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Stearoyl coenzyme A desaturase 1 expression and activity are increased in the liver during iron overload

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Abstract

In humans, hepatic iron overload can lead to hepatocellular carcinoma development. Iron related dysregulation of hepatic genes could play a role in this phenomenon. We previously found that the carbonyl-iron overloaded mouse was a useful model to study the mechanisms involved in the development of hepatic lesions related to iron excess. The aim of the present study was to identify hepatic genes overexpressed in conditions of iron overload by using this model. A suppressive subtractive hybridization was performed between hepatic mRNAs extracted from control and 3% carbonyl-iron overloaded mice during 8 months. This methodology allowed us to identify stearoyl coenzyme A desaturase 1 (SCD1) mRNA overexpression in the liver of iron loaded mice. The corresponding enzymatic activity was also found to be significantly increased. In addition, we demonstrated that both SCD1 mRNA expression and activity were increased in another iron overload model in mice obtained by a single iron-dextran subcutaneous injection. Moreover, we found, in both models, that SCD1 mRNA was not only influenced by the quantity of iron in the liver but also by the duration of iron overload since SCD1 mRNA upregulation was not detected in earlier stages of iron overload. In addition, we found that cellular repartition likely influenced SCD1 mRNA expression. In conclusion, we demonstrated that iron excess in the liver induced both the expression of SCD1 mRNA and its corresponding enzymatic activity. The level and duration of iron overload, as well as cellular repartition of iron excess in the liver likely play a role in this induction. The fact that the expression and activity of SCD1, an enzyme adding a double bound into saturated fatty acids, are induced in two models of iron overload in mice leads to the conclusion that iron excess in the liver may enhance the biosynthesis of unsaturated fatty acids.

Keywords: Iron overload; Gene expression; Liver; Stearoyl coenzyme A desaturase 1; Mouse

1. Introduction

Iron is crucial for cellular life. However, its excess can induce organ damage. The liver, which is the main iron storage site in the organism, is a specific target. During iron overload diseases, liver fibrosis, cirrhosis, and hepatocellular carcinoma are frequently observed [1–5]. In addition, many authors suggest that during alcoholic liver diseases and

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chronic viral hepatitis, liver iron excess may exacerbate hepatic damage [6–9].

Molecular mechanisms involved in the development of hepatic lesions in conditions of liver iron excess are not well characterized. On the one hand, it has been reported that high amounts of hepatic iron increase the production of free radical hydroxyls thereby inducing lipid and DNA peroxidation [10–13]. On the other hand, it must also be considered that expression of some hepatic genes may be influenced by liver iron overload, thereby leading to the disturbance of biological or metabolic pathways. Until now, only few mRNAs have been identified as modulated by iron excess in the liver. It is well known that the transferrin receptor mRNA steady-state level is decreased during iron overload in the liver due to the presence of an iron responsive element in its 3′ untranslated region [14–16]. In addition, in vivo and in vitro studies performed in experimental models have reported an increase of transforming growth factor β and procollagen I mRNAs, both involved in the development of fibrosis, under the effect of iron overload [17–19]. In in vivo models of iron overload, it has been demonstrated that the expression of γ-glutamyl transpeptidase [20] and heme oxygenase 1 [21], two proteins potentially involved in oxidative stress, are also upregulated in the liver. Fimmel et al. [22] also reported an increase in the amount of complement C4 mRNA in hepatic stellate cells isolated from the liver of carbonyl-iron overloaded rats as compared to those isolated from control rats.

Taking these data together, it appears that identification of hepatic genes modulated by iron excess is crucial because they may participate in the development of hepatic lesions related to iron excess in humans.

With this aim, we performed suppressive subtractive hybridization (SSH) between livers from iron overloaded and control mice and studied hepatic genes which were induced during iron overload. This recently developed methodology allows the analysis of differential gene expression in two given conditions [23]. Using this technology on the carbonyl-iron overloaded mouse model, we highlighted the upregulation of stearoyl coenzyme A desaturase 1 (SCD1) mRNA, which encodes an enzyme involved in lipid metabolism. This overexpression was confirmed in another model of iron overload in mice using iron-dextran. In both models of iron overload, we also found that the increase of SCD1 mRNA expression was accompanied by an enhancement of SCD1 activity.

2. Materials and methods

2.1. Animals

In this study, 5 week old BALB/cJ male mice were used (CERJ, Le Genest St Isle, France). They were maintained under standard conditions of light, atmosphere, and temperature according to French law and regulations. They had free access to tap water and standard AO3 diet (UAR, France). The composition of the diet was 21, 5.5, and 51% of proteins, lipids, and glucids respectively and the iron content was 280 mg/kg of diet. The animals were sacrificed by cervical dislocation. Livers were removed immediately after sacrifice and conserved as necessary for the different investigations. Iron overload was performed using carbonyl-iron or iron-dextran.

2.1.1. Carbonyl-iron overload

In a first set of experiments, mice were iron overloaded for 8 months by addition of 0.5, 1.5, or 3% carbonyl-iron to the diet. Animals receiving no carbonyl-iron in the diet were used as controls. We have previously characterized this model [24]. Liver iron concentrations (LICs) in 3, 1.5, and 0.5% carbonyl-iron supplemented mice were respectively on average ten, four, and two times as high as the control value [24].

In another similar experiment, 3% carbonyl-iron was added to the diet for 2 or 8 months to confirm the results and to study the relationship between duration of iron overload and level of mRNA overexpression.

