Glucosidase and Mannosidase Inhibitors Mediate Increased Secretion of Mutant α1 Antitrypsin Z*

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It is now well known that the addition and trimming of oligosaccharide side chains during post-translational modification play an important role in determining the fate of secretory, membrane, and lysosomal glycoproteins. Recent studies have suggested that trimming of oligosaccharide side chains also plays a role in the degradation of misfolded glycoproteins as a part of the quality control mechanism of the endoplasmic reticulum (ER). In this study, we examined the effect of several inhibitors of carbohydrate processing on the fate of the misfolded secretory protein α1 antitrypsin Z. Retention of this misfolded glycoprotein in the ER of liver cells in the classical form of α1 antitrypsin (α1-AT) deficiency is associated with severe liver injury and hepatocellular carcinoma and lack of its secretion is associated with destructive lung disease/emphysema. The results show marked alterations in the fate of α1 antitrypsin Z (α1-ATZ). Indeed, one glucosidase inhibitor, castanospermine (CST), and two mannosidase inhibitors, kifunsesine (KIF) and deoxymannojirimycin (DMJ), mediate marked increases in secretion of α1-ATZ by distinct mechanisms. The effects of these inhibitors on secretion have interesting implications for our understanding of the quality control apparatus of the ER. These inhibitors may also constitute models for development of additional drugs for chemoprophylaxis of liver injury and emphysema in patients with α1-AT deficiency.

Recent studies have provided further evidence that asparagine-linked oligosaccharide side chains play an important role in intracellular transport of glycoproteins. For one, mannose-6-phosphate modification is a key determinant of sorting to the lysosome (1). Second, a number of studies have now shown that transport of secretory and membrane glycoproteins from the ER to their appropriate destination depends on the interaction of the innermost glucose residue of the oligosaccharide side chains with the resident ER molecular chaperones calnexin and calreticulin (2, 3). This means that trimming of the N-glycan by glucosidases I and II and interaction with calnexin and calreticulin facilitate the proper folding and translocation of wild type glycoproteins. There is also evidence that trimming of glucose residues by glucosidases and of mannose residues by ER mannosidases is involved in the degradation of misfolded, unassembled, or mutant glycoproteins (4–10). Third, Nichols et al. (11) have shown that ERGIC-53, a lectin which is specifically localized in the ER-Golgi intermediate compartment, is mutated in the combined deficiency of coagulation factors V and VIII. These results suggest that a lectin-like mechanism involving the interaction of carbohydrate side chains with ERGIC-53 is required for secretion of factors V and VIII.

In the classic type of α1 antitrypsin (α1-AT) deficiency, the most common genetic cause of emphysema in adults and of liver disease in children, the mutant glycoprotein α1-ATZ is retained in the ER of liver cells rather than secreted into the extracellular fluid (12). The mutant α1-ATZ molecule is characterized by a single amino acid substitution, which results in its polymerization in the ER (13, 14). However, the mutant protein retains ~80% of the functional activity of its wild type counterpart, inhibition of neutrophil elastase (15, 16). Because of the lack of this elastase inhibitor in the lung, deficient individuals often develop destructive lung disease/emphysema (12). A subgroup of α1-AT-deficient individuals, predominantly infants and children, also develop chronic liver disease apparently because of the hepatotoxic effect of the mutant α1-ATZ molecule retained in the ER (12). Recent studies have indicated that this subgroup is “susceptible” to liver injury by virtue of a lag in ER degradation resulting in greater accumulation of the misfolded hepatotoxic α1-ATZ molecule in liver cells (17). There are at least two and perhaps more pathways responsible for degradation of α1-ATZ in the ER. One pathway involves stable binding of α1-ATZ to calnexin, conjugation of ubiquitin to the cytoplasmic tail of complexed calnexin, and degradation of the α1-ATZ-polyubiquitinated calnexin complex by the proteasome (18). There also appears to be a ubiquitin-independent proteasomal mechanism for degradation of α1-ATZ in the ER (19).

