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Correlation between Hepatocarcinogenic Effect of Estragole and Its Influence on Glucocorticoid Induction of Liver-Specific Enzymes and Activities of FOXA and HNF4 Transcription Factors in Mouse and Rat Liver

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Abstract—It is known that the carcinogenic effect of estragole, a component of essential oils of many spicy plants, is characterized by species, tissue, and sex specificity. It causes mainly liver tumors in female mice but is not carcinogenic for male mice and for rats. In this work, the estragole hepatocarcinogenicity was shown for female mice of previously not studied ICR line. The strict correlation between estragole hepatocarcinogenicity and its ability to decrease the level of glucocorticoid induction of liver-specific enzymes tyrosine aminotransferase (TAT) and tryptophan oxygenase (TO) was found. Inhibition of TAT and TO inducibility by estragole takes place only in female mice but not in male mice and in rats. Studying the estragole effect on DNA-binding activity of transcription factors, present mainly in liver and regulating expression of genes encoding liver-specific proteins, has shown that estragole decreases FOXA and HNF4 activities but not activities of C/EBP and HNF1, and this happens only in female mice, for which this substance is hepatocarcinogen, but not in male mice and in rats. Pentachlorophenol, preventing hepatocarcinogenic effect of estragole, abolishes inhibitory influence of the latter on the TAT and TO glucocorticoid induction and restores DNA-binding activity of FOXA and HNF4. Thus, a correlation was revealed between the estragole hepatocarcinogenic effect and decrease in DNA-binding activity of transcription factors FOXA and HNF4, which might be indicative of the role of these factors in tumor suppression mechanisms in liver.

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Key words: hepatocarcinogens, estragole, tryptophan oxygenase, tyrosine aminotransferase, glucocorticoid induction, HNF4, FOXA

It was noted over 40 years ago that in rats fed the hepatocarcinogenic azo dye N,N-dimethyl-4-aminoazobenzene, the level of glucocorticoid induction of adaptive liver enzymes tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5; TAT) and tryptophan oxygenase (L-tryptophan:oxygen-2,3-oxidoreductase, EC 1.13.11.11; TO) sharply decreases during the period preceding tumor development [1, 2]. Later the inhibitory effect on glucocorticoid induction of both enzymes was revealed in rat liver for a number of different classes of carcinogens causing liver tumors in these animals [3, 4]. In 1979, we reported for the first time that the mouse-specific hepatocarcinogen *ortho*-aminoazotoluene (OAT) decreases TAT induction in mouse liver, and this is observed only in lines sensitive to the tumor-inducing effect of OAT [5]. Further study showed that the OAT effect is realized at the level of *TAT* gene transcription [6] and is due to lowering total DNA-binding activity of FOXA family (family of A proteins containing fox domain (forkheadbox A)) transcription factors (HNF3 in an older nomenclature) [6-8] playing the key role in establishment of the amplitude of induction of this gene by glucocorticoid [9].

Correlation between the carcinogen effects on TAT glucocorticoid induction and DNA-binding activity of

Abbreviations: C/EBP, CCAAT/enhancer binding protein; DENA, diethylnitrosamine; FOXA, family of A proteins containing fox domain (forkheadbox A); HNF1, -4, hepatocyte nuclear factors 1 and 4; i.p., intraperitoneal; 3′-MeDAB, 3′-methyl-4-dimethylaminobenzene; OAT, *ortho*-aminoazotoluene; TAT, L-tyrosine:2-oxoglutarate aminotransferase; TO, L-tryptophan:oxygen-2,3-oxidoreductase.

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FOXA family transcription factors in liver and their ability to induce in the latter tumor development was revealed by us on other models as well. In particular, 3′-methyl-4-dimethylaminobenzene (3′-MeDAB), a species-specific hepatocarcinogen for rats, and diethylnitrosamine (DENA), a hepatocarcinogenic both for rats and mice, inhibited TAT induction and activity of FOXA proteins in rat liver, and DENA did the same in mice [6]. Since transcription factors of the FOXA family (proteins FoxA1, FoxA2, and FoxA3) are important for differentiation, phenotype maintenance, and inhibition of hepatocyte proliferation [10, 11], the data suggest that inhibition of the function of these regulatory proteins is essential for hepatocarcinogenesis induced by the studied substances, and the decrease in the TAT glucocorticoid induction can be considered as a marker of following development of liver tumors in response to chemical compounds.

Estragole (1-allyl-4-methoxybenzene), an aromatic compound present in some food plants, exhibits both species and sex specificity of liver tumor induction. It is carcinogenic for female mice but not for male mice and is not carcinogenic for rats [12]. Therefore, it seemed very interesting to study the effect of this compound on TAT glucocorticoid induction and total DNA-binding activity of FOXA proteins in the liver of animals of these groups as well as its effect on glucocorticoid induction of another liver-specific enzyme, tryptophan oxygenase.

MATERIALS AND METHODS

Materials. The following reagents were used in this work: [α-^{32}P]deoxy-ATP (specific activity 6000 Ci/mmol) (Amersham, Great Britain); Tris, Hepes, spermine, spermidine, dithiothreitol (DTT), bromphenol blue, and dexamethasone phosphate (Sigma, USA); *E. coli* DNA polymerase I Klenow fragment (SibEnzyme, Russia); DEAE-81 paper (Whatman, Great Britain); acrylamide, N,N′-bis-acrylamide (Reanal, Hungary); estragole, EDTA, EGTA, sucrose, and glycerol (Serva, Germany). Other reagents of chemically pure and of special purity grades were from Russia.