2.1.2. Iron-dextran overload

A single subcutaneous injection of iron-dextran (Sigma, France) was performed at a dose of 1 g/kg body weight on 5 week old mice as previously described by Carthew et al. [25]. Control mice received a single injection of a mixture containing dextran

and phenol at the same concentration as in the iron-dextran solution. Animals were sacrificed 4 months later.

To study the effect of iron overload duration on SCD1 mRNA expression, animals were sacrificed 1 week, 3 weeks, or 2 months after iron-dextran injection. Three animals were included in each group at each point.

2.2. Histological study

For histological studies, livers were fixed in 4% formalin, and paraffin embedded thereafter. Slices of liver (5 μm) were stained using Perls' staining for iron assessment. Hepatic lobular and cellular localization of iron deposits has been reported for each case on Perls' stain using the total iron score (TIS) [26,27]. TIS is the sum of HIS (hepatocytic iron score), SIS (sinusoidal iron score), and PIS (portal iron score). HIS reflects the quantity of iron deposited in hepatocytes, whereas SIS reflects the quantity of iron deposited in sinusoidal cells. Finally, PIS reflects the presence of iron in the portal space, including extracellular space, biliary canals, and endothelial cells.

2.3. Hepatic iron concentration

LIC was evaluated biochemically from livers previously fixed in 4% formalin according to Barry and Sherlock's method [28].

2.4. Total RNAs and poly(A)$^+$ RNA extraction

Total RNAs were extracted from each liver specimen previously frozen in liquid nitrogen using the Promega kit SV total RNA isolation system (Madison, WI, USA). To perform the SSH, total RNAs were pooled for each group of mice and poly(A)$^+$ RNAs were generated (Invitrogen's FastTract kit).

2.5. Suppressive subtractive hybridization

SSH was performed using the PCR-Select cDNA Subtraction kit (Clontech, Palo Alto, CA, USA) according to the manufacturer's instructions. Briefly, double strand cDNAs were prepared from 2 μg of poly(A)$^+$ RNAs from livers of control mice ($n=4$) (driver population) and 3% carbonyl-iron overloaded mice ($n=4$) (tester population). The cDNAs were then digested with *Rsa*I. Tester cDNA was ligated to adapter 1 and to adapter 2 in two separate ligations. In the first hybridization, excess of driver cDNA was firstly hybridized for 8 h at 68°C with tester cDNA ligated to adapter 1 in reaction 1 and with cDNA ligated to adapter 2 in reaction 2. Secondly, reactions 1 and 2 were hybridized together in the presence of fresh driver cDNA. This second hybridization was performed overnight at 68°C. The subtractive product was amplified by PCR using oligonucleotides which were complementary to adapters 1 and 2 respectively. PCR was performed according to the following parameters: 75°C for 5 min; 27 cycles at (94°C for 30 s; 66°C for 30 s; 72°C for 1.5 min). Then, a nested PCR was performed as follows: 12 cycles at (94°C for 30 s; 68°C for 30 s; 72°C for 1.5 min). The final PCR product was named iron induced cDNAs and corresponded to the gene population overexpressed in the liver by iron excess. In the same manner, we performed the reverse subtractive hybridization in order to obtain genes repressed in the liver by iron excess. This final product was named iron repressed cDNA.

2.6. Cloning and selection of iron induced cDNAs

Iron induced cDNAs obtained from the nested PCR were cloned into a T/A pCR 2.1 plasmid (The Ligator, R&D Systems, Wiesbaden-Nordenstadt, Germany). Transformation efficiency was approximately 10^7 colonies per μg of starting DNA. Five thousand clones were plated onto LB selection medium plates. Replicas were performed on nitrocellulose filters and prepared for hybridization. Iron induced cDNAs and iron repressed cDNAs were labeled with [α-^{32}P]dCTP (3000 Ci/mmol, Amersham, Buckinghamshire, UK) using the Rediprime II kit (Amersham). Filters were successively hybridized with labelled iron induced cDNAs and following dehybridization, with labelled iron repressed cDNAs. Filters were then autoradiographed and stored at −80°C. Differentially expressed clones were selected by comparing and overlaying autoradiograms obtained with the two probes. Clones that showed little or no detectable hybridization with the iron repressed cDNA probe but a strong hybridization

with the iron induced cDNA probe were isolated. Clones which hybridized similarly with the two probes were considered false positives and were eliminated. Following the initial screening, selected clones were picked up onto a new LB selection plate and transferred onto new nitrocellulose filters. A second round of selection was then performed as described above. Finally, the two rounds of selection allowed us to identify 150 clones which were potentially induced by iron. Plasmids from selected colonies were purified using the Wizard PCR Preps DNA purification system (Promega, Madison, WI, USA). Fifty randomly selected clones were sequenced using the Thermosequenase kit (Amersham, Buckinghamshire, UK) with α-^{33}P radiolabelled dideoxynucleotides (1500 Ci/mmol). Samples were analyzed on a 5% polyacrylamide urea gel. DNA identity scores were generated by matching the query sequences to a subject found in the GenBank and EMBL databases using the BLAST algorithm. Seven different cDNAs were identified.

