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RESEARCH ARTICLE

Adenovirus-mediated apo(a)-antisense-RNA expression efficiently inhibits apo(a) synthesis in vitro and in vivo

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Apo(a) is a very atherogenic plasma protein without apparent function, which is highly expressed in humans. The variation in plasma Lp(a) concentration among individuals is considerable. Approximately 10–15% of the white population exhibit plasma Lp(a) concentrations above the atherogenic cut-off value of approximately 30 mg/dl. Since there is currently no safe way of treating those patients with drugs, we have tested the possibility of interfering with apo(a) biosynthesis by adenovirus-mediated expression of antisense apo(a) mRNA comprising the 5′ UTR, the signal sequence and the first three kringles of native apo(a). Transduction of rat hepatoma McA RH 7777 cells which stably expressed apo(a) with 18 kringle IV (KIV) domains with apo(a)-antisense adenovirus (AS-Ad) at multiplicity of infection (MOI) of 30 reduced apo(a) synthesis to 23% as compared with control cells. As apo(a) is not synthesized in laboratory animals, we induced biosynthesis of the N-terminal fragments of apo(a) in mice by adenovirus-mediated gene transfer. Cotransduction of these mice with AS-Ad, which expressed up to eight times higher amounts of apo(a) than stable transgenic apo(a) mice, led to an almost complete disappearance of apo(a) from plasma. We conclude that the proposed AS-construct is very efficient in interfering with apo(a) biosynthesis in vivo. The strategy of inducing the synthesis of a nonexpressed protein followed by knocking it out by AS technology may also be applicable to other systems. Gene Therapy (2001) **8,** 425–430.

Keywords: *transgenic apo(a) mice; adenovirus; antisense-RNA; atherosclerosis*

Introduction

Lp(a) is a lipoprotein occurring in plasma of humans and primates, but not in other animals, with concentrations ranging from <1 to >200 mg/dl.[1] In humans, Lp(a) values are under strict genetic control and amount to 8–9 mg/dl (medians) and 18–20 mg/dl (means), respectively. Elevated plasma concentrations above 25–30 mg/dl are considered to contribute to the risk for thrombo-atherogenic diseases.[2–4] Lp(a) is a complex plasma lipoprotein formed extracellularly through the covalent binding of free apolipoprotein(a) [apo(a)], being synthesized in the liver, with apoB-100 of low density lipoprotein (LDL).[5] Small amounts of apo(a) are also present in plasma in its free form. Apo(a) is a high-molecular weight glycoprotein consisting of repetitive domains, so called kringles (K), which share a striking homology with plasminogen KIV.[6] KIVs type-1 (T1) and KIV T3–T10 are present in a single copy in apo(a)[7] whereas the number of 'repetitive kringle' KIV T2 varies from 3 to 43[8] and is responsible for the genetically determined size polymorphism.[8,9] In addition, apo(a) contains one copy of a kringle which is homologous to kringle-V, and an analogue of the protease domain of plasminogen.[6] In addition to humans,[2–4,10] the atherogenicity of apo(a) and Lp(a) has been proven in transgenic mice.[11,12]

Atherogenic potential of free apo(a) was demonstrated in transgenic mice expressing human apo(a). These mice developed atherosclerosis due to focal apo(a) accumulation in the vessel wall leading to inhibition of transforming growth factor-β (TGF-β) activity, activation of smooth muscle cells, and subsequent accumulation of lipids in the vessel wall.[11,13,14]

Additionally, the atherogenic potential of Lp(a) was demonstrated in double transgenic mice expressing both human apo(a) and human apoB-100.[12]

Due to its great atherogenicity, many attempts were made in the past to medicate individuals with increased Lp(a) levels with drugs or diets. None of those proved to be of value, as almost all drugs such as statins or bile acid sequestrants, which lower plasma LDL by increasing specific receptors in the liver, have very inconsistent effects and sometimes even cause an increase of Lp(a) concentrations.[15] Nicotinic acid and its derivatives may lower Lp(a) levels by up to 30%,[16,17] yet they are not widely used because of their side-effects. Anabolic sterols with a Lp(a) lowering capacity of 70%[18] are obviously not suitable for treatment of elevated Lp(a) plasma levels. Many other agents reported in the literature such as alcohol, polyunsaturated fatty acids, aspirin[19] or ascorbic acid have an inconsistent effect on Lp(a).

