α-Glucosidase inhibitors have a prolonged antiviral effect against hepatitis B virus through the sustained inhibition of the large and middle envelope glycoproteins

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Previous work has shown that the secretion of enveloped hepatitis B virus (HBV) DNA and the HBV middle envelope protein (MHBs) are sensitive to glucosidase inhibition. Here, it is shown that HBV DNA secretion remains depressed after the removal of the glucosidase inhibitor and long after glucosidase function returns to normal. For example, glyco-processing and the secretion of α1 anti-trypsin returned to normal within 3 h of the removal of the glucosidase inhibitor. In contrast, the secretion of HBV did not return to normal for more than 7 days after the removal of the inhibitor. Consistent with the inhibition of HBV virion secretion, the levels of HBV L and HBV M proteins were also reduced by treatment with the glucosidase inhibitor and remained reduced for 7 days after compound withdrawal. The implications of the prolonged antiviral effect against HBV and the use of glucosidase inhibitors as antiviral agents are discussed.

Keywords: antiviral, glucosidase inhibitors, glycosylation, hepatitis B virus, protein degradation

Introduction

Hepatitis B virus (HBV) remains a major world problem. There is a clear need for the introduction of safe and effective therapies for chronic HBV. Worldwide, more than 350 million people are chronically infected with HBV, and between 15% and 40% of these individuals will die from serious liver diseases, if left untreated (El-Serag and Mason, 1999). The major complication is the development of primary hepatocellular carcinoma (HCC) that causes an estimated 500,000 deaths annually (El-Serag et al., 2001).

Current antiviral approaches have used nucleoside/nucleotide based agents that have targeted the viral polymerase protein as the primary therapeutic target. The long-term benefit of this approach is unclear as drug-resistant mutants have emerged for most of the nucleoside/nucleotide based antivirals. Recently, there have been several reports that have indicated that animals or people given a strong antiviral agent that can reduce both viraemia and the levels of circulating subviral particles (hepatitis B surface antigens; HBsAg) could mount beneficial immunological responses to the virus (Boní et al., 2001; Menne et al., 2002). Reductions in HBsAg are thought to be largely a secondary consequence of reductions of viraemia, which limits the mediated spread of the virus and requires a very long period of treatment (Nowak et al., 1996). The beneficial immunological response seen under these conditions can be boosted by vaccination using a simple subunit vaccine (Menne et al., 2002).

We have shown that safe and well-tolerated doses of glucosidase inhibitor can reduce the amount of virus secretion within days and weeks in tissue culture and in woodchucks, respectively. (Block et al., 1994; Block et al., 1998). Glucosidases I and II, in the endoplasmic reticulum (ER), mediate processing of N-linked glycan on glycoproteins (see Figure 1A). This processing is necessary for the interaction of many glycoproteins with the protein folding chaperon, calnexin. MHBs glycoprotein folding and secretion appears to have an obligate requirement for calnexin mediated folding and processing by ER glucosidases (Prange et al., 1999). In addition, as many other viral glycoproteins appear to have a requirement for this interaction, glucosidase inhibitors have been postulated as potentially useful broad spectrum antiviral agents (Mehta et al., 1998; Courageot et al., 2000; Whitby et al., 2004).

Since the folding of MHBs, synthesized in the human 2.2.15 hepatoblastoma (Hep G2 2.2.15) cells producing virus, is mediated by calnexin, it seemed likely that biogenesis of MHBs would be the most sensitive HBV glycoprotein
**Figure 1.** The action of glucosidase I and II enables the interactions between folding glycoproteins and the endoplasmic reticulum chaperones, calnexin and calreticulin.

(A) Glycosylation pathway. α-Glucosidase I co-translationally removes the terminal α-1-2 glucose (Glu) residue, which is followed by the removal of the exposed α-1-3 residue by glucosidase II. The resulting monoglucosylated glycoprotein is recognized by and binds to calnexin and/or calreticulin. Removal of the last α-1-3 linked glucose residue mediates release from calnexin. Misfolded proteins can be reglucosylated by glycoprotein glucosyltransferase, which allows them to rebind calnexin. In the glycan structures, triangles represent glucose residues.