2.7. RNA blot analysis

Total liver RNAs (10 μg) were loaded on a denaturing 1.2% agarose gel and then transferred to Hybond N^+ filters (Amersham, Buckinghamshire, UK). Filters were hybridized with selected [α-^{32}P]dCTP (3000 Ci/mmol) radiolabelled cDNAs (Rediprime II, Amersham, Buckinghamshire, UK). Filters were autoradiographed and stored at −80°C. The quantification of mRNA abundance was performed by densitometry and lane loading differences were measured using a β-actin probe. The mouse cytoskeletal β-actin cDNA (GenBank accession number X03672) used in this study was generated by RT-PCR using oligonucleotides 5′-TGTGCTGTCCCTGTATGCCT-3′ and 5′-TAGGAGCCAGAGCAGTAATC-3′. The generated cDNA fragment was 553 bp in length.

2.8. Stearoyl coenzyme A desaturase 1 activity assay

Fresh livers from each mouse overloaded with 3% carbonyl-iron for 8 months and each mouse overloaded with iron-dextran (sacrificed 4 months later) were homogenized in four volumes of a solution of 0.25 M sucrose and 50 mM phosphate buffer (pH 7.4) [29]. Livers from the respective control mice were processed identically. The solution was then centrifuged at $10\,000 \times g$ for 10 min. An aliquot of the supernatant was stored at −20°C for protein measurement. The supernatant was used as the enzymatic solution for the stearoyl coenzyme desaturase 1 assay, and incubated at 37°C in the presence of coenzyme A (0.54 mM), ATP (7.2 mM), NADH (0.8 mM), $MgCl_2$ (6 mM), and phosphate buffer (102 mM, pH 7.16). The reaction was initiated by the addition of 30 nmol [^{14}C]stearic acid (440 GBq/mol) in 3 μl ethanol and stopped 20 min later by adding 2 mmol KOH in ethanol. Each assay mixture was heated 30 min at 70°C. The fatty acids were liberated by acidification, extracted with diethylether and dried. Fatty acid methyl esters were prepared by heating at 70°C for 30 min with a methanol:H_2SO_4 (10:1, v/v) solution and then extracted with pentane. They were later separated by thin layer chromatography on silver nitrate impregnated silica gel H (12 g/100 g) plates using a mixture of diethylether: hexane (10:90, v/v). Spots corresponding to the substrate stearic acid and product oleic acid were visualized by dichlorofluorescein and radioactivity was counted (Packard Tri-carb 1600 TR, Meriden, CT, USA). Enzymatic activity was determined from the amount of radioactivity and expressed as nmol stearic acid converted to oleic acid/min/mg protein. Protein concentration was determined by a modified Lowry procedure [30] in the supernatant described above.

2.9. Statistical analysis

Results were expressed as mean ± S.D. The Mann–Whitney test was used for the estimation of statistical significance when appropriate. A P value less than 0.05 was considered statistically significant.

3. Results

3.1. Identification of hepatic overexpression of SCD1 mRNA in carbonyl-iron overloaded mice

Performing SSH between cDNAs from the liver of 3% carbonyl-iron overloaded mice and control mice, we isolated a cDNA of 338 bp. A query of the GenBank database using the BLAST algorithm revealed

