Original Article

Alpha-1 Anti-trypsin Exerts a Hepatoprotective Effect on Immune-mediated Hepatitis and Acetaminophen-induced Liver Injury

Yehudit Shabat, Ami Ben Ya'acov and Yaron Ilan*

Gastroenterology and Liver Units, Department of Medicine, Hebrew University-Hadassah Medical Center, Jerusalem, Israel

Abstract

Background and Aims: The serine proteinase inhibitor alpha-1 anti-trypsin (AAT) protects the body against protease activity. Several functions of AAT beyond those attributed to its anti-protease activity have been described, among them immunomodulatory and anti-inflammatory properties. The present study aimed to determine the efficacy of AAT for the treatment of immune-mediated liver injury using the models of concanavalin A-induced immune-mediated hepatitis and acetaminophen -induced liver damage. ***Methods:*** AAT was administered to mice subjected to concanavalin A-induced immune-mediated hepatitis or 2 h after acetaminophen-induced liver damage. Mice were followed for changes in serum levels of liver enzymes, liver histology, and for interferon gamma serum levels. ***Results:*** Treatment with AAT alleviated concanavalin A-induced immune-mediated liver damage, as demonstrated by a reduction in the serum levels of liver enzymes and interferon gamma, and an improved lymphocyte infiltration into the liver on liver biopsies. Moreover, treatment with AAT was associated with alleviation of the acetaminophen-induced liver injury. ***Conclusions:*** AAT exerts an hepatoprotective effect on immune-mediated and drug-induced liver damage. The data support its potential use in patients with immune-associated liver disorders.

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Introduction

The serine proteinase inhibitor (serpin) alpha-1 anti-trypsin (AAT) protects the body against protease activity.[1] AAT is the most abundant circulating serpin and acts as an acute phase reactant.[2] Mutations in the AAT gene (SERPINA1) that lead to a deficiency in AAT are associated with chronic obstructive pulmonary disease. The Z mutation encodes a misfolded variant of AAT that is not efficiently secreted and accumulates intracellularly in the endoplasmic reticulum of AAT-producing hepatocytes.[1]

Multiple functions of AAT beyond those attributed to its anti-protease activity have been described.[2] AAT plays a role in immunomodulation, inflammation, proteostasis, and apoptosis.[2] AAT also displays properties affecting a wide range of inflammatory cell types.[3] The tolerogenic properties of AAT are associated with the induction of T-regulatory lymphocytes (also known as regulatory T cells, Tregs) in a B cell-dependent manner.[4]

AAT protects pancreatic islets from allorejection and autoimmune damage.[5] AAT administered as monotherapy induces anti-inflammatory conditions in the setting of pancreatic islet transplantation, which favors the development of antigen-specific Tregs.[6] Neutrophil elastase, which is secreted by activated neutrophils, circulates in the plasma, further promoting inflammation and fibrosis. AAT has been shown to interfere with disease progression in experimental autoimmune encephalomyelitis and collagen-induced arthritis models.[5]

Patients with systemic sclerosis displayed a deficiency in serum AAT levels, supporting its anti-inflammatory effect.[7] AAT deficiency has also been demonstrated as correlated with antineutrophil cytoplasmic antibody-associated vasculitis.[8] Its levels are also decreased in Wegener's granulomatosis patients, and may be involved in disease pathogenesis and the worsening of these patients' clinical manifestations. AAT contributes to the pathogenesis of ankylosing spondylitis, as well, by up-regulating gene expression in synovial tissues.[9] Finally, in patients with acute myocardial infarction, the left ventricular ejection fraction is inversely correlated with AAT concentrations in the serum, suggesting that systolic dysfunction is associated with an inflammatory response.[10]

The hepatoprotective role of AAT has been previously described. Systemic treatment with AAT decreased Jo2-induced liver cell apoptosis in the Jo2 FAS/CD95 activating liver failure model. Increased survival and a reduction of apoptotic hepatocytes were also observed in the alpha-amanitin and acetaminophen (APAP)-induced liver injury mouse models.[11] The effect of A1AT therapy was determined in A1AT or A1AT plus oncostatin M treated primary human hepatocytes isolated from liver tissues obtained from A1AT-deficient patients. In a dose-dependent manner, purified A1AT lowered SERPINA1 expression in the hepatocytes. This latter effect was more prominent in hepatocytes stimulated with oncostatin M.

