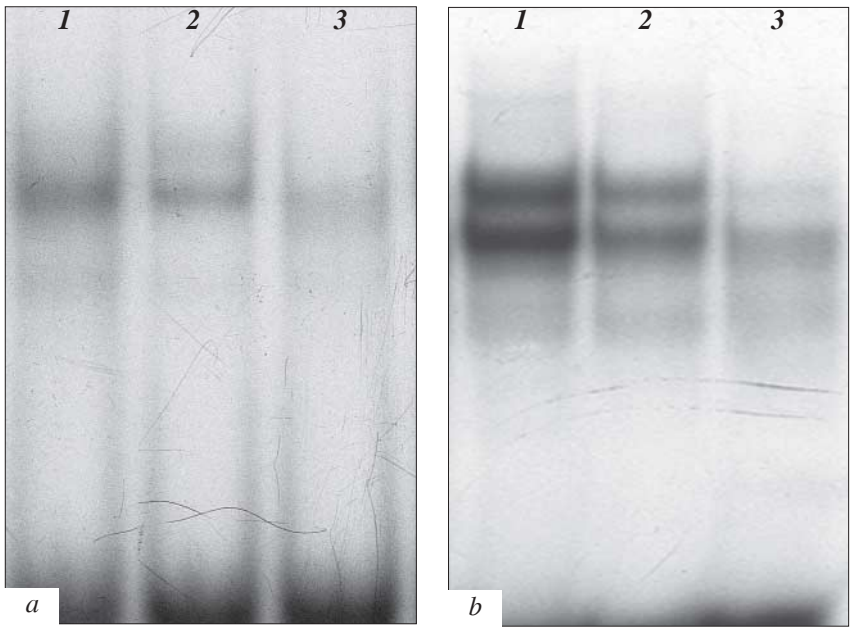

Fig. 1. Effects of 3'-MeDAB (*2*) and OAT (*3*) on DNA-binding activity of FOXA proteins in 12-day-old (*a*) and 3-month-old ICR mice (*b*). Control (*1*). Results of 1 of 3 independent experiments. The liver nuclear extract from 3 mice was studied in each point of each experiment.

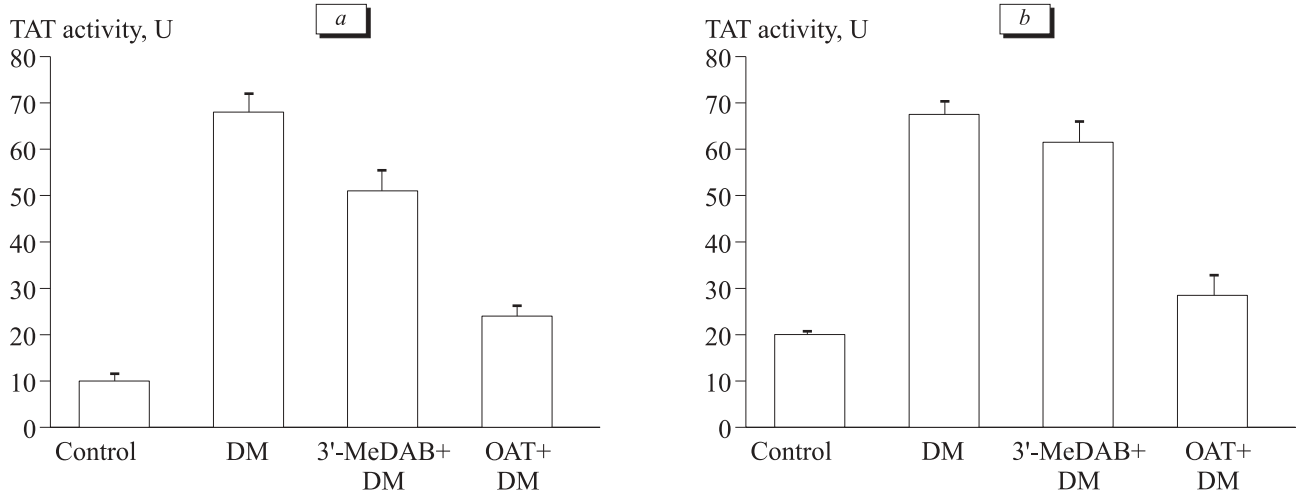

Fig. 2. Effects of 3'-MeDAB and OAT on glucocorticoid induction of TAT in 12-day-old (*a*) and 3-month-old ICR mice (*b*). Each bar represents the average data for 6 mice. DM, dexamethasone.

stration of OAT and 3'-MeDAB to 12-day-old mice, the reduction of FOXA protein activity was accompanied by a decrease in glucocorticoid induction of TAT. It should be emphasized that OAT was more potent than 3'-MeDAB in modulating the induction of TAT and activity of FOXA protein. 3'-MeDAB had no effect on FOXA activity and did not decrease glucocorticoid induction of TAT in adult mice. Similarly to 12-day-old mice, administration of OAT significantly decreased FOXA activity and glucocorticoid induction of TAT in adult animals. A relationship between glucocorticoid induction of TAT and DNA-binding activity of FOXA was revealed after administration of OAT to mice sensitive and resistant to the hepatocarcinogenic effect of this compound. OAT significantly decreased glucocorticoid induction of TAT and DNA-binding activity of FOXA in the liver of sensitive mice (A and SWR), but not in resistant animals (CC57BR and AKR) [4]. These data suggest that FOXA proteins determine the amplitude of glucocorticoid induction of TAT in mice (similarly to rats [8]).

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