In the current study, we used glucosidase and mannosidase inhibitors to examine the role of oligosaccharide side chain trimming in the fate of α1-ATZ. Previous studies have shown that glucosidase and mannosidase inhibitors inhibit secretion of wild type α1-AT (20). Studies of another mutant α1-AT molecule which is retained and degraded in the ER, α1-ATHONG KONG, have shown that glucosidase and mannosidase inhibitors alter ER degradation—accelerated by glucosidase inhibitors and delayed by mannosidase inhibitors (4, 5). However, there are no previous reports of the effect of these inhibitors on mutant α1-ATZ. The results show that there are effects on ER degradation but, to our surprise, several glucosidase and...
mannosidase inhibitors mediated an increase in secretion of α1-ATZ. Because the mutant α1-ATZ molecule partially retains functional activity (15, 16) and because clinical studies have suggested that only partial correction is needed for prevention of both liver and lung injury in α1-AT deficiency (17, 21), the effects of this class of drugs are of therapeutic as well as pathobiologic interest.

EXPERIMENTAL PROCEDURES

Materials—Neutrophil elastase was obtained from Athens Research and Technology, Inc. (Athens, GA). Kifunensine (KIF), castanospermine (CST), and N-methyldeoxynojirimycin (MDNJ) were obtained from Toronto Research Chemicals (Ontario, Canada). N-butyldexyozojirimycin (BDNJ) was a generous gift from Dr. G. Jacob (Monsanto, St. Louis, MO). 1,4-Dideoxy-1,4-imino-α-mannitol hydrochloride (DIM) was obtained from Sigma. Deoxymannojirimycin (DMJ) was obtained from Calbiochem. Endoglycosidase H (Endo H) and N-glycosidase F (PNGase F) were obtained from Roche Molecular Biochemicals.

Cell Lines, Metabolic Labeling, Immunoprecipitation, SDS-PAGE—The human fibroblast cell line CJZ12B engineered for stable expression of mutant α1-AT was derived from an α1-AT-deficient patient (17). The method of Butcher et al. (18) was used. Purified α1-ATZ mRNA was used to program insecticidal microsomes for translation over 60 min at 37 °C. The microsomal vesicles were then isolated by centrifugation and incubated in a proteolysis-primed lysate at 37 °C for the indicated time intervals. Aliquots were taken at the indicated time intervals, and the samples were resolved on 10% SDS-PAGE gels. Co-immunoprecipitation with anti-GRP78 antibody was done using a nondenaturing buffer of 1% Nonidet P-40 detergent, 7.5 mM CHAPS in 50 mM HEPES, pH 7.5, with 200 mM NaCl (HBS) and apryrase 50 units/ml.

Endoglycosidase H and N-Glycosidase F Digestion—Samples were immunoprecipitated with antibody to α1-AT, and the immunoprecipitates were resuspended in 20 μl of 50 mM Tris, pH 6.8, 0.5% SDS, and 0.1 mM β-mercaptoethanol. Samples were heated at 90–100 °C for 5 min. For Endo H digestion, the 20-μl samples were diluted with 17 μl of 0.15 M NaOAc, pH 6, and 0.5 μl of 0.1 μM PMSF. Endo H was added using 3 μl of a 1 units/μl solution. For N-glycosidase F digestion, the resuspended samples were added to 8 μl of 1 M NaPO4 buffer, pH 8.3, 5 μl of 14% Nonidet P-40 detergent, 7.5 μl of 250 units/μl N-glycosidase F, and 0.5 μl of 0.1 mM PMSF. All samples were incubated overnight at 37 °C and then dissolved in sample buffer for SDS-PAGE.

Formation of Complexes with Neutrophil Elastase—Cells were subjected to a pulse-chase protocol, and the EC medium was harvested
The absence of CST. The half-times for disappearance of $a$ of the newly synthesized $a$ between 2 and 4 h of the chase period, but in this case there was interaction with anticalnexin (center panels). 

Panel A: The reaction mixture was preincubated for 60 min at 30 °C in the absence (Control, left panel; Post-CST, right panel) or presence of CST (Pre-CST, center panel). Aliquots of the reaction were run directly (left panels) or homogenized under nondenaturing conditions and subjected to immunoprecipitation with anti-1-ATZ (center panels) or anti-BiP (right panels).

Panel B: The untrimmed slower migrating 1-ATZ polypeptide also disappears from IC after 6 h of the chase period. This medium was incubated for 30 min at 37 °C with neutrophil elastase, in amounts from 1 ng to 5 μg in 0.5 ml of 50 mM Tris buffer, pH 8.0, and then subjected to immunoprecipitation with anti-1-AT for SDS-PAGE.