It has been suggested that augmentation with native M-A1AT protein and a parallel reduction in expression of dysfunctional mutant Z-A1AT may be beneficial for severe

Keywords: Alpha-1 anti-trypsin; Chronic liver disease; Acetaminophen; Drug-induced liver injury.

Abbreviations: AAT, alpha-1 anti-trypsin; ALT, alanine aminotransferase; APAP, acetaminophen; AST, aspartate aminotransferas; ConA, concanavalin A; DILI, drug-induced liver injury; IFN_{γ}, interferon gamma; NASH, nonalcoholic steatohepatitis; serpin, serine protease inhibitor; T1D, type 1 diabetes; Tregs, regulatory T cells.

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***Correspondence to:** Yaron Ilan, Department of Medicine, Hebrew University-Hadassah Medical Center, P.O.B 12000, Jerusalem, IL-91120, Israel. Tel: +972-2-6778231, Fax: +972-2-6431021, E-mail: ilan@hadassah.org.il

AAT-deficient liver, and this motivates further studies.[12] The present study aimed to determine the potential efficacy of AAT for the treatment of immune-mediated liver injury using models of concanavalin A (ConA)-induced hepatitis and APAP-induced liver damage to determine its potential systemic immunomodulatory effect.

Methods

Animals

Male C57BL/6 mice (11–12 weeks old) were obtained from Harlan Laboratories (Jerusalem, Israel) and maintained in the Animal Core of the Hadassah-Hebrew University Medical School. Mice received standard laboratory chow and water *ad libitum* and were housed in a 12-hour light/dark cycle. Animal experiments were performed according to the guidelines and with the approval of the Hebrew University-Hadassah Institutional Committee for Care and Use of Laboratory Animals.

Induction of ConA-induced hepatitis

ConA (MP Biomedicals, Santa Ana, CA, USA) was dissolved in a solution consisting of 50 mM Tris pH 7, 150 mM NaCl, and 4 mM $CaCl_2$, and was injected into the tail vein at a dose of 500 μg/mouse (15 mg/kg). Mice were sacrificed 15 h after ConA injection.

Experimental groups

Two consecutive experiments were conducted. In the first study, four groups of mice were used (n = 5/group). Mice in the control groups were treated with 0.35 mg/mouse double-distilled water or dexamethasone 2 h before ConA injection; the two AAT-treated groups (AAT A-9024; Sigma, St. Louis, MO, USA) were orally administered with 1.0 or 0.2 mg/mouse of AAT. In the second experiment, 5 groups (n = 6/group) were studied. Mice were treated with double-distilled water or with dexamethasone, or with one of three AAT dosages (0.35, 1.0 or 2.0 mg/mouse) administered intraperitoneally.

Induction of APAP-mediated hepatotoxicity

APAP-mediated hepatotoxicity was induced in mice via oral administration of 4 mg of APAP. Liver toxicity was determined based on the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels.

Experimental groups

Three groups of mice were used (n = 5/group). At 2 h after the oral administration of APAP, the mice were treated with double-distilled water or with one of two AAT dosages (25 or 0.5 mg/mouse) administered intraperitoneally.

Assessment of the effect of AAT treatment on liver damage

Liver enzymes. Serum was obtained from individual mice. Serum AST and ALT levels were determined using an automated analyzer.

Cytokine measurement. Serum interferon-gamma (IFN-γ) levels were measured in each animal using a commercially available "sandwich" ELISA kit (Quantikine; R&D Systems, Minneapolis, MN, USA).

Histological examination of the liver. Paraffin-embedded liver sections were prepared from each mouse. Organs were sliced into 4–5-μm-thick parts, and these sections were stained with hematoxylin-eosin. The sections were scored according to the extent of liver damage using a previously described method[13,14] with the following parameters: lymphocyte adhesion to hepatic and portal veins and sinusoids, the number of infiltrating leukocytes into the liver parenchyma, and the number of necrotic lesions (all per 10X high-power field).

Statistical analysis

The comparison of two independent groups' means was performed using a two-sided student's t-test. A p value of <0.05 was considered significant.

