Antisense gene delivered by an adenoassociated viral vector inhibits iron uptake in human intestinal cells: Potential application in hemochromatosis☆

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Abstract

Hereditary hemochromatosis (HH) is a condition in which intestinal iron absorption is greatly elevated. Present treatment is weekly phlebotomy, affecting quality of life and leading to recurrent infections. The iron transporter divalent metal transporter-1 (DMT-1) of enterocytes is responsible for iron uptake from the intestinal lumen; iron is further extruded into the blood by the basolateral transporter ferroportin-1. A therapeutic approach for HH could start with a long-term reduction of iron transport by reduction of DMT-1 levels. We designed an AAV vector coding for a short antisense RNA (AAV-DMT-1-AS) against DMT-1, which reduced iron uptake by 50–60% in human intestinal cells (Caco-2). At low infection levels, DMT-1 mRNA virtually disappeared, suggesting RNAi-like and/or RNase H antisense effects. DMT-1 mRNA levels returned to normal at higher infection levels, indicating that an additional mechanism of mRNA occupation, able to block *DMT-1* translation and to avoid feedback regulation by iron responsive elements (IRE), also exists. Cell morphology was normal in all cases and no increases in the interferon-related responses, measured by (a) 2′-5′ A oligo synthetase (b) IFITM1 and (c) ISGF3γ mRNA levels, were observed. Studies presented herein indicate that enterocyte targeting with a gene coding for a short antisense against iron transport blocks enterocyte iron uptake, which may have therapeutic value.

Keywords: Hemochromatosis; Antisense; Adenoassociated vector; Gene therapy; Iron; Caco-2 cells

1. Introduction

While iron is an essential element required for growth and survival, iron overload leads to cell injury. Unbound iron catalyzes the generation of oxygen radicals, which oxidize lipids, proteins and DNA. Since the ability of mammals to eliminate iron is low, iron absorption by the small intestine must be carefully controlled [1].

Alterations in iron homeostasis lead to pathological disorders in humans. Hereditary hemochromatosis (HH) is a condition in which intestinal iron absorption is greatly elevated [2]. About one million individuals in the US are affected by this disease [3], exceeding the prevalence of cystic fibrosis and muscular dystrophy combined [4]. The disease is caused by the continued intestinal absorption of dietary iron despite adequate or raised body iron stores. Absorbed iron is progressively accumulated in organs such as liver, pancreas and heart. Siderosis causes cirrhosis and liver failure, diabetes mellitus, cardiomyopathy and other complications that lead to premature death [4–6]. Present treatment is a weekly phlebotomy with removal of 450–500 ml of blood [7] affecting quality of life, and leading to neutropenia and to recurrent infections. High blood iron levels also potentiate other pathological conditions; individuals with HH who abuse alcohol, a condition in which oxygen radical generation is per se increased, are at a nine-fold greater risk of developing cirrhosis [8].

The bipolar enterocyte absorbs ferrous ions from the intestinal lumen and subsequently releases these to the

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portal blood. Two iron transporters at each pole of the enterocyte are responsible for the transepithelial transport of iron; the divalent metal transporter-1 (DMT-1) at the luminal side incorporates iron into the enterocyte and at the basolateral side the iron transporter ferroportin-1 extrudes it into the circulation [9,10]. The apical iron transporter DMT-1 is greatly increased in HH patients [11]. Studies by several groups have shown that 80% of patients with clinical features of HH are homozygous for a single mutation in the *HFE* gene, which results in the substitution of tyrosine for cysteine at amino acid 282 (C282Y) that eliminates a disulphide bond in the protein [12–14]. Another mutation in *HFE* results in the substitution of aspartate for histidine at amino acid 63 (H63D) [12,14]. The normal HFE protein is thought to inhibit the activity of the apical DMT-1 iron transporter by transcytosis [15], but other mechanisms such as the maturation of crypt cells into a high *DMT-1* expression enterocyte have also been proposed [16]. In patients presenting increased levels of apical DMT-1, high iron absorption, typical of this disease [11,17], is observed.