Oligonucleotides. Double-stranded oligonucleotides corresponding to known binding sites of transcription factors (the second strand is not shown) were used in this work: HNF1, 5′-cagtGGTTAGTGTGGTTAATGATC-TACAGTT-3′ from the rat albumin gene promoter [13]; FOXA, 5′-cagtCGAGTTGACTAAGTCAATAATCA-GAATCAGTCG-3′ from the mouse transthyretin gene promoter [14]; Ets, 5′- cagtTCGAACTTCCTGCTCGA-3′ from −2.5 kb enhancer of the rat *TAT* gene [15]; HNF4, 5′-cagtCGCTGCTGCTCTTTGATCTGTAGG-CG-3′ from −3.5 kb enhancer of the rat *TAT* gene [16]; C/EBP, 5′-cagtGATCCATATTAAGGACATGCCG-3′ from the rat *c-fos* gene promoter [17].

The added protruding ends, at which after annealing oligonucleotides were labeled in the presence of [α-^{32}P]deoxy-ATP using the *E. coli* DNA polymerase I Klenow fragment, are designated in lower case. Oligonucleotides were synthesized on an ASM-102I automatic synthesizer (Biosset, Russia) using the H-phosphonate technique.

Animals. We used 2-3-month-old ICR male and female mice and female Wistar rats of 180-200 g bred in the vivarium of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences. Animals were kept in plastic cages, six to eight animals in each, under natural lighting and with free access to water and food (briquette food PK120-1; Laboratorsnab, Moscow). All manipulations were carried out in accordance with the International Rules for Work with Animals (European Communities Council Directive; 86/609/EEC).

Investigation of carcinogenic activity of estragole. Estragole was dissolved in olive oil (15 mg/ml) and introduced into mice intraperitoneally (i.p.) (1 ml/100 g body weight) once a week for 17 weeks. Mice were under observation for one and a half years (65.5% of the animals died during this time) and the rest were decapitated at the age of 537 days. All naturally dieing or decapitated mice were dissected, the presence of macroscopic tumors was registered, and affected organs were fixed in 10% formaldehyde. Fixed material was used to prepare paraffin sections that were stained with hematoxylin and eosin and studied under a microscope.

Determination of enzyme activity. Carcinogens were introduced into animals by a single i.p. injection in olive oil 24 h prior to sacrifice (estragole at a dose of 300 mg/kg, OAT at 225 mg/kg). The solvent was injected into control animals. Pentachlorophenol solution in olive oil (0.22%) was injected into animals i.p. 45 min prior to estragole, 0.5 ml/100 g body weight. Dexamethasone phosphate was injected i.p. at a dose of 5 mg/kg body weight, 19 h after carcinogens. Animals were decapitated, livers were taken, and TAT and TO activities were determined as described previously [8, 18]. Enzyme activities were expressed for TAT in μmoles of *p*-hydroxyphenyl pyruvate/100 mg protein per hour, for TO in μmoles of kynurenine/gram liver per hour.

Isolation of protein extracts of liver cell nuclei. Extracts of liver cell nuclei were prepared using the modified Gorsky–Shapiro technique [19]. Animals were decapitated 20 h after estragole injection, the liver was perfused with cooled buffer solution containing 10 mM Hepes, pH 7.6, 25 mM KCl, 1 mM EDTA, and homogenized in 8-10 volumes of buffer solution containing 10 mM Hepes, pH 7.6, 25 mM KCl, 1 mM EDTA, 0.15 mM spermine, 0.5 mM spermidine, 2 M sucrose, and 10% glycerol (w/v). The homogenate was layered on a 10 ml cushion of the same buffer in the tubes of a SW28 ultracentrifuge rotor (Beckman L8-50 M/E; Beckman, USA) and centrifuged for 40 min at 24,000 rpm. The supernatant was discarded, and the pellet of nuclei was suspended in 4 ml of buffer containing 25 mM Hepes, pH 7.6, 100 mM KCl, 0.1 M

EDTA, 3 mM $MgCl_2$, 1 mM DTT, and 10% glycerol (w/v). The nuclei were lysed by addition of 0.4 ml of saturated (at 0°C) ammonium sulfate solution. The lysate was transferred into tubes of a SW-50 rotor and centrifuged for 90 min at 34,000 rpm. For protein precipitation, 0.882 g ammonium sulfate was added in small portions with stirring to 3.5 ml supernatant, and then the mixture was centrifuged for 30 min at 34,000 rpm. The precipitate was dissolved in 200-400 µl of buffer solution containing 25 mM Hepes, pH 7.6, 100 mM KCl, 0.2 mM EDTA, 0.2 mM EGTA, 1 mM DTT, and 10% glycerol (w/v), and the solution was dialyzed twice for 45 min against 250 ml of the same buffer solution. After dialysis, extracts was divided into aliquots and stored at −70°C.

Retardation in gel of nuclear protein complexes with specific DNA probes. The DNA-binding activity of proteins was estimated by the gel retardation technique [19]. The reaction mixture contained 25 mM Hepes, pH 7.6, 100 mM KCl, 0.2 mM EDTA, 0.2 mM EGTA, 1 mM DTT, 10% glycerol (w/v), 2 ng labeled oligonucleotide, and 4 µg of the nuclear extract protein incubated in advance for 15 min with sonicated calf thymus DNA (1 µg/6.5 µg protein). After incubation for 10 min at 20°C, the mixture was separated by electrophoresis in 4.5% native polyacrylamide gel, the gel was dried, and the banding pattern was visualized on a Molecular Imager FX Pro Plus phosphoimager (BioRad, USA). Protein concentration was measured on a Eppendorf biophotometer (Germany) at 280 nm with the coefficient indicating the nucleic acid contaminant in the sample. Statistical data processing was carried out in the program package STATISTICA 6.0 using the Student's *t*-criterion.

