Glycoconjugate J (1989) 6:229-240

Effects of the α-Mannosidase Inhibitors, 1,4-Dideoxy-1,4-imino-D-mannitol and Swainsonine, on Glycoprotein Catabolism in Cultured Macrophages

PETER F DANIEL[1*], DAVID S NEWBURG[1], NANCY E O'NEIL[1], PAUL W SMITH[2] and GEORGE W J FLEET[2]

[1] *Department of Biochemistry, E.K. Shriver Center, 200 Trapelo Road, Waltham, MA 02254 U.S.A.*
[2] *Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford OX1 3QY, England*

Received May 9/November 11, 1988.

Key words: α-mannosidase inhibitors; oligosaccharide processing; macrophages; swainsonine; 1,4-dideoxy-1,4-imino-D-mannitol; reversed phase HPLC

Thioglycollate-stimulated murine peritoneal macrophages were cultured for eight days in the presence of swainsonine, or 1,4-dideoxy-1,4-imino-D-mannitol (DIM), or both of these competitive α-mannosidase inhibitors together. Analysis of accumulated high-mannose oligosaccharides by reversed phase HPLC after perbenzoylation revealed that DIM- and DIM-plus swainsonine-treated macrophages contained larger amounts of $Man_7GlcNAc$, $Man_8GlcNAc$ and $Man_9GlcNAc$, while swainsonine-treated macrophages contained relatively more $Man_3GlcNAc$ and $Man_5GlcNAc$. These results are consistent with the known inhibitory effects of DIM and swainsonine on Golgi mannosidases I and II, respectively, and on lysosomal α-mannosidase. Depletion of stored oligosaccharides to control values was complete within seven days of terminating swainsonine treatment.

Swainsonine, a naturally-occurring alkaloid, is a specific and reversible competitive inhibitor of lysosomal α-mannosidase (EC 3.2.1.24) [1]. When ingested, it produces a toxicosis reminiscent of the genetic α-Mannosidoses seen in animals and humans [2], including accumulation of intracellular oligosaccharides and inhibition of lysosomal α-mannosidase [3, 4]. Swainsonine is also a potent inhibitor of Golgi mannosidase II [5] and because this inhibition results in altered processing of asparagine-linked glycans, cultured cells treated with this inhibitor have been used for studying oligosaccharide processing [6]. Because of the dual effects of swainsonine on processing and lysosomal catabolism, treated cells can also be used for comparison with cells from individuals having Mannosidosis, to explore further the pathobiology of this rare genetic disease [7].

Another naturally-occurring structural analogue of mannose, 1,5-dideoxy-1,5-imino-D-mannitol (abbreviation DMM, derived from the trivial name 1-deoxymannojirimycin) has

*Author for correspondence.

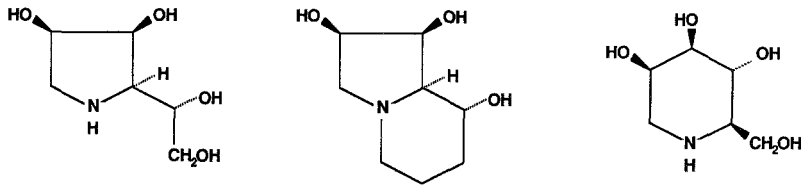

Figure 1. Structures of α-mannosidase inhibitors. See text for abbreviations.

been isolated from the seeds of a tropical legume, *Lonchocarpus sericeus* [8]. DMM was synthesized recently [9, 10] and was shown to be a specific inhibitor of Golgi mannosidase I [11], which resulted in the presence of $Man_9GlcNAc_2$ as the predominant *N*-linked glycan [12] and in impaired secretion of glycoproteins [13]. Because the structure of swainsonine is closer to that of an azafuranose derivative of mannose than to the six-membered ring of DMM (see Fig. 1), a new inhibitor was synthesized containing the features of DMM on a five-membered ring [14, 15] to test the possibility that this might be an even stronger inhibitor. This compound, 1,4-dideoxy-1,4-imino-D-mannitol (DIM), is a potent inhibitor *in vitro* of Jack Bean and lysosomal α-mannosidases as well as of Golgi mannosidase I [16].

We have developed a sensitive HPLC procedure that has enabled us to quantify directly the small amounts of oligosaccharides accumulated in cultured cells with induced or natural storage disorders [17]. In this study, we have compared the effects of DIM and swainsonine on cultured macrophages with respect to the amount and chain length of high mannose oligosaccharides that accumulate. We have also monitored the disappearance of stored oligosaccharides after discontinuation of swainsonine treatment. A preliminary account of this work has been presented [18].