A possible new therapeutic approach for the treatment of all forms of HH, independent of its etiology and mechanism, is to reduce the expression of the apical *DMT-1* transporter gene, thus inhibiting iron uptake by the absorptive enterocyte. One possible strategy to inhibit gene expression is the administration of pre-formed antisense oligonucleotides or of short interfering RNA oligonucleotides (siRNA). However, these small molecules are easily hydrolyzed by the nucleases present in the cells, which reduce the half-lives of their therapeutic effects. This problem could be overcome by generating an antisense RNA or a hairpin siRNA coded by a gene under the control of an appropriate promoter, for the long-term generation of the therapeutic molecules in the enterocyte itself.

The polarized human intestinal epithelial Caco-2 cell line is a well-recognized model to study intestinal iron absorption by the apical DMT-1 transporter [18]. In the present studies, we have transduced Caco-2 cells with a short antisense-generating gene carried by an adenoassociated viral vector (AAV). The AAV is a human parvovirus carrying a small single-stranded DNA. This vector is currently considered to be one of the most promising viral vector systems for gene therapy because of a unique combination of properties, including the lack of association with any human disease [19], which gives AAV one of the highest biosafety ratings among all gene transfer viral vectors [20]. The virus infects a wide range of cell types [21] and has the ability to induce long-term gene expression because its recombinant genome establishes stable episomal forms and may also integrate into the host genome [22]. The AAV virus is also resistant to physiological temperatures and to extreme pH conditions [23]. Studies by During et al. [24] showed that the oral administration of an AAV vector carrying a therapeutic gene maintained a stable expression of the transgene in rat intestine for at least 6 months. Since the turnover of enterocytes is 3–5 days, such a protracted expression indicates that stem cells within the crypts were transduced by the AAV vector. Recent studies have also demonstrated that transfection of reporter genes into intestinal cells can be accomplished by oral administration of plasmids encapsulated in chitosan, a polymer of D-glucosamine [25].

In the present studies we have generated an AAV vector carrying a short antisense gene against an internal region of DMT-1 mRNA and have evaluated the effect of this construct on iron uptake by Caco-2 cells, a model of human intestinal epithelial cell transport. Studies demonstrate that the AAV vector carrying the antisense gene blocks *DMT-1* gene expression and inhibits iron uptake.

2. Materials and methods

2.1. Cell lines

HEK-293 cells (American Type Culture Collection CRL-1573, Rockville, MD) and Caco-2 cells (American Type Culture Collection HTB-37) were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Gibco Life Technologies, Auckland, NZ). Caco-2 cells, a model of epithelial intestinal cells, express a polarized phenotype with high levels of GLUT-5, a glucose transporter found in the brush border cells of the small intestine but absent from colon cells [15,26]. Caco-2 cells have an active iron responsive element/iron responsive protein (IRE/IRP) system that regulates apical iron uptake depending on the levels of intracellular iron [27].

2.2. Plasmid constructs

A plasmid containing the *DMT-1* antisense gene flanked by the viral ITRs of adenoassociated virus was generated by cloning a fragment of 295 bp (nucleotides 624–920) of the DMT-1 cDNA (GenBank accession NM000617), in pAAV-MCS (Stratagene, Cedar Creek, TX) with the use of *Eco*RI and *Xba*I restriction enzymes and T4 DNA ligase (Promega, Madison, WI). The *DMT-1* antisense gene was preceded by a CMV promoter and flanked by an hGF polyA signal. This construct, pAAV-DMT-1-AS, was confirmed by DNA sequencing.

2.3. Vector production

To generate the AAV-DMT-1 antisense viral vector, low-passage subconfluent HEK-293 cells (in 10 cm dishes) cultured in DMEM without serum were cotransfected by calcium phosphate [28] with 10 μg of each pAAV-DMT-1-AS (*DMT-1* antisense gene), pAAV-RC (*rep* and *cap* genes) and pAAV-helper (adenoviral genes). AAV-LacZ was similarly generated by cotransfection with 10 μg of each

pAAV-LacZ, pAAV-RC and pAAV-helper. The medium was changed 24 h after cotransfection, and cells were harvested 3 days after cotransfection. All the original plasmids were obtained from Stratagene (Cedar Creek, TX).