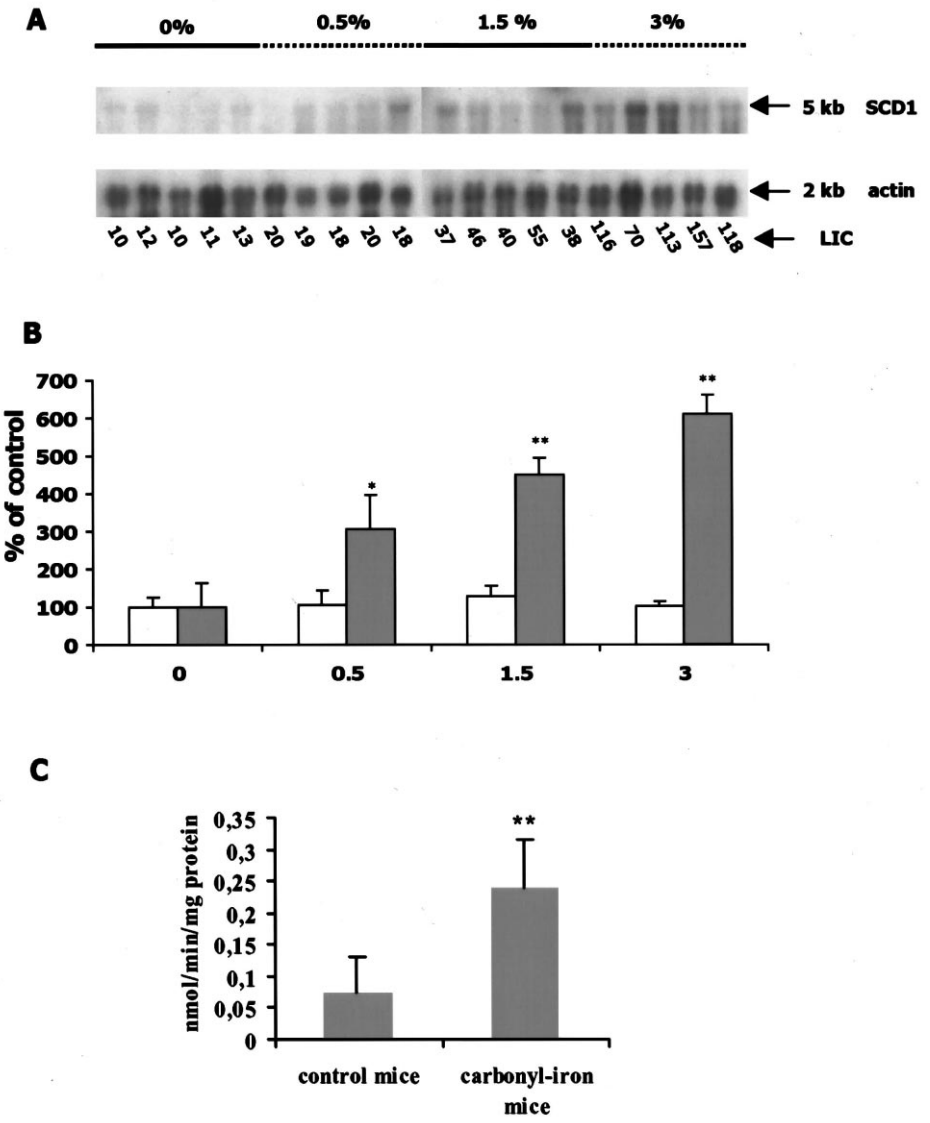

Fig. 1. SCD1 expression in carbonyl-iron overloaded mice. (A) Expression of SCD1 mRNA analyzed by Northern blot in livers of mice overloaded with 0.5, 1.5, and 3% carbonyl-iron for 8 months and control mice. Each lane corresponds to one animal. SCD1 mRNA was greatly overexpressed in the liver of iron overloaded mice. Hybridization with the β-actin probe is also presented in order to evaluate lane loading differences. LICs expressed as μmol/g of dry liver are mentioned. (B) Densitometry analysis of the autoradiographs. The average for each group of iron overload is presented in histograms (white bars: actin; gray bars: SCD1 mRNAs). Densitometry values obtained in control mice were arbitrarily expressed as 100% for each mRNA. Statistical analysis is reported as $^{*}P < 0.05$ and $^{**}P < 0.01$ between overloaded mice and control mice. (C) SCD1 activity measured in the livers of iron overloaded mice with 3% carbonyl-iron for 8 months ($n = 6$) and their controls ($n = 6$).

that this cDNA was 100% identical in its overlapping region to exon 6 of SCD1 (GenBank accession number M21285), also named delta 9 desaturase, an enzyme of lipid metabolism. Six other cDNAs were also identified. Preliminary Northern blot analysis showed that the mRNA overexpression was clear only for two of them including SCD1 mRNA and an mRNA that we cloned and characterized separately [31]. In addition, as some data of the literature suggested a link between iron and lipid metabolisms, we decided to further study the relationship between iron overload and SCD1 mRNA expression.

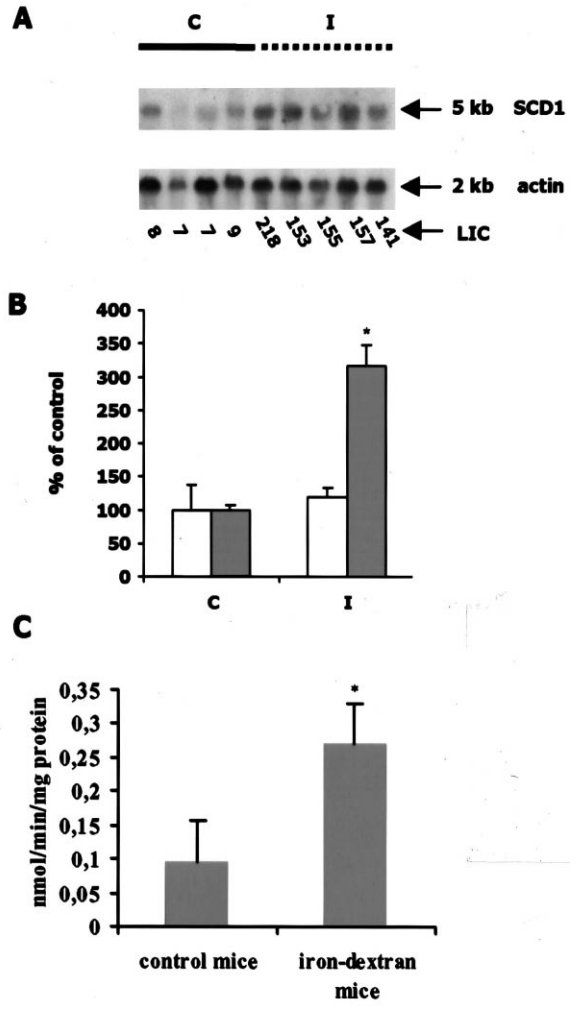

Fig. 2. SCD1 expression in iron-dextran overloaded mice. (A) Hepatic expression of SCD1 mRNA in livers of iron-dextran injected mice ($n=5$) and control mice ($n=4$). SCD1 mRNA was upregulated by iron excess whereas β-actin mRNA was not. LICs expressed as μmol/g of dry liver are mentioned. (B) The average densitometry of autoradiographs is presented in histograms (white bars: actin; gray bars: SCD1). Densitometry values obtained in control mice were arbitrarily expressed as 100% for each mRNA. Statistical analysis is reported as $*P<0.01$ between overloaded mice and control mice. (C) SCD1 activity (delta 9 desaturase) measured in the livers of 4 months iron-dextran overloaded mice ($n=5$) and their controls ($n=4$).

