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Decreased CYP3A2 expression and activity in senescent male Wistar rats: Is there a role for HNF4α?

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Abstract

The effect of ageing on CYP3A2, a male specific isoform, was examined in adult (9 months) and senescent (24 months) male rats. A significant decrease (65%) of CYP3A2-related activity (midazolam oxidation) was observed in all senescent rats. Half of these rats still express CYP3A2 suggesting that decreased activities in these rats are due to post-translational modifications. The other senescent male rats did not express CYP3A2 anymore, indicating an impairment of transcription. These transcriptional modifications are due to the previously shown continuous secretion of GH in senescent male rats. GH also regulates HNF4α, a hepatocyte nuclear factor, essential for the basal transcriptional activation of the CYP3A2 gene. In senescent rats, a drastic reduction (76%) of HNF4α protein content and a decrease in DNA binding activity were observed. When these parameters were assessed in male and female rats of the same age (3 months), a higher HNF4α DNA binding activity and a higher HNF4α protein content (38%) were observed in female rats. Our results show that in male senescent rats (1) the decrease of HNF4α is not consistent with the continuous secretion of GH, and (2) the suppression of CYP3A2 expression is not dependent to the HNF4α binding activity.
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1. Introduction

Age-related changes in pharmacokinetics are probably multi-factorial. Indeed, ageing is accompanied by marked changes in the physiology of many organs, as well as in their constituent cells which may, in turn, affect processes such as drug disposition. The elimination of drugs that undergo hepatic oxidative metabolism by the microsomal mono-oxygenases is compromised in the elderly (Sotaniemi et al, 1997; Turnheim, 2004). This age-related decline in drug clearance results in increased plasma concentrations, prolongation of the plasma half-life of these drugs and therefore a higher risk of drug toxicity in elderly patients.

The reasons why hepatic clearance is reduced with age have not been satisfactorily elucidated. Among various factors, it appears that liver size and blood flow decrease with ageing (Kinirions and Crome, 1997; Schmucker, 2001). However, the influence of age in humans on the intrinsic activities of metabolizing drug enzymes is still controversial and particularly complicated by the considerable number of factors (alcohol, drugs, tobacco, diseases, gender, genetic differences, nutritional intake, etc.) which can influence drug metabolism. Studies with animal models allow to limit certain sources of variability.

Cytochrome P450s (CYPs) constitute a superfamily of haem-proteins that play an important role in the detoxification of numerous xenobiotics as well as endogenous compounds such as steroids, fatty acids, prostaglandins, and leukotrienes (Nebert et al., 1996; Gonzalez, 1988;

Abbreviations: BSA, bovine serum albumin; CYP, cytochrome P450; GH, growth hormone; HNF, hepatocyte nuclear factor; EMSA, electromobility shift assay; ARP-1, apolipoprotein AI regulatory protein I.

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Guengerich, 1990). Recent studies demonstrated that some P450 isoforms were suppressed in senescent male rats (Agrawal and Shapiro, 2003; Dhir and Shapiro, 2003), most probably due to a change in growth hormone (GH) secretion profile during ageing. It should be noted that the expression of P450 enzymes is also influenced by gender. Indeed, rat liver contains at least a dozen sex-dependent isoforms of P450. This sexual dimorphism is also mediated by the GH secretion profile (Legraverend et al., 1992; Shapiro et al., 1995; Dhir and Shapiro, 2003). In rats as well as other species, GH is secreted in a sex dependent manner (Legraverend et al., 1992). In the adult male rat, GH is secreted by the pituitary gland in a highly regular, pulsatile manner, with high peaks of hormone (200–300 ng GH/ml plasma) occurring every 3.5 h, separated by periods during which GH levels are undetectable ($\leqslant$2 ng/ml). By contrast, pituitary GH release in the adult female rat is more frequent and results in a near continuous presence of GH in the circulation at concentrations typically ranging from 15 to 40 ng/ml (Jansson et al., 1985). In senescent male rats, three changes have been reported: (1) a decrease of pulse height ($\sim$30%); (2) a shortening of interpulse periods ($\sim$15%); and (3) elevated nadirs by a continuous presence of GH in the circulation in senescent male rats (15 ng/ml in senescent versus 0 ng/ml in adult rats) (Dhir and Shapiro, 2003).

The sex-dependent plasma GH profiles regulate the sexually dimorphic expression of liver CYPs through intracellular signalling networks that are still unclear. Some of these regulatory pathways may include a group of structurally diverse transcription factors termed hepatocyte-enriched nuclear factors (HNFs) (Wiwi and Waxman, 2004).