Materials and Methods

Cell Culture Conditions

Peritoneal macrophages were elicited by injecting adult C57BL/6J mice (Jackson Laboratories, Bar Harbor, MA, USA) intraperitoneally with 1 ml of 4% Brewer's thioglycollate medium (Difco, Detroit, MI, USA). After four days the mice were killed by cervical dislocation. Macrophages were harvested by peritoneal lavage with Medium 199 and cultured in 35 mm polystyrene dishes at approximately 3×10^6 cells/plate in 2 ml of medium, as described [19]. Cells harvested from a single mouse peritoneum were routinely plated on three dishes and incubated for two hours at 37°C in 5% CO_2 in air to allow the macrophages to adhere to the dish. They were washed by vigorous agitation with medium and allowed to remain in medium for two days. The medium was changed and after three days of additional growth the cells were treated with the inhibitors. Protein was measured by the method of Lowry *et al.* [20].

Treatment with Inhibitors

Swainsonine and DIM were synthesized and purified according to published procedures [15, 21]. Media were formulated to contain 0.1 mM, 1 mM and 5 mM DIM, or 0.1 mM swainsonine, or a combination of 5 mM DIM and 0.1 mM swainsonine; medium without inhibitors was used for control cultures. Three plates of macrophages were grown in each of these media for four days and they were given fresh medium for an additional four days, whereupon the cells were harvested. After removal of the medium, cells were washed thrice with 2 ml of 0.9% saline at 37°C, harvested by scraping with a rubber policeman in 0.3 ml of ice-cold saline, and transferred with a 0.3 ml rinse to a small conical tube. The cells were disrupted by sonication on ice with a microprobe.

Recovery from Swainsonine Treatment

The ability of cells to catabolize accumulated oligosaccharides after the termination of swainsonine treatment was studied in macrophages that had been cultured in medium containing 0.1 mM swainsonine for seven days, with a single medium change on day four. Cells were then harvested from three dishes (zero time point of recovery). The remaining dishes were changed to inhibitor-free medium and the cells were harvested after one, two, four or seven days of recovery. Additional medium changes were made on days two and four to hasten the release of swainsonine into the medium. Cells cultured throughout the entire experimental period in inhibitor-free medium were harvested as untreated controls.

HPLC Analysis of Oligosaccharides

Oligosaccharides were analyzed from aliquots of cell homogenate as described [17]. The sample was deionized on mixed bed resins, and the neutral oligosaccharide fraction was reduced and benzoylated. Derivatized samples were dissolved in acetonitrile, injected on a Microsorb C8 column (4.6 mm x 100 mm; Rainin Instruments, USA) and eluted with a linear gradient of acetonitrile/water, 4/1 by vol, changing to pure acetonitrile over 15 min. Burdick and Jackson HPLC grade solvents were purchased from Rainin Instruments. HPLC was performed with a Varian Instrument Model 5020 (Varian Associates, USA). The output from a model SF 770 variable wavelength detector operating at 230 nm (Kratos/Schoeffel, USA) was connected to a model 4270 integrator (Spectra-Physics, USA). Perbenzoylated raffinose (11 benzoyl groups) was used as an external standard for quantification as described [17]. Areas were normalized to 11 benzoyl groups and then divided by the raffinose response; the molar extinction per benzoyl group is approx.12,600.

Sources of Standard Oligosaccharides

Manα1-3Manβ1-4GlcNAc, Manα1-2Manα1-3Manβ1-4GlcNAc and Manα1-2Manα1-2Manα1-3Manβ1-4GlcNAc were isolated from urine of patients with α-Mannosidosis [22]; Manα1-6Manβ1-4GlcNAc was isolated from bovine Mannosidosis urine [17], as were Manα1-6[Manα1-3]Manβ1-4GlcNAc and Manα1-3Manα1-6Manβ1-4GlcNAc after digestion with endoglucosaminidase D and H, respectively; Manα1-3Manα1-6[Manα1-3]Manβ1-4GlcNAc and Manα1-6[Manα1-3]Manα1-6[Manα1-3]Manβ1-4GlcNAc were obtained from urine of sheep with locoweed toxicosis [23] after digestion with endoglucosaminidase H.