There is ample evidence that plasma Lp(a) levels >30

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mg/dl contribute to the risk of cardiovascular diseases and stroke.[10] We therefore hypothesize that interfering with the expression of apo(a) in turn will lead to a reduction of plasma Lp(a) levels and might ultimately reduce the atherogenic risk.

Since there is no safe drug available for this purpose at the present time, we aimed at interfering with apo(a) expression in a molecular way *in vitro* by delivering an apo(a)-antisense construct to stably transfected liver cells which produced large amounts of apo(a). In addition, *in vivo* studies were conducted using transduced mice expressing recombinant human apo(a), which were also treated with the apo(a)-antisense construct.

Results

Expression of recombinant adenovirus in transduced cells

McA-RH 7777 rat hepatoma cells were infected with recombinant adenovirus AS-apo(a)-Ad at multiplicity of infection (MOI) of 30. RNA was isolated from transduced cells 5, 10 and 20 h after infection and analyzed by Northern blot using AS-apo(a)-cDNA as a probe. A very efficient expression of antisense-apo(a)-RNA was observed already 5 h after infection. The abundance of AS-apo(a)-RNA strikingly increased over a time period of 20 h (Figure 1). We have also found that raising the MOI to 50 or 100, respectively, was detrimental to the cells resulting in cytopatic appearance 20 h after transduction (data not shown).

Effect of AS-apo(a)-RNA expression on apo(a) synthesis

In order to assess the efficiency of adenovirus-mediated transduction, the secretion of apo(a) into the medium by cultivated McA-RH 7777-XL cells was followed using immunoquantification. McA-RH 7777-XL cells stably expressing apo(a) with 18 KIV domains were transduced with AS-apo(a)-Ad, and LacZ-Ad as a control, at MOI 30. To test the effect of LacZ-Ad transduction on apo(a) secretion, nontransduced cells (NT) were used as an additional control. AS-apo(a)-Ad transduction caused a significant reduction in apo(a) secretion into the medium as compared with LacZ-Ad transduced cells. A reduction by a factor of 2.6 was observed 24 h after transduction: (301 ± 14.1 ng/ml *versus* 798 ± 16.5 ng/ml) (Figure 2a). The difference between AS-apo(a)-Ad- and LacZ-Ad-transduced cells became even more pronounced 36 h after transduction had been started (Figure 2a). At the latter time-point, AS-apo(a)-Ad-transduced cells produced only 23% of apo(a) compared with control LacZ-Ad-transduced cells (346 ± 18.9 ng/ml *versus* 1497 ± 45.1 ng/ml). The amount of apo(a) secreted by nontransduced cells after 24 h and 36 h was slightly but not significantly increased compared with LacZ transduced cells (820 ± 10.8 ng/ml *versus* 798 ± 16.5 ng/ml and 1548 ± 28.9 ng/ml *versus*. 1497 ± 45 ng/ml, respectively). To examine whether antisense expression led to a possible formation of truncated forms of apo(a), aliquots of the cell medium from the above-described experiment were analyzed by Western blot. Figure 2b shows that the integrity of apo(a)-XL was retained upon AS-apo(a)-Ad or LacZ-Ad transduction when compared with nontransduced cells.