The aim of this work was to investigate the influence of ageing on CYP3A2, a male specific isoform that is expressed early after birth in males and females but is suppressed after three weeks in females (Ribeiro and Lechner, 1992). Since in senescent male rats the GH secretion profile is characterized by a continuous secretion of GH leading to the loss of typical male free-GH periods becoming closer to the one observed in females (Dhir and Shapiro, 2003), we postulate that senescent rats will lose their CYP3A2. To verify this hypothesis we measured the activity, protein content and mRNA level on CYP3A2 in male Wistar rats at the age of 9 (adult rats) and 24 (senescent rats) months. Since an essential role of HNF4α, and/or HNF4α related nuclear factors, in the regulation of the basal transcriptional activation of the CYP3A2 gene in liver cells has been described (Miyata et al., 1995; Ogino et al., 1999), we focused on HNF4α as potential molecular cell signaling explaining the suppression of CYP3A2 expression during ageing. Therefore, we investigated both HNF4α DNA binding activity and protein content in rats at the age of 9 and 24 months. Indeed, since GH regulates HNF4α (Lahuna et al., 2000), it is tempting to speculate that the age-related change in the GH secretion profile may modify the HNF4α DNA binding activity that could explain the suppression of CYP3A2 expression. Moreover, since CYP3A2 is a male specific isoform, we decided to compare these results with those obtained in both male and female rats of the same age (3 months).

2. Materials and methods

2.1. Animals

Male Wistar rats at the age of 9 and 24 months were purchased from Harlan (The Netherlands). The lifespan of male Wistar rats is 26 months. Male and female Wistar rats at the age of 3 months old were bought from the University Central Animal Breeding Facilities. Animals were housed in individual cages in a temperature- and light-controlled room (12 h dark/light cycle). They received a standard diet (A03 UAR, France) and water *ad libitum*. After one week of acclimation, rats were weighed and killed under pentobarbital anesthesia (60 mg/kg i.p.). The liver was removed. One part was used to prepare liver microsomes and nuclear extracts and the other part was frozen at −80 °C.

2.2. Chemicals

Midazolam and flunitrazepam were purchased from Roche (Grenzach-Wylhen, Germany). 4-Hydroxy-midazolam was provided by Hoffmann-La Roche (Basel, Switzerland). Rabbit polyclonal antibodies against CYP3A2 were from Chemicon (Temecula, USA), rabbit polyclonal anti-HNF4α was from Cell Signaling and goat polyclonal anti-ARP-1 was from Santa Cruz. Primers for RT-PCR were synthesized at the Laboratoire d'Hématologie (Université Catholique de Louvain, Bruxelles, Belgium). Solvents used were of HPLC grade and all other chemicals were of the purest quality available.

2.3. Preparation of liver microsomes

Hepatic microsomal fractions were prepared by differential centrifugation as described previously (Leclercq et al., 1997). The amount of protein was determined by the method of Lowry using BSA as standard (Lowry et al., 1951).