2.4. Vector purification

Viral vectors were purified by the single-step column purification (SSCP) protocol described by Auricchio et al. [29]. Cells in one 10 cm dish were resuspended in 2.5 ml DMEM, frozen and thawed twice and incubated with 0.1 mg of both DNAse I (Sigma, St. Louis, MO) and RNAse A (Calbiochem, Darmstadt, Germany) for 30 min at 37 °C. After 15 min of centrifugation at 3000 × *g* at 4 °C, the supernatant was transferred to a new tube and incubated in 0.5% sodium deoxycholate (Sigma) for 30 min at 37 °C and sequentially filtered through a 5 μm pore size filter (Millipore, Carrigtwohill, CO, SLSV R25 LS) and a 0.8 μm pore size filter (Millipore). The cleared crude lysate was then applied on a heparin affinity column (Sigma) equilibrated with 25 ml of phosphate buffer saline solution (PBS) pH 7.4. After all the lysate had passed through, the column was washed twice with 25 ml of PBS pH 7.4 containing 0.1 M NaCl. The virus was then eluted with 15 ml PBS pH 7.4 plus 0.4 M NaCl. The eluate was concentrated to 1 ml by centrifugation on a Millipore (Amicon Ultra 30 K MWCO) filter. To adjust the NaCl concentration to physiological levels the filter device was refilled with 25 ml of PBS pH 7.4 and the virus was concentrated to 1 ml again.

2.5. Vector titration

The number of genome copies of the AAV vectors was determined by semi-quantitative PCR, comparing the intensity of the amplicons generated by different dilutions of the viral stock to the intensity of the amplicons generated by a known concentration of the plasmid used to produce the viral vector.

2.6. Transduction of Caco-2 cells and iron uptake determination

Caco-2 cells at 70% confluence were incubated at different MOI with the AAV-DMT-1 antisense or AAV-LacZ viral vectors in DMEM without serum for 8 h, after which normal serum levels were restored. The cells were incubated an additional 10 days to achieve the differentiation that characterizes bipolar epithelial cells. Differentiated Caco-2 cells were washed with saline buffer (50 mM HEPES; 94 mM NaCl; 7.4 mM KCl; 0.74 mM $MgCl_2$ and 1.5 mM Ca Cl_2; pH 7), and incubated at 37 °C for 60 min in saline buffer supplemented with 0.2 μM $^{55}FeCl_3$–sodium nitrilotriacetate complex (^{55}Fe-NTA 1:2 molar ratio). This iron complex is reduced to ferrous iron by a luminal reductase (DCYTB) [30]. Iron uptake was stopped by washing the cells three times with ice-cold PBS supplemented with 1 mM EDTA to eliminate extracellular ^{55}Fe binding. Cells were resuspended in buffer (40 mM Tris–HCl; 100 mM NaCl and 1 mM EDTA; pH 7.5) and the radioactivity was determined in a liquid scintillation counter. Iron uptake was expressed as pmol of iron per milligram of protein. Total protein concentration was determinated by the Bradford method [31].

