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TARGETING OF ANTIVIRAL DRUGS TO T_4-LYMPHOCYTES

ANTI-HIV ACTIVITY OF NEOGLYCOPROTEIN–AZTMP CONJUGATES *IN VITRO*

GRIETJE MOLEMA,*† ROBERT W. JANSEN,† RUDI PAUWELS,‡ ERIK DE CLERCQ‡ and DIRK K. F. MEIJER†

†Department of Pharmacology & Therapeutics, University Centre for Pharmacy, Groningen University, The Netherlands; and ‡Division of Microbiology, Department of Human Biology, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

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Abstract—The delivery of the anti-HIV agent 3′-azido-3′-deoxythymidine (AZT), in its 5′-monophosphate form, (in)to human T-lymphocyte MT-4 cells *in vitro* through covalent coupling to neoglycoproteins was investigated. *In vivo* application of this drug targeting concept may lead to increased efficacy and/or diminished side effects caused by AZT during the treatment of AIDS and ARC patients. The rationale for the design of the neoglycoprotein carriers is based on the existence of sugar recognizing lectins on T-lymphocytes. Using a phenyl-linkage between sugar and Human Serum Albumin (HSA), various mannose-, fucose-, galactose- and glucose-containing neoglycoproteins were synthesized. The intrinsic anti-HIV activity of these neoglycoproteins was tested *in vitro* in HIV-1 infected MT-4 cells. Only the derivative having 40 moles mannose per mole protein ($Man_{40}HSA$) shows pronounced anti-HIV-1 activity itself. This effect may be caused by interference of the $Man_{40}HSA$ with the gp120-CD4 mediated virus/MT-4 cell interaction. After conjugation with AZTMP, the mannose- as well as the fucose- and galactose-containing conjugates exhibited a pronounced activity. Conjugates of glucose–HSA and HSA displayed much less activity in spite of the fact that drug loading was considerably higher, compared with the galactose, mannose and fucose derivatives. In the series of mannose-neoglycoproteins, the $Man_{22}HSA$–AZTMP conjugate was shown to be more than 30 times as active against HIV-1 compared to HSA–AZTMP. Selectivity indices of Man_7- and $Man_{22}HSA$–AZTMP were exceeding the AZT and AZTMP indices, indicating that these conjugates possess a more selective action. Stability experiments indicate that the potent action of the galactose–, mannose– and fucose–HSA–AZTMP conjugates is not due to a complete extracellular hydrolysis of the covalent drug–protein bond. Since $Man_{22}HSA$ has no intrinsic activity in the concentration range used, the antiviral effect is unlikely to be explained by synergism of extracellular released AZTMP/AZT and the carrier. Possibly, delivery of AZTMP through recognition of the neoglycoprotein by a component of the cell membrane and subsequent internalization and release of the drug from the conjugate may play a role.

Nucleoside analogues are potent but relative toxic antiviral agents, that are good candidates for specific delivery to various cell types in the liver [1–3] as well as blood cells [4].

Human Immunodeficiency Virus (HIV) is the causative agent of the acquired immune deficiency syndrome (AIDS) and a spectrum of related disorders [5, 6]. The supposed target of HIV is the CD4-molecule present on the helper/inducer subset of lymphocytes [7–9]. This CD4-molecule seems to be an essential component of the receptor for the AIDS retrovirus [7]. Some Epstein–Barr Virus transformed lymphocytes [10], epidermal Langerhans cells [11] and cells in brain tissue [12] may also express this cell surface molecule. Recent studies have shown that HIV can also be detected in monocytes/macrophages of AIDS patients [13–16].

Essential for the interaction between the virus and the CD4-molecule appear to be the carbohydrate chains of the virus envelope glycoprotein gp120 [17, 18]. Structure analysis of the oligosaccharide substituents of gp120 from HIV-1 revealed that this glycoprotein carries predominantly oligomannosidic glycans [19, 20]. The results of Lifson *et al.* [21] suggest that the mannose-containing carbohydrate moieties on the viral envelope glycoprotein are involved in interaction with the CD4-molecule.

In vitro inhibition of infectivity and cytopathic effects of HIV caused by the thymidine analogue AZT (3′-azido-3′-deoxythymidine) was reported by Mitsuya *et al.* [22]. After permeation of the cell membrane by non-facilitated diffusion [23], AZT needs to be phosphorylated to its triphosphate form (AZTTP) to be antivirally active [24]. AZTTP inhibits HIV-1 reverse transcriptase competitively with respect to the natural substrate dTTP [25]. It may also serve as an alternative substrate for the reverse transcriptase, thus preventing further DNA chain elongation [25]. So far, more than 20,000 individuals with severe AIDS and AIDS Related Complex (ARC) have been treated with AZT [26]. Usually, this resulted in clinical and immunological improvement [25, 26]. However, severe side effects,

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* Correspondence to: G. Molema, Department of Pharmacology & Therapeutics, University Centre for Pharmacy, Groningen University, Ant. Deusinglaan 2, 9713 AW Groningen, The Netherlands.

particularly bone marrow suppression, have been reported following the administration [27, 28]. These side effects strongly limit its therapeutic use and may even give rise to cessation of therapy [27].