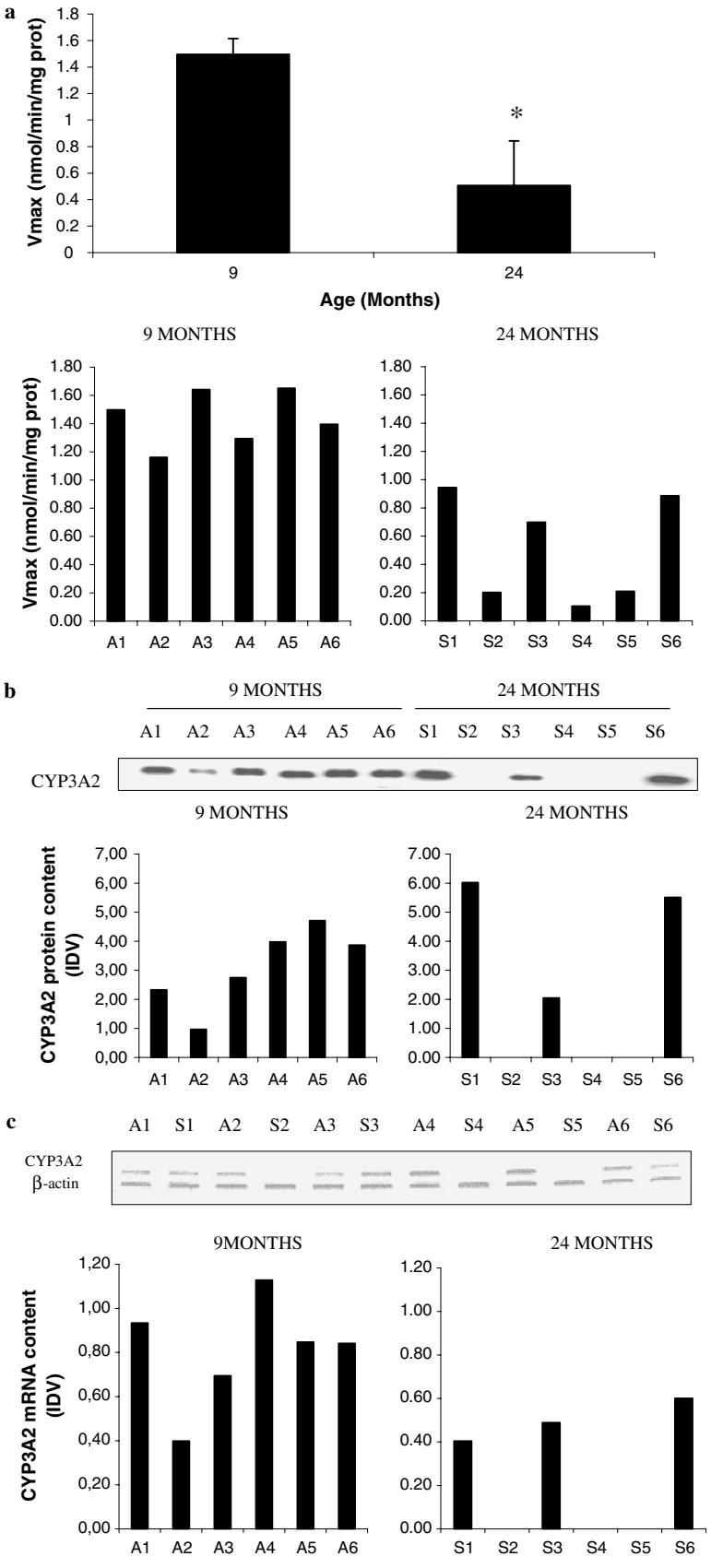
2.4. Liver nuclear extract preparation

Liver nuclear extracts were prepared from 100 mg of tissue using a Nuclear extract kit (Active Motif) according to manufacturer's instructions.

2.5. Midazolam metabolism

The rate of midazolam hydroxylation by rat liver microsomes was quantified by measuring the formation of 4-hydroxy-midazolam (Eeckhoudt et al., 1998). Briefly, 0.6 mg of liver microsomal protein, 0.1 M phosphate buffer pH 7.4, 0.1 M KCl, 1 mM EDTA, 6 mM

$MgCl_2 \cdot 6H_2O$, 0.3 U of isocitric dehydrogenase, 10 mM isocitric acid, were pre-incubated at 37 °C for 5 min with different concentrations of midazolam ranging from 1 to 100 μM. The incubation was started by the addition of a solution of 0.2 mM NADPH, 0.2 mM NADP. After 16 min, the reaction was terminated by 40 μl of NaOH 1 N and placed on



ice. Flunitrazepam (*Grenzach-Wylhen, Germany*) was added as internal standard. Midazolam and its metabolites were extracted with 4.5 ml of cyclohexane–ether (31:69). After mixing and centrifugation for 10 min at 2000*g*, the organic layer was evaporated and conserved at −20 °C. The residue was dissolved in water–acetonitrile (95:5) and injected into the capillary HPLC column (150 mm × 0.8 mm ID, LC Packings).

2.6. Western immunoblotting

The proteins (1.5 μg liver microsomal proteins for CYP3A2 and 24 μg of liver nuclear extracts for HNF4α and ARP-1) were subjected to (SDS)–polyacrylamide gel electrophoresis (12% separating gel) followed by electroblot to a nitrocellulose membrane. The membranes were blocked for 1 h at room temperature in TBS buffer (pH 7.4) containing 5% (w/v) of powdered milk protein. Next, they were probed for 2 h with polyclonal rabbit anti-rat CYP3A2 antibody (1/8000) or rabbit anti-HNF4α (1/1000) or goat anti-ARP-1 (1/500) at 25 °C in a fresh solution of BSA (5% w/v) in TBS–Tween buffer. The membranes were washed and reprobed with goat anti-rabbit antibody coupled to horseradish peroxidase (1/10,000) at room temperature. Immunodetection was performed using the ECL™ detection kit (Amersham, UK). The film was scanned and the density of the bands was calculated using the program Image Master (Pharmacia Biotech Benelux, Roosendaal, The Netherlands). Prior to the measurements of samples, linear conditions between protein concentrations and optical density were established. In addition, when possible, the band corresponding to targeted proteins was checked by using protein standards (rat supersomes). The results are expressed as integrated density values (IDV). Protein loading was assessed by staining the blots with Ponceau S.