(B) Design of rebound study. Duplicate cultures of Hep G2 2.2.15 cells were either left untreated (UNT), treated with the glucosidase inhibitor deoxynojirimycin (DNJ), or lamivudine (3TC) for 7 days. After 7 days of treatment, the inhibitor was removed, the media collected (rebound day 0) and replaced with either fresh drug containing medium (one set) or with plain culture medium (another set). Culture medium collections occurred at 0, 3, 5 and 7 days after initial drug removal (rebound day 0, 3, 5 and 7). DNJ⁶, rebound DNJ; 3TC⁶, rebound lamivudine.
to glucosidase inhibitors. Indeed, previous work from our group and others has shown that secretion of MHBs is drastically reduced from cells in which glucosidase is inhibited and it appeared to accumulate within the intracellular compartment, as inferred by detection in antigen capture (ELISA) type assays (Lu et al., 2001).

In the present study, we have explored virion secretion and envelope protein degradation as a function of time during and following relief from inhibitors of ER glucosidase (see Figure 1B). Surprisingly, HBV secretion from 2.2.15 cells remained suppressed for 7 days following the removal of glucosidase inhibition, despite demonstration that glycan processing had completely returned to pre-inhibition function within 3 h. As expected, MHBs secretion was also sensitive to glucosidase inhibition. Previous work has suggested that viral glycoprotein MHBs accumulated in glucosidase-inhibited cells. However, in our recent work, it has been determined that in glucosidase-inhibited cells, LHBs and MHBs are degraded (Simsek et al., 2005) with levels not returning to pretreatment levels for days after the removal of the inhibitor. As LHBs has been shown to be essential for virus formation (Bruss and Ganem, 1991) and the sensitivity of LHBs tracks with that of the virus, we suggest a model in which LHBs is primarily responsible for the glucosidase mediated inhibition of HBV.

Materials and methods

Cells and compounds

Hep G2 cells, a stable tissue culture line derived from a human hepatoblastoma, purchased from the American Type Culture Collection (Rockville, MD, USA), were grown in RPMI 1640 (Gibco-BRL, Rockville, MD, USA) containing 10% fetal bovine serum (Gibco-BRL). Hep G2 2.2.15 cells were kindly provided by Dr George Acs (Mt. Sinai Medical College, New York, NY, USA) and maintained as Hep G2 cells, but with the addition of 200 µg/ml of G418 (Gibco-BRL). N-Butyldexamonojirimycin (NB-DNJ) and deoxynojirimycin (DNJ) were provided by Monsanto Searle (St. Louis, MO, USA) and Synergy Pharmaceuticals, Inc. (Eddison, NJ, USA), respectively. Lamivudine (3TC) was purchased from Moravek Biochemicals (Brea, CA, USA).

Drug treatment

Quadruplet cultures of Hep G2 2.2.15 cells were either left untreated (UNT) or treated with DNJ (4.52 mM) or with 3TC (4.7 µM). For both DNJ and 3TC, two sets of samples were used, one that would remain drug treated and another in which drug would be removed (rebound). Media and drug were changed every 2 days. After 7 days of treatment, media was either replaced with fresh media containing drug (DNJ or 3TC) or with just media for rebound analysis (DNJ-R or 3TC-R). This initial day is referred to as rebound day 0. The rebound was continued for 3, 5 and 7 days after drug removal.