RESULTS AND DISCUSSION

Effect of Glucosidase Inhibitors on the Fate of 1-ATZ—First, we examined the effect of several glucosidase inhibitors, CST and MDNJ, on the relative electrophoretic migration of 1-ATZ (Fig. 1A). In the absence of drug, 1-ATZ migrates at $\sim$ 52 kDa. Previous studies have shown that this 52-kDa polypeptide represents a biosynthetic intermediate with high-mannose-type oligosaccharide side chains (17). In the presence of CST or MDNJ, 1-ATZ migrates slightly more slowly, reflecting inhibition of glucose removal. Next we examined the effect of CST on the fate of 1-ATZ in pulse-chase experiments (Fig. 1B). The results show that in the absence of CST, the 52-kDa 1-ATZ polypeptide is retained for 1 h but begins to disappear from IC between 2 and 4 h, with only trace amounts of the 55-kDa mature 1-AT polypeptide appearing in EC. In the presence of CST, the untrimmed 1-ATZ polypeptide also disappears from IC between 2 and 4 h of the chase period, but in this case there is a marked increase in the amount of mature 55 kDa 1-ATZ secreted into the EC medium. By 6 h into the chase period, 31 ± 2% of the newly synthesized 1-ATZ was secreted into the medium in the presence of CST as compared with 17 ± 3% in the absence of CST. The half-times for disappearance of 1-ATZ from IC were 0.92 ± 0.35 h in the absence and 0.90 ± 0.26 h in the presence of CST. These results indicate that there is increased secretion of 1-ATZ in the presence of CST.

We also examined the effect of the glucosidase inhibitor MDNJ on the fate of 1-ATZ in C172 cells (Fig. 1C). The results also show that the untrimmed 1-ATZ generated in the presence of MDNJ disappears from IC more rapidly but, in contrast to CST, there was no increase in the secretion of 1-ATZ. These results indicate that the effect of CST on secretion of 1-ATZ is specific. Experiments with a third glucosidase inhibitor, BDNJ, show that it also does not increase secretion of 1-ATZ (data not shown). It is not clear at this time why CST, but not DNJ or MDNJ, mediates increased secretion. Previous studies have suggested that there may be differences in the efficiency of the glucosidase inhibition and in the number of glucose residues on the three oligosaccharide side chains of wild type 1-AT after treatment with CST, DNJ, and MDNJ (20, 22). Alternatively, CST may mediate the increased secretion of 1-ATZ through a secondary effect or through an effect that is unrelated to glucosidase inhibition.

Next, we examined the effect of CST on ER degradation of 1-ATZ. CST is known to prevent the interaction of glycoproteins with calnexin (2, 3). Our previous studies have indicated that interaction with calnexin is, at least in part, required for proteasomal degradation of 1-ATZ and that it is the 1-ATZ-calnexin complex which is targeted by the ubiquitin system and the proteasome (18). Although careful accounting for radioactivity in the pulse-chase experiments in intact cells in Fig. 1B suggests that there is no difference in the disappearance of 1-ATZ from IC in the presence of CST, we chose to more specifically determine the effect of CST on degradation of 1-ATZ here using the cell-free microsomal translocation system. Previous studies have indicated that 1-ATZ is specifically degraded in this cell free system by a mechanism which involves the proteasome and which closely recapitulates the degradation of 1-ATZ in intact cells (18). In Fig. 2A, left panel, the 52-kDa 1-ATZ polypeptide begins to disappear between 15–30 min and is completely degraded between 30–45 min of the chase period. When the reaction is preincubated with CST (center panel), the untrimmed slower migrating 1-ATZ polypeptide is degraded slightly more slowly, beginning 30 min and only completed by 45–90 min of the chase period. When the
A. little a subjected to immunoprecipitation (left panel) using a protocol similar to the one in Fig. 1A. Compared with the control, a slightly lower mobility of a1-ATZ is seen with DMJ or KIF treatment but not with DIM treatment. B–D. fate of a1-ATZ in the presence of DMJ 1 mM (B), KIF 0.3 mM (C), or DIM 1 mM (D). A pulse-chase protocol similar to that in Fig. 1F was used. With DMJ and KIF, intracellular and secreted forms of a1-ATZ have approximately the same mobility. With DIM, the secreted a1-ATZ has a mobility intermediate between that in control and that in the presence of KIF or DMJ. In three separate experiments in each case, the half-times for disappearance of a1-ATZ from IC were 0.95 ± 0.22 h in the absence and 2.78 ± 1.1 h in the presence of KIF. The fraction secreted was 0.11 ± 0.05 in the absence and 0.31 ± 0.09 in the presence of KIF. The half-times for disappearance of a1-ATZ from IC were 0.82 ± 0.06 h in the absence and 2.12 ± 0.65 h in the presence of DMJ. The fraction secreted was 0.17 ± 0.06 in the absence and 0.28 ± 0.03 in the presence of DMJ.