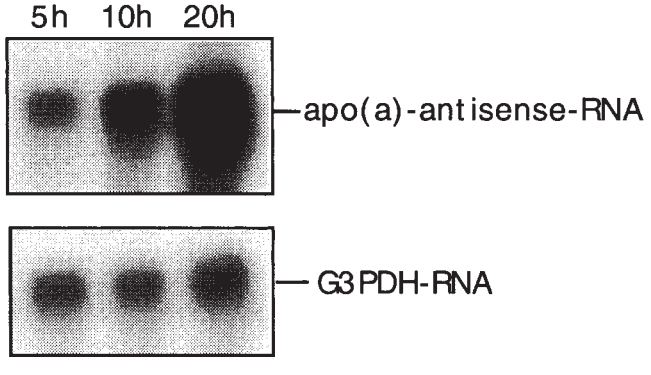

Figure 1 *Antisense apo(a) mRNA expression in McA-RH 7777 cells: McA-RH 7777 cells were transduced with AS-Ad at MOI 30. RNA was isolated from the cells 5, 10 and 20 h, respectively, after transduction. Northern blot was performed as described in Materials and methods using AS-apo(a)-cDNA as a probe. In addition the blot was stripped and reprobed with labeled glyceraldehyde-3-phosphate dehydrogenase (G3PDH) cDNA that served as a control for RNA loading.*

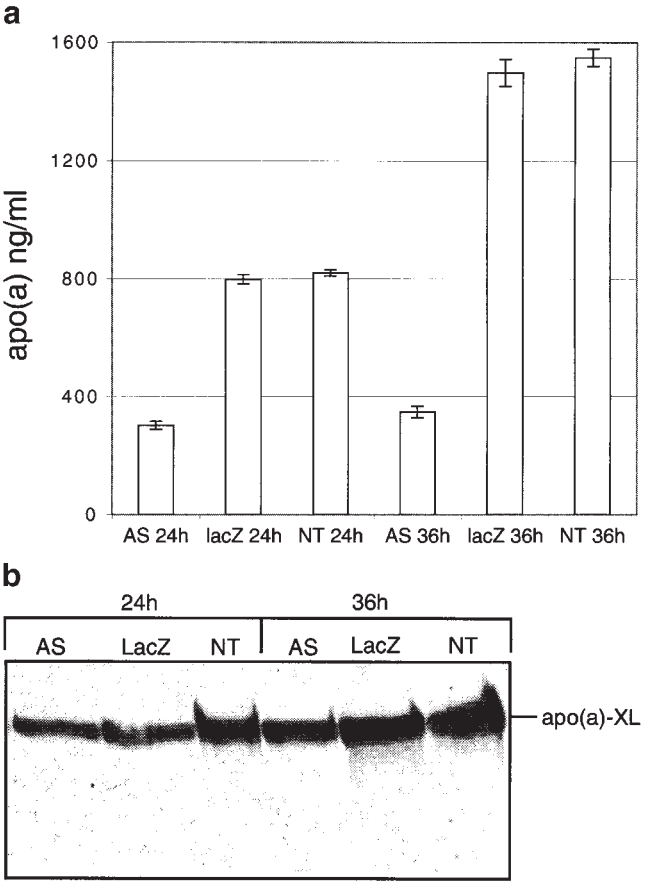

Figure 2 *Effect of apo(a)-antisense on apo(a) concentration in cell medium of McA-RH 7777-XL cells: (a) McA-RH 7777-XL cells were transduced with AS-Ad and LacZ-Ad, respectively, at MOI 30. Nontransduced (NT) cells were used as a control for LacZ transduction. Medium was collected from the transduced and nontransduced control cells 24 h and 36 h after transduction. Apo(a) concentration was measured in medium by DELFIA. Results are mean ± s.d. of triplicate analyses. (b) Western blot analysis of the cell medium described under (a). Cell medium was subjected to 3.75–12.5% SDS-PAGE under reducing conditions followed by Western blotting as described in Materials and methods. Detection was performed by the ECL method using polyclonal anti-apo(a) from rabbit and a horseradish peroxidase-labelled goat anti-rabbit IgG conjugate.*