Cytotoxicity (cell viability) assay

The viability of Hep G2 2.2.15 cell cultures treated with the indicated concentration of imino sugar (DNJ or NB-DNJ at 4.52 mM) inhibitor was measured by trypan blue dye exclusion staining at Day 0, 3, 5 and 7 post drug removal. Viable cells were coloured white and were counted on a haemocytometer by a light microscope. In addition, the cytotoxicity of the glucosidase inhibitors was also evaluated by the mitochondrial toxicity test (MTT; Sigma Aldrich, St. Louis, MO, USA) and the lactate dehydrogenase (LDH) assay (Promega, Madison, WI, USA; Lu et al., 1995).

Detection of secreted viral DNA

Analysis of DNA secreted from tissue culture cells was performed as before using a method that would discriminate between enveloped and unenveloped virions (Wei 1996; Delaney et al., 1999; Mehta et al., 2001). DNA was separated by electrophoreses on a 1.0% agarose gel, transferred to a nylon membrane and probed with 32P labelled HBV probes, as we have done previously (Mehta et al., 2001). Briefly, virus in the culture medium was concentrated by pelleting through 20% sucrose for 16 h (SW 41 rotor, 36,000 RPM). Virus was resuspended in 200 µl of 10 mM TRIS (pH 7.9), 10 mM EDTA (pH 8.0), and 10 mM MgCl₂. Proteinase K was added to a final concentration of 750 µg/ml and the samples incubated for 1 h at 37°C. After 1 h, SQ1 DNase (Promega) was added to each tube to a final concentration of 50 units/ml and incubated at 37°C for 1 h. Sodium dodecyl (SDS) and Proteinase K were added to a final concentration of 1% and 500 µg/ml, respectively, and the reaction allowed to proceed at 37°C for 4 h. DNA was purified by phenol/chloroform extraction followed by isopropanol precipitation. Viral DNA was separated by electrophoreses on a 1.0% agarose gel, transferred to a nylon membrane and probed with 32P labelled HBV probes. HBV specific bands were subsequently identified and quantified using a Bio-Rad Personal FX phosphoimager (Bio-Rad, Hercules, CA, USA).

Detection of α-1 anti-trypsin (AAT) by immunoprecipitation

HepG2 and HepG2 2.2.15 cells were used to detect the secretion of a cellular glycoprotein, AAT, after treatment with and removal of the glucosidase inhibitor. Briefly, the cells were treated with 4.52 mM DNJ for 7 days. After the treatment, DNJ was removed at 1 h, 3 h or 1 day. The cells were then incubated with 35S methionine (200 uCi/ml) for 4 h. The culture supernatant was aspirated and clarified at 37°C for 4 h. The culture supernatant was aspirated and clarified at 37°C for 4 h.
Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 0.05% SDS and 0.2% NP-40 (final concentrations) and used in an immunoprecipitation assay. The clarified radiolabelled medium was incubated with human monoclonal antitrypsin antibody (Santa Diagnostics, Inc., NJ, USA) at 4°C overnight. The immune complexes were then precipitated using protein-G agarose (Roche Diagnostics Corp., IN, USA). Following washing with lysis buffer, the immune complexes were released from protein-G agarose using SDS-polyacrylamide gel electrophoresis (PAGE) denaturation buffer and boiled for 5 min. Samples were resolved by SDS-PAGE (12% polyacrylamide) and the labelled proteins were detected using a Bio-Rad Personal FX phosphoimager.

**Detection of hepatitis B large and middle surface antigens by Western blot**

The proteins from culture medium were resolved by 12% SDS-PAGE, transferred to PVDF (Millipore, Bedford, MA, USA) membranes, and blocked with 5% powdered milk in 0.1% Tween-20/PBS at room temperature for 1 h. After incubation with primary polyclonal pre-S2 antibody (Research Diagnostics, Inc. NJ, USA) as a 1:1,000 dilution in 0.1% Tween-20 with 2% BSA for 1 h at room temperature, blots were washed three times with 0.1% Tween-20/PBS. The blots were then incubated with secondary antibody (peroxidase-conjugated donkey anti-rabbit IgG serum) as a 1:5,000 dilution in 0.1% Tween-20 with 2% BSA for 1 h at room temperature. After being washed three times with 0.1% Tween-20/PBS, the blots were developed using an enhanced chemiluminescence (ECL; Amersham corporation, Arlington heights, IL, USA) as the detection reagent.