Results

AAT alleviated the immune-mediated liver damage induced by ConA

The immunomodulatory effect of AAT was assessed in the ConA-induced hepatitis model via the measurement of liver enzymes and pathology. Fig. 1 shows the impact of

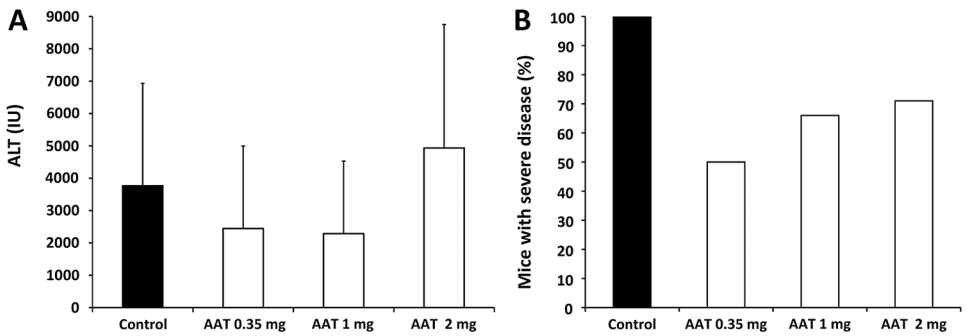

Fig. 1. Effect of AAT on immune-mediated liver damage in the ConA-induced hepatitis model. A. Mice were treated intraperitonally with three dosages of AAT following ConA administration; after 15 h, serum ALT levels were measured. B. Percentage of mice developing the significant disease (ALT >1000 IU) was reduced in the mice treated with 0.35 mg AAT.

Abbreviations: AAT, alpha-1 anti-trypsin; ALT, alanine aminotransferase; ConA, concanavalin A.

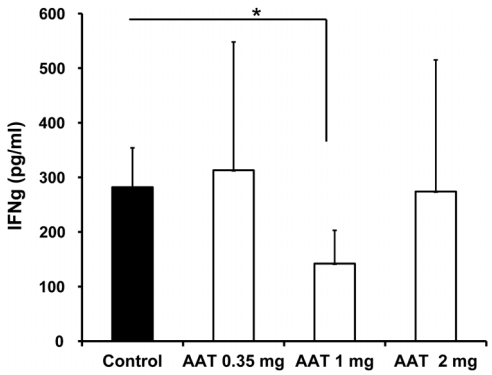

Fig. 2. Effect of AAT on the inflammatory response in the ConA-induced hepatitis model. Serum IFNγ levels were measured via ELISA and were significantly reduced in the mice treated with 0.35 mg AAT.

Abbreviations: AAT, alpha-1 anti-trypsin; ConA, concanavalin A.

intraperitoneal administration of AAT on serum ALT levels (Fig. 1A). Intraperitoneal administration of AAT exerted a dose-dependent beneficial effect on liver damage. A trend for reduction in the liver enzymes was detected for the 0.35 mg and 1 mg treated groups but not for the 2 mg treated group. AST levels corresponded to the ALT levels (p = non-significant).

Fig. 1B shows that the number of mice developing severe disease (ALT >1000 IU) was markedly reduced from 100% in the controls to 50% in the 1 mg parenteral AAT-treated groups ($p < 0.005$). No effect was noted in orally treated mice.

Fig. 2 shows the effect of AAT on the serum IFNγ levels. A significant reduction in IFNγ serum levels was observed in mice treated with the intraperitoneal dose of 1.0 mg ($p < 0.05$), but not for those receiving the low (0.35 mg) or high (2.0 mg) dosages.

Table 1 shows the effect of the intraperitoneal administration of AAT on the histological score. The mice receiving an intraperitoneal dose of 0.35 mg of AAT exhibited a significant improvement in the histological score for infiltrating lymphocytes ($p < 0.05$). Fig. 3 shows representative sections from mice in the AAT-treated and saline-treated control groups, indicating a decrease in lymphocyte infiltration to the liver.

AAT alleviated APAP-mediated liver damage

The hepatoprotective effect of AAT was tested in the APAP-mediated liver damage model. Mice were treated with one of two dosages of AAT at 2 h after the induction of injury. Fig. 4 shows that both the 0.25 mg and 0.5 mg dosages of AAT were associated with a trend for a decrease in the serum AST levels (p = non-significant). A similar trend on the serum ALT levels was observed (p = non-significant).