Effect of apo(a)-antisense-RNA on apo(a)-XL mRNA abundance

Subsequently the effect of antisense-RNA on the abundance of apo(a)-XL-mRNA was studied by Northern blot analysis of total RNA. McA-RH 7777-XL cells transduced with AS-apo(a)-Ad or LacZ-Ad and incubated for 24 or 36 h were investigated. Upon hybridization bands representing 7.4 kb apo(a)-XL mRNA could be detected in RNA samples obtained from LacZ-Ad, but not from AS-apo(a)-Ad-transduced cells (Figure 3a). Weak apo(a)-XL-mRNA bands obtained from AS-apo(a)-Ad transduced cells were visible only after overexposure of the blot. These results drew us to the conclusion that AS-apo(a)-mRNA expression led to an active degradation of apo(a)-XL-mRNA. They were further substantiated by RT-PCR of the RNA samples (Figure 3b).

In vivo effect of apo(a)-antisense-RNA on apo(a) plasma levels in mice

The capacity of AS-apo(a)-Ad to reduce apo(a) synthesis and excretion into plasma was also tested *in vivo*. Since mice do not produce Lp(a), a fragment of human apo(a) was expressed in mice by adenovirus-mediated gene transfer. Six mice were transduced with an adenovirus, N-Ad, coding for N-terminal portion of apo(a) consisting of signal sequence, KIV T1 and five copies of KIV T2. Three mice out of the six were additionally transduced with AS-apo(a)-Ad and three with LacZ-Ad.

The control mice transduced with N-Ad and LacZ-Ad produced large quantities of N-apo(a) with maximal apo(a) plasma concentration on days 5 (18.4 ± 3.5 mg/dl) and 6 (17.8 ± 3.6 mg/dl). In contrast, the co-transduction of mice with AS-apo(a)-Ad in addition to N-Ad led to only minimal secretion of apo(a) into plasma, reaching maximally 1.5% that of controls. A maximal plasma apo(a) level was seen on day 2 of the latter experiment (Figure 4).

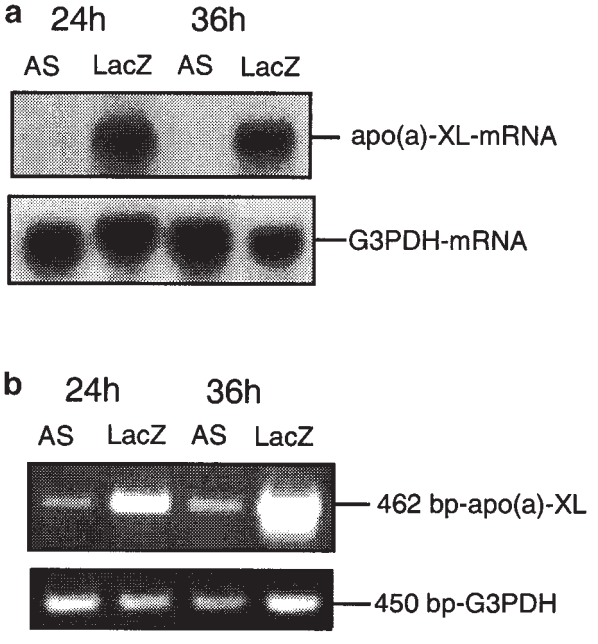

Figure 3 *Effect of apo(a)-antisense expression on apo(a)-XL-mRNA content in McA-RH 7777-XL cells. (a) McA-RH 7777-XL cells were transduced with AS-apo(a)-Ad (AS) and LacZ-Ad (LacZ) respectively, at MOI 30. RNA was isolated 24 and 36 h upon transduction and analyzed by Northern blot using 850-bp PstI–KpnI fragment (described in Materials and methods) as probe. The blot was reprobed with radiolabelled G3PDH cDNA. The positions of apo(a)-XL-mRNA and G3PDH are indicated on the right of the blots (b). RNA samples described under (a) were also analyzed by RT-PCR using apo(a) specific primers AS1 and AS2 as well as G3PDGf and G3PDHr as described in Materials and methods. The positions of 462 bp apo(a)-XL- and 450 bp G3PDH-amplification products are indicated on the right.*