RESULTS

Hepatocarcinogenic effect of estragole. Female mice of ICR line tolerated well weekly injections of 150 mg/kg estragole, which was shown both by the absence of lethality and by progressive increase of the body mass by more than 50% by the end of injections. The first tumor was registered in the liver of a mouse that died at the age of 324 days. Among 30 animals that survived to this age, tumors were found in 24 (80%). Since frequency of spontaneous liver tumors in females of ICR line is close to zero [20], all tumors in our case were obviously induced by estragole. Liver tumors were found upon dissection as single (in five animals) or more often as multiple nodules and tumors (4.8 ± 0.5 per mouse) from 2 to 15 mm in diameter. Histologically all of them were typical hepatocellular adenomas and carcinomas, usually highly or moderately differentiated.

Estragole effect on TAT and TO glucocorticoid induction. Data on estragole effect on glucocorticoid induction of tyrosine aminotransferase (TAT) and tryptophan oxygenase (TO) in mouse and rat livers are shown in Table 1. Estragole introduction significantly decreased TAT induction in female mice sensitive to the tumor-inducing effect, but did not influence the level of glucocorticoid induction of this enzyme either in rats or male mice resistant to the hepatocarcinogenic effect of estragole. These results fully coincide with previous data on different carcinogens (DENA, OAT, 3′-MeDAB), which also inhibited glucocorticoid induction of TAT only in animals that develop liver tumors in response to these compounds [19, 21].

Table 1. Effect of estragole on glucocorticoid induction of tyrosine aminotransferase (TAT) and tryptophan oxygenase (TO) in liver of female and male mice and female rats

Experimental conditions	Enzyme activity		
	mice		rats
	female	male	
TAT, µmol *p*-hydroxyphenyl pyruvate/100 mg protein per hour			
Basal level	19 ± 2.3 (6)	26.1 (1)	18.6 ± 0.5 (3)
Glucocorticoid induction	112 ± 5.0 (10)	105 ± 4.1 (4)	68.5 ± 3.2 (8)
Induction + estragole	54 ± 3.3* (10)	100 ± 3.3 (8)	59 ± 6.4 (8)
TO, µmol kynurenine/gram liver per hour			
Basal level	1.8 ± 0.21 (6)	2.7 (1)	1.6 ± 0.12 (3)
Glucocorticoid induction	5.3 ± 0.2 (10)	5.9 ± 0.35 (4)	8.1 ± 0.49 (8)
Induction + estragole	1.2 ± 0.2* (10)	5.8 ± 9.35 (8)	7.5 ± 0.49 (8)

Note: Number of animals is shown in parentheses.

* Significant ($p < 0.001$) decrease in the level of glucocorticoid induction in response to estragole.

Table 2. Effect of OAT on glucocorticoid induction of TAT and TO in the livers of female mice

	TAT, μmol *p*-hydroxyphenyl pyruvate/100 mg protein per hour	TO, μmol kynurenine/gram liver per hour
Basal level	26.6 (1)	2.52 (1)
Glucocorticoid induction	116 ± 4.6 (4)	8.9 ± 0.36 (4)
Induction + OAT	70 ± 8.4* (4)	5.4 ± 0.46* (4)

Note: Number of animals is shown in parentheses.
* Significant ($p < 0.001$) decrease in the level of glucocorticoid induction in response to OAT.

Table 3. Effects of estragole and pentachlorophenol on glucocorticoid induction of TAT and TO in livers of female mice

Group and effect	Enzyme activities in liver	
	TAT, units/100 mg protein per hour	TO, units/gram liver per hour
Basal level[&]	32.4; 23.6	2.0; 2.3
Glucocorticoid induction[#]	102 ± 8.2	6.0 ± 0.68
Induction + estragole[#]	49 ± 4.6*	1.5 ± 0.38*
Induction + pentachlorophenol[#]	92 ± 6.6	5.2 ± 0.28
Induction + pentachlorophenol + estragole[#]	101 ± 3.3	5.4 ± 0.15

[#] Four animals in each group.
[&] Results of independent experiments for two animals.
* Significant ($p < 0.001$) decrease in level of glucocorticoid induction in response to estragole.

Estragole significantly decreased the level of glucocorticoid induction of another liver-specific enzyme (TO) in female mice, in which this compound stimulates development of liver tumors, but not in rats and in male mice (Table 1) for which it is not carcinogenic. Glucocorticoid induction of TO also decreased in response to another hepatocarcinogen, *ortho*-aminoazotoluene (OAT), and also only in animals sensitive to its tumor-inducing effect (Table 2).

However, comparison of effects of estragole and OAT on induction of liver-specific enzymes shows that while the level of TAT induction decreases approximately by half in response to both OAT and estragole, a different situation is observed in the case of TO. Estragole decreases almost five-fold the glucocorticoid induction of this enzyme, whereas it decreases it only by half in response to OAT (Tables 1 and 2).

It is known that carcinogenic effect of estragole requires its metabolic activation, in which sulfotransferase, an enzyme of xenobiotic metabolism system, plays a significant role [22, 23]. In our experiments, the use of the sulfotransferase-specific inhibitor pentachlorophenol completely eliminated the effect of inhibition by estragole of TAT and TO glucocorticoid induction (Table 3). Since introduction of pentachlorophenol into animals prevents the development of estragole-induced liver tumors [23], our results show again that the effect of abrogation of TAT and TO glucocorticoid induction is associated with the subsequent development of liver tumors.