Discussion

The data of the present study show that treatment with AAT exerts a hepatoprotective effect on animal models of immune-mediated hepatitis and APAP-induced liver damage. AAT treatment is currently under examination in several clinical trials for its potential beneficial impact on a variety of diseases in which the immune system plays a role in pathogenesis.[15] The immune system plays a significant role in the pathogenesis of several liver disorders. In addition to the "classical" immune-mediated liver disorders, including autoimmune hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis, the immune system also plays a role in alcoholic and nonalcoholic steatohepatitis (NASH).[16–18]

In the present study, intraperitoneal administration of AAT exerted a dose-dependent beneficial effect on the ConA immune-mediated hepatitis model, as demonstrated by a reduction in serum levels of liver enzymes and IFNγ. The number of mice developing severe disease was markedly reduced from 100% in controls to 50% in AAT-treated mice. Mice treated with the 0.35 mg intraperitoneal dose displayed a significant improvement in the histological score for infiltrating lymphocytes. As liver enzymes and IFNγ serum levels change over time in this model, following them along the experimental course may have shown better correlation. The lack of an observed effect of orally administered AAT may be attributed to the lack of its absorption or the lack of a result of AAT on the immune cells in the gut.

Drug-induced liver injury (DILI) is a common cause for drug withdrawal from the market and can result in severe clinical outcomes.[19] Idiosyncratic DILI development is mediated by multiple events, including reactive metabolite formation, oxidative stress, and signaling pathway inductions, and may involve innate and adaptive immune responses.[20] Cytotoxic T lymphocytes were proposed to play an essential role in the pathogenesis of the liver damage mediated by several drugs.[21] Immunomodulatory agents are being developed as potential treatments for NASH and DILI.[22–27] APAP is a commonly used antipyretic and analgesic agent. APAP overdose and the resulting hepatotoxicity constitute a significant health problem. APAP overdose may lead to severe and even fatal hepatotoxicity.[28] The APAP-mediated liver injury is characterized by high serum levels of liver enzymes and may invovle immune mediated injury.[29] Our data suggest a potential for AAT to exert a hepatoprotective effect on the APAP-mediated liver damage model. Both the 0.25 mg and 0.5 mg dosages were associated with a trend for a decrease in the serum liver enzyme levels.

Although AAT is not a "classic" anti-inflammatory or immunosuppressive agent, it exerts several immunomodulatory

Table 1. Effect of AAT on the histological score in the ConA-induced hepatitis model (mean ± SD)

Group	Treatment	Lymphocyte adhesion, %	Infiltrating leukocytes, %	Necrotic lesions, %
A	Control	2.53 ± 13	3.4 ± 8.5	0.98 ± 1.83
B	Dexamethasone	0.58 ± 2.5*	0.58 ± 2.5*	0*
C	AAT 0.35 mg IP	2.8 ± 11.89	2.45 ± 8.33*	1 ± 1.67

*$p < 0.05$.

Abbreviations: AAT, alpha-1 anti-trypsin; ConA, concanavalin A; IP, intraperitoneal.

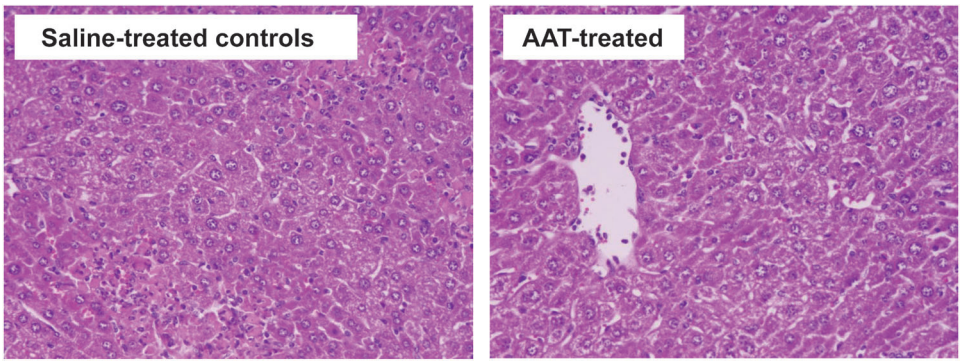

Fig. 3. Effect of AAT on histology in the ConA-induced hepatitis model. A. Saline-treated control mice displayed lymphocyte adhesion to veins and sinusoids, necrotic foci, and leukocyte infiltration into the parenchyma. B. AAT-treated mice displayed reduced leukocyte infiltration into the parenchyma.