In order to confirm SCD1 mRNA overexpression in the liver of mice overloaded with 3% carbonyl-iron for 8 months, Northern blot analysis was performed using SCD1 cDNA, obtained by SSH, as a probe. Moreover, mRNA expression was analyzed in the liver of mice fed intermediate doses of carbonyl-iron (0.5 and 1.5%) to search for a possible dose dependent expression of SCD1 mRNA. As presented in Fig. 1A, the SCD1 probe hybridized with a 5 kb mRNA. This length corresponded to the expected size of SCD1 mRNA. In the liver of carbonyl-iron overloaded mice, the SCD1 mRNA amount was increased in a dose dependent manner. Its expression was maximal in the livers of 3% carbonyl-iron overloaded mice. It was indeed 6.1 fold more than in control mice which had a carbonyl-iron free diet (Fig. 1B). Despite the fact that the level of SCD1 mRNA was heterogeneous from one mouse to another one, this overexpression was statistically significant (Fig. 1B; $P<0.01$).

Therefore, we measured SCD1 activity to see if the upregulation of SCD1 mRNA, observed in the livers of iron overloaded mice, was associated with an enhancement of SCD1 enzymatic activity. This investigation was performed on livers obtained from mice overloaded with 3% carbonyl-iron for 8 months for which we found SCD1 mRNA induction. Assays demonstrated a 3.3 fold increase of SCD1 activity in the liver of 3% carbonyl-iron overloaded mice as compared to control mice (Fig. 1C). This increase was statistically significant ($P<0.01$).

3.2. *Overexpression of SCD1 mRNA in the liver of iron-dextran overloaded mice*

In order to determine whether the overexpression of SCD1 mRNA was effectively related to iron excess and not to other nutritional disturbances induced by oral carbonyl-iron supplementation, we analyzed SCD1 mRNA expression in another in vivo model of iron overload in mice using iron-dextran injection. In these animals, which were sacrificed 4 months after the injection, we found a 3 fold increase of SCD1 mRNA steady-state level as compared to control mice ($P<0.01$; Fig. 2A,B). In these mice, the LIC was 21 fold higher in iron-dextran treated mice than in control mice (165.4 ± 30.3 versus 7.8 ± 0.8 μmol iron/g dry weight, respectively). The overexpression of SCD1 mRNA was associated with a 2.8 fold ($P<0.02$) increase of SCD1 enzymatic activity in the liver of iron-dextran mice compared to control mice (Fig. 2C).

Table 1
Study of SCD1 mRNA expression and corresponding iron load parameters in carbonyl-iron overloaded mice

	2 months		8 months	
	control, $n=4$	iron loaded, $n=4$	control, $n=4$	iron loaded, $n=4$
SCD1 mRNA level	100 ± 80	110 ± 70	100 ± 98	320 ± 80*
LIC	5.5 ± 1	72 ± 9	10.5 ± 1	120 ± 20**
TIS	0 ± 0	15.3 ± 1.9	1.8 ± 2.3	22.5 ± 1.7**
HIS/TIS	nc	60 ± 7	nc	43 ± 3**
SIS/TIS	nc	21 ± 1	nc	25 ± 3
PIS/TIS	nc	19 ± 6	nc	32 ± 1**

SCD1 mRNA is expressed as 100% of the control values obtained at the same time. LIC is expressed as μmol iron/g dry weight liver. TIS, which is the sum of the HIS, SIS, and PIS, is expressed in arbitrary units. HIS, SIS, and the ratio PIS/TIS are expressed as percentages. *Significantly different from the control value obtained at the same time. **A significant increase or decrease of the corresponding parameter was observed in the iron loaded group. nc = not calculated (TIS = 0).

3.3. *Influence of iron overload duration and hepatic cellular repartition of iron excess on SCD1 mRNA expression*

Then, we studied the expression of SCD1 mRNA at earlier stages of iron overload for both models.

For the carbonyl-iron overload model, when iron was administered for only 2 months, the amount of SCD1 mRNA was quite identical in the liver of control mice and mice fed 3% carbonyl-iron although the LIC was 14 fold higher in the latter group (Table 1). After 8 months, corresponding to a LIC of the order of 120 μmol iron/g dry tissue in iron overloaded mice, SCD1 mRNA induction was similar to that observed in animals used for SSH. Then, to compare iron repartition in the liver at 2 and 8 months, we evaluated iron deposits in hepatic cells using histological iron scores. We observed that TIS increased concomitantly with LIC. In addition the rise of the PIS/TIS ratio and the drop of the HIS/TIS ratio reflect that the relative weights of portal and hepatocytic iron deposits in the TIS calculation respectively increased and decreased between 2 and 8 months (Table 1). Thus, the carbonyl-iron model after 2 and 8 months differs in terms of both liver iron load and hepatic cellular repartition of iron excess.