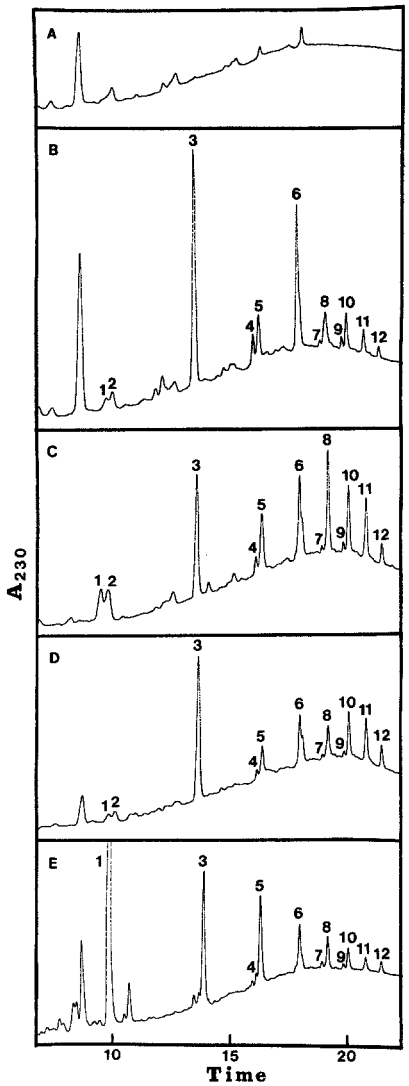

Figure 2. Comparison of the effects of DIM and swainsonine on the HPLC elution profiles of perbenzoylated oligosaccharide alditols isolated from cultured murine macrophages.
Oligosaccharides were isolated from macrophages after culture for eight days in the absence of inhibitors (panel A) or in the presence of 0.1 mM swainsonine (panel B), 5 mM DIM (panel C), or a combination of 0.1 mM swainsonine and 5 mM DIM (panel D). The HPLC profile of standard oligosaccharides from human α-Mannosidosis urine is shown in panel E. For peak identification see Table 1. One eighth of the total neutral oligosaccharides isolated from *ca.* 9 x 10^6 cells was injected.

Results

Comparison of DIM and Swainsonine

When macrophages were treated with DIM at concentrations of 1 mM or less, the oligosaccharide profile obtained by HPLC did not differ from that of control macrophages (Fig. 2A). However, at a concentration of 5 mM, the lysosomal α-mannosidase was sufficiently inhibited for appreciable amounts of high mannose oligosaccharides to accumulate (Fig. 2C). The profile was similar to that obtained from urine of patients with α-Mannosidosis (Fig. 2E) and consisted of oligosaccharides with two to nine mannose residues and a single *N*-acetylglucosamine residue, indicating the presence of an active lysosomal endo-β-*N*-acetylglucosaminidase [24] in mouse macrophages. Swainsonine and combined swainsonine and DIM treatment also resulted in the accumulation of high mannose oligosaccharides (Fig. 2B and D). However, the oligosaccharides accumulating as a result of each of these treatments differed in their relative abundance, as shown in Table 1. Treatment with DIM caused a greater accumulation of the larger oligosaccharides ($Man_{6-9}GlcNAc$) than did treatment with swainsonine. $Man_5GlcNAc$ and $Man_3GlcNAc$ were the principal oligosaccharides that accumulated in swainsonine treated macrophages. In macrophages treated with both inhibitors, $Man_7GlcNAc$, $Man_8GlcNAc$ and $Man_9GlcNAc$ accumulated to a greater extent than with either of the inhibitors alone, suggesting that inhibition of processing and lysosomal α-mannosidase(s) was incomplete in the presence of 5 mM DIM alone.

The quantitative data presented in Table 1 are consistent with the presence of an additional lysosomal α-mannosidase in mouse macrophages that degrades branched $Man_3GlcNAc$ to $Man_2GlcNAc$. This enzyme is inhibited by swainsonine but not by DIM. As a result, the two isomers of $Man_2GlcNAc$ (for structures see Table 2) comprise 26.4% of the total oligosaccharides in cells treated with DIM alone, but only 11% in cells treated with swainsonine alone or DIM plus swainsonine, with a concomitant increase in the amount of $Man_3GlcNAc$. Cenci di Bello *et al.* [7] originally described a similar enzyme in human skin fibroblasts. A single isomer of $Man_2GlcNAc$ ($Man_2GlcNAc$-I) is observed in swainsonine-treated control human fibroblasts and then only during the recovery period after removal of swainsonine; however, it is the major oligosaccharide accumulated in untreated Mannosidosis fibroblasts [17].