Abbreviations: AAT, alpha-1 anti-trypsin; ConA, concanavalin A.

effects.[15] AAT was reported to affect key immune-related signaling pathways in models of disease in which it has been shown to be therapeutically effective.[3] The anti-inflammatory activity of AAT may result from inhibiting neutrophil enzymes.[15] However, AAT also exhibits immune tolerogenic activities that are not straightforwardly explained by serine protease inhibition or by suppressed inflammatory function.[15] These tolerogenic activities are also beyond reduced inflammatory parameters.[15] It was recently suggested that unlike immunosuppression, AAT might assist the immune system to distinguish between beneficial responses against threats and unwanted immune-mediated reactions which may lead to target organ damage.[15]

At the molecular level, AAT docks to cholesterol-rich lipid-rafts and circulating lipid particles and directly binds to IL-8, ADAM17, and damage-associated molecular patterns.[15] It may also exert an effect on autophagy, thereby affecting endoplasmic reticulum function.[30] At the cellular level, peripheral blood T lymphocytes stimulated with a mitogen display granular cytoplasmic expression of AAT.[31] AAT reduces a detrimental tumor necrosis factor alpha-dependent effect on lymph nodes in adipose and pancreatic tissues.[32] It reduces levels of inflammatory markers, promoted the lipopolysaccharide-induced dendritic cell phenotype, facilitates Treg expansion, and protects pancreatic islets from alloimmune and autoimmune responses.[33] AAT-treated stimulated dendritic cells displayed reduced levels of MHC class II, CD40, CD86, and IL-6, and increased levels IL-10 and sustained levels of inducible CCR7. AAT-treated cells also exhibited enhanced chemokine-dependent migration and low surface levels of CD40.[33] However, AAT does not block dendritic cell activities, nor does it promote viral and tumor susceptibilities.[15] Therefore, AAT is neither a typical anti-inflammatory nor a direct immunosuppressive compound.[15] AAT increases IL-1 receptor antagonist expression in human mononuclear cells and promotes Tregs in animal models.[5] *In vitro*, AAT suppressed lipopolysaccharide-induced secretion of pro-inflammatory cytokines such as tumor necrosis factor alpha and IL-1beta, enhanced the production of the anti-inflammatory cytokine IL-10, and impaired the translocation of NF-kappaB in dendritic cells.[34] AAT also plays a role in organ defense against damage-associated molecular patterns and pathogen-associated molecular patterns caused by cigarette smoke or infections.[2]

In vivo, administration of AAT after bone marrow transplantation decreased mortality in three models of acute graft-versus-host disease, and reduced serum levels of pro-inflammatory cytokines in transplant recipients.[34] AAT treatment reduced the expansion of alloreactive effector T cells but enhanced the recovery of Tregs, thereby altering the ratio of donor T effector cells to Tregs.[34] AAT reduced the bacterial burden after infection via the modulation of the host immune response.[35]

AAT has been shown to prevent type 1 diabetes (T1D) development, to prolong islet allograft survival and to inhibit pancreatic B cell apoptosis *in vivo*.[5] Short-term treatment with AAT in non-obese diabetic mice, which are afflicted with both T1D and type 2 diabetes, restores euglycemia, immune tolerance to self-islets, and insulin signaling.[32] In humans with T1D, significantly lower plasma concentrations of AAT were reported, suggesting a potential role of AAT in the pathogenesis of T1D.[36] In these patients, circulating levels of the heat-shock protein gp96 are elevated and are bound to AAT. Recent data suggested that the deficiency of AAT is associated with an increased risk of developing type 2 diabetes.[36–38]

The results of the present study support the hepatoprotective effect of AAT. Further studies are required to elucidate the mechanism of action in different models. In light of recent

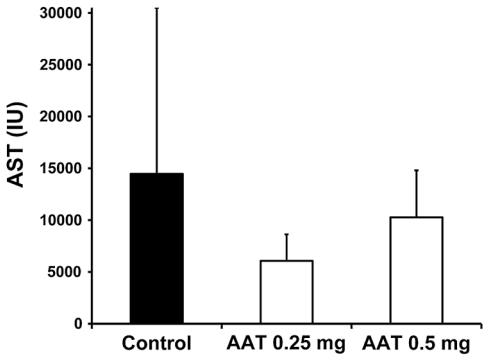

Fig. 4. Effect of AAT on APAP-induced liver damage. Mice were treated with three different intraperitoneal dosages of AAT at 2 h after APAP administration, followed by measurement of ALT serum levels.

Abbreviations: AAT, alpha-1 anti-trypsin; ALT, alanine aminotransferase; APAP, acetaminophen.

ongoing trials of AAT in patients with diabetes,[37,38] and considering its high clinical safety record, the data of the present study suggest its potential use for liver disorders in which the immune system is involved in the pathogenesis of, including NASH, and for prevention or treatment of DILI.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Designed the study (YI, ABY), wrote the manuscript (YI, ABY, YS), conducted the experiments (ABY).

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