Using a carrier molecule to specifically target AZT to HIV-infected cells, the problem of systemic toxicity of the drug could be substantially diminished. Moreover, efficient targeting of AZT may lead to smaller doses of the drug required for therapeutic success.

Taking the helper/inducer T lymphocyte subset as the primary target for antiviral therapy, we designed neoglycoproteins with various sugar molecules based on the existence of different lectins on lymphocytes. Lectins with demonstrated sugar specificity for galactose [29–33], mannose [30, 33, 34] and fucose [35] were reported to be present on lymphocytes. A lectin with two combining sites for both mannose and galactose, acting synergistically, was also proposed [33].

Various *p*-aminophenylsugar derivatives were covalently coupled to Human Serum Albumin (HSA) by thiophosgene activation of the sugar [36, 37]. Conjugation of the obtained neoglycoproteins with the 5′-monophosphate derivative of AZT was performed using a (water soluble) carbodiimide mediated reaction [38], leading to neoglycoprotein–drug conjugates (Fig. 1). The advantage of using a phosphorylated derivative may be that conversion to the active triphosphate form occurs more readily. Also, conjugation of the 5′-monophosphate nucleoside derivative was shown to result in a more efficient degree of substitution compared with the parent compound [39]. At the same time, resistance problems based on deficient phosphorylation may be circumvented.

In the present study, the anti-HIV-1 activity of the neoglycoprotein carriers as well as the AZTMP-carrier conjugates were tested *in vitro* in HIV-1 infected MT-4 cells as described by Pauwels *et al.* [40].

MATERIALS AND METHODS

Chemicals. Human Serum Albumin (HSA, fraction V), *p*-aminophenylsugar derivatives and ECDI (1-ethyl-3(3-dimethylaminopropyl)carbodiimide) were obtained from the Sigma Chemical Co (St Louis, MO, U.S.A.). Thiophosgene was obtained from Janssen Chimica (Beerse, Belgium). AZT (3′-azido-3′-deoxythymidine) and AZTMP (the 5′-monophosphate derivative of AZT) were obtained from the Rega Institute, Leuven, Belgium. All other chemicals were of analytical grade or the best grade available.

Synthesis of neoglycoproteins. Neoglycoproteins were prepared by coupling thiophosgene activated *p*-aminophenylsugars to HSA [36, 37]. A detailed description of synthesis, purification and analysis of the obtained products will be published elsewhere.*†

Conjugation of AZTMP to neoglycoproteins. Using ECDI as coupling agent [38], AZTMP was conjugated to various neoglycoproteins. Analysis of the products with regard to the molar ratio drug to protein was performed using a combined acid hydrolysis/HPLC assay (as described by Molema *et al.*†).

Cell cultures. All experiments concerning the antiviral activity of the preparations were performed at the Rega Institute, Leuven, Belgium.

The T_4-cell line which was used in this study is the HTLV-I transformed T_4-cell line, MT-4, and was kindly provided by Dr N. Yamamoto, Yamaguchi University, Yamaguchi, Japan. The MT-4 cells were grown in RPMI 1640 DM ("Dutch Modification") medium (Flow Laboratories, Irvine, U.K.), supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS) and 20 μg/mL gentamycin (E. Merck, Darmstadt, F.R.G.). The cells were maintained at 37° in a humidified atmosphere of 5% CO_2 in air. Every 3–4 days, cells were spun down and seeded at 2×10^5 cells/mL in new cell culture flasks. At regular time intervals, the MT-4 cells were analysed for the presence of mycoplasma and consistently found to be mycoplasma free.

Virus. HIV-1 (strain HTLV-III_B) which was kindly provided by Dr R. C. Gallo (National Cancer Institute, Bethesda, MD, U.S.A.) was obtained from the culture supernatant of a persistently HIV-infected HUT-78 cell line (HUT-78/HTLV-III_B). The virus titer of the supernatant was determined in MT-4 cells. The virus stock was stored at −70° until used.

Anti-HIV-1 assay. The procedure to determine the anti-HIV-1 activity in MT-4 cells was carried out as described previously [40, 41]. Briefly, exponentially growing MT-4 cells were either infected with HIV-1 or mock-infected. After resuspension at 4×10^5cells/mL in complete medium, 100-μL volumes were added in 96-well microtiter trays to serial five-fold dilutions of the compounds to be assayed. The cell cultures were incubated at 37° in a humidified atmosphere of 5% CO_2 in air. Five days after infection the viability of mock- and HIV-infected cells was examined by a colorimetric assay (the MTT method). The 50% inhibitory concentration (IC_{50}) was defined as the concentration of the compound that protected HIV infected cells by 50%, whereas the 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the compound that reduced the viability of mock-infected cells by 50%. The selectivity index (SI) is defined as the ratio $CC_{50}:IC_{50}$.