Recovery from Swainsonine

Macrophages cultured in medium containing 0.1 mM swainsonine for seven days were allowed to recover from α-mannosidase inhibition in inhibitor-free medium. After seven days of swainsonine treatment, macrophages had accumulated $Man_2GlcNAc$ through $Man_9GlcNAc$, with a preponderance of $Man_3GlcNAc$ and $Man_5GlcNAc$ (Fig. 3A). Surprisingly, during the first four days of recovery the total amount of high mannose oligosaccharides remained constant (Table 3), but the relative amounts of $Man_3GlcNAc$, $Man_5GlcNAc$ and $Man_{7-9}GlcNAc$ decreased, with a concomitant increase in the abundance of the $Man_2GlcNAc$ and $Man_4GlcNAc$ isomers. During the recovery process the relative isomeric composition of $Man_5GlcNAc$ (peak 6) changed, as shown by the appearance of a shoulder on the descending edge. Unmarked peaks in Fig. 3 panels B and C, eluting between peaks 3 and 4, did not co-elute with Manα1-2Manα1-3Manβ1-4GlcNAc, Manα1-6[Manα1-

Table 1. Relative abundance of oligosaccharides determined by HPLC.
Isolated oligosaccharides were reduced, perbenzoylated and quantified by reversed phase HPLC on a C8 column, employing an acetonitrile-water gradient for elution. Key: SW, swainsonine.

Peak[a]	Component[b]	Treated macrophages			
		Mannosidosis urine	0.1 mM SW	5 mM DIM	5 mM DIM + 0.1 mM SW
		Mol%			
1	$Man_2GlcNAc$-I	68.8	4.7	11.9	4.7
2	$Man_2GlcNAc$-II	-	6.0	14.5	6.4
3	$Man_3GlcNAc$	-	44.0	23.3	41.2
3[c]	$Man_3GlcNAc$-M	14.2	-	-	-
4	$Man_4GlcNAc$-I	0.7	4.3	2.3	2.0
5[c]	$Man_4GlcNAc$-M	8.0	-	-	-
5	$Man_4GlcNAc$-II	-	8.6	10.2	7.6
6	$Man_5GlcNAc$	4.4	20.0	13.9	14.3
7	$Man_6GlcNAc$-I	0.3	0.5	0.6	0.6
8	$Man_6GlcNAc$-II	1.8	5.4	10.8	6.2
9	$Man_7GlcNAc$-I	0.2	0.8	0.5	0.7
10	$Man_7GlcNAc$-II	1.1	2.9	5.7	8.1
11	$Man_8GlcNAc$	0.7	2.0	5.1	6.3
12	$Man_9GlcNAc$	0.4	0.7	1.3	2.1
nmol/mg protein			4.0	3.7	3.8

[a] See Fig. 2.
[b] See Table 2 for probable structures of $Man_{2-5}GlcNAc$.
[c] A slight difference in retention times was observed for peaks 3 and 5 from Mannosidosis urine and the corresponding peaks from mouse macrophages.

3]Manβ1-4GlcNAc or Manα1-3Manα1-6Manβ1-4GlcNAc, and may represent non-carbohydrate contaminants. After seven days of recovery, the oligosaccharide profile had changed dramatically and did not differ substantially from that of the control (cf. Fig. 3D and E). Such a rapid recovery was expected on the basis of an earlier study on swainsonine-poisoned sheep, in which the amount of oligosaccharides in the urine declined rapidly and reached baseline levels within 12 days of discontinuing swainsonine administration [25].

Discussion

Earlier studies [14] demonstrated that DIM is a ten-fold better inhibitor than swainsonine of Jack bean α-mannosidase *in vitro* (K_i 0.76 and 9.5 μM, respectively). In contrast, the six-membered ring mannose analogue, DMM, is a very weak inhibitor of Jack bean α-mannosidase with a reported K_i of 400 μM [9]. Inhibition of intact cells is dependent upon the penetration of the cells by the inhibitor as well as by its relative inhibitory potency. Chotai *et al.* [26] have shown that swainsonine is internalized rapidly by cultured fibroblasts in a