Discussion

All attempts to lower plasma Lp(a) satisfactorily in patients suffering from vascular diseases remained elusive so far. We therefore pursued a molecular approach for lowering apo(a) production by expression of apo(a) antisense-RNA in *in vivo* and *in vitro* systems. After having tested several possibilities, an antisense apo(a) construct containing the signal sequence, KIV T1, and two copies of KIV T2, proved to inhibit very effectively the *in vitro* apo(a) production in cultured rat hepatoma cells which stably expressed apo(a) with 18 KIV domains. *In vivo*, antisense-RNA was evenly efficient. Since mice do not contain endogenous apo(a), we first initiated apo(a) production by transduction with adenovirus coding for the N-terminal apo(a) fragment containing 6 KIV domains. This led to a highly efficient expression of a truncated apo(a) yielding plasma levels of >10 mg/dl. We preferred this model to stably expressing transgenic mice, since in the latter model plasma apo(a) levels reach only <5 mg/dl.

Our apo(a) antisense construct was delivered and expressed by adenovirus-mediated gene transfer approach. We obtained transient but very efficient reductions of apo(a) synthesis upon transduction both *in vitro* in cell culture and *in vivo* in mice transiently expressing N-terminal apo(a) fragments. RNA analysis revealed a strong reduction in apo(a)-mRNA upon AS-apo(a)-Ad transduction suggesting RNA duplex formation with concomitant RNA degradation. Double-stranded RNA-specific endoribonucleases were described in bacteria[20] and yeast.[21] Database search revealed homologues of this

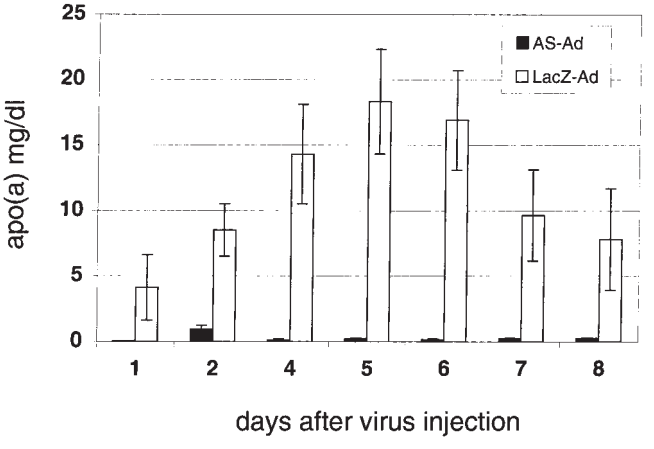

Figure 4 *In vivo effect of apo(a)-antisense on apo(a) production. Three mice out of six were injected into the tail vein with a mixture containing 5×10^8 p.f.u. of N-Ad and 10^8 p.f.u. of AS-apo(a)-Ad in total volumen of 117 μl. Three control mice were injected with a mixture containing 5×10^8 p.f.u. of N-Ad and 10^8 p.f.u. of LacZ-Ad. Blood was collected at indicated time-points and the apo(a) concentration in the plasma samples was determined by DELFIA. Values are mean ± s.d.*

enzyme in mouse and man.[22] It remains open if such a homologue is responsible for double-stranded RNA cleavage in our system. We cannot exclude that in addition to apo(a)-mRNA degradation the interference of antisense mRNA with initiation or propagation of translation might be operative in our system.