CST is only added during the chase period (right panel), a condition which does not interfere with initial trimming by glucosidase but does prevent dissociation of glycoproteins from calnexin (23), the untrimmed 52-kDa a1-ATZ polypeptide is degraded at a rate which is not slowed but rather is very close to that in control with disappearance beginning between 15–30 min and completed between 30–45 min.

In Fig. 2B, microsomal vesicles from these reactions were homogenized under nondenaturing conditions and subjected to immunoprecipitation with antibodies to calnexin and GRP78/ BiP. This protocol was designed to determine whether a1-ATZ could be co-immunoprecipitated by anticalnexin and/or anti-BiP and, if so, to determine the kinetics of a1-ATZ-calnexin and a1-ATZ-BiP association and dissociation. The results show that trimmed a1-ATZ is co-immunoprecipitated by anticalnexin early in the chase period but this complex disappears between 30–45 min of the chase period (center panel, control). The kinetics of disappearance of the a1-ATZ-calnexin complex is similar to those of a1-ATZ in reactions that have not been subjected to immunoprecipitation (left panel, control). Very little a1-ATZ is co-precipitated by anticalnexin in reactions that were preincubated with CST (center panel, CST). Although very little a1-ATZ is co-precipitated with anti-BiP under control conditions (right panel, control), some untrimmed a1-ATZ is immunoprecipitated with anti-BiP after preincubation with CST (right panel, CST). This a1-ATZ-BiP complex is detected at the beginning of the chase period and very rapidly disappears. Previous studies have shown that misfolded proteins may aggregate in association with BiP when glucose trimming is inhibited (24). This data suggests that in the presence of CST there is less, or negligible, interaction of untrimmed a1-ATZ with calnexin, that a certain proportion of the untrimmed a1-ATZ has bound to BiP, and that the untrimmed a1-ATZ-BiP complex is rapidly dissociated or degraded. Thus, when CST inhibits glucose trimming and, in turn, interaction with calnexin, a1-ATZ is degraded rapidly by an alternative degradative pathway that involves an untrimmed a1-ATZ-BiP complex. This alternative degradative pathway appears to also involve the proteasome because it is significantly abrogated by the proteasomal inhibitors MG132 in the cell free system (data not shown) as well as in CJZ12B cells (Fig. 3). This alternative degradative mechanism also probably explains the relatively modest inhibition of a1-ATZ degradation in the cell free system after preincubation with CST. Taking into consideration differences in fluorographic exposure time for the panels in Fig. 2B, we estimate that only 5–10% of total a1-ATZ is co-immunoprecipitated with anti-BiP antibody in the presence of CST. This implies that one or more additional mechanisms are involved in degradation of a1-ATZ under these conditions.

Effect of Mannosidase Inhibitors on the Fate of a1-ATZ—Several recent studies have indicated that ER mannosidases play a role in the processing of carbohydrate side chains of some glycoproteins in the ER (4, 5). Here (Fig. 4) we examined the effect of a mannosidase I inhibitor, KIF, a mannosidase II inhibitor, DIM, and an inhibitor of both mannosidases I and II, DMJ (25). The effect of these inhibitors on the relative electrophoretic mobility