Stability of the conjugates in cell culture media. In order to establish the percentage of covalently bound AZTMP to be released from the conjugate during the period of antiviral assay, the conjugates were incubated in cell culture medium under the same conditions as the antiviral assays were performed [40, 41]. The neoglycoprotein carriers, AZT and AZTMP were incubated under the same conditions, as a control experiment. Because of the probability of cell lysis during the anti-HIV assay and the resulting release of various intracellular enzymes,

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[*] Jansen RW, Molema G, Ching TL, Oosting R, Harms G, Moolenaar F, Hardonk MJ and Meijer DKF, Hepatic endocytosis of various types of mannose-terminated albumins: what is important, sugar recognition, net charge or the combination of these features. *J Biol Chem*, submitted.

[†] Molema G, Jansen RW, Visser J, Herdewijn P, Moolenaar F and Meijer DKF, Neoglycoproteins as carriers for antiviral drugs: synthesis and analysis of protein–drug conjugates. *J Med Chem*, in press.

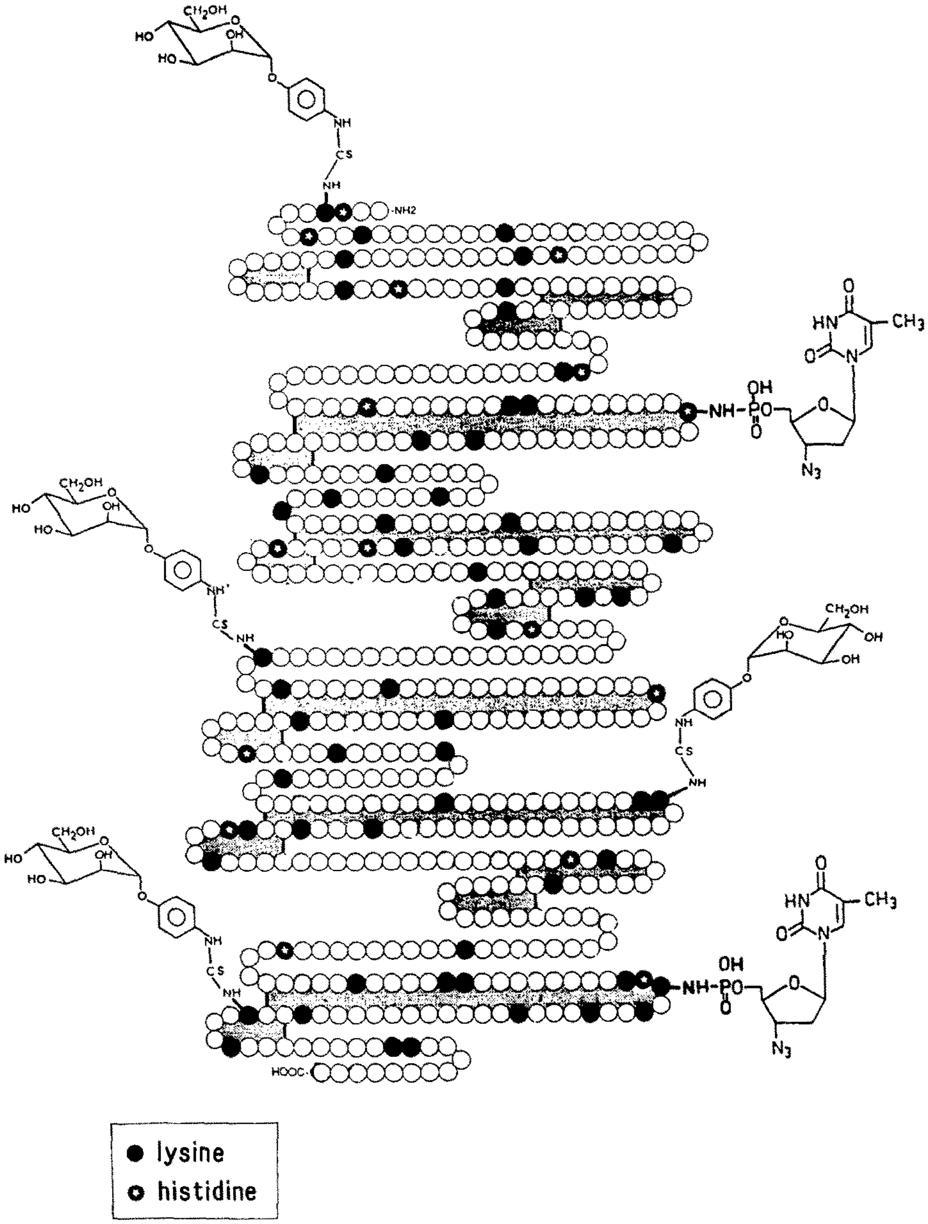

Fig. 1. Schematic representation of a neoglycoprotein–AZTMP conjugate: *p*-aminophenyl-mannose is coupled to lysine groups of HSA, AZTMP is bound to lysine and histidine. Circles represent amino acids.

the same stability experiments were performed in cell culture medium with contents of lysed MT-4 cells.

RESULTS

Table 1 shows the results of the neoglycoprotein syntheses. By varying the molar ratio *p*-aminophenyl-mannopyranoside to protein during the synthesis, it was possible to produce a series of neoglycoproteins with molar ratios mannose to protein ranging from 7:1 to 40:1. Using a constant excess of the various sugars during synthesis (420:1 sugar to protein on molar basis), the resulting neoglycoproteins differed in the number of bound sugar molecules, being 10 for fucose, 32 for galactose, 26 for glucose and 40 for mannose.