Effect of estragole on DNA-binding activity of transcription factors in liver. We have shown earlier that the decrease in glucocorticoid induction of TAT in response to hepatocarcinogens DENA, OAT, and 3′-MeDAB depends on inhibition of the FOXA family transcription factors, total DNA-binding activity of which is almost halved. The FOXA family transcription factors are involved in differentiation and maintenance of the mature hepatocyte phenotype, and just these functions are deteriorated in cancer cell [10]. Therefore, it can be supposed that inhibition of activity of these proteins as factors of differentiation is responsible for tumor development. Since estragole, like other hepatocarcinogens, decreases the level of glucocorticoid induction of TAT, we supposed

Table 4. Effect of estragole on DNA-binding activity of transcription factors in mouse and rat livers

	FOXA (HNF3)	HNF4	C/EBP	Ets
Female mice	0.55 ± 0.08* (3)	0.20 ± 0.02* (3)	0.96 ± 0.14 (3)	1.00 ± 0.15 (3)
Male mice	1.09 ± 0.04 (3)	1.08 ± 0.20 (3)	1.04 ± 0.07 (3)	0.95 ± 0.12 (3)
Rats	0.95 ± 0.15 (2)	1.00 ± 0.05 (2)	1.00 ± 0.05 (2)	1.05 ± 0.07 (2)

Note: Results of quantitative data processing after autoradiogram densitometry are shown. Typical autoradiograms are shown in Figs. 1 and 2. Data are given in relative units (DNA-binding activity of transcription factors in the liver of control animals, not treated with estragole, is taken as the activity unit). The number of independent experiments is shown in parentheses. Two mice or one rat were used in each experiment.
* Significant ($p < 0.01$) difference from the level of DNA-binding activity in the liver of animals not treated with estragole.

that in this case the activity of FOXA transcription factors should also decrease. In fact, estragole decreases by half the total DNA-binding activity of FOXA proteins in female mice compared to control (Table 4 and Fig. 1). In this case DNA-binding activity of FOXA proteins does not change in animals for which estragole is not carcinogenic (in rats and male mice) (Figs. 1 and 2). Thus, DNA-binding activity of these transcription factors decreases in response to estragole only in animals sensitive to its tumor-inducing effect.

Besides lowering the glucocorticoid inducibility of TAT, estragole also decreased the level of TO induction to a significantly greater extent, five times for TO, compared to lowering by half for TAT. In contrast, OAT equally inhibited (by half) the glucocorticoid inducibility of TAT and TO (Tables 1 and 2). Owing to this, we supposed that

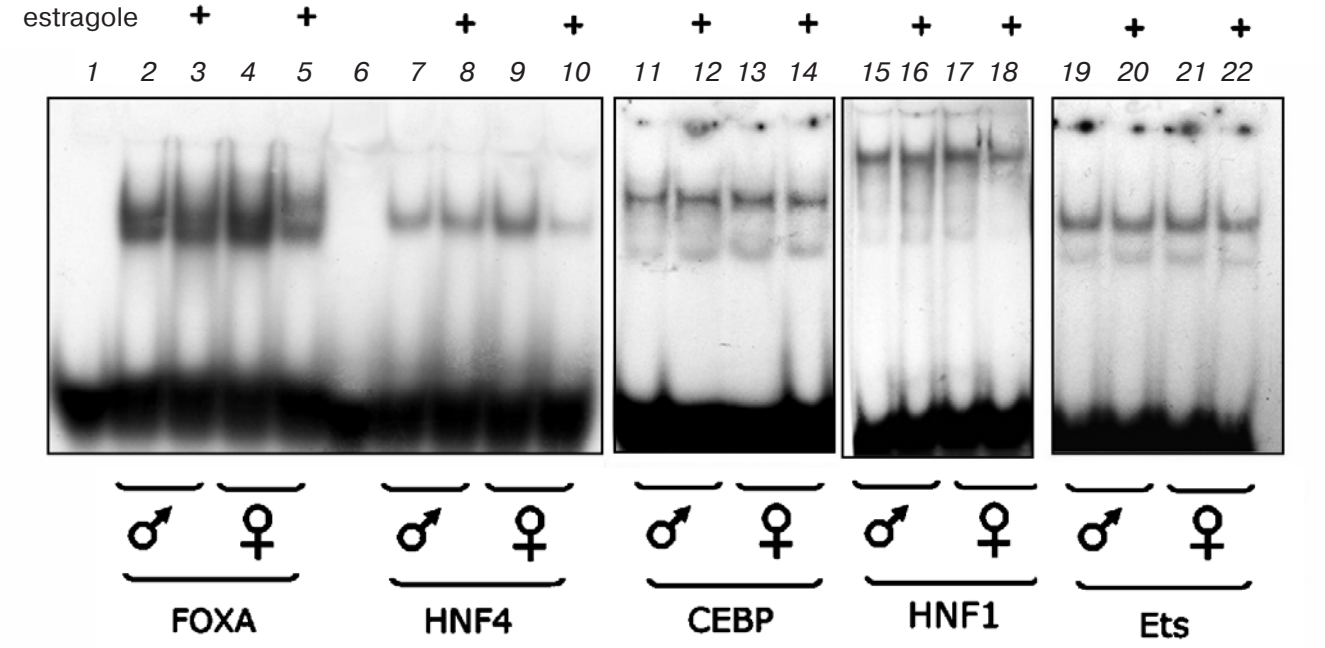

Fig. 1. DNA-binding activity of FOXA, HNF4, HNF1, and C/EBP transcription factors in the liver of male (♂) and female (♀) mice in control and after estragole injection (+). Typical results of one of three independent experiments are shown. Two mice per point were used in each experiment.