**Detection of HBsAg by ELISA**

Two hundred microlitres of media from the same studies was used to determine the level of HBsAg in the culture medium of untreated and treated cells. Analysis was performed using the Abbott Diagnostics AUSZYME Monoclonal Diagnostic kit (Abbott Laboratories, North Chicago, IL, USA).

**Results**

**Glucosidase inhibitors have a prolonged antiviral effect against HBV**

We have previously shown that the secretion of HBV from tissue cultures is reduced by glucosidase inhibitors (Block et al., 1994; Mehta et al., 1997a; Block et al., 1998). These observations were confirmed in the experiments shown in Figures 2A & 2B, in which the amount of enveloped viral DNA detected in the culture medium of Hep G2 2.2.15 cells was determined after 7 days of incubation with DNJ, a competitive inhibitor of ER glucosidases. As glucosidase inhibitors such as DNJ primarily inhibit the secretion of enveloped viral DNA and not unenveloped viral particles (Block et al., 1994), an assay that distinguishes between these two sources of viral DNA was used to determine antiviral activity (Wei, 1996). Briefly, in this assay, fully enveloped viral particles are protected from limited protease and DNase digestion by the presence of the viral envelope (Wei, 1996; Delaney and Isom 1998). In contrast, unenveloped viral particles are sensitive to this digestion (Wei, 1996). As Figure 2A shows, untreated Hep G2 2.2.15 cells secrete DNA that is insensitive to DNase digestion, suggesting that this DNA was within enveloped viral particles. Treatment with the glucosidase inhibitor DNJ caused the reduced secretion of HBV DNA. The majority of the HBV DNA secreted from glucosidase-inhibited cells were sensitive to DNase treatment, suggesting that it was contained within unenveloped viral particles. The source of these unenveloped particles is not known, but may be related to inhibition of the viral glycoproteins and the intracellular accumulation of unenveloped viral particles, which has been previously shown (Mehta et al., 1997b). Figure 2B shows that glucosidase inhibitors caused a 95% (±13%; n=6) reduction of enveloped HBV DNA in the culture medium after 7 days of treatment. Surprisingly, this reduction persisted for over 7 days after the drug had been removed from the culture medium. As this result may be indicative of cell death, the toxicity of the cultures was measured by trypan blue staining of the cells at multiple time points. As Figure 2C shows, no toxicity was observed at any time point. In contrast, the overnight treatment of cultures with 10% ethanol treatment caused a >95% reduction in culture viability (data not shown). Since there was no evidence of cytotoxicity as determined by trypan blue staining, MTT or LDH assays, it is concluded that DNJ selectively inhibited the secretion of HBV, under conditions where cell functions were not detectably affected.