2.7. RT-PCR

Total RNA was extracted from approximately 30 mg of rat liver using a SV TOTAL RNA ISOLATION kit (Promega) according to manufacturer's instructions. The cDNA was synthesized in a total volume of 20 μl of reaction mixture containing 1 μg of RNA in the RT reaction buffer (Invitrogen), 10 nmol/l dithiothreitol, 25 pg of oligo(dT) primer, 0.5 mmol/l each deoxyribonucleoside triphosphate (dNTP), 200 units of superscript II Rnase H^- Reverse Transcriptase (Invitrogen). Reaction mixtures were incubated for 1 cycle at 42 °C for 2 min, 42 °C for 50 min and were terminated by heating at 70 °C for 15 min and then chilled to 4 °C. PCR mixture consisted of PCR buffer (200 mM Tris–HCl, 500 mM KCl, pH 8.4), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 μM of specific primers for CYP3A2 and β-actin (CYP3A2: forward primer 5′-AGTAGTGACGATTCCAACATAT-3′ and reverse primer 5′-TCAGAGGTATCTGTGTTT-3′; β-actin: forward primer 5′-CTGACCGAGCGTGGCTACAG-3′ and reverse primer 5′-GGTGCTAGGAGCCAGGGCAG-3′), 2 μl of cDNA, and 1 unit of *Taq* DNA polymerase (Invitrogen). PCR mixtures were incubated at 94 °C for 3 min followed by the respective number of cycles (22 for CYP3A2 and 23 cycles for β-actin) at 94 °C for 45 s, then for 30 s at their corresponding annealing temperatures (54 °C for CYP3A2 and 62 °C for β-actin), and 72 °C for 1 min 30 s. Afterwards, the extension step was followed for 10 min at 72 °C. The levels of mRNA were expressed as arbitrary units obtained by densitometry using the program Image Master (Pharmacia Biotech Benelux, Roosendaal, The Netherlands) after normalization to β-actin mRNA levels.

2.8. EMSA

The following double-stranded oligonucleotides synthesized by Eurogentec were used as probe of HNF4α: APF1, 5′-GAG-TAC-CAA-AGT-CCA-GGT-GA-3′ which codes the HNF4α DNA binding site of apolipoprotein CIII gene (15). They were labeled with [γ^{32}P]-ATP (Amersham Pharmacia Biotech) by T4 polynucleotide kinase (Fermentas) and purified by ChomaSpin columns (BD Biosciences). Binding reactions involved mixing 10 fmols of probes (5×10^4 cpm), 3 μg of poly(dI–dC) (Amersham Pharmacia Biotech) with 3 μg rat liver nuclear extracts in binding buffer (12.5 mM Tris–HCl (pH 7.5), 5% glycerol, 1.25 mM $MgCl_2$, 625 μM EDTA and 625 μM DTT) adjusted to 15 μl. After 45 min incubation at room temperature, the DNA–protein complexes were separated at room temperature on a pre-electrophoresed 6% polyacrylamide gel in 0.25× TBE buffer. DNA-bound factors were identified with immune serum or unlabeled probe for competitions (100-fold molar excess) added to liver nuclear extracts, on ice, 15 min prior to addition of the labeled probe.

Fig. 1. Effect of ageing on 4-hydroxy-midazolam formation (a), on the amount of CYP3A2 proteins (b) and CYP3A2 mRNA levels (c). (a) Midazolam oxidation was quantified by recording (HPLC) the formation of 4-OH-midazolam after 16 min incubation of rat liver microsomes at 37 °C. Upper panel represents the mean value of $V_{max} \pm$ SEM ($n = 6$) of rats at the age of 9 and 24 months. Lower panel represents individual values expressed as V_{max} (nmol metabolite produced/min/mg protein) for each adult rat at the age of 9 (A) and 24 (S) months. *$p < 0.05$ as compared to animals at the age of 9 months. (b) The content of CYP3A2 protein was assessed by immunoblotting. Western Blot showing CYP3A2 detection by rabbit polyclonal anti-CYP3A2 antibody of each adult (A) and senescent (S) rat. The amount of protein loading in each lane was assessed by staining the blots with Ponceau S (data not shown). Lower panel represents the individual integrated density values (IDV) of CYP3A2 content in the blots. (c) The levels of CYP3A2 mRNA were evaluated by semi-quantitative RT-PCR. RT-PCR analysis of CYP3A2 prepared from total RNA isolated from each adult (A) and senescent (S) rat. Lower panel represents the CYP3A2 mRNA levels that were measured by densitometry and normalized to β-actin mRNA expression level. Results were expressed as integrated density values (IDV) of CYP3A2 mRNA level for each rat.

2.9. Analysis of kinetic data

Kinetic data (V_{max} and K_m) were determined by the non-linear regression program WinNonLin (SCI, Software, Lexington, KY, USA) assuming single enzyme Michaelis–Menten kinetics.

2.10. Statistical analysis

Each group was composed of six rats. The results presented in the tables correspond to the mean ± SEM. Data were analyzed using *t*-test to determine the statistical significance among the two groups. The level of significance was set at $p < 0.05$.

3. Results

3.1. Metabolic studies

In order to evaluate the influence of ageing on CYP3A2 activities, we measured the rate of formation of the 4-hydroxy-midazolam, a reaction mainly catalyzed by the CYP3A2 (Kobayashi et al., 2002). Fig. 1a shows that ageing is influencing the CYP3A2 activity. The rate of formation of 4-OH is decreased by 65% (1.44 ± 0.16 to 0.51 ± 0.33 nmol/min/mg protein, $p < 0.05$) in senescent male rats. It must be noted that K_m was not modified in rats from 9 to 24 months (data not shown).