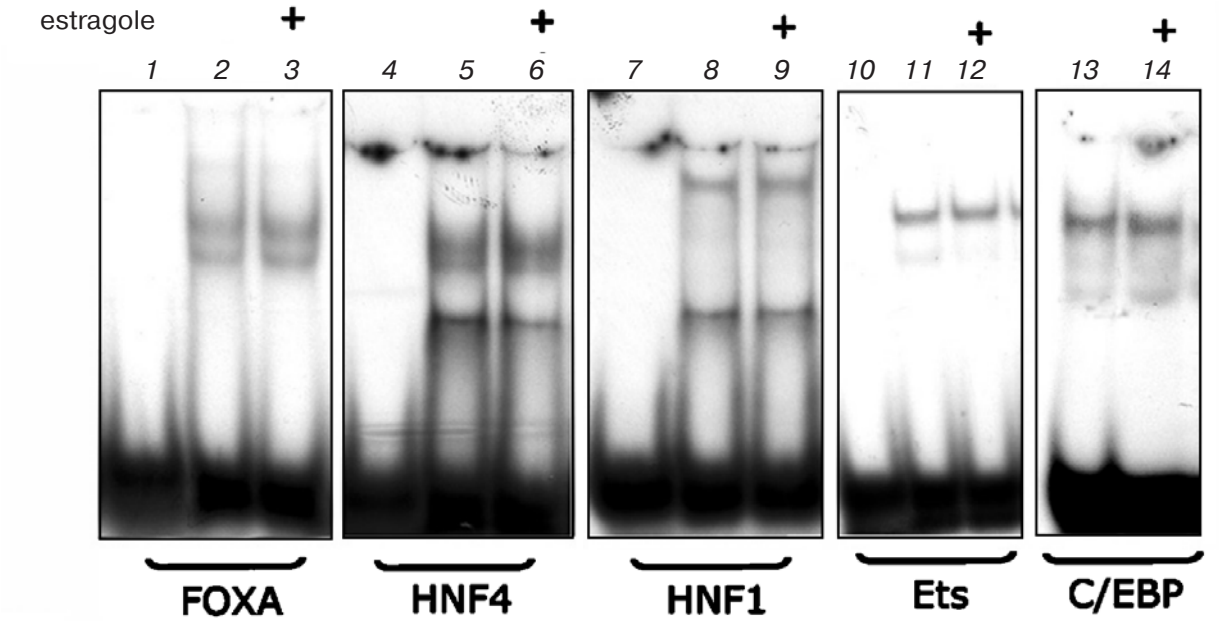

Fig. 2. DNA-binding activity of FOXA, HNF4, HNF1, and C/EBP transcription factors in the liver of female rats in control and after estragole injection (+). Typical results of one of two independent experiments are shown.

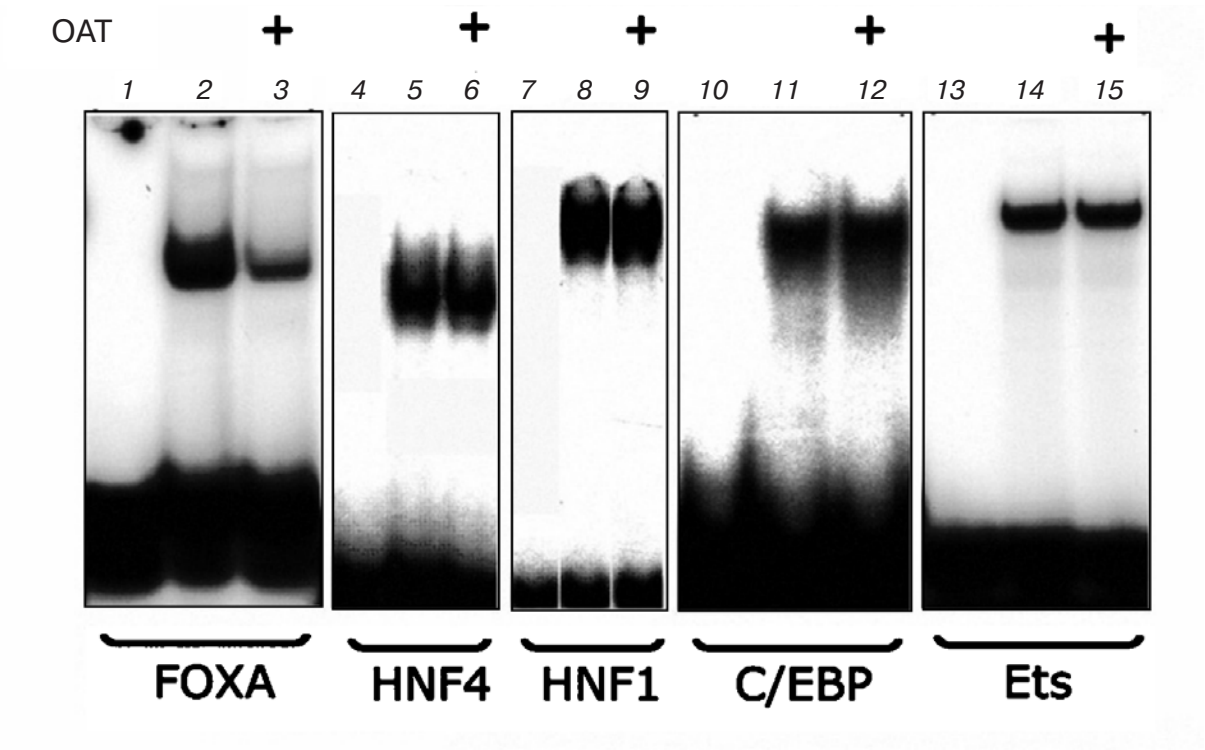

Fig. 3. DNA-binding activity of HNF4, HNF1, and C/EBP transcription factors in mouse liver in control and after OAT injection (+). Typical results of one of three independent experiments are shown. Two mice per point were used in each experiment.