2.7. Determination of mRNA levels

Total RNA was isolated from control and transduced Caco-2 cells with Trizol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The first strand of cDNA was obtained by reverse transcription (M-MLV reverse transcriptase, Promega, Madison, WI) from 5 μg of total RNA using the appropriate reverse primers. Ten percent of the RT reaction was then used for PCR amplification with Taq DNA polymerase (Promega). Initial studies were conducted to demonstrate that the bands generated and quantitated by scanning were in the linear range of mRNA levels. The PCRs of β-actin, DMT-1 sense, DMT-1 antisense, transferrin receptor-I, ferroportin-1, 2′-5′ A oligo synthetase, IFITM-1 and ISGF3γ were performed at 94 °C for 30 s, 60 °C for 60 s and 72 °C for 60 s for 20–30 cycles. The PCR products were run on appropriate gels depending on product size, evidenced by ethidium bromide, scanned and quantified using the SCION software [32]. The primers used for de RT-PCR reactions were 5′-TTG ACT AAG GCA GAA TGC AGG-3′ and 5′-CGT TCT CAT CAC CAT TGC AG3-′ for DMT-1 sense and antisense amplifications. These primers amplify the IRE and the non-IRE forms of DMT-1. 5′-CTT CTA CAA TGA GCT GCG TG3-′ and 5′-GAG GAT CTT CAT GAG GTA GTC-3′ for β-actin amplifications, 5′-TGG CTC GGC AAG TAG ATG G-3′ and 5′-TCA CTG GAG ACT CGG TTC C-3′ for transferrin receptor-1 amplifications, 5′-CTA GTA ACA GGA TAG CAA CAG-3′ and 5′-TTG GCT TGC TCG TAT TGA TTT-3′ for ferroportin-1 amplifications, 5′-AGG TGG TAA AGG GTG GCT CC-3′ and 5′-ACA ACC AGG TCA GCG TCA GAT-3′ for 2′–5′ A oligo synthetase amplifications, 5′-AGC ATC CGG ACA CCA CAG C-3′ and 5′-ACG TCG CCA ACC ATC TTC C3′ for IFITM-1 amplifications and 5′CAG AAC TGC ACA CTC AGT CC-3′ and CCT CTC AAG CTG GCT CAG C-3′ for ISGF3γ amplifications.

2.8. Statistical analysis

All variables were tested in triplicates, and the experiments were repeated at least twice. *t*-Test or ANOVA was used to test differences in means by the SISSA software [33]. Differences were considered significant at $p < 0.05$.

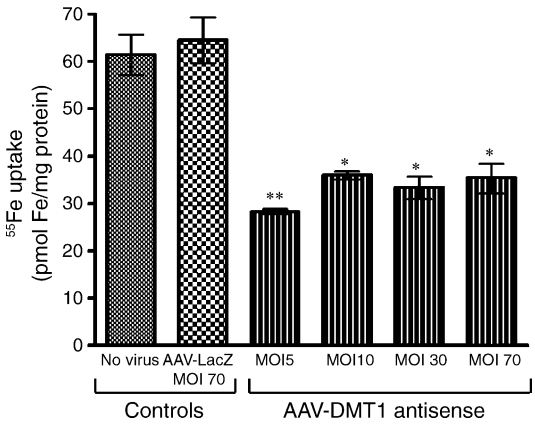

Fig. 1. Inhibition of iron uptake by Caco-2 intestinal epithelial cells transduced with an AAV vector carrying a short antisense gene against DMT-1: Caco-2 cells were transduced at different multiplicities of infection (MOIs) with AAV-DMT-1 antisense viral vector for 48 h. Cells were then cultured for 8 additional days to achieve cell differentiation and incubated with 0.2 μM of $^{55}FeCl_3$-sodium nitrilotriacetate (NTA). Iron uptake by the cells was determinated 1 h after of ^{55}Fe addition ($^{**}p < 0.01$; $^{*}p < 0.05$ vs. control AAV or vs. no vector. $n = 3$ independent experiments; means ± S.D.).

3. Results

The inhibitory effect on ^{55}Fe uptake by Caco-2 cells transduced with a short anti *DMT-1* antisense-generating gene is shown in Fig. 1. Pretreatment of Caco-2 cells with the antisense-generating gene carried by an adenoassociated viral vector markedly inhibited (55%; $p < 0.01$) the uptake of iron by the cells, even at low levels of viral infection (viral particles per cell: MOI = 5). At higher MOIs, ^{55}Fe uptake remained inhibited at similar levels. Fig. 2 shows that at the low level of infection (MOI = 5) DMT-1 mRNA levels are greatly reduced (60–70%). At higher levels of infection (MOIs of 10–70) DMT-1 mRNA levels return to normal, despite a continuously inhibited iron transport, suggesting a compensatory effect on gene transcription. However, such an increase in DMT-1 message is not translated into higher protein levels, possibly due to an occupation of DMT-1 mRNA resulting from increased levels of antisense RNA. Indeed, we determined that the levels of the antisense RNA were actually increased at increasing levels of infection (MOI from 5 to 70). Fig. 3 shows that higher levels of antisense mRNA are present at higher MOIs. Neither ferroportin mRNA levels nor transferin receptor-1 mRNA levels were elevated at any of the MOIs (Fig. 4; see Section 4). Treatment with AAV carrying the *DMT-1* antisense gene did not affect the expression of the gene for β-actin, a housekeeping protein.