By looking at individual values of 4-hydroxy-midazolam formation, 2 populations of senescent rats can be identified. The rats belonging to the first one (S1, S3 and S6) lost by more than 45% their enzymatic activity. This decreased activity cannot be related to a decrease in protein content since these rats always expressed CYP3A2 at the same or even higher extent to adult rats, as shown by their protein amounts (Fig. 1b) and mRNA levels (Fig. 1c). This suggests post-translational modifications of CYP3A2 proteins during ageing. On the other hand, the second group of rats (S2, S4, and S5) lost more than 90% of their enzyme activities. In these rats, the loss of CYP3A2 protein content (Fig. 1b) is well correlated to the transcription level of CYP3A2 mRNA (Fig. 1c), suggesting that, in this case, the loss of enzyme activity is due to transcriptional modifications.

Since the inter-animal variability was determined to be low for enzyme activity, protein and mRNA levels in rats at the age of 3 months (data not shown), the differences observed in senescent rats are not due to this variability but related to ageing.

3.2. HNF4α DNA binding activity is modulated by age

To determine whether ageing affected the level of HNF4α DNA-binding activity, EMSA was performed using the APF1 oligonucleotide, which corresponds to the HNF4α binding site of apoCIII gene promoter. Both HNF4α and the apolipoprotein AI regulatory protein I (ARP-1 or COUP-TFII) have been shown previously to bind this oligonucleotide (Sladek et al., 1990). Two bands were detected in the EMSAs with rat liver nuclear protein extracts. The upper band corresponds to HNF4α, and the lower corresponded to ARP-1, as determined by supershift analysis with antibodies specific for HNF4α and ARP-1 (Fig. 2a). Although supershift with nuclear extract (Fig. 2a) from adult rats is very light (lane 5), the supershift is much more intense in senescent rats (lane 8). Moreover, it should be noted that the level of ARP-1 protein content was shown to remain constant from 9 to 24 months old (Fig. 2b).

EMSA analysis of nuclear extracts from adult and senescent rats revealed a difference in the mobility of the protein:DNA complex (Fig. 3a). In senescent rats, this difference corresponds to a decrease of intensity of the upper band and an increase of the lower band. It can be concluded that HNF4α DNA binding activity significantly decreased whereas ARP-1 DNA binding activity is significantly increased in senescent male rats. To check whether this decrease of DNA binding activity may be attributed to changes in HNF4α proteins, we measured them by immunoblotting. HNF4α protein was significantly decreased by 76% in senescent rats as compared to adult rats (Fig. 3b). As a consequence, the decrease of HNF4α DNA binding activity is most probably due to the decrease in HNF4α protein content and may explain the switch between HNF4α and ARP-1 for APF1.

3.3. HNF4α DNA binding activity is modulated by sex

In senescent male rats there is a change in the GH secretion profile (Dhir and Shapiro, 2003). To verify whether the decrease of HNF4α expression is due to the continuous secretion of GH in senescent male rats, we investigated its DNA binding activity in female rats. When APF1 oligonucleotide was incubated with liver nuclear extracts from male and female rats of the same age (3 months), a higher DNA binding activity was observed in females as compared to males (Fig. 4a). To see if this higher activity of binding is due to a change in the amount HNF4α proteins, we measured them by Western blot. Results show that the amount of HNF4α protein is 38% higher in female as compared to male rats (Fig. 4b, 1.90 ± 0.21 arbitrary units for male versus 2.65 ± 0.47 arbitrary units for female rats, $p < 0.05$). Therefore, the increased signal intensity observed in female nuclear extracts was clearly due to an increase in protein concentration.

4. Discussion

To examine the effect of age on CYP3A2 activity, protein and mRNA content, we compared adult and senescent rats. We observed a decrease of CYP3A2-related activity in all senescent male rats. This age-related decrease of midazolam oxidation can be explained in two different ways: by post-translational modifications that diminish or inactive the enzyme efficiency, and by transcriptional modifications