In the iron-dextran model, despite the fact that the SCD1 mRNA level was not significantly modified between 1 and 3 weeks after iron injection, its steady-state level was clearly increased in iron treated mice which were sacrificed 2 months after the injection. In this model, LIC and TIS reached a plateau as soon as 1 week. However, there was a progressive rise of the HIS/TIS ratio and a concomitant decrease of the SIS/TIS ratio (Table 2). These data mean that,

Table 2
Study of SCD1 mRNA expression and corresponding iron load parameters in iron-dextran overloaded mice

	1 week		3 weeks		2 months	
	control, $n=3$	iron loaded, $n=3$	control, $n=3$	iron loaded, $n=3$	control, $n=3$	iron loaded, $n=3$
SCD1 mRNA	100 ± 38	110 ± 42	100 ± 91	213 ± 106	100 ± 98	402 ± 100*
LIC	5.6 ± 0.8	198 ± 51	5.7 ± 1.2	211 ± 24	6 ± 1	177 ± 8
TIS	0 ± 0	17 ± 4.1	0 ± 0	22.5 ± 1.7	0 ± 0	21 ± 0
HIS/TIS	nc	28 ± 7	nc	40 ± 4**	nc	57 ± 0**
SIS/TIS	nc	56 ± 2	nc	40 ± 2**	nc	24 ± 0**
PIS/TIS	nc	16 ± 7	nc	20 ± 3	nc	19 ± 0

SCD1 mRNA is expressed as 100% of the control values obtained at the same time. LIC is expressed as μmol iron/g dry weight liver. TIS, which is the sum of the HIS, SIS, and PIS, is expressed in arbitrary units. HIS, SIS, and the ratio PIS/TIS are expressed as percentages. *Significantly different from the control value obtained at the same time. **A significant increase or decrease of the corresponding parameter was observed in the iron loaded group. nc = not calculated (TIS = 0).

in this model, iron content is stable over time, but iron repartition varies with, respectively, an increase and a drop of hepatocytic and sinusoidal iron deposits.

4. Discussion

Mechanisms involved in the development of hepatic fibrosis and hepatocellular carcinoma during iron overload diseases in humans are not well understood. We hypothesized that abnormal expression of some hepatic genes during iron overload may be involved in these phenomena.

In a previous work we characterized a new murine model of hepatic iron overload obtained by supplementation of 3% carbonyl iron in the diet [24]. This model exhibited a strong iron overload of the liver associated with the following hepatocyte nuclear abnormalities: (i) presence of iron containing ferritin inclusions, (ii) enlargement of hepatocyte nucleus size, and (iii) increase of mitotic index with presence of abnormal mitotic figures, particularly well established after 12 months of iron exposure. In addition, some iron free hepatocyte foci were found. Except for their smaller size, they were comparable to iron free foci found in humans during genetic hemochromatosis which could predict hepatocellular carcinoma development [32]. Taking these data together, we concluded that this murine iron overload model was useful to study the molecular mechanisms involved in the development of hepatic lesion related to iron excess, especially hepatocellular carcinoma.

In order to identify hepatic genes abnormally overexpressed during iron overload and possibly involved in the development of hepatic lesions, we performed SSH between cDNAs obtained respectively from the livers of control and 8 months 3% carbonyl-iron overloaded mice. This method allowed us to identify an increase of the SCD1 mRNA steady-state level in carbonyl-iron overloaded mice as compared to control mice. SCD1 mRNA encodes the first enzyme involved in fatty acid desaturation meaning biosynthesis of unsaturated fatty acids [33]. This process mainly occurs in the liver. Since recent observations obtained in humans suggest interactions between iron and lipid metabolisms [34] we decided to further study the relationship between liver iron excess and both SCD1 mRNA expression and activity. This enzyme is part of an enzymatic complex including cytochrome *b*5 reductase and cytochrome *b*5 in addition to the SCD1 enzyme [33]. This complex catalyzes the oxidative conversion of stearoyl coenzyme A, a saturated fatty acid, to oleyl coenzyme A by adding a double bond between carbons 9 and 10 of stearoyl coenzyme A. Concerning SCD1, it has been previously demonstrated that SCD1 mRNA expression can be modulated by many factors. Indeed, it is well known that SCD1 expression can be increased by insulin [35,36], fructose [35], glucose [37], a lipid poor diet [38], and peroxisome proliferators [39]. Conversely, polyunsaturated fatty acids and glucagon were able to decrease the mRNA level [36,40]. In our work, we found a large amount of SCD1 mRNA in the livers of mice which were overloaded with 3% carbonyl-iron for 8 months as compared to control mice. This result suggests that iron excess is another factor which is able to modulate SCD1 mRNA expression.

To confirm the role of iron itself in the mRNA overexpression, we used another model of iron overload in mice using iron-dextran injection. By using the parenteral route, we aimed to avoid any indirect mechanisms of upregulation due to possible disturbance in nutrient absorption, related to the oral loading with carbonyl-iron. We clearly observed an increase of the SCD1 mRNA steady-state level in parenterally iron overloaded animals at 2 months as well as 4 months after iron injection, thereby suggesting that iron excess itself is responsible for the overexpression. This result was reinforced by the induction of the SCD1 mRNA in an iron dose dependent manner when carbonyl-iron was administered for 8 months.