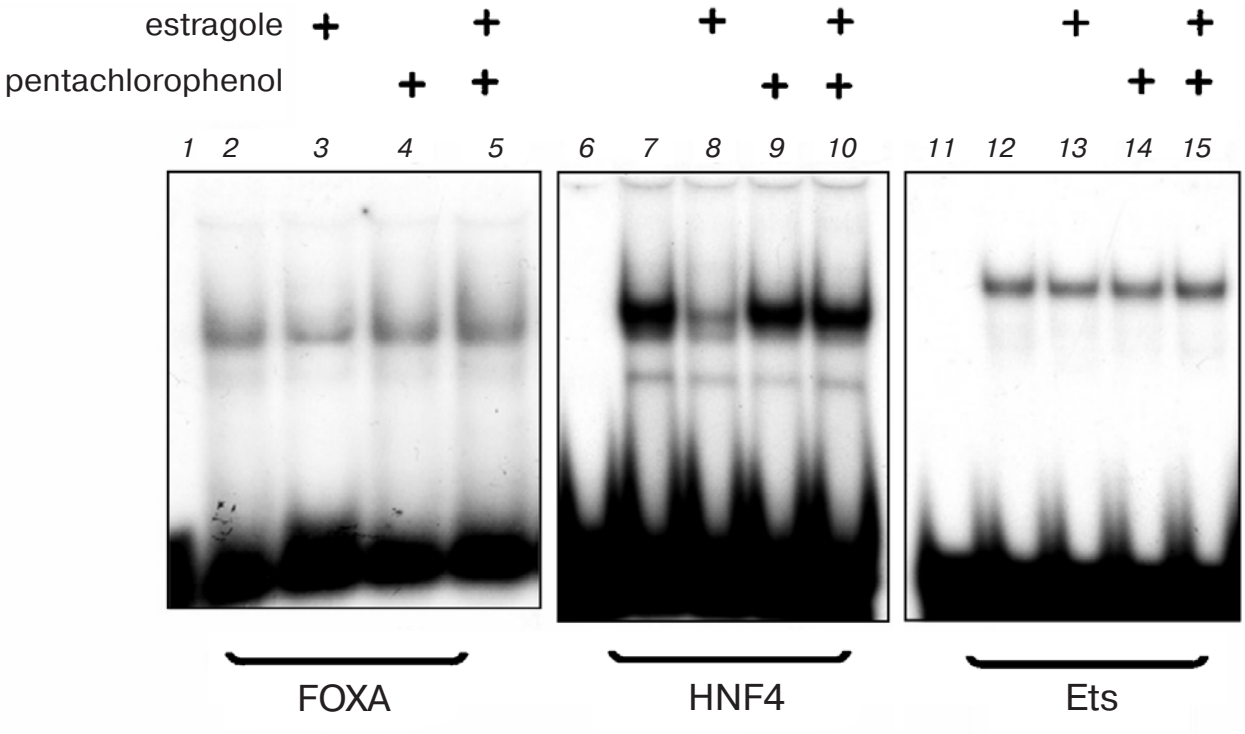

Fig. 4. Effect of pentachlorophenol and estragole on DNA-binding activity of FOXA and HNF4 transcription factors in the liver of female mice. Typical results of one of three independent experiments are shown. Two mice per point were used in each experiment.

in addition to FOXA proteins, other transcription factors sensitive to estragole but not to OAT are involved in regulation of *TO* gene expression by glucocorticoids.

Regulatory regions of the *TO* gene are practically unstudied [24]. However, it is known that glucocorticoid regulation of genes, including TO, whose expression takes place only in liver, is carried out with obligatory participation of transcription factors expressed mainly in this organ (liver-enriched transcription factors): C/EBP, HNF1, FOXA, HNF4, and HNF6 [25]. Owing to this, we compared the effects of estragole and OAT on total DNA-binding activities of transcription factors of the C/EBP, HNF1, and HNF4 families.

It was found that OAT had no effect on DNA-binding activity of these transcription factors (Fig. 3). As positive control, we studied DNA-binding activity of FOXA proteins inhibited by OAT [19], and as negative control we used the DNA-binding activity of Ets protein family that, as shown earlier, is insensitive to hepatocarcinogens [8, 26]. On the contrary, introduction of estragole resulted in significant decrease in DNA-binding activity of HNF4 proteins. Figure 1 shows a typical autoradiogram; quantitative processing of the data of several experiments showed that estragole caused an 80% decrease in activity of HNF4 family transcription factors (Table 4). In this case the decrease in the HNF4 DNA-binding activity was observed only in animals for which estragole was hepatocarcinogenic, i.e. in female mice. In male mice and in rats, for which estragole is not carcinogenic, DNA-binding activity of HNF4 did not change. At the same time, estragole had no effect on activity of C/EBP and HNF1 families of transcription factors. Activity of the Ets protein family also did not change, which makes it possible to use it also as a control in the case of estragole.

The use of pentachlorophenol abrogated the inhibitory effect of estragole on DNA-binding activity of HNF4 and FOXA proteins (Fig. 4). Together with previous data on abrogation by pentachlorophenol of the effect of estragole on TAT and TO glucocorticoid inducibility, this result supports the idea that the decrease in DNA-binding activity of these factors stimulates a decrease in glucocorticoid inducibility of these enzymes.