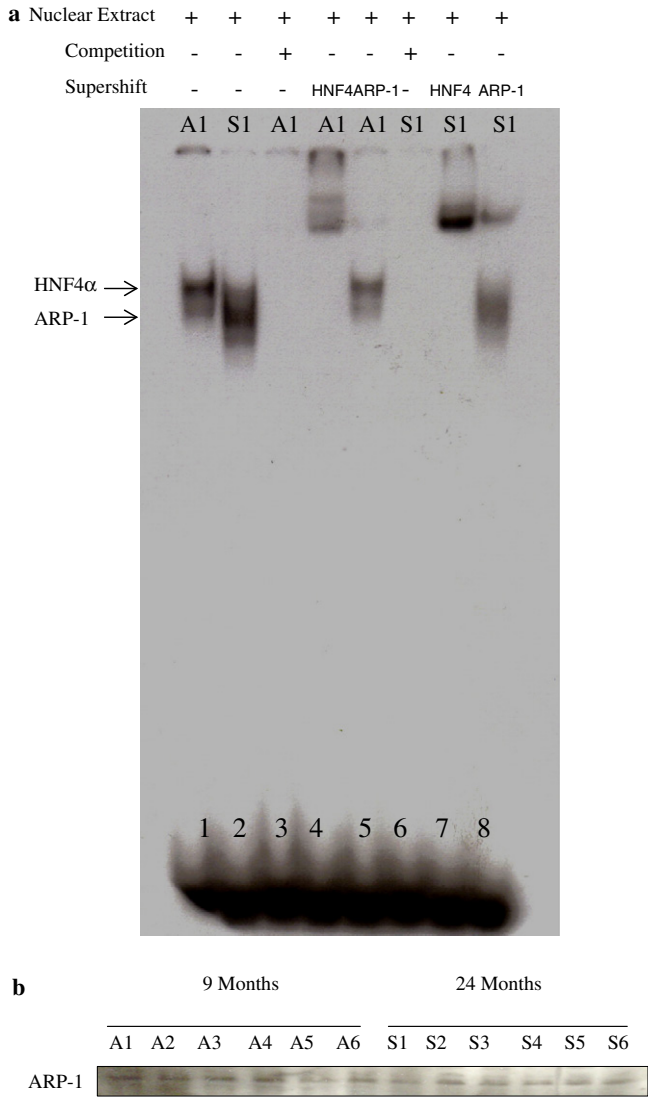


Fig. 2. Supershifts on HNF4α DNA binding activity and ARP-1 protein content in liver of rats at the age of 9 and 24 months. (a) EMSA were performed using APF1 and 9 μg of liver nuclear extract from a adult rat (A1) (lanes 1, 3, 4 and 5) and a senescent rat (S1) (lanes 2, 6, 7 and 8). A 100-fold molar excess of unlabeled APF1 oligonucleotide was used as a specific competitor (lanes 3 and 6). Supershifts were performed using antisera HNF4α (lanes 4 and 7) or ARP-1 (lanes 5 and 8). (b) The protein content of ARP-1 was assessed by immunoblotting. Western blot showing ARP-1 protein detection by a goat polyclonal anti-ARP-1 antibody of liver nuclear extracts prepared from livers of individual animals at the age of 9 (adults, A) and 24 months (senescent, S).

that suppress the CYP3A2 expression leading to a decrease of the enzyme activity. Such an increased variance in old animals and appearance of some major changes in some but not all animals of the same age has already been reported (Schmucker, 2001). Indeed, this decrease is associated to a reduced amount of CYP3A2 protein in some senescent rats whereas in the other rats the protein content does not decrease. This last case suggests that, in these senescent male rats, CYP3A2 proteins are expressed at the same or even a higher level as compared to adult rats but they are not entirely functional. We have observed a similar situation, that is decreased enzyme activity without changes in the amount of protein, for another CYP isoform, i.e. the CYP2E1 (Wauthier et al., 2004a) and also for a phase II enzyme, sulfotransferase SULT1A1 (Wauthier et al., 2004b). These results suggest that CYP3A2 proteins undergo post-translational modifications during ageing. The nature of such modifications remains, however, to be investigated.

On the other hand, we observed that in the other half of senescent rats, the decrease of CYP3A activities is well correlated with a drastic reduction of CYP3A2 expression. In this case, the decrease of CYP3A activities is due to transcriptional modifications. These results are in agreement with previous studies (Imaoka et al., 1991; Agrawal and Shapiro, 2003; Dhir and Shapiro, 2003) showing that P450 isoforms in liver of senescent male rats are both demasculinized (suppression of male-dependent P450 enzymes expression), and feminized (induction of female-dependent P450 enzymes expression). These processes (demasculinization and feminization) are due to a change in the male GH profile in senescent male rats and more specifically to the loss of the GH-devoid interpulse leading to a continuous secretion of growth hormone like in female rats (Dhir and Shapiro, 2003).

To investigate cellular mechanisms involved in the suppression of CYP3A2 expression, we focused on HNF4α, a homodimeric orphan nuclear receptor. Indeed, in addition regulating CYP3A2, HNF4α has been reported to play a central role in regulating sex-dependent mouse liver Cyp gene expression in the liver of HNF4α-deficient mice (Wiwi et al., 2004). Moreover, since HNF4α is regulated by GH (Lahuna et al., 2000), a change in the secretion profile of GH may influence its DNA binding activity and, therefore explain the suppression of CYP3A2 expression. Accordingly, EMSA analysis with liver nuclear extracts from rats at the age of 9 and 24 months revealed an important reduced binding activity of HNF4α, which was correlated to a drastic reduction (76%) in HNF4α protein content in all senescent animals. This reduction of protein content is in accord with a recent study that reported a decrease of 74% in HNF4α protein content in the liver of male Sprague–Dawley rats at the age of 18 months compared to rats at the age of 3 months (Sanguino et al., 2005). Nevertheless, it is difficult to explain the suppression of the CYP3A2 expression in the senescent rats only by the decrease of HNF4α DNA binding affinity. Indeed, the reduced HNF4α protein content and DNA binding affinity is observed in all senescent rats but the suppression of CYP3A2 expression occurs only in half of these rats.