Table 2. Probable structures of stored oligosaccharides in mouse macrophages

Peak[a]	Component	Structure
1	$Man_2GlcNAc$-I	Manα1-3Manβ1-4GlcNAc
2	$Man_2GlcNAc$-II	Manα1-6Manβ1-4GlcNAc
3	$Man_3GlcNAc$	Manα1-6Manβ1-4GlcNAc 3 Manα1
3[b]	$Man_3GlcNAc$-M	Manα1-2Manα1-3Manβ1-4GlcNAc
4	$Man_4GlcNAc$-I	Manα1-3Manα1-6Manβ1-4GlcNAc 3 Manα1
5[b]	$Man_4GlcNAc$-M	Manα1-2Manα1-2Manα1-3Manβ1-4GlcNAc
5	$Man_4GlcNAc$-II	Manα1-6Manα1-6Manβ1-4GlcNAc[c] 3 Manα1
6	$Man_5GlcNAc$	Manα1-6Manα1-6Manβ1-4GlcNAc 3 3 Manα1 Manα1

[a] Peak numbers refer to Fig. 2.
[b] A slight difference in retention times was observed for peaks 3 and 5 from Mannosidosis urine and the corresponding peaks from mouse macrophages. Structures of Mannosidosis urine oligosaccharides $Man_3GlcNAc$-M and $Man_4GlcNAc$-M were taken from Nordén *et al.* [22]. Probable structures of mouse macrophage oligosaccharides are based on co-elution with authentic standards on HPLC, and on results obtained from permethylation studies of oligosaccharides isolated from swainsonine-treated human skin fibroblasts [17].
[c] Tentative structure, as no standard was available.

time- and concentration-dependent manner and is concentrated inside the lysosomes by ion trapping. DIM shares structural features and weak basicity with swainsonine and is also taken up by cells, but less readily than swainsonine [27]. The inhibition by these competitive enzyme inhibitors is reversible, which complicates direct measurement of the degree of inhibition of lysosomal α-mannosidase due to the considerable dilution of the lysosomal contents following cell lysis. Therefore, we are reporting only indirect measurements of their relative potency, as measured by oligosaccharide accumulation. The main thrust of this study was to test whether DIM is a more potent inhibitor of lysosomal α-mannosidase than swainsonine when given to cultured mammalian cells, because treatment of cultured human fibroblasts with 100 μM swainsonine has been shown to produce a storage disorder closely resembling Mannosidosis [7, 17].

The results presented here show that DIM is about fifty-fold less potent than swainsonine in the mouse macrophage test system that we employed, because no accumulation of oligosaccharides was observed at concentrations of DIM less than 5 mM. This relative

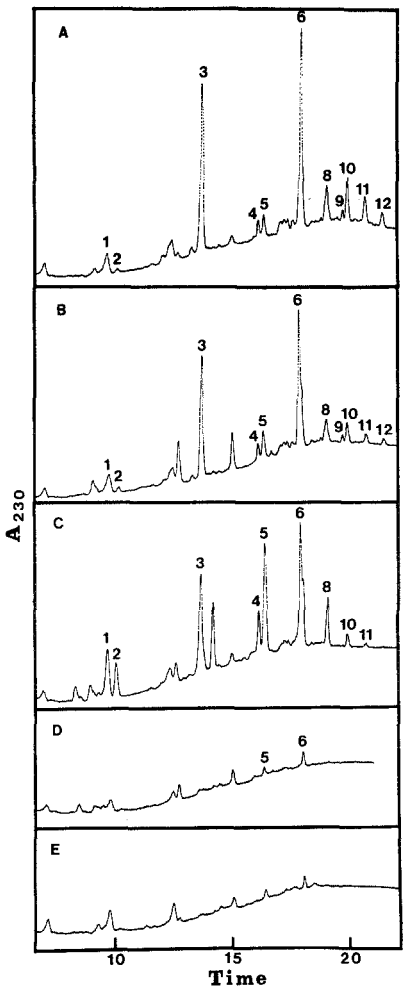

Figure 3. Changes in the HPLC elution profiles of oligosaccharides isolated from cultured murine macrophages during recovery from swainsonine treatment. Macrophages were treated with 0.1 mM swainsonine for seven days (panel A) and then allowed to recover in fresh medium for one day (panel B), four days (panel C), and seven days (panel A) and then allowed to recover in fresh medium for one day (panel B), four days (panel C), and seven days (panel D); panel E: control, untreated macrophages after 14 days in culture. Isolated oligosaccharides were reduced and benzoylated prior to HPLC analysis. For peak identification see Table 1. Major unmarked peaks in panels B and C did not co-elute with any available mannose oligosaccharide standard. One twenty-fifth of the total neutral oligosaccharides isolated from three petri dishes was injected.

potency of DIM and swainsonine on mouse macrophages *in vitro* is consistent with earlier results on rat liver lysosomal α-mannosidase: DIM gave 50% inhibition at a level of 6-12 μM [16] compared to reported values for swainsonine [5] and DMM [9] of 0.2 μM and 380 μM. It should be noted that these relative inhibitory potencies are approximate, as values for 50% inhibition are dependent on the substrate concentration employed.