In cell culture experiments apo(a)-mRNA abundance and protein synthesis were drastically reduced but not abolished despite the fact that at MOI 30, much more antisense mRNA than apo(a)-XL-mRNA was found in the cells. One possible explanation could be that not all apo(a)-XL mRNAs are accessible to antisense-RNA and escape duplex formation. Another explanation is based on our observation that upon transduction 5–15% of the cells remained non-transduced as was revealed by X-gal staining of LacZ-Ad transduced cells (not shown).

In vivo experiments in transduced mice provide further evidence that efficient delivery of the virus, ie efficient transduction, can almost fully abolish protein expression. As shown in Figure 4 the mice cotransduced with AS-apo(a)-Ad synthesized only a minor fraction of apo(a) as compared with controls although we applied five times more N-Ad than AS-apo(a)-Ad into each mouse. These results demonstrate that the expression of the particular antisense apo(a) used in our study by recombinant adenovirus represents a very efficient way of reducing apo(a) synthesis *in vivo*.

In view of the fact that the Lp(a) plasma concentration is determined by the rate of apo(a) synthesis[5] a reduction of apo(a) synthesis by adenovirus-mediated antisense approach could be a tool for preventing atherosclerosis in patients with high plasma Lp(a). One has also to bear in mind that even second-generation adenovirus vectors which were used in this study are highly toxic and not suitable for clinical use.[23,24] There are, however, promising attempts using helper-dependent adenovirus vectors[25] with negligible hepatotoxicity in mice,[26] which may become applicable for humans in future. Alternatively, antisense oligodeoxynucleotides (ODNs) conjugated with asialoglycoprotein[27] could be applied to reduce apo(a) expression. However, in our opinion expression of an antisense-RNA using adenovirus-mediated approach is advantegous in comparison to ODNs for two reasons. First, upon an efficient adenovirus-mediated delivery of the antisense construct, high level expression of antisense-RNA can be achieved in the targeted cells. Second, the fact that more than 95% of injected recombinant adenovirus are taken up by the liver, the site of apo(a) synthesis, in combination with high level expression proves our adenovirus-mediated antisense approach to be a suitable method for reducing apo(a) synthesis and in turn Lp(a) plasma levels.

Here two consideration may be worth noting: (1) All studies so far have failed to prove any physiological function for Lp(a), thus even a complete abolition of apo(a) might have no negative side-effect. (2) Apo(a) is a glycoprotein, which is highly expressed in a fraction of the human population, and it appears promising to develop a strategy following the present approach to abolish apo(a) production strategy. Finally, our experimental approach in animals to test the efficacy of reducing the expression of human genes may also prove useful for other highly expressed potential pathological plasma proteins.

Materials and methods

Construction of an antisense apo(a)-cDNA vector

A 1049 bp *Eco*RI–*Hha*I fragment from λ 18 clone[6] containing 5′-untranslated region (UTR), signal sequence, KIV T1, two copies of KIV T2 and 294 pb of the following KIV T2 was ligated with a 48 bp *Hha*I–*Bam*HI fragment derived by annealing and extension of oligonucleotides K and L, respectively, as described before[28] into pBluescript. The final construct contained 5′ UTR, signal sequence, KIV T1 followed with two copies of KIV T2 and was called antisense (AS)-apo(a)-cDNA.

Construction of N-apo(a)-cDNA

The 2010 bp fragment containing 5′-(UTR), signal sequence, KIV type (T) 1, five copies of KIV T2 and the first 225 bp of the next KIV T2 was obtained by digestion of the clone pSG5-XL[29] with *Xho*I followed by partial digestion with *Bam*HI. The 121 bp *Bam*HI–*Xba*I fragment encompassing the last 117 bp of KIV T2 followed by a stop codon was obtained by PCR amplification by using primers A (5′ gagggatcccggtgtcaggtgg 3′) and B (5′ tatctagatttatgttcggaagg 3′) and a sequence of the λ 18 cDNA lacking KIV T1 as a template followed by *Bam*HI/*Xba*I digestion of the PCR product. Finally, a 2010 bp *Xho*I/*Bam*HI fragment and 121 bp *Bam*HI/*Xba*I fragment were cloned into *Xba*I/*Xho*I digested pBKCMV (Stratagene, La Jolla, CA, USA).