**Glucosidase function returns to normal within 3 h after removal of glucosidase inhibitor**

As imino sugar glucosidase inhibitors such as DNJ and NB DNJ are competitive inhibitors of the enzyme, it was not clear for how long the inhibition of the enzyme would last after drug removal. The measurement of glucosidase function in Hep G2 cells has proved to be problematic based upon the presence of the shut endomannosidase pathway. This pathway, which is present in most cell types, prevents the secretion of glycoproteins containing glucosylated N-linked glycan. While other cells that lack the endomannosidase pathway could be used, we firmly believed that an assay using the same cells, and under the same conditions would provide more information. Thus, an assay that allowed for the measurement of glucosidase inhibition in Hep G2 cells was used (Tan et al., 1991). Briefly, mature AAT recovered from the culture medium of cells with fully functioning glucosidase migrates as a 53 KD polypeptide (Figure 3). In contrast, a species of AAT characteristic of
A polypeptide bearing aberrant glycan can be detected in the culture medium of cells in which glucosidase has been inhibited (Tan et al., 1994). As shown in Figure 3, within 3 h of removal of glucosidase inhibitors, the wild type, fully processed AAT is the only species of AAT detected. This result is consistent with analysis by others using various glucosidase inhibitors and other glycoproteins such as α-1 acid glycoprotein (Tan et al., 1991). This analysis was conducted with both Hep G2 2.2.15 cells and the parental, HBV free, Hep G2 cells to observe the possibility that virus production, itself, might have an influence upon the activity of glucosidase enzyme and their inhibitors. Since there was no detectable difference in the fate of AAT in glucosidase-inhibited Hep G2 2.2.15 and Hep G2 cells, HBV production appears to have no effect upon glucosidase function or AAT synthesis, itself, and only Hep G2 2.2.15 cells were used in subsequent studies. Taken together, the results of the analysis of AAT in cells removed from the glucosidase-inhibitor suggests that glucosidase function has returned to pretreatment levels within 3 h after the removal of the competitive enzyme inhibitor and also implicates a more specific effect upon HBV virion secretion.

Figure 2. The secretion of HBV DNA in endoplasmic reticulum glucosidase-inhibited Hep G2 2.2.15 cells

(A) Southern blot analysis of the level of enveloped hepatitis B virus (HBV) virus in the culture medium of Hep G2 2.2.15 cells either left untreated or treated with deoxynojirimycin (DNJ; 4.52 mM) or lamivudine (3TC; 4.7 mM) for 7 days. HBV relaxed circular DNA is indicated (rcDNA). Samples from left to right are as follows: untreated (UNT), 3TC, 3TC® (rebound 3TC, that is, where 3TC was replaced with plain media after 7 days of treatment), DNJ and DNJ® (rebound DNJ, that is, where DNJ was replaced with plain media after 7 days of treatment). One-half of each sample was digested sequentially with Proteinase K and DNAse to digest unenveloped, unencapsidated DNA (labelled as + DNAse in Figure). DNA was then extracted from all samples and analysed by Southern blotting. (B) Graphical representation showing the level of virus secreted into the culture medium 3, 5 or 7 days after drug removal. Briefly, after the initial 7 day treatment, the inhibitor was removed and the level of virus in the culture media measured as explained in Figure 2A. Y-axis represents the level of HBV in the culture medium as compared to the untreated controls. (C) The toxicity of the drug treatment as determined by trypan blue dye exclusion analysis. For each time point, the viability of each treatment is given as a function of untreated controls. X-axis represents drug treatment and time after drug removal and Y axis represents the percentage of viable cells, as compared to untreated controls. RBD rebound day.
The inhibition of LHBs and MHBs proteins correlates with the prolonged antiviral activity of the glucosidase inhibitors

We have previously shown that glucosidase inhibitors prevented the secretion of MHBs protein in Hep G2 2.2.15 cells. However, the long-term effect on MHBs protein secretion and the effect on LHBs was unknown. Therefore, Hep G2 2.2.15 cells were incubated in the absence and presence of glucosidase inhibitor for 7 days, followed by washing and replacement of culture medium free of any glucosidase inhibitor. The amount of secreted LHBs and MHBs present during glucosidase inhibition and following removal of inhibitor was determined by Western blot with a polyclonal antibody specific for the pre-S2 epitope. Since the pre-S2 epitope is present on both LHBs and MHBs, both glycoproteins are detected by this antibody, and the results are shown in Figure 4. As the LHBs are a minor species, Figure 4A is presented in two exposures to visualize both the glycosylated and unglycosylated LHBs. Thus, the top panel in Figure 4A shows the 33 and 36 kDa, mono glycolsylated and di-glycosylated MHBs polypeptides, respectively, resolved from the pair of un-glycosylated and mono glycosylated LHBs polypeptides (Figure 4A, ‘U’ as ‘UNT’). As this figure shows, the level of the MHBs protein in the culture medium is reduced following treatment with the glucosidase inhibitor and remains repressed until 7 days after drug removal. Two MHBs isoforms, gp33 and gp36, do not resolve clearly from each other, as a result of heterogenous N-linked glycans processing and the presence of O-linked glycans (Werr and Prange 1998; Tai et al., 2002). The bottom panel of Figure 4A shows the overexposure of the same gel to allow for visualization of the glycosylated LHBs species. As this figure clearly shows, the level of glycosylated LHBs secreted into the culture medium is reduced following treatment with the glucosidase inhibitor and remains repressed until 7 days after drug removal. The loading of samples was controlled using an antibody specific to human albumin, which is secreted by the Hep G2 and Hep G2 2.2.15 cells (data not shown).