Then, we confirmed that the increase in the mRNA level was associated with a rise of the corresponding SCD1 activity in the livers of mice overloaded with carbonyl-iron or iron-dextran. In addition to the ability of iron to control SCD1 mRNA expression that we highlighted in this paper, it has been previously demonstrated that the activity of SCD1 can also be modulated at the post-translational level by iron status. Indeed, it is well established that SCD1 is an enzyme containing one iron atom [41] and that its activity is decreased in situations of cellular iron deprivation induced by iron chelation

[42,43]. However, the mechanism involved in the SCD1 mRNA overexpression in the livers of iron loaded animals is not known. Considering that, in the iron-dextran model, the SCD1 mRNA steady-state level increases only a few weeks after the injection, whereas LIC rises quickly (a few days) it is unlikely that the effect of iron on SCD1 expression and activity is a direct one. This view may be supported by the fact that we did not find an overexpression of SCD1 mRNA in mouse hepatocytes exposed to citrate iron (data not shown). Conversely, it is likely that the development of liver iron overload is able to induce cellular or biochemical alterations which in turn could lead to and/or participate in the SCD1 mRNA overexpression. In such a case, SCD1 mRNA overexpression could reflect a compensatory mechanism in response to a cellular need for renewing unsaturated fatty acids. Indeed, it has been reported in different in vivo models of iron overload that hepatic iron excess usually results in hepatic lipid peroxidation meaning unsaturated fatty acid degradation [44–46].

In order to study if SCD1 mRNA expression is enhanced at early stages of iron overload, we performed different durations of overload for both carbonyl-iron and iron-dextran models. We found that in the carbonyl-iron model, the induction was late (after 8 months of iron exposure), occurring when LIC was very high (120 μmol iron/g dry tissue). However, from data obtained in the iron-dextran model, it is clear that a strong iron amount in the liver was not sufficient to lead to SCD1 mRNA induction. Indeed, in this model, even though the LIC found 1 week after the injection was higher than that found after 8 months of carbonyl-iron supplementation, no upregulation of SCD1 mRNA was found at this time. Nevertheless, SCD1 mRNA overexpression was found 2 months after iron injection whereas LIC was quite similar to that measured after 1 week. We therefore propose that other factor(s) may influence the expression of SCD1 mRNA. In the carbonyl-iron model, it is well established that iron deposits are mainly located in hepatocytes [24,47], whereas in the iron dextran model iron deposits are mainly located in non-parenchymal cells in the earlier stages of iron overload [25]. In this latter model, there is a modification of cellular hepatic iron repartition over time. Indeed, we observed an increase of the HIS/TIS ratio concomitant with a decrease of the SIS/TIS ratio. Thus, a change of iron repartition in the liver in addition to a high LIC likely participates in SCD1 overexpression during iron overload. Analysis of the data showed that, for the two models, SCD1 mRNA overexpression was observed only when the HIS/TIS ratio was higher than 40% suggesting that abundant and prolonged iron deposits in hepatocytes are required for the overexpression of SCD1 mRNA. Such features of iron deposits are observed in human pathology.

In conclusion, performing SSH, we demonstrated that the SCD1 mRNA level and its corresponding enzymatic activity were increased in chronically carbonyl-iron and iron-dextran overloaded mice. The level of iron overload in the liver and the hepatic cellular types involved in the storage of iron excess are both likely to play a role. By influencing SCD1 expression and activity, iron excess in the liver may enhance the biosynthesis of unsaturated fatty acids. The disturbance of this metabolic pathway could play a role in the development of hepatic injuries triggered by iron excess.

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References

[1] R.A. Risdon, M. Barry, D.M. Flynn, J. Pathol. 116 (1975) 83–95.
[2] C. Niederau, R. Fischer, A. Sonnenberg, W. Stremmel, H.J. Trampisch, G. Strohmeyer, New Engl. J. Med. 313 (1985) 1256–1262.
[3] M.L. Bassett, J.W. Halliday, L.W. Powell, Hepatology 6 (1986) 24–29.
[4] O. Loréal, Y. Deugnier, R. Moirand, L. Lauvin, D. Guyader, H. Jouanolle, B. Turlin, G. Lescoat, P. Brissot, J. Hepatol. 16 (1992) 122–127.