Construction and purification of recombinant adenovirus

The recombinant adenovirus AS-apo(a)-Ad was prepared by cotransfection of pAvCvSv[30] and pJM17[31] into HEK-293 cells. The *Bam*HI/*Kpn*I AS-apo(a)-cDNA fragment was subcloned into *Bgl*II/*Kpn*I digested pAvCvSv in opposite, antisense orientation and the resulting shuttle plasmid (5 μg) was cotransfected with 5 μg of pJM17 into human embryonic kidney (HEK)-293 cells, plated on to a 60-mm culture dish the day before transfection at a density of 2×10^6 cells per dish, by calcium phosphate coprecipitation method.[32] Ten days after transfection recombinant adenoviral plaques were picked, propagated on HEK-293 cells and screened for the presence of AS-apo(a)-cDNA construct by PCR. Positive adenoviral vector clones were plaque-purified twice more on HEK-293 cells. Large-scale production of high titer recombinant adenovirus was performed as described.[33] LacZ-Ad contained β-galactosidase cDNA instead of the AS-apo(a)-cDNA.[33] In N-Ad, AS-apo(a)-cDNA was replaced with N-apo(a)-cDNA.

RNA analysis

Northern blot analysis: Total RNA was prepared from either noninfected or AS-apo(a)-Ad or LacZ-Ad infected McA-RH 7777 and McA-RH 7777 -XL cells by using RNeasy kit (Qiagen, Vienna, Austria). The RNAs were quantified by spectrophotometry, and 15 μg of total cellular RNA were size-fractionated on a 1% formaldehyde-agarose gel in MOPS buffer, transferred to a nylon membrane (Biodyne B, PALL, Vienna, Austria), incubated for 6 h in prehybridization buffer (0.15 M sodium phosphate pH 7.2, 1 mM EDTA, 7% SDS, and 1% BSA) and then further incubated with radiolabelled 850-bp *Pst*I–*Kpn*I fragment encompassing the last 165 bp of KIV T10, KV

and the first 430 bp of protease domain[6] or with radiolabelled AS-apo(a)-cDNA as a probe. After 16-h incubation at 65°C, the filters were washed for 10 min in 0.5% (w/v) SDS, 2 × SSC (0.3 M NaCl and 0.03 M sodium citrate), pH 7.0, at 23°C, and then for 10–15 min in 0.5% SDS, 0.3 × SSC at 65°C before exposing to CRONEX medical X-ray film (Sterling Diagnostic Imaging, Newark, DE, USA). In addition the blots were stripped and reprobed with labeled glyceraldehyde-3-phosphate dehydrogenase (G3PDH) cDNA (Clontech Laboratories, Palo Alto, CA, USA) which served as a control for RNA loading.

Reverse-transcriptase-polymerase chain reaction (RT-PCR): Total RNA (3 μg) was treated with RQ1 Rnase-free Dnase I (Promega, Mannheim, Germany) for 15 min at 37°C and subsequently used as a template for first strand cDNA synthesis in a 30-μl reaction. The reaction mixture contained 0.5 mM dNTPs, 15 units of RNAguard, 3.3 μM random hexamer primers (Pharmacia Biotech, Vienna, Austria), 10 mM DTT, 1 × first strand buffer and 200 units Moloney murine leukemia virus reverse transcriptase (Life Technologies, Vienna, Austria). Reactions were incubated for 1 h at 37°C, heated to 75°C for 10 min and 2.5 μl of the completed reactions were used as template for PCR. 50- and 100-μl PCR reactions contained 0.2 mM dNTPs, 10 μM of oligonucleotide primers, 1 × PCR buffer and 1 unit of DyNAzyme II DNA polymerase (Finnzyme Oy, Vienna, Austria). Apo(a) cDNA was amplified in 23 cycles (94°C, 5 min; 94°C, 30 s; 60°C, 30 s; 72°C, 1 min) using primers AS1 (5′- ACAACCG ATCCGTGTGTGAG-3′) and AS2 (5′-GTTCAGAAGG AGGCCCTAGG-3′), respectively, corresponding to nucleotides 3561–3580 and 4004–4023, respectively, in clone λ 41.[6] G3PDH cDNA from McA-RH 7777-XL cells was amplified in 20 cycles in total volumes of 50 μl under identical conditions as described above using primers G3PDH f (5′-ACCACAGTCCATGCCATCAC-3′) and G3PDH r (5′-TCCACCACCCTGTTGCTGTA-3′).