Fig. 4. Effect of a mannosidase I and II inhibitors on the fate of a1-ATZ. A, mobility of a1-ATZ in the absence of mannose trimming. CJZ fibroblasts were incubated with serum-free control medium or serum-free medium supplemented with DMJ (1 mM), KIF (0.3 mM), DIM (1 mM), or a mannosidase inhibitor, CST (23). B–D, fate of a1-ATZ in the presence of DMJ 1 mM (B), KIF 0.3 mM (C), or DIM 1 mM (D). A pulse-chase protocol similar to that in Fig. 1F was used. With DMJ and KIF, intracellular and secreted forms of a1-ATZ have approximately the same mobility. With DIM, the secreted a1-ATZ has a mobility intermediate between that in control and that in the presence of KIF or DMJ. In three separate experiments in each case, the half-times for disappearance of a1-ATZ from IC were 0.95 ± 0.22 h in the absence and 2.78 ± 1.1 h in the presence of KIF. The fraction secreted was 0.11 ± 0.05 in the absence and 0.31 ± 0.09 in the presence of KIF. The half-times for disappearance of a1-ATZ from IC were 0.82 ± 0.06 h in the absence and 2.12 ± 0.65 h in the presence of DMJ. The fraction secreted was 0.17 ± 0.06 in the absence and 0.28 ± 0.03 in the presence of DMJ.
of α1-ATZ is shown in Fig. 4A. There is slightly slower electrophoretic migration in the presence of KIF and DMJ but not in the presence of DIM. Next, we examined the effect of these inhibitors on the fate of α1-ATZ in pulse-chase experiments (Fig. 4, B–D). In the presence of KIF and DMJ, the untrimmed α1-ATZ polypeptide is retained IC for a longer period of time than in control, and there is a marked increase in the amount of α1-ATZ that appears EC. The α1-ATZ polypeptide that is secreted in the presence of KIF and DMJ is 52 kDa as compared with the 55-kDa polypeptide secreted in the presence of CST. In contrast, the mannosidase II inhibitor DIM has no effect on degradation or secretion of α1-ATZ (Fig. 4D). DIM is active in these experiments as evidenced by the fact that the small amount of α1-ATZ that reaches the EC fluid has a more rapid relative electrophoretic mobility at ~53 kDa. These data indicate that DMJ and KIF mediate a marked decrease in degradation and an increase in secretion of α1-ATZ. The effect of DMJ and KIF on secretion of α1-ATZ probably involves a different mechanism than the effect of CST because the α1-ATZ that is secreted in each of these cases has a different electrophoretic mobility. The data also implicate ER mannosidase I but not ER mannosidase II in playing an important role in the fate of the misfolded α1-ATZ molecule.

Characterization of Carbohydrate Side Chains of α1-ATZ Secreted in the Presence of CST or KIF—To determine whether α1-ATZ secreted in the presence of CST or KIF has high mannose- or complex-type oligosaccharide side chains, we examined the effect of Endo H and PNGase F (Fig. 5A). In HepG2 cells, the 55-kDa wild type α1-AT in the extracellular fluid was resistant to digestion with Endo H but cleaved to an ~46-kDa polypeptide by PNGase F as expected (left panel). In CJZ12B cells treated with CST, the 55-kDa α1-AT polypeptide was partially resistant and partially sensitive to Endo H (center panel). A 46-kDa Endo H-sensitive polypeptide probably represents the cleavage product of α1-ATZ with high-mannose carbohydrate. The ~48- and ~52-kDa partially Endo H-sensitive polypeptides probably represent cleavage products of α1-ATZ with high-mannose carbohydrates on one or two of its three carbohydrate side chains. The 55-kDa Endo H-resistant polypeptide probably represents α1-ATZ with complex carbohydrates at all three of its side chains. This pattern was not because of incomplete digestion because it did not change with higher concentrations of Endo H (data not shown). An identical result has previously been observed for the 55-kDa wild type α1-AT polypeptide secreted by hepatocytes in the presence of CST (20). Maturation of one, two, or all three carbohydrate side chains to the complex type in the presence of CST is probably a result of the cell type-specific expression in Golgi of endo-α-d-mannosidase which can deglucosylate glycoproteins during inhibition of glucosidases (26). In CJZ12B cells treated with CST, the 55-kDa α1-ATZ polypeptide was cleaved to 46 kDa by PNGase F as expected (center panel).

In CJZ12B cells treated with KIF, the 52-kDa α1-ATZ polypeptide was completely Endo H-sensitive being cleaved to 46 kDa (right panel), indicating that it is an intermediate with high-mannose carbohydrate side chains.