DISCUSSION

Estragole is a component of essential oils of many spicy plants (dill, basil, sweet bay, etc.) used as food dressings in cookery and as perfume ingredients in production of perfumery, detergents, etc. Just this explains the interest shown to it (as well as to different components of essential oils of above-mentioned plants) from the community and services monitoring the safety of consumer goods. The latter is largely enhanced by reports concerning the carcinogenicity of estragole, safrole, and other alkenyl benzenes for laboratory animals. Carcinogenic potentials of estragole were detected in the laboratory of E. and J. Miller on CD-1 line suckling mice [27] and then on adult female mice of the same line [12]. In the first case, liver tumors were found in 23-39% of animals that survived to the age of 15 months (with 12% in control); in the second case tumors were detected in 56 and 71% of mice that obtained estragole with food for 12 months. The compound 1′-hydroxyestragole, considered as the primary estragole derivative on the pathway of its metabolic activation, did not differ from estragole in the frequency of liver tumor induction and in their number per mouse [12]. In this case, neither 1′-hydroxyestragole nor 1′-hydroxyestragole-2′,3′-oxide, another proximal estragole metabolite, exhibited hepatocarcinogenic activity on rats. Estragole was also not hepatocarcinogenic for adult male mice [12].

The ICR line mice, for which sensitivity to a number of different carcinogens was shown elsewhere, were used in our experiments [20]. In these animals, in the case of a significantly shorter time of treatment (17 weeks against 12 months) and correspondingly lower total estragole dose (by more than one order of magnitude), liver tumors emerged at a higher frequency (80% against 56-71%) and in a higher number per animal (4.8 against 1.8) than in CD-1 mice [12]. Besides liver tumors, lymphomas developed in our experiments in 30% of the mice. The results obtained on CD-1 mice, in which lymphoma frequency exceeded 17%, are also indicative of estragole carcinogenicity for mouse lymphoid tissue [12].

Estragole causes mainly emergence of liver tumors and only in female mice, but not in rats and male mice, so it demonstrates species, tissue, and sex specificity of its carcinogenicity. Aminoazo dyes OAT and 3′-MeDAB exhibit similar specificity. Earlier, in the course of their investigation, a marker of hepatocarcinogenicity, a decrease in TAT glucocorticoid induction was discovered, which was observed only in animals for which this compound was hepatocarcinogenic [19, 21]. The same decrease in glucocorticoid inducibility of TAT appeared to be a specific feature of estragole hepatocarcinogenicity. It decreased TAT inducibility only in female mice in which it caused liver tumors, but not in rats or male mice. Besides, inhibition of glucocorticoid induction of another liver enzyme, tryptophan oxygenase, by hepatocarcinogens OAT and estragole was found. The decrease in TO induction was observed in response to OAT in mice, for which it is hepatocarcinogenic and in response to estragole only in female mice for which it exhibits tumor-inducing effect, but not in rats and male mice. Thus, besides TAT, inhibition of TO glucocorticoid induction can be an additional marker indicative of hepatocarcinogenicity of these compounds.

The TAT and TO encoding genes are expressed only in hepatocytes [28, 29]. Glucocorticoid regulation of such genes requires the presence of transcription factors expressed mainly in liver (liver-enriched transcription factors), whose binding sites are located on DNA near those of glucocorticoid receptors [25]. It was shown in our pre-

vious works that hepatocarcinogenic compounds inhibit activity of FOXA family transcription factors (HNF3 according to the old nomenclature) [6-8, 30], multiple binding sites of which are located within the glucocorticoid-dependent enhancers of *TAT* genes [31], and it is likely that just this causes the decrease in the level of glucocorticoid induction of this gene in response to such compounds. This work deals with the study of effects of estragole and OAT on total DNA-binding activity of proteins of FOXA and some other transcription factor families expressed mainly in liver, like C/EBP proteins whose binding sites are also numerous in the glucocorticoid-dependent enhancers of the *TAT* gene [31], as well as HNF1 and HNF4 proteins. It appeared that OAT decreases DNA-binding activity only of the FOXA family. Introduction of estragole results in lowering of the DNA-binding activity of FOXA and HNF4 and only in animals for which estragole serves as hepatocarcinogen. In this case the FOXA activity decreased by half in response to both compounds, whereas the HNF4 activity decreased five-fold in response to estragole. We have revealed a correlation between changes in enzyme and transcription factor activities. OAT caused equal decrease by half of the level of TAT and TO glucocorticoid induction and only activity of FOXA proteins changed. In response to estragole, the glucocorticoid inducibility of TO decreased much more pronouncedly (five times) and in addition to FOXA activity, and the DNA-binding activity of HNF4 proteins decreased as well. Therefore, it can be supposed that in glucocorticoid regulation of TO, in addition to FOXA proteins, transcription factors of the HNF4 family play a significant role, and just this provides for the high amplitude of the change in the induction of the enzyme.

The role of HNF4 proteins in liver tumor induction by estragole is not clear. However, it is known that transcription factors of the HNF4 family are involved in liver cell differentiation and regulate expression of a number of liver-specific genes [32, 33] whose products are responsible for cell phenotype formation. It can be supposed that the alteration of HNF4 function, like that of the previously detected FOXA proteins [6, 8], results in change in liver cell differentiation and stimulates tumor development.