Apo(a) quantitation by DELFIA: Apo(a) concentrations in cell culture medium and mouse plasma were determined by dissociation enhanced fluorescence immunoassay (DELFIA), as described previously.[29] Briefly, polyclonal antibody against apo(a) was used for coating 96-well microtiter plates. 50–3000-fold diluted samples were incubated on 96-well microtiter plates for 3 h. Following extensive washing, an Europium-labelled polyclonal anti-apo(a) antibody was added and further measurements were performed in the DELFIA reader (Pharmacia) as recommended by the manufacturer. The antibodies were affinity purified and showed no cross-reactivity with plasminogen. Purified Lp(a) with a defined number of KIV repeats was used as a standard. The DELFIA assay was sensitive to approximately 5 ng apolipoprotein. The between-run precision of the assay was ±6%.

Western blot analysis: Cell medium obtained from virus infected cells was mixed 1:1 with loading buffer (20% (w/v) glycerol, 5% w/v) SDS, 0.15% (w/v) bromophenol blue, 63 mmol/l Tris-HCl, pH 6.8) and incubated for 10 min at 95°C. Samples (50 μl aliquots) were fractionated by SDS-PAGE (3.75–12.5%) for 1 h at 150 V, transferred to nitrocellulose followed with the visualization by an enhanced chemiluminescence assay (ECL; Amersham Pharmacia Biotech, Little Chalfont, UK) after incubation with specific antibodies as described previously.[29,34]

Cell culture and recombinant adenovirus infection

McArdle RH 7777 rat hepatoma cells stably expressing apo(a)-XL with 18 KIV domains (McA-RH 7777-XL)[29] were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum (FCS), 50 units/ml penicillin, 50 mg/ml streptomycin and 400 μg/ml G418. McA-RH 7777 cells were cultivated under similar conditions but without G 418. The cells were plated on to six-well or 24-well dishes 16 h before infection and were infected with 10 or 30 MOI (multiplicity of infection), respectively, of AS-apo(a)-Ad or LacZ-Ad as indicated in each experiment. The cells were infected in culture medium containing 2% FCS for 1 h at 37°C. Upon infection fresh medium with 10% FCS was added to the cells. Medium was collected after indicated time-points and replaced with fresh medium. Apo(a) concentration in the collected medium was measured by DELFIA.

In vivo experiments in mice

The apo(a) negative mice used in this experiment were described previously.[29] The animals were housed at 22°C under a constant light–dark cycle and had free access to water and rodent chow (4.5% fat; 21% protein) (Ssniff-Soest, Germany). During injection into the tail vein, as well as during bleeding by retro-orbital puncture, the mice were anesthetized with Isoflurane (Pharmacia & Upjohn, Guyancourt, France). Three mice out of six were injected into the tail vein with a virus mixture containing 5×10^8 p.f.u. of N-Ad and 10^8 p.f.u. of AS-apo(a)-Ad in total volumes of 117 μl. Three control mice were injected with a mixture similar to the one above but containing 10^8 p.f.u. of LacZ-Ad instead of AS-apo(a)-Ad. Blood was collected after indicated time-points and apo(a) concentration in the plasma samples was determined by DELFIA.

Acknowledgements

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