In addition, to verify this interpretation, we further analysed the HNF4α protein levels and DNA binding activity in females and males of the same age (3 months). Indeed, the suppression of CYP3A2 expression in both female (3 months) and male senescent (24 months) rats is due to a continuous secretion of GH (Dhir and Shapiro, 2003).

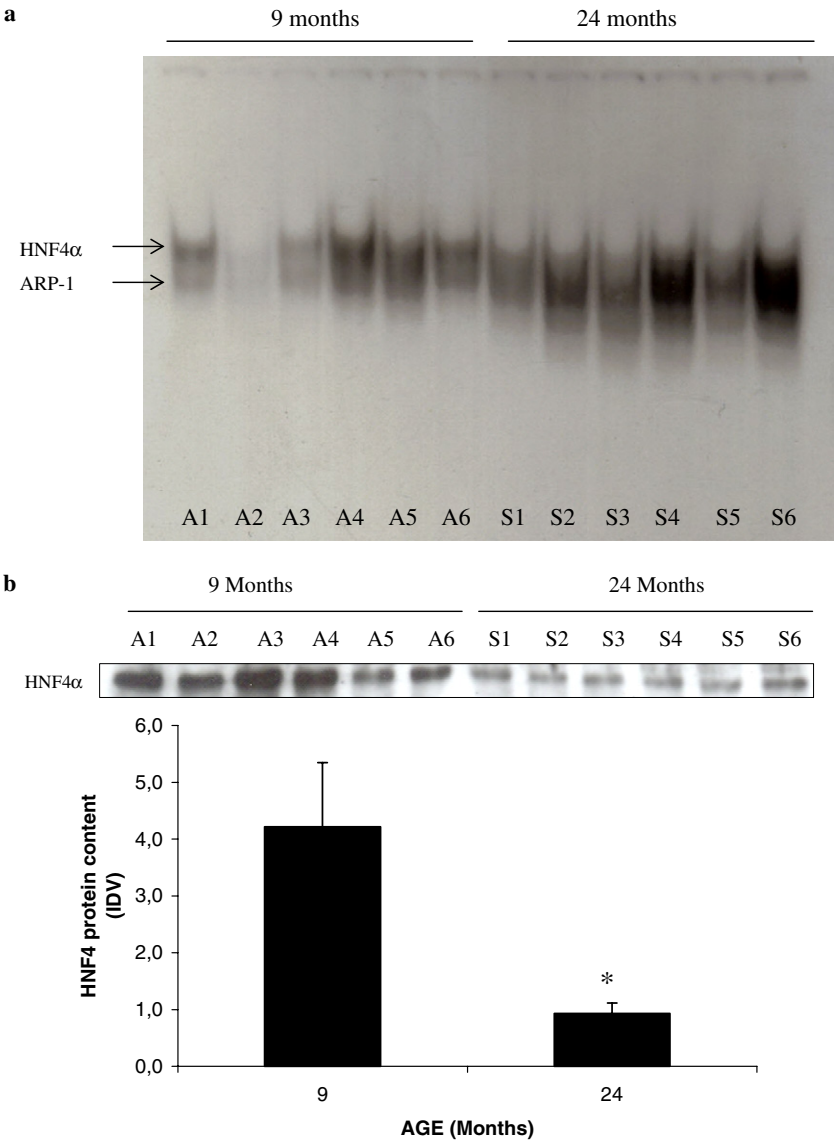


Fig. 3. Influence and age on HNF4α on DNA binding activity and protein content in the liver of rats at the age of 9 and 24 months. (a) EMSA analysis of HNF4α was performed using APF1 probe and 6 μg of liver nuclear extracts from livers of individual adult (A) and senescent (S) rats. (b) The content of HNF4α protein was assessed by immunoblotting. Representative Western blot showing HNF4α detection by rabbit polyclonal anti-HNF4α antibody in the nuclear extracts prepared from rats at the age of 9 (adults, A) and 24 (senescent, S) months. The amount of protein loading in each lane was assessed by staining the blots with Ponceau S (data not shown). Lower panel: HNF-4 contents in the blots are expressed as integrated density values (IDV) after densitometry of the respective electrophoretic bands. Results represent the mean ± SEM ($n = 6$). *$p < 0.05$ as compared to animals at the age of 9 months.