As can be seen from the mannose–neoglycoprotein series, the more sugar molecules were coupled, the less AZTMP could be attached to the neoglycoprotein (Table 2). The covalent coupling of the sugar derivatives to the protein involves the lysine ε-NH_2 groups of the protein [36]. However, conjugation of AZTMP also takes place using these ε-NH_2 groups, along with one of the imidazole nitrogens of histidine [38, 42]. So, in general, the more sugar molecules are bound to the protein, the less ε-NH_2 groups are available for AZTMP conjugation, although small deviations are observed.

After synthesis, the products were extensively

Table 1. Influence of the molar ratio sugar to protein in the reaction mixture during the synthesis of neoglycoproteins using thiophosgene activated *p*-aminophenyl-sugars

Sugar	Molar ratio sugar: protein in reaction mixture	Molar ratio bound sugar: protein in the neoglycoprotein product
Mannose	10:1	7:1
	25:1	22:1
	420:1	40:1
Galactose	420:1	32:1
Fucose	420:1	10:1
Glucose	420:1	26:1

washed with distilled water using the Amicon Stirred Cell equipped with a PM10 membrane (Amicon B.V., the Netherlands). Very small amounts of non-covalently bound AZTMP were recovered after lyophilization.

The results of the anti-HIV-1 assays show that the $\mathrm{Man_{40}HSA}$* neoglycoprotein itself exhibits some protective action against viral infectivity, whereas the other mannosylated, galactosylated, fucosylated and glucosylated neoglycoproteins do not (Table 3).

A considerable increase in antiviral activity of the neoglycoprotein was observed after conjugation with AZTMP (Table 2 and 3). While the $\mathrm{IC_{50}}$ for the neoglycoprotein $\mathrm{Man_{22}HSA}$ was $>3.3\,\mu\mathrm{M}$, after conjugation with AZTMP the $\mathrm{IC_{50}}$ value is reduced 5000-fold to $0.60 \times 10^{-3}\,\mu\mathrm{M}$. Moreover, this conjugate is more than 30 times as potent as HSA conjugated with AZTMP. A similar result, although to a lesser extent, is encountered in the case of the other two AZTMP–mannosyl albumins and also for the galactose and fucose conjugates. On the other hand, the $\mathrm{Gluc_{26}HSA}$–AZTMP conjugate is not significantly more antivirally active than the HSA conjugate.

All compounds, neoglycoproteins as well as their AZTMP conjugates, do not show significant toxicity in the assay. The $\mathrm{CC_{50}}$ values range from $>1.21\,\mu\mathrm{M}$ to $>6.55\,\mu\mathrm{M}$: the precise determination of these values was limited by the solubility of the various compounds. Together with the low $\mathrm{IC_{50}}$ values, this lack of toxicity leads to selectivity indices of more than 5000 for the mannose- and galactose-containing conjugates. The $\mathrm{Man_{22}HSA}$–AZTMP conjugate even possesses an SI of more than 10,000, therefore being at least about two-fold more selective than AZTMP (SI 7038) and four-fold more selective than AZT (SI 2600). *p*-Aminophenyl-mannopyranoside and HSA do not have any antiviral activity or toxic effects themselves.

During incubation of the conjugates in the two types of cell culture media, the phosphoamide bond is slowly hydrolysed, leading to the release of AZT. After the first 24 hr 18% and 22% of the covalently bound drug is released in RPMI 1640 DM and RPMI 1640 DM + lysed MT-4 cell contents, respectively (Fig. 2). AZTMP itself is rapidly hydrolysed to AZT: hydrolysis in RPMI 1640 DM comes to completion within 18 hr, whereas in RPMI 1640 DM + MT-4 cell contents it only takes less than 6 hr. AZT is stable in both media (data not shown).

DISCUSSION

In order to investigate whether AZT can be targeted to MT-4 cells, we conjugated the drug, in its 5′-monophosphate form, to various neoglycoproteins.

The study presented here shows the results of *in vitro* testing of various neoglycoproteins and their AZTMP conjugates regarding their anti-HIV-1 activity.

When evaluated for their inhibitory effects on HIV-1 induced cytopathogenicity in MT-4 cells *in vitro*, the neoglycoprotein containing 40 mannose molecules per HSA molecule was effective itself, in contrast with the other carriers. This may be explained by the fact that the interaction between HIV-1 gp120 envelope proteins and the CD4 molecules of $\mathrm{T_4}$-lymphocytes is mandatory for infection to take place [7, 18, 43, 44]. gp120 is a heavily glycosylated glycoprotein carrying predominantly oligomannosidic glycans [19]. Possibly, the $\mathrm{Man_{40}HSA}$ neoglycoprotein interferes with the binding of gp120 to the CD4 molecule. This interference may be accomplished by interaction with the CD4 molecule or by binding to a particular membrane component in the near vicinity of the CD4 molecule.