[5] Y.M. Deugnier, D. Guyader, L. Crantock, J.M. Lopez, B. Turlin, J. Yaouanq, H. Jouanolle, J.P. Campion, B. Launois, J.W. Halliday et al., Gastroenterology 104 (1993) 228–234.
[6] J.K. Olynyk, K.R. Reddy, A.M. Di Bisceglie, L.J. Jeffers, T.I. Parker, J.L. Radick, E.R. Schiff, B.R. Bacon, Gastroenterology 108 (1995) 1104–1109.
[7] H. Tsukamoto, W. Horne, S. Kamimura, O. Niemela, S. Parkkila, S. Yla-Herttuala, G.M. Brittenham, J. Clin. Invest. 96 (1995) 620–630.
[8] B. Turlin, F. Juguet, R. Moirand, D. Le Quilleuc, O. Loréal, J.P. Campion, B. Launois, M.P. Ramée, P. Brissot, Y. Deugnier, Hepatology 22 (1995) 446–450.
[9] A. Piperno, A. Vergani, I. Malosio, L. Parma, L. Fossati, A. Ricci, G. Bovo, G. Boari, G. Mancia, Hepatology 28 (1998) 1105–1109.
[10] B.R. Bacon, R.S. Britton, Chem. Biol. Interact. 70 (1989) 183–226.
[11] R.S. Britton, Semin. Liver Dis. 16 (1996) 3–12.
[12] R. Meneghini, Free Radical Biol. Med. 23 (1997) 783–792.
[13] V. Abalea, J. Cillard, M.P. Dubos, J.P. Anger, P. Cillard, I. Morel, Carcinogenesis 19 (1998) 1053–1059.
[14] K.K. Rao, D. Shapiro, E. Mattia, K. Bridges, R. Klausner, Mol. Cell. Biol. 5 (1985) 595–600.
[15] J.L. Casey, M.W. Hentze, D.M. Koeller, S.W. Caughman, T.A. Rouault, R.D. Klausner, J.B. Harford, Science 240 (1988) 924–928.
[16] E.W. Mullner, B. Neupert, L.C. Kuhn, Cell 58 (1989) 373–382.
[17] A. Pietrangelo, E. Rocchi, L. Schiaffonati, E. Ventura, G. Cairo, Hepatology 11 (1990) 798–804.
[18] R. Gualdi, G. Casalgrandi, G. Montosi, E. Ventura, A. Pietrangelo, Gastroenterology 107 (1994) 1118–1124.
[19] K. Houglum, G.A. Ramm, D.H. Crawford, J.L. Witztum, L.W. Powell, M. Chojkier, Hepatology 26 (1997) 605–610.
[20] K.E. Brown, M.T. Kinter, T.D. Oberley, M.L. Freeman, H.F. Frierson, L.A. Ridnour, Y. Tao, L.W. Oberley, D.R. Spitz, Free Radical Biol. Med. 24 (1998) 545–555.
[21] G. Montosi, C. Garuti, S. Martinelli, A. Pietrangelo, Hepatology 27 (1998) 1611–1622.
[22] C.J. Fimmel, K.E. Brown, R. O'Neill, R.D. Kladney, J. Immunol. 157 (1996) 2601–2609.
[23] L. Diatchenko, Y.F. Lau, A.P. Campbell, A. Chenchik, F. Moqadam, B. Huang, S. Lukyanov, K. Lukyanov, N. Gurskaya, E.D. Sverdlov, P.D. Siebert, Proc. Natl. Acad. Sci. USA 93 (1996) 6025–6030.
[24] C. Pigeon, B. Turlin, T.C. Iancu, P. Leroyer, J. Le Lan, Y. Deugnier, P. Brissot, O. Loréal, J. Hepatol. 30 (1999) 926–934.
[25] P. Carthew, E.R. Edwards, A.G. Smith, B. Dorman, J.E. Francis, Hepatology 13 (1990) 534–539.
[26] Y.M. Deugnier, O. Loréal, B. Turlin, D. Guyader, H. Jouanolle, R. Moirand, C. Jacquelinet, P. Brissot, Gastroenterology 102 (1992) 2050–2059.
[27] B. Turlin, I. Robert, S. Maugendre, F. Le Gall, P. Brissot, M.P. Ramée, Y. Deugnier, Ann. Pathol. 17 (1997) 329–332.
[28] M. Barry, S. Sherlock, Lancet 2 (1971) 100–103.
[29] P. Legrand, D. Catheline, M.C. Fichot, P. Lemarchal, J. Nutr. 127 (1997) 249–256.
[30] A. Bensadoun, D. Weinstein, Anal. Biochem. 70 (1976) 241–250.
[31] C. Pigeon, G. Ilyin, B. Couselaud, P. Leroyer, B. Turlin, P. Brissot, O. Loréal, J. Biol. Chem. (in press).
[32] Y.M. Deugnier, P. Charalambous, D. Le Quilleuc, B. Turlin, J. Searle, P. Brissot, L.W. Powell, J.W. Halliday, Hepatology 18 (1993) 1363–1369.
[33] H.G. Enoch, A. Catala, P. Strittmatter, J. Biol. Chem. 251 (1976) 5095–5103.
[34] R. Moirand, A.M. Mortaji, O. Loréal, F. Paillard, P. Brissot, Y. Deugnier, Lancet 349 (1997) 95–97.
[35] K.M. Waters, J.M. Ntambi, J. Biol. Chem. 269 (1994) 27773–27777.
[36] P. Lefevre, C. Diot, P. Legrand, M. Douaire, Arch. Biochem. Biophys. 368 (1999) 329–337.
[37] B.H. Jones, M.K. Standridge, K.J. Claycombe, P.J. Smith, N. Moustaid-Moussa, Biochem. J. 335 (1998) 405–408.
[38] J.M. Ntambi, Prog. Lipid Res. 34 (1995) 139–150.
[39] C.W. Miller, J.M. Ntambi, Proc. Natl. Acad. Sci. USA 93 (1996) 9443–9448.
[40] K.T. Landschulz, D.B. Jump, O.A. MacDougald, M.D. Lane, Biochem. Biophys. Res. Commun. 200 (1994) 763–768.
[41] J. Shanklin, E. Whittle, B.G. Fox, Biochemistry 33 (1994) 12787–12794.
[42] P. Strittmatter, L. Spatz, D. Corcoran, M.J. Rogers, B. Setlow, R. Redline, Proc. Natl. Acad. Sci. USA 71 (1974) 4565–4569.
[43] G.A. Rao, R.T. Crane, E.C. Larkin, Lipids 18 (1983) 573–575.
[44] B.R. Bacon, A.S. Tavill, G.M. Brittenham, C.H. Park, R.O. Recknagel, J. Clin. Invest. 71 (1983) 429–439.
[45] B.R. Bacon, R.S. Britton, Hepatology 11 (1990) 127–137.
[46] L.G. Valerio Jr., D.R. Petersen, Toxicol. Lett. 98 (1998) 31–39.
[47] C.H. Park, B.R. Bacon, G.M. Brittenham, A.S. Tavill, Lab. Invest. 57 (1987) 555–563.