Studies reported in 1987 suggested that long RNA–RNA hybrids may activate the interferon response [34]. Although these studies have not been reproduced, these are generally accepted in the field. Recent studies have also shown that short RNA–RNA hybrids (siRNAs) may also activate up to 400-fold the synthesis of 2′–5′ A oligo synthetase, an enzyme that is part of the interferon cascade

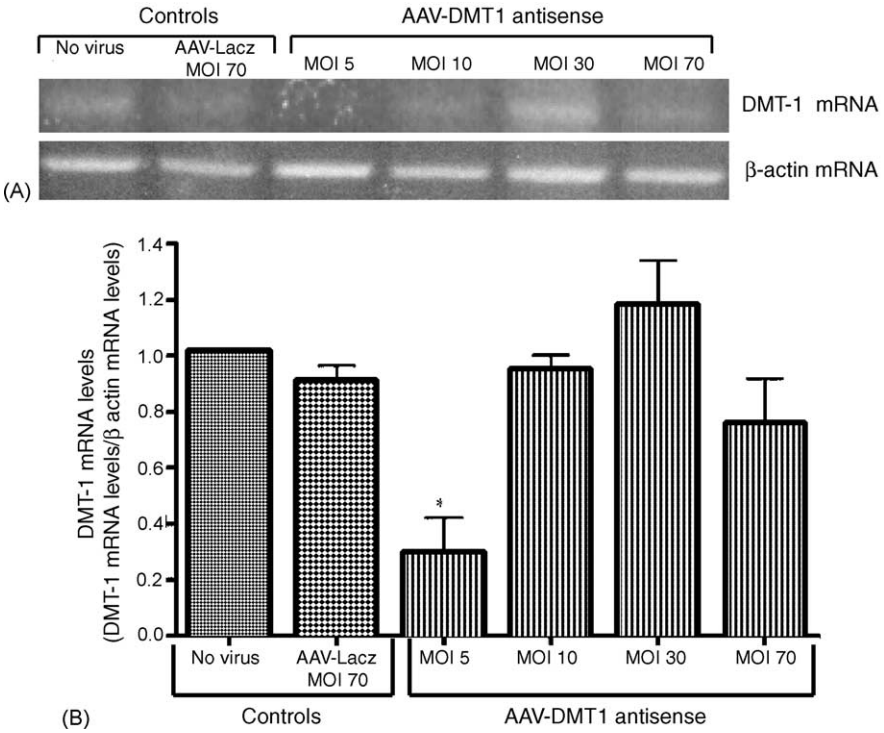

Fig. 2. DMT-1 mRNA levels in Caco-2 intestinal epithelial cells transduced with an AAV vector carrying a short antisense gene against DMT-1: (A) DMT-1 mRNA and β actin mRNA from transduced and control cells amplified by RT-PCR (representative experiment; see quantitation below) (B) Amplicons of DMT-1 mRNA and β actin mRNA were scanned, quantified and normalized to 1.0 with the SCION software ($^{*}p < 0.03$; vs. control AAV or vs. no vector. $n = 3$ independent experiments; means ± S.D.).

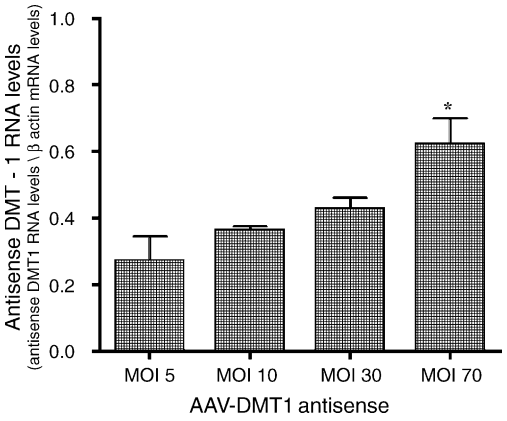

Fig. 3. Expression of the *DMT-1* antisense gene in Caco-2 intestinal epithelial cells transduced with an AAV vector carrying a short antisense gene against DMT-1: RNA extracted from transduced intestinal epithelial cells was amplified by RT-PCR with specific primers for the antisense DMT-1 RNA ($n = 3$ independent experiments; means ± S.D.). Increases in MOI led to significant increases in DMT-1 antisense RNA (MOI 5 vs. MOI 70 $p < 0.05$).