Therefore, if the decrease of CYP3A2 expression in senescent male rats is due to a lower HNF4α DNA binding resulting from a continuous GH secretion, we should also observe a lower HNF4α DNA binding activity in female as compared to male rats. However, our results revealed a significant increase in the binding of HNF4α to its DNA target in female compared to male rats. This was confirmed by the protein content that is more pronounced in females compared to males. Regarding this lack of correlation between HNF4α and CYP3A2 expression in females and in half of senescent rats (rats in which CYP3A2 is still maintained), we suggest that HNF4α is unlikely to play a unique and direct role in the suppression of CYP3A2 expression in senescent male rats. Since CYP3A2 is expressed at high levels in the hypophysectomized rats, disappears when GH is secreted constantly and is partially suppressed under influence of episodic GH (Waxman et al., 1995), GH must have an inhibitory role. As a consequence, HNF4α can be involved in the positive expression of CYP3A2 but probably another GH-related factor plays an inhibitory role on the transactivation of CYP3A2 and thus suppresses its expression.

Further studies are required to elucidate the nature of a GH-related factor that is either lacking in senescent rats or is able to suppress the expression of CYP3A2 in response to a constant GH secretion.

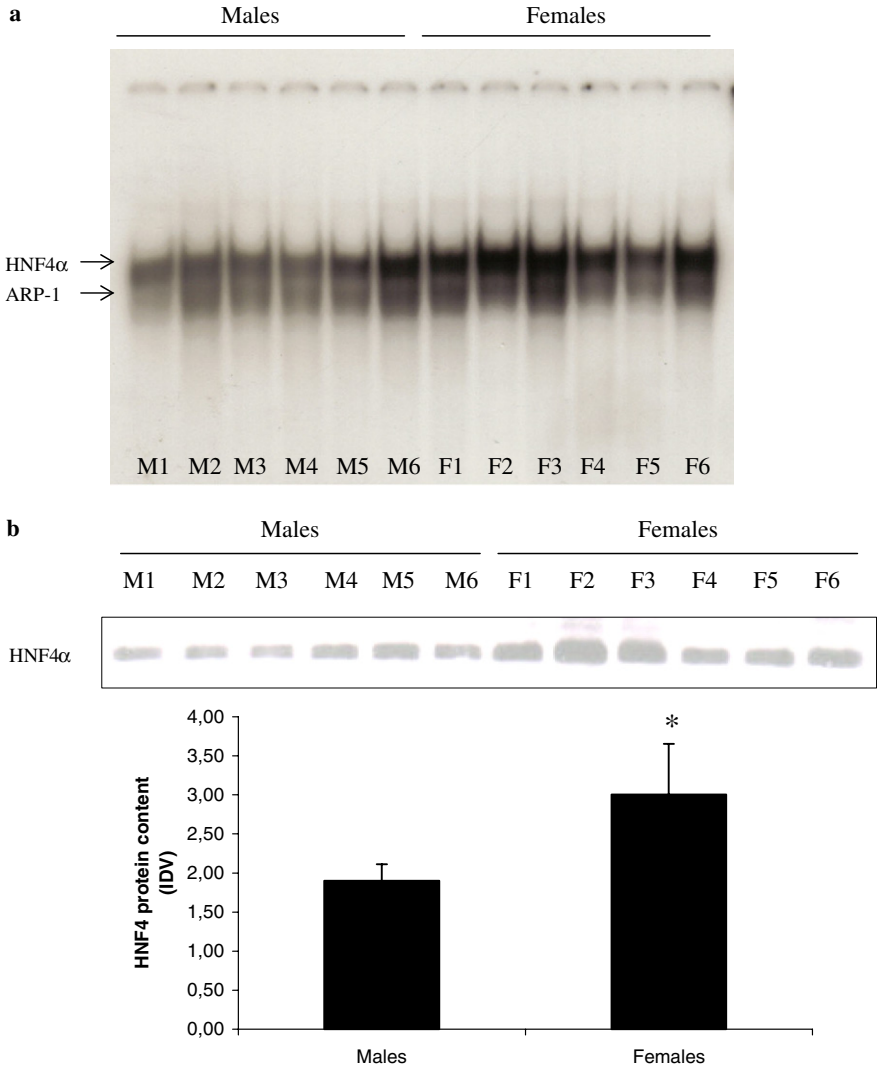


Fig. 4. Influence of sex on HNF4α DNA binding activity and protein content in rat liver of male (M) and female (F). (a) EMSA analysis of HNF4α was performed using APF1 probe and 6 μg of liver nuclear extracts from six individual male (M) and six individual female rats (F). (b) The content of HNF4α protein was assessed by immunoblotting. Representative Western blot showing HNF4α detection by rabbit polyclonal anti-HNF4α antibody in the nuclear extracts prepared from the livers of individual animals. The amount of protein loading in each lane was assessed by staining the blots with Ponceau S (data not shown). Lower panel: HNF4α contents in the blots are expressed as integrated density values (IDV) after densitometry of the respective electrophoretic bands. Results represent the mean ± SEM ($n = 6$). *$p < 0.05$ as compared to males.

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