Table 3. HPLC quantification of oligosaccharides isolated from macrophages recovering from swainsonine treatment.

Peak[a]	Component	Days after discontinuing swainsonine treatment			
		Day 0	Day 1	Day 4	Day 7
		nmol/mg protein[b]			
1	$Man_2GlcNAc$-I	0.30	0.27	0.74	-
2	$Man_2GlcNAc$-II	0.03	0.05	0.36	-
3	$Man_3GlcNAc$	1.48	1.06	0.84	-
4	$Man_4GlcNAc$-I	0.10	0.11	0.23	-
5	$Man_4GlcNAc$-II	0.16	0.20	0.69	0.04
6	$Man_5GlcNAc$	1.21	0.98	0.74	0.06
8	$Man_6GlcNAc$-II	0.23	0.14	0.15	-
9	$Man_7GlcNAc$-I	0.03	0.03	-	-
10	$Man_7GlcNAc$-II	0.19	0.09	0.04	-
11	$Man_8GlcNAc$	0.11	0.04	0.01	-
12	$Man_9GlcNAc$	0.06	0.02	-	-
Total oligosaccharides		3.9	3.0	3.8	0.1

[a] Peak numbers refer to Fig. 3.
[b] Pooled oligosaccharides isolated from three petri dishes. For experimental details see the footnote to Table 1.

Palamarczyk *et al.* [16] tested the effects of DIM on glycoprotein processing in influenza virus-infected MDCK cells and established that it is an inhibitor of Golgi mannosidase I with 50% inhibition at 3-6 μM. DMM is a slightly better inhibitor of mannosidase I (50% inhibition of the rat liver enzyme at 1-2 μM) [11]); however, the exact effect on cultured cells depended not only on the virus strain used but also on the host cell and the length of incubation [12]. Thus, each of the three mannose analogues that has been tested has an effect on glycoprotein processing: DMM and DIM both inhibit Golgi mannosidase I, whereas swainsonine is a powerful inhibitor of Golgi mannosidase II (50% inhibition at 0.2 μM) [4]. Although DIM is a close structural analogue of swainsonine (see Fig. 1), its action more closely resembles DMM in inhibiting mannosidase I rather than mannosidase II. However, because the product of mannosidase I becomes a substrate for mannosidase II, DIM may also inhibit the latter enzyme [16] without this being reflected in the nature of the oligosaccharides accumulated in DIM-treated macrophages. Cenci di Bello *et al.* [27] have shown that DIM is a strong inhibitor of the residual α-mannosidase activity at pH 5 in Mannosidosis fibroblasts, which is predominantly due to Golgi mannosidase II.

Mice, like most other rodents and also humans, possess an endo-β-*N*-acetylglucosaminidase [22] that is active on *N*-linked glycans, with the result that inhibition of lysosomal α-mannosidase leads to storage of mannose-containing oligosaccharides with a single *N*-acetylglucosamine residue at the reducing terminus. This is in contrast to the situation in

swainsonine-intoxicated sheep or in cattle with genetic α-Mannosidosis, where the majority of the storage oligosaccharides have a $GlcNAc_2$ moiety at the reducing terminus [17, 28] due to the absence of endo-β-*N*-acetylglucosaminidase in these species [29].

Treatment of mouse macrophages with 100 μM swainsonine caused the accumulation of oligosaccharides containing two to nine mannose residues and a single glucosamine residue. The HPLC elution profile was very similar to that obtained from swainsonine-treated human skin fibroblasts [17], with $Man_5GlcNAc$ (derived from hybrid glycans produced as a result of swainsonine inhibition of mannosidase II) and branched $Man_3GlcNAc$ (derived from complex glycans) as the major components.