[35,36]. Thus, we determined if the AAV-DMT-1 generated antisense activated the expression of the 2′–5′ A oligo synthetase gene. The expression of two other genes that are part of the interferon cascade were also measured: interferon-inducible transmembrane protein (IFITM-1) and the transcriptional regulator ISGF3-γ Fig. 5 shows that neither the levels of 2–5′ A oligo synthetase mRNA nor of IFITM-1 or ISGF3-γ were different in cells transduced with the AAV vector carrying the antisense gene from those of the control AAV vectors carrying the *LacZ* gene, thus indicating that in this system the interferon mechanism is not activated.

4. Discussion

Hemochromatosis is the most prevalent genetic disease in Caucasian populations [4]. About 80% of HH patients present a substitution of tyrosine for cysteine at amino acid 282 (C282Y), eliminating a disulphide bond in the protein [12–14]. Another mutation results in the substitution of aspartate for histidine at amino acid 63 (H63D) [12,14]. Hemochromatosis also occurs in a smaller percentage (20%) of patients with a normal HFE [37–41], indicating that other control mechanisms may lead to a greater absorption of iron. Overall, six different genetic abnormalities have been described that increase the intestinal uptake of iron and lead to hemochromatosis [37]. Notably, iron accumulation also potentiates the effect of other toxins where oxygen radicals play an important role in the generation of TNF-α, including alcoholic cirrhosis in humans [8] and alcohol induced liver injury in animals [42]. The divalent metal transporter DMT-1 has been recently shown to also transport copper ions [43], thus anti DMT-1 antisense coding genes may be of value in reducing liver injury in Wilson's disease patients, a condition in which copper export from cells is reduced. Conversely, in a therapy against hemochromatosis involving a reduction of DMT-1 levels, oral supplementation with other divalent trace metals should be in place.

Data obtained showed that an antisense gene carried by an AAV vector reduces by 50–60% the uptake of iron in a model system of intestinal epithelium [15,26]. The inhibition of DMT-1 remained constant at MOIs of 5–70 virions/cell, despite the marked increases in antisense transcript levels at the higher MOIs. These data suggest that the maximal inhibition of this transporter was already reached at the lower MOIs. The residual ^{55}Fe uptake may correspond to mechanisms not related to DMT-1, including facilitated diffusion of ^{55}Fe into the cells. Thus, the real extent of the inhibition of the DMT-1 carrier itself may be underestimated. In cells of patients with hemochromatosis, DMT-1 is markedly increased [11] and thus, this transporter would play a greater role in iron uptake by the enterocytes of HH patients.

Unexpectedly, the level of DMT-1 mRNA was markedly reduced at the lower MOIs (five virions/cell) while these levels returned to normal or even exceeded the control levels at higher MOIs (10–70 virions/cell), despite higher levels of antisense RNA transcript. A reduction in DMT-1