The mannose containing neoglycoproteins show marked increase in antiviral activity when conjugated with AZTMP. The same holds for $\mathrm{Gal_{32}HSA}$–AZTMP and $\mathrm{Fuc_{10}HSA}$–AZTMP, although to a lesser extent. The $\mathrm{Man_{22}HSA}$–AZTMP conjugate was shown to be the most potent preparation, with an antiviral activity, based on the units AZTMP present in the conjugates, exceeding that of HSA–AZTMP for more than 30 times. These compounds are not only more antivirally active, but also show less toxicity than the antiviral drug itself, resulting in higher selectivity indices.

The stability experiments indicate that hydrolysis of the phosphoamide bond between AZTMP and the protein leads to release of AZT. Whether AZTMP or AZT is released depends on the pH at which hydrolysis takes place: at pH 4 the nitrogen involved in the phosphoamide bond is protonated. This makes the N—P bond more hydrolysis sensitive,

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* Abbreviations: Fuc, fucose; Gal, galactose; Gluc, glucose; Man, mannose. The number denotes the number of sugar molecules per protein molecule.

Table 2. Anti-HIV-1 activity of (neoglyco)protein–AZTMP conjugates compared to AZTMP and AZT

Conjugate	Molar ratio AZTMP : protein	N	IC_{50} conjugate* ($\times 10^{-3}$ μM)	IC_{50} expressed as AZTMP ($\times 10^{-3}$ μM)	Potency (per unit AZTMP) vs HSA–AZTMP	CC_{50} conjugate† (μM)	SI‡
HSA–AZTMP	5.8	4	5.93 ± 1.84	34.4 ± 10.7	1.0	>1.45	>245
$Gluc_{26}$HSA–AZTMP	5.6	4	1.85 ± 1.47	10.4 ± 8.2	3.3	>1.21	>654
Man_7HSA–AZTMP	2.8	2	0.48 ± 0.01	1.34 ± 0.03	25.7	>3.53	>7354
Man_{22}HSA–AZTMP	1.5	2	0.60 ± 0.21	0.90 ± 0.31	38.2	>6.55	>10,917
Man_{40}HSA–AZTMP	1.3	4	1.14 ± 0.70	1.48 ± 0.91	23.2	>6.18	>5421
Gal_{32}HSA–AZTMP	1.9	5	1.23 ± 0.22	2.34 ± 0.42	14.7	>6.44	>5236
Fuc_{10}HSA–AZTMP	3.5	2	0.57 ± 0.24	2.00 ± 0.84	17.2	>1.38	>2421
AZTMP	—	7	3.68 ± 3.67	—	—	25.9 ± 6.9	7038
AZT	—	3	5.0 ± 5.0	—	—	13.0 ± 6.0	2600

N, number of experiments.
* Fifty per cent inhibitory concentration.
† Fifty per cent cytotoxic concentration.
‡ Selectivity Index: CC_{50}/IC_{50}.

Table 3. Anti-HIV-1 activity of neoglycoproteins and control compounds

	IC_{50}^{*} (μM)
HSA	>3.62
$Gluc_{26}$HSA	>3.29
Man_7HSA	>3.62
Man_{22}HSA	>3.33
Man_{40}HSA	0.54
Gal_{32}HSA	>6.44
Fuc_{10}HSA	>3.50
p-a.p.mannose†	>922.51

All data represent average values for at least two separate experiments.
* Fifty per cent inhibitory concentration.
† *p*-Aminophenyl-mannopyranoside.

leading to release of AZTMP. At pH 7.4, the phosphate oxygens are deprotonated causing high electron delocalization over the phosphate part of the bond. In this case AZT is released. After 24 hr of incubation, being the period of time in which the AZTMP is supposed to exhibit its antiviral activity, 18% and 22% of the covalently bound drug is released in fresh medium and medium with lysed cell contents, respectively. The fact that HSA–AZTMP and $Gluc_{26}$HSA–AZTMP show some antiviral activity, may be explained by such a release. One should consider the possibility of an accelerated release after recognition of the neoglycoprotein by a specific lectin on the cell surface. The microclimate of the plasma membrane of the cells may provide the required hydrolytic conditions (e.g. lower pH, excreted or membrane bound enzymes) for a more rapid degradation of the phosphoamide bond between drug and neoglycoprotein. If this would be a major mechanism, it is clear that the HSA–AZTMP and $Gluc_{26}$HSA–AZTMP should exhibit a potency similar to the amount of AZTMP present in the conjugate. However, this is clearly not the case (Table 2). In conclusion, extracellular drug release cannot solely account for the antiviral activity observed.

In the case of Man_{40}HSA–AZTMP, one could think of a synergistic effect of the neoglycoprotein carrier itself (interference with gp120–CD4 interaction) and AZTMP/AZT. A synergistic effect of dextransulfate with AZT has been observed by Ueno and Kuno [45]. Dextransulfate is thought to act on HIV-1 adsorption by interference with the gp120–CD4 interaction as well as to inhibit reverse transcriptase [25, 46–48]. In our study, however, the IC_{50} of Man_{40}HSA is 0.54 μM and that of the conjugate 1.14×10^{-3} μM, corresponding with a 480-fold lower concentration of the carrier. It is highly questionable whether this concentration of the neoglycoprotein could induce a synergistic effect.