The corresponding intracellular levels of the LHBs and MHBs are shown in Figure 4B. Our previous work has shown that glucosidase inhibition causes the proteasomal degradation of the LHBs and MHBs (Simsek et al., 2005). Consistent with our previous analysis, Figure 4B shows that treatment with the glucosidase inhibitor DNJ caused the degradation of the LHBs and MHBs proteins (Simsek et al., 2005). LHBs was reduced by 66% ±37% (n=6), whereas MHBs protein was reduced by 86% ±8% (n=6). By 3 days after the removal of glucosidase inhibitor, the intracellular reductions of LHBs and MHBs was not statistically significant (Figure 4B) suggesting that protein stability had returned to normal.

Figure 4C shows that the levels of HBV small envelope protein (SHBs) secreted into the culture medium does not change with glucosidase inhibition, which indicates the specific sensitivity of the LHBs and MHBs to the glucosidase inhibitors. Although the S domain is shared with both the LHBs and the MHBs, the vast excess of SHBs, as compared to the level of the LHBs and MHBs, overwhelms the contribution of the other envelope proteins in this assay.

The quantification of these experiments for both virion secretion and the secretion of the LHBs and MHBs protein are shown as a function of time in Figure 4D. As this figure shows, LHBs, MHBs and virus secretion are reduced between 80–90% following 7 days of treatment with the glucosidase inhibitor. Removal of glucosidase
Figure 4. The production of LHBs and MHBs proteins in endoplasmic reticulum glucosidase-inhibited cells

(A) Hep G2 2.2.15 cells were treated with deoxynojirimycin (DNJ) for 7 days as in Figure 1B, the level of the large protein (LHBs) and middle protein (MHBs) in the culture media determined as a function of drug removal over time. For each time point, matching drug-containing samples are used as controls. The level of secreted LHBs and MHBs proteins were detected by immuno-blot using a polyclonal pre-S2 specific antibody. Top panel contains a lighter exposure that allows for the analysis of the unglycosylated LHBs protein, whereas the bottom panel provides an overexposure of the same blot that is required to visualize the glycosylated LHBs species. (B) The intracellular level of LHBs and MHBs as a function of glucosidase inhibition. As in panel A, Hep G2 2.2.15 cells were treated with DNJ for 7 days and the level of the LHBs and MHBs protein in cell lysates was determined as a function of drug removal over time. Actin is used as a loading control and presented below the main figure. (C) The level of small protein (SHBs) secreted into the culture medium as a function of drug treatment and removal. SHBs levels were detected via ELISA using the commercially available hepatitis B surface antigen ELISA kit. (D) Correlation between the inhibition of virus secretion and the secretion of LHBs and MHBs as a function of drug treatment. For graph, X-axis represents the time during and after drug removal; Y-axis represents the level of either secreted viral DNA, or of each individual envelope proteins as compared to untreated controls. DN, DNJ treated; gl, glycosylated large protein; HBV DNA, hepatitis B virus; pL, unglycosylated large protein; R0, rebound day 0; R3, rebound day 3; R5, rebound day 5; R7, rebound day 7; UN, untreated.
inhibitor causes the slow return of both virus secretion and the secretion of the LHBs and MHBs protein over a 7-day period. As the LHBs are essential for the formation and secretion of HBV virion, it is assumed that the prolonged effect on the LHBs is responsible for the prolonged antiviral activity seen with the glucosidase inhibitors.