Functional Activity of α1-ATZ Secreted in the Presence of CST or KIF—We examined the possibility that α1-ATZ secreted in the presence of CST, KIF, or DMJ could form an SDS-stable complex with neutrophil elastase (Fig. 5B). The results show that wild type α1-AT from HepG2 cells forms ~66- and ~75-kDa high molecular mass complexes with elastase (left panel). Complexes begin to form at 0.1 μg of elastase added, with complete conversion by 0.5 μg of elastase added. The ~66-kDa band probably represents complexes that have undergone partial hydrolysis during the reaction or during processing/gel electrophoretic analysis. The ~51-kDa band represents cleaved α1-ATZ. The α1-ATZ secreted from CST-treated CJZ12B cells also forms high molecular mass com-
plexes with elastase that migrate to ~75 kDa. Here, however, complexes start to become apparent at 0.5 μg of elastase added, and complete conversion to the complex form requires 2 μg of elastase. The α1-ATZ secreted from KIF-treated or DMJ-treated C1Z12B also forms complexes with elastase. In this case, the complex migrates at ~70 kDa. Because α1-ATZ secreted in the presence of KIF or DMJ is ~52 kDa, the ~70-kDa complex probably corresponds to the ~75 kDa complex formed with wild type α1-AT from HepG2 cells. The α1-ATZ from KIF-treated or DMJ-treated C1Z12B cells only starts to form complexes when 0.5 μg of elastase is added and only at 2 μg of elastase is it completely converted to the complex form. These data indicate that α1-ATZ secreted in the presence of CST, KIF, or DMJ is functionally active although, in each case, it is apparently slightly lower in activity than wild type α1-AT secreted by HepG2 cells. Similar results have been described for wild type human α1-AT secreted with high-mannose type carbohydrate side chains by Saccharomyces cerevisiae and methylotrophic yeasts (27, 28).

Taken together, the results of these studies indicate that the glucosidase inhibitor CST mediates increased secretion of α1-ATZ and that the mannosidase inhibitors KIF and DMJ mediate both decreased degradation and increased secretion of α1-ATZ. The results, therefore, suggest that there are mechanisms, at least two, by which steps in oligosaccharide side chain trimming can be circumvented and a cohort of misfolded glycoproteins permitted to traverse the secretory pathway. In one previous study, CST has been shown to increase secretion of carboxyl-terminal truncated fragments of influenza virus hemagglutinin (24), but there are no previous reports of a similar effect for KIF or DMJ. It is not clear at this time whether the alterations in the structure of the oligosaccharide side chain prevent the side chain from interacting with molecules responsible for its retention, permit the side chain to interact with molecules that can facilitate the folding of the mutant α1-ATZ protein, or result in alterations in the conformation of α1-ATZ in such a way that its folding is facilitated. In the case of α1-ATZ generated in the presence of mannosidase inhibitors KIF and DMJ, the ERGIC-53 cycling pathway is an excellent candidate for mediating increased secretion. Mousalli et al. (29) have recently shown that ERGIC-53 mediates secretion of coagulation factors V and VIII by specific recognition of fully glucose-trimmed, mannose 9 oligosaccharide side chains. Interaction with the ERGIC-53 cycling pathway would not, however, provide an explanation for the delay in degradation that also occurs in the presence of KIF and DMJ. Several previous studies have suggested the possibility that uncharacterized “carbohydrate-binding chaperones” are responsible for stabilization of untrimmed secretory and membrane proteins during treatment with mannosidase inhibitors (6, 9). Nevertheless, comparison of different studies in the literature (4–10, 27–29) suggests that there are species-specific and substrate-specific differences in the effects of glucosidase and mannosidase inhibitors and that there may also be differences in their effects depending on whether the protein substrate is membrane-bound, secretory, wild type, unassembled, or mutant.

The results also provide further evidence that physiologic and pharmacologic perturbations have separate and independent effects on ER degradation and secretion of the misfolded α1-ATZ molecule. Glyceraldehyde 3-phosphate dehydrogenase (PMA) mediates increases in secretion of α1-ATZ without affecting its degradation (30). Lactacystin, cycloheximide, and lowering of temperature to 27 °C are associated with a decrease in degradation of α1-ATZ without any change in secretion (30). KIF, DMJ (Fig. 4), and elevation of temperature to 42 °C are associated with decreased degradation and increased secretion (30).

Because the mutant α1-ATZ retains functional activity and because clinical studies have suggested that only partial correction of the secretory defect is needed for prevention of both liver and lung disease in α1-AT deficiency, any drug which enhances secretion is a candidate for chemoprophylaxis in patients with this deficiency. Our recent studies have shown that a chemical chaperone PBA is an excellent candidate (30). The current study suggests that CST may be another potential chemoprophylactic agent. Indeed, a compound based on CST, 6-O-butanoyl CST is currently in clinical trials as an adjuvant treatment for HIV infection (31). The mannosidase inhibitors KIF and DMJ are also less attractive drugs for chemoprophylaxis because they delay degradation of α1-ATZ as well as increase its secretion. Thus, drugs based on these compounds have the potential to prevent lung injury but to increase the likelihood of liver injury in α1-AT-deficient patients.

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