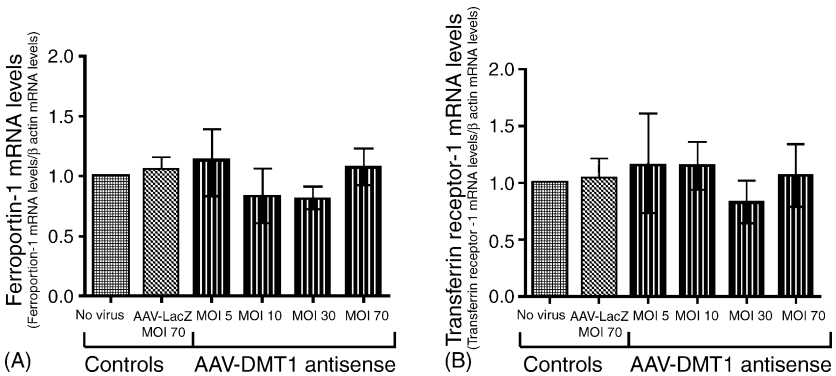

Fig. 4. Levels of mRNA/actin mRNA for (A) ferroportin-1 and (B) transferrin receptor-1 in Caco-2 intestinal epithelial cells transduced with an AAV vector carrying a short antisense gene against DMT-1. RNA extracted from transduced intestinal epithelial cells was amplified by RT-PCR with specific primers for ferroportin-1 and transferrin receptor-1 mRNA ($n = 3$ independent experiments; means ± S.D.).

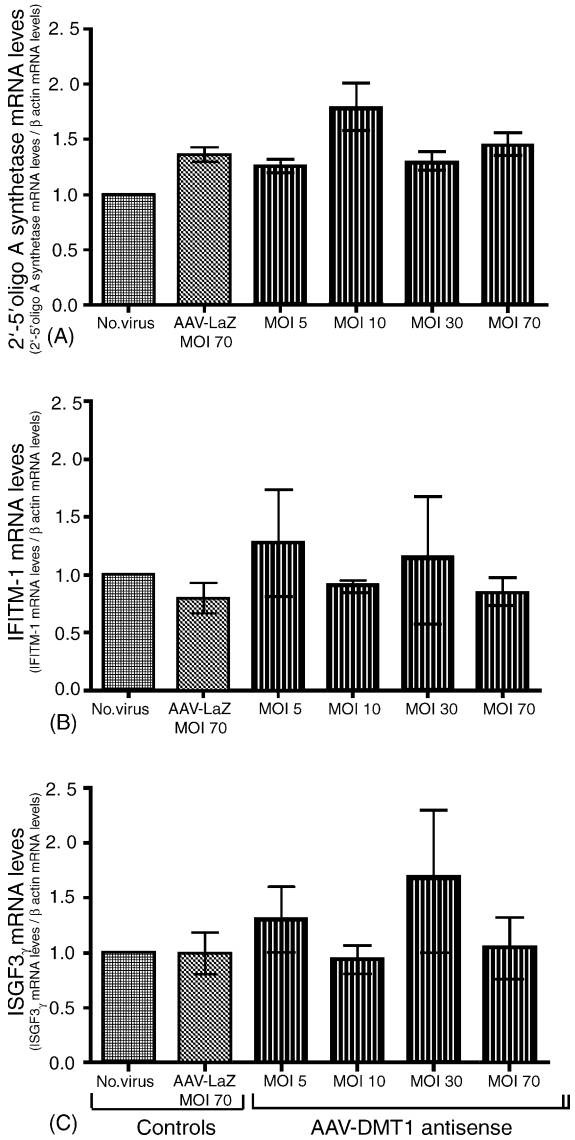

Fig. 5. Lack of interferon cascade activation by a short antisense gene against DMT-1. Bars show the levels of mRNA/actin mRNA for (A) 2′-5′ A oligo synthetase (B) IFITM-1 and (C) ISGF3γ in Caco-2 intestinal epithelial cells transduced with an AAV vector carrying a short antisense gene against DMT-1. RNA extracted from transduced intestinal epithelial cells was amplified by RT-PCR with specific primers for (A) 2′-5′A oligo synthetase, (B) IFITM-1 and (C) ISGF3γ ($n = 3$ independent experiments; means ± S.D.).

mRNA at low levels of antisense mRNA could be due to an RNAi-like effect in which the dsRNA hybrid formed by the sense and antisense moieties might be processed by the ribonuclease Dicer [44]. Alternatively, the single strand DNA in AAV might act via an RNase H mediated antisense mechanism. Increased levels of antisense RNA anti DMT-1 mRNA were clearly observed at increased MOIs. At high levels of anti DMT-1 antisense RNA the effect appears to be primarily one of occupation (and nuclease protection) of the DMT-1 mRNA transcript. However, RNAi or RNase H mechanisms might coexist. An occupation blockade, existing at high levels of the antisense transcript, is in line with the fact that the return of DMT-1 mRNA to normal levels was not expressed as a higher transport of ^{55}Fe.