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REFERENCES

1. Anderson, R. A., Raina, P. N., and Milholland, R. J. (1966) *Oncologia*, **20**, 153-166.
2. Fiala, S., and Fiala, A. E. (1959) *Brit. J. Cancer*, **13**, 236-250.
3. Kensler, T. W., Busby, W. F., Davidson, N. E., and Wogan, G. N. (1976) *Cancer Res.*, **36**, 4647-4651.
4. Horikoshi, N., Tashiro, F., Tanaka, N., and Ueno, Y. (1988) *Cancer Res.*, **48**, 5188-5192.
5. Kaledin, V. I., and Zakharova, N. P. (1984) in *Investigations on Malignant Tumor Induction and Metastasis in Experimental Animals* (Gruntenko, E. V., ed.) [in Russian], Novosibirsk, pp. 146-185.
6. Merkulova, T. I., Kropachev, K. Y., Timofeeva, O. A., Vasiliev, G. V., Levashova, Z. B., Ilnitskaya, S. I., Kobzev, V. F., Pakharukova, M. Y., Bryzgalov, L. O., and Kaledin, V. I. (2005) *Mol. Carcinog.*, **44**, 223-232.
7. Merkulova, T. I., Kaledin, V. I., Kropachev, K. Y., Kobzev, V. F., and Vasiliev, G. V. (1998) *Doklady RAN*, **361**, 700-703.
8. Kropachev, K. Y., Kaledin, V. I., Kobzev, V. F., Plisov, S. Y., and Merkulova, T. I. (2001) *Mol. Carcinog.*, **31**, 10-15.
9. Roux, J., Pictet, P., and Grange, T. (1995) *DNA Cell Biol.*, **14**, 17-20.
10. Schrem, H., Klempnauer, J., and Borlak, J. (2002) *Pharmacol. Rev.*, **54**, 129-158.
11. Nakamura, T., Mura, T., Saito, K., Ohsawa, T., Akiyoshi, H., and Sato, K. (1998) *Biochem. Biophys. Res. Commun.*, **253**, 352-357.
12. Miller, E. C., Swanson, A. B., Phillips, D. H., Fletcher, T. L., Liem, A., and Miller, J. A. (1983) *Cancer Res.*, **43**, 1124-1134.
13. Cereghini, S., Raymondjen, M., Carranca, A., Herbomel, P., and Yaniv, M. (1987) *Cell*, **50**, 627-638.
14. Lai, E., Prezioso, V., Smith, E., Litvin, O., Costa, R., and Darnell, J. (1990) *Genes Dev.*, **4**, 1427-1436.
15. Espinas, M., Roux, J., Ghysdael, J., Pictet, R., and Grange, T. (1994) *Mol. Cell Biol.*, **14**, 4116-4125.
16. Nitch, D., Boshart, M., and Schutz, G. **(**1993) *Proc. Natl. Acad. Sci. USA*, **90**, 5479-5483.
17. Diehl, A. M., and Yang, S. Q. (1994) *Hepatology*, **19**, 447-456.
18. Kaledin, V. I., Klimova, N. V., Vasiliev, G. V., Ivanova, E. A., Vasilieva, E. D., and Ilnitskaya, S. I. (2003) *Byul. Eksp. Biol. Med.*, **136**, 435-437.
19. Merkulova, T. I., Kropachev, K. Y., Timofeeva, O. A., Vasiliev, G. V., Ilnitskaya, S. I., Levashova, Z. B., Kobzev, V. F., and Kaledin, V. I. (2003) *Biochemistry (Moscow)*, **68**, 520-528.
20. Kaledin, V. I., Vasyunina, E. A., Ovchinnikova, L. P., Ronichevskaya, G. M., Zvereva, L. N., and Ilnitskaya, S. I. (2002) *Vestnik VOGIS*, **21/22**, 11-19.
21. Kaledin, V. I., Glazko, T. T., and Zakharova, N. N. (1979) *Doklady RAN*, **244**, 233-237.
22. Boberg, E. W., Miller, E. C., Miller, J. A., Poland, A., and Liem, A. (1983) *Cancer Res.*, **43**, 5163-5173.
23. Bock, K. W., and Schirmer, G. (1987) *Arch. Toxicol. Suppl.*, **10**, 125-135.
24. Kaltschmidt, C., Muller, M., Brem, G., and Renkawitz, R. (1994) *Mech. Dev.*, **45**, 203-210.
25. Schoneveld, O. J., Gaemers, I. C., and Lamers, W. H. (2004) *Biochim. Biophys. Acta*, **1680**, 114-128.
26. Kropachev, K. Y., Pakharukova, M. Yu., Bryzgalov, L. O., Kaledin, V. I., Kobzev, V. F., and Merkulova, T. I. (2004) *Doklady RAN*, **397**, 694-696.
27. Drinkwater, N. R., Miller, E. C., Miller, J. A., and Pitot, H. C. (1976) *J. Nat. Cancer Inst.*, **57**, 1323-1331.
28. Granner, D. K., and Hargrove, J. L. (1983) *Mol. Cell. Biochem.*, **53/54**, 113-128.
29. Schmid, W., Scherer, G., Dabtsch, H., Patric, M., and Schutz, G. (1982) *EMBO J.*, **1**, 1287-1293.
30. Pakharukova, M. Yu., Smetanina, M. A., Kaledin, V. I., Kobzev, V. F., Romanova, I. V., and Merkulova, T. I. (2007) *Byul. Eksp. Biol. Med.*, **144**, 313-316.
31. Grange, T., Roux, J., Rigaud, G., and Pictet, R. (1991) *Nucleic Acids Res.*, **19**, 131-139.
32. Schrem, H., Klempnauer, J., and Borlak, J. (2004) *Pharmacol. Rev.*, **56**, 291-330.
33. Abelev, G. I., and Lazarevich, N. L. (2006) *Adv. Cancer Res.*, **95**, 61-113.