Another explanation for the observed results could be the involvement of an endocytotic process taking place after recognition by a cell bound lectin. Endocytosis is a general term for various uptake mechanisms [49, 50], performed by different cell types for a wide variety of functions, e.g. nutrition, host

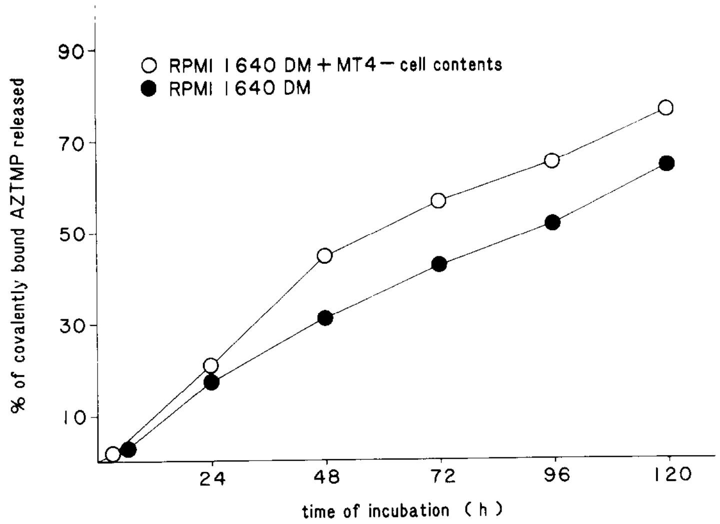

Fig. 2. Stability of $Man_{22}HSA$–AZTMP in cell culture media during 120 hr under conditions described.

defence and transport [51]. Considering the fact that the $Gluc_{26}HSA$–AZTMP and HSA–AZTMP conjugates are considerably less potent in inhibiting HIV-1 infection compared to the other compounds, adsorptive- and/or receptor-mediated endocytosis are possibly involved. The lectins thought to be present on lymphocyte membranes recognize galactose, mannose and fucose [29–35]. These receptors could interact with the sugar moieties of the neoglycoproteins, inducing a pinocytotic mechanism or receptor–ligand internalization. This internalization could lead to sequestration in acidic compartments such as endosomes and lysosomes [3]. Both the acidic environment as well as the presence of proteases and phosphorylases could then lead to release of AZTMP or AZT. The question remains, whether this release is followed by passive diffusion of the drug through the lysosomal membrane and transfer to the cytoplasmic compartment. Lanzavecchia *et al.* [52] showed that T_4 cells are fully capable of capturing soluble (mannose-containing) gp120. Also, they can process the gp120 and display processed parts on their outer surface [52].

Experiments to unravel the mechanism(s) responsible for the antiviral activity exhibited by the various neoglycoprotein–AZTMP conjugates are in progress. They include *in vitro* uptake experiments in MT-4 cells with ^{125}I-labeled neoglycoproteins and their AZTMP–conjugates and studies with regard to the influences on the intracellular transport and degradation by substances such as cytochalasin B, colchicine and chloroquine [53]. *In vivo* distribution studies in rats of the neoglycoproteins and their AZTMP–conjugates may lead to the development of preparations that only slowly leave the bloodstream due to capture by the RES and hepatic cells and provide an optimal exposure time for the various blood cell types.

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REFERENCES

1. Meijer DKF, Jansen RW and Molema G, Disposition of macromolecules in the liver in relation to drug targeting. In: *Current Topics in Gastroenterology and Hepatology* (Eds. Tytgat GNJ and van Blankenstein M), pp. 374–391. Georg Thieme Verlag, Stuttgart, 1990.
2. Meijer DKF, Molema G, Jansen RW and Moolenaar F, Design of cell-specific drug targeting preparations for the liver: where cell biology and medicinal chemistry meet. In: *Trends in Drug Research* (Ed. Claassen V), pp. 303–332. Elsevier, Amsterdam, 1990.
3. Meijer DKF and van der Sluijs P, Covalent and non-covalent protein binding of drugs: implications for hepatic clearance, storage and cell-specific drug delivery. *Pharm Res* **6**: 105–118, 1989.
4. Monsigny M, Roche A-C and Midoux P, Uptake of neoglycoproteins via membrane lectin(s) of L1210 cells evidenced by quantitative flow cytofluorometry and drug targeting. *Biol Cell* **51**: 187–196, 1984.
5. Barré-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, Dauguet C, Axler-Blin C, Vézinet-Brun F, Rouzioux C, Rozenbaum W and Montagnier L, Isolation of a T-lymphotropic retrovirus from a patient at risk for Acquired Immune Deficiency Syndrome (AIDS). *Science* **220**: 868–871, 1983.
6. Popovic M, Sarngadharan MG, Read E and Gallo RC, Detection, isolation and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **224**: 497–500, 1984.
7. Dalgleish AG, Beverley PCL, Clapham PR, Crawford DH, Greaves MF and Weiss RA, The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* **312**: 763–767, 1984.
8. Klatzmann D, Barré-Sinoussi FB, Nugeyre MT, Dauguet C, Vilmer E, Griscelli C, Brun-Vezinet F, Rouzioux C, Gluckman JC, Chermann J-C and Montagnier L, Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. *Science* **225**: 59–63, 1984.
9. McDougal JS, Kennedy MS, Sligh JM, Cort SP, Mawle A and Nicholson JKA, Binding of HTLV-III/LAV to T4+ T cells by a complex of the 110K viral protein and the T4 molecule. *Science* **231**: 382–385, 1986.
10. Levy JA, Shimabukuro J and McHugh T, AIDS-associated retroviruses (ARV) can productively infect other

cells besides human T helper cells. *Virology* **147**: 441, 1985.