Intestinal cells are known to increase the translation of DMT-1 mRNA when intracellular levels of iron are low [45]. Normally, iron ions bind to endogenous ‘‘iron response proteins’’ which inhibit DMT-1 synthesis by reducing the stability of DMT-1 mRNA. In the absence of iron, DMT-1 mRNA is stabilized by the IRP proteins, which allows an increased translation of DMT-1 thus increasing the ability of the cell to incorporate iron [45]. Clearly, from the data obtained, such a mechanism was obliterated by both transcript degradation at low MOI levels and by transcript occupation at high MOI levels. Avoiding the IRE–IRP feedback mechanism, as shown here, is essential for the success of this type of therapy. Noteworthy, neither ferroportin mRNA levels nor transferin receptor mRNA levels, proposed to respond to IRE–IRP elements [46], were elevated after the prolonged treatment (10 days) that decreased DMT-1 activity. These data suggest that the intracellular iron concentration returns to normal values, conceivably by the entry of the iron-loaded transferrin receptor-1 via the basolateral route. Such a system would not be able to reduce the net transepithelial iron transport.

In a recent study, Galli et al. [47] lipofected human macrophages with short double stranded oligoribonucleotides (siRNA) against ferroportin mRNA. The studies showed marked increases in macrophage iron. Although in enterocytes this mechanism might generate iron overload effects, it might be considered as an adjunct to the present therapeutic approach.

As indicated earlier, in our cell model we chose to deliver a short antisense gene by AAV administration since During et al. [24] had shown that oral administration of AAV was able to transduce rat small intestine epithelial cells with the β-galactosidase gene for periods exceeding 6 months, thus most likely targeting stem cells in the intestinal crypt. Senescent rats, which lose much of their intestinal β-galactosidase, show significant weight reductions when lactose is given as the only source of calories. The studies [24] showed that animals transduced with the intestinal β-galactosidase gene gained weight while fed on lactose. Tissues not in the gastrointestinal tract were not transduced by oral administration of the AAV. Recent studies have shown that it is also possible to transfect the intestine with the gene coding for β-galactosidase by oral administration of a plasmid carrying the β-galactosidase gene protected by chitosan, an aminosugar polymer [25].

It has been reported that long RNA–RNA hybrids may activate the interferon cascade. The expression of three genes that are part of the interferon cascade: (i) 2′–5′ A oligo synthetase gene, (ii) interferon-inducible transmembrane protein (IFITM-1) and (iii) transcriptional regulator ISGF3-γ were not elevated in cells transduced with the AAV vector carrying the antisense gene, thus indicating

that potentially toxic effects of the interferon system were not induced by administration of the short antisense coding gene carried by the AAV vector.

Hemochromatosis is usually diagnosed after the development of symptoms in the fourth to sixth decades of life, when patients have significant iron overload. The only presently accepted treatment for this disease is periodic bleeding (phlebotomy). Initially one or two units of blood (500–1000 ml), each containing 200–250 mg of iron, are removed weekly until serum ferritin levels are reduced below 50 ng/ml and transferrin saturation drops to a value below 30%, requiring 2–3 years [48,49]. It is unlikely that in the absence of an initial aggressive phlebotomy in these patients, iron levels can return to normal even a complete inhibition of intestinal iron uptake. Thus, any therapy that inhibits iron uptake at the enterocyte levels, whether by antisense or RNAi should follow an initial depletion of the iron accumulated for decades.

Overall, we report that a short antisense gene against *DMT-1* gene expression carried by an AAV vector markedly inhibits the transport of iron in a human intestinal cell line. Noteworthy, the treatment overcomes the natural intracellular iron regulatory system that increases DMT-1 levels when iron ions are reduced in the enterocyte. To our knowledge, work presented constitutes the first model of gene therapy aimed at hemochromatosis. The findings may have therapeutic potential in the treatment of divalent metal accumulation and of oxygen radical related diseases.

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