**Discussion**

Previous work had shown that HBV is sensitive to glucosidase inhibition. Here we show that glucosidase inhibitors cause the prolonged repression of HBV virus secretion. That is, HBV virion secretion only returns to normal 7 days after removal of glucosidase inhibitors from tissue culture. In addition to the prolonged antiviral effect, glucosidase inhibitors also caused the prolonged inhibition of the secretion of LHBs and MHBs proteins. It is noted that a prolonged antiviral effect against HBV in tissue culture has been observed with compounds such as 3TC, but those results came from the accumulation of the active compound within the cell (Abdelhamed et al., 2002). In contrast, when glucosidase inhibitors were removed from culture, glucosidase function returned within 3 h. It was possible that toxicity could be a possible cause and this was examined carefully by MTT assay, trypan blue staining and LDH toxicity analysis (data not shown). In all circumstances no toxicity was observed. In addition, the continued secretion of host glycoproteins and the SHBs protein suggested that no toxicity was present.

Our results imply that the glucosidase inhibitors primarily work by reducing the level of LHBs protein, which is essential for virus secretion (Bruss and Ganem, 1991). There are several possibilities as to how this reduction results in the prolonged antiviral effect of glucosidase inhibitors. It is possible that the continued antiviral effect is the result of the creation of some long-lived product that could have a dominant negative effect on virus secretion. Prior reports have suggested that MHBs protein accumulated in the presence of glucosidase inhibitors (Lu et al., 1997; Lu et al., 2001). However, recent work has suggested that it is not intact M protein that accumulates (Simsek et al., 2005). It is important to point out that prior reports have used MHBs only systems and/or antibodies to the S domain. Consistent with this, recent analysis using a MHBs only system has shown the accumulation of an S epitope reactive species in the presence of glucosidase inhibitors, whereas pre-S2 reactive species have declined. It is hypothesized that in the presence of glucosidase inhibitors, the LHBs and MHBs proteins are retained and partially degraded resulting in the disappearance of the pre-S2 epitope and the accumulation of a polypeptide containing the S epitope (manuscript in preparation).

It is also possible that there exists a ‘threshold’ level of LHBs that is required for virus formation and that this is the mechanism for the prolonged inhibition of virus secretion by the glucosidase inhibitors. That is, the levels of LHBs protein remain low for 5 days after drug removal. Indeed, these levels may be too low to drive the formation of virus. However, it is interesting to point out that the low levels of both LHBs and MHBs appear to be the result of continued degradation of these proteins via the proteasome (Simsek et al., 2005). Studies on how this is mediated and if the antiviral activity of the glucosidase inhibitors is the result of the inhibition of the LHBs or MHBs proteins are underway. As the LHBs protein has been shown to be essential for virus formation and secretion (Bruss and Ganem, 1991), it can be assumed that the reduction of the LHBs protein would have a negative impact on virus formation and secretion. In contrast, the MHBs protein is not thought to be essential for virus formation and secretion, and hence its reduction may not have a dramatic effect on virus morphogenesis (Bruss and Ganem, 1991).

In conclusion, we have shown here that the glucosidase inhibitors have a prolonged effect on virion secretion, and on the secretion of the LHBs and MHBs proteins. This prolonged antiviral effect continues well after the removal of drug and return of enzyme function. As glucosidase inhibitors are being postulated for potential uses against hepatitis C virus (Whitby et al., 2004) and flaviviruses (Courageot et al., 2000; Whitby et al., 2005), it will be important to study the prolonged effect of these compounds in those systems as well.

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Durable suppression of HBV with glucosidase inhibitors


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