11. Wood GS, Warner NL and Warnke RA, Anti-leu-3/T4 antibodies react with cells of monocyte/macrophage and Langerhans lineage. *J Immunol* **131**: 212, 1983.
12. Pert CB, Ruff MR, Ruscetti F, Farrar WL and Hill JM, HIV receptor in brain and deduced peptides that block viral infectivity. In: *Psychological, Neuropsychiatric and Substance Abuse Aspects of AIDS* (Eds. Bridge TP *et al.*), pp. 73–83. Raven Press, NY, 1988.
13. Gartner S, Markovits P, Markovitz DM, Kaplan MH, Gallo RC and Popovic M, The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science* **233**: 215–219, 1986.
14. Ho DH, Rota TR and Hirsch MS, Infection of monocyte/macrophages by Human T Lymphotropic Virus Type III. *J Clin Invest* **77**: 1712–1715, 1986.
15. Pauza CD and Price TM, Human Immunodeficiency virus infection of T cells and monocytes proceeds via receptor-mediated endocytosis. *J Cell Biol* **107**: 959–968, 1988.
16. Salahuddin SZ, Rose RM, Groopman JE, Markham PD and Gallo RC, Human T lymphotropic virus type III infection of human alveolar macrophages. *Blood* **68**: 281, 1986.
17. Gruters RA, Neefjes JJ, Tersmette M, de Goede REY, Tulp A, Huisman HG, Miedema F and Ploegh HL, Interference with HIV-induced syncytium formation and viral infectivity by inhibitors of trimming glucosidases. *Nature* **330**: 74–77, 1987.
18. Matthews TJ, Weinhold KJ, Lyerly HK, Langlois AJ, Wigzell H and Bolognesi DP, Interaction between the human T-cell lymphotropic virus type IIIB envelope glycoprotein gp120 and the surface antigen CD4: role of carbohydrate in binding and cell fusion. *Proc Natl Acad Sci USA* **84**: 5424–5428, 1987.
19. Geyer H, Holschbach C, Hunsmann G and Schneider J, Carbohydrates of Human Immunodeficiency Virus. Structures of oligosaccharides linked to the envelope glycoprotein 120. *J Biol Chem* **263**: 11760–11767, 1988.
20. Robinson WE, Montefiori DC and Mitchell WM, Evidence that mannosyl residues are involved in Human Immunodeficiency Virus Type 1 (HIV-1) pathogenesis. *AIDS Res Hum Retroviruses* **3**: 265–282, 1987.
21. Lifson J, Coutré S, Huang E and Engleman E, Role of envelope glycoprotein carbohydrate in human immunodeficiency virus (HIV) infectivity and virus-induced cell fusion. *J Exp Med* **164**: 2101–2106, 1986.
22. Mitsuya H, Weinhold KJ, Furman PA, St Clair MH, Nusinoff Lehrman S, Gallo RC, Bolognesi D, Barry DW and Broder S, 3′-Azido-3′-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus in vitro. *Proc Natl Acad Sci USA* **82**: 7096–7100, 1985.
23. Zimmerman TP, Mahony WB and Prus KL, 3′-Azido-3′-deoxythymidine. An unusual nucleoside analogue that permeates the membrane of human erythrocytes and lymphocytes by nonfacilitated diffusion. *J Biol Chem* **262**: 5748–5754, 1987.
24. Furman PA, Fyfe JA, St Clair MH, Weinhold K, Rideout JL, Freeman GA, Nusinoff Lehrman S, Bolognesi DP, Broder S, Mitsuya H and Barry DW, Phosphorylation of 3′-azido-3′-deoxythymidine and selective interaction of the 5′-triphosphate with human immunodeficiency virus reverse transcriptase. *Proc Natl Acad Sci USA* **83**: 8333–8337, 1986.
25. De Clercq E, Chemotherapeutic approach of AIDS. *Verhandelingen van de Koninklijke Academie voor Geneeskunde van België* **L nr 2**: 166–217, 1988.
26. Hirsch MS, Azidothymidine. *J Infect Diseases* **157**: 427–431, 1988.
27. De Clercq E, Perspectives for the chemotherapy of AIDS. *Anticancer Res* **7**: 1023–1038, 1987.
28. Kennedy CJ, AZT in the treatment of AIDS and ARC. In: *HIV and Other Highly Pathogenic Viruses* (Ed. Smith RA), pp. 81–93. Academic Press, Harcourt Brace Jovanovich, 1987.
29. Apgar JR and Cresswell P, Expression of cell surface lectins on activated human lymphoid cells. *Eur J Immunol* **12**: 570–576, 1982.
30. Boldt DH and Armstrong JP, Rosette formation between human lymphocytes and sheep erythrocytes. *J Clin Invest* **57**: 1068–1078, 1976.
31. Decker JM, Lectin-like molecules on murine thymocytes and splenocytes-I. Molecules specific for the oligosaccharide moiety of the fetal α-globulin fetuin. *Mol Immunol* **17**: 803–808, 1980.
32. Gowda SD, Stein BS, Mohagheghpour N, Benike CJ and Engleman EG, Evidence that T cell activation is required for HIV-1 entry in $CD4^+$ lymphocytes. *J Immunol* **142**: 773–780, 1989.
33. Kieda CMT, Bowles DJ, Ravid A and Sharon N, Lectins in lymphocyte membranes. *FEBS Lett* **94**: 391–396, 1978.
34. Kieda C, Roche A-C, Delmotte F and Monsigny M, Lymphocyte membrane lectins. Direct visualization by the use of fluoresceinyl-glycosylated cytochemical markers. *FEBS Lett* **99**: 329–332, 1979
35. Stoolman LM and Rosen SD, Possible role for cell-surface carbohydrate-binding molecules in lymphocyte recirculation. *J Cell Biol* **96**: 722–729, 1983.
36. Kataoka M and Tavassoli M, Synthetic neoglycoproteins: a class of reagents for detection of sugar-recognizing substances. *J Histochem Cytochem* **32**: 1091–1094, 1984.
37. Monsigny M, Roche A-C, Kieda C, Midoux P and Obrenovitch A, Characterization and biological implications of membrane lectins in tumor, lymphoid and myeloid cells. *Biochimie* **70**: 1633–1649, 1988.
38. Fiume L, Bassi B, Busi C and Mattioli A, Preparation of a lactosaminated albumin–ara–AMP conjugate which remains soluble after lyophilization. *Pharm Acta Helv* **60**: 318–320, 1985.
39. Halloran MJ and Parker CW, The preparation of nucleotide–protein conjugates: carbodiimides as coupling agents. *J Immunol* **96**: 373–378, 1966.
40. Pauwels R, Balzarini J, Baba M, Snoeck R, Schols D, Herdewijn P, Desmyter J and De Clercq E, Rapid and automated tetrazolium-based colorimetric assay for detection of anti-HIV compounds. *J Vir Meth* **20**: 309–321, 1988.
41. Pauwels R, Balzarini J, Schols D, Baba M, Desmyter J, Rosenberg I, Holy A and De Clercq E, Phosphonylmethoxyethyl purine derivatives, a new class of anti-Human Immunodeficiency Virus agents. *Antimicrobial Agents Chemother* **32**: 1025–1030, 1988.
42. Fiume L, Bassi B and Bongini A, Conjugates of 9-β-D-arabinofuranosyladenine 5′-monophosphate (ara-AMP) with lactosaminated albumin. Characterization of the drug–carrier bonds. *Pharm Acta Helv* **63**: 137–139, 1988.
43. Maddon PJ, Dalgleish AG, McDougal JS, Clapham PR, Weiss RA and Axel R, The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* **47**: 333–348, 1986.
44. Sattentau QJ and Weiss RA, The CD4 antigen: physiological ligand and HIV receptor. *Cell* **52**: 631–633, 1988.
45. Ueno R and Kuno S, Dextran sulphate, a potent anti-HIV agent *in vitro* having synergism with zidovudine. *Lancet* June 13: 1379, 1987.
46. Mitsuya H, Looney DJ, Kuno S, Ueno R, Wong-Staal F and Broder S, Dextran sulfate suppression of viruses in the HIV family: inhibition of virion binding to CD4+ cells. *Science* **240**: 646–649, 1988.

47. Nakashima H, Yoshida O, Baba M, De Clercq E and Yamamoto N, Anti-HIV activity of dextran sulphate as determined under different experimental conditions. *Antiviral Res* **11**: 233–246, 1989.
48. Baba M, Pauwels R, Balzarini J, Arnout J, Desmyter J and De Clercq E, Mechanism of inhibitory effect of dextran sulfate and heparin on the replication of human immunodeficiency virus *in vitro*. *Proc Natl Acad Sci USA* **85**: 6132–6136, 1988.
49. Shepherd VL, Intracellular pathways and mechanisms of sorting in receptor-mediated endocytosis. *TIPS* **10**: 458–462, 1989.
50. Silverstein SC, Steinman RM and Cohn ZA, Endocytosis. *Annu Rev Biochem* **46**: 669–722, 1977.
51. Stahl P and Schwartz AL, Receptor-mediated endocytosis. *J Clin Invest* **77**: 657–662, 1986.
52. Lanzavecchia A, Roosnek E, Gregory T, Berman P and Abrignani S, T cells can present antigens such as HIV gp120 targeted to their own surface molecules. *Nature* **334**: 530–532, 1988.
53. Freed BM, Lempert N and Lawrence DA, The inhibitory effects of *N*-ethylmaleimide, colchicine and cytochalasins on human T-cell functions. *Int J Immunopharm* **11**: 459–465, 1989.