We had anticipated that $Man_9GlcNAc$ would be an abundant storage oligosaccharide in DIM-treated macrophages because DIM is an inhibitor of both mannosidase I and lysosomal α-mannosidase. However, compared to swainsonine-treated macrophages, the relative abundance of $Man_9GlcNAc$ was only doubled from 0.7% to 1.3% in cells treated with 5 mM DIM (Table 1). There are three possible explanations for this unexpected result: (i) mannosidase I is completely inhibited but lysosomal α-mannosidase is not, allowing considerable intralysosomal degradation of $Man_9GlcNAc$; (ii) mannosidase I is not completely inhibited and so processing can proceed further; (iii) additional processing and/or lysosomal α-mannosidases are present that are unaffected by DIM. In an attempt to eliminate the first possibility, we examined the oligosaccharide profile after culturing mouse macrophages in the presence of both 5 mM DIM and 100 μM swainsonine, on the premise that the combination of the two inhibitors would totally inhibit any residual lysosomal α-mannosidase activity. Under these conditions the relative abundance of $Man_9GlcNAc$ was increased to 2.1% and there were also increased amounts of $Man_7GlcNAc$ and $Man_8GlcNAc$, as compared to the values with either DIM or swainsonine alone. This very modest increase in $Man_9GlcNAc$ and the consistency in the amount of stored oligosaccharides at *circa* 4 nmol/mg protein, suggests that extensive processing of high mannose glycoproteins must be occurring prior to their lysosomal catabolism, but does not distinguish between possibilities (ii) and (iii).

We currently favour the third alternative since, in addition to Golgi mannosidases IA, IB and II [30, 31], at least four other non-lysosomal mammalian α-mannosidases have been identified. These include two endoplasmic reticulum (ER) α-mannosidases, which probably play a significant role in mannose trimming reactions. The ER mannosidase removes one specific mannose residue from $Man_9GlcNAc_2$-protein to form a single isomer of $Man_8GlcNAc_2$-protein [32]. A second ER α-mannosidase removes up to three α(1-2)-linked mannose residues from $Man_9GlcNAc_2$-protein to form $Man_6GlcNAc_2$-protein [33]. A cytosolic α-mannosidase active at neutral pH has been purified [34], but subsequently it was shown to be immunologically related to an ER α-mannosidase [35], suggesting that they are two forms of the same enzyme and that the soluble form is derived from the ER membrane α-mannosidase by proteolysis. Recently, an endomannosidase has been found in rat liver Golgi membranes which trims *N*-linked $Glc_1Man_9GlcNAc_2$ to $Man_8GlcNAc_2$ with the release of Glcα1-3Man [36].

This study demonstrates that DIM is an inhibitor of both mannosidase I and lysosomal α-mannosidase in mouse macrophages and differs from both swainsonine and DMM in its

specificity. The amount of DIM needed to inhibit lysosomal α-mannosidase in cultured cells is disappointingly high compared to results obtained from *in vitro* studies. New or modified inhibitors with greater uptake and specificity would contribute greatly toward further progress in understanding the role of the many α-mannosidases that have been described in eukaryotic cells.

Acknowledgements

The authors thank Dr. Christopher Warren for his critical review of the manuscript. This investigation was supported by grants HD-16942 and HD-05515 from the National Institutes of Health, and by the Department of Mental Retardation of the Commonwealth of Massachusetts (contract # 3403-8403-306).

References

1 Dorling PR, Huxtable CR, Colegate SM (1980) Biochem J 191:649-51.
2 Dorling PR, Huxtable CR, Vogel P (1978) Neuropathol Appl Neurobiol 4:285-95.
3 Tulsiani DRP, Touster O (1983) Arch Biochem Biophys 224:594-600.
4 Tulsiani DRP, Broquist HP, Touster O (1985) Arch Biochem Biophys 236:427-34.
5 Tulsiani DRP, Harris TM, Touster O (1982) J Biol Chem 257:7936-39.
6 Elbein AD, Pan YT, Solf R, Vosbeck K (1983) J Cell Physiol 115:265-75.
7 Cenci di Bello I, Dorling PR, Winchester B (1983) Biochem J 215:693-96.
8 Fellows LE, Bell EA, Lynn DG, Pilkiewicz F, Miura I, Nakanishi K (1979) J Chem Soc Chem Commun 977-78.
9 Legler G, Julich E (1984) Carbohydr Res 128:61-72.
10 Fleet GWJ, Ramsden NG, Witty DR (1988) Tetrahedron Lett 29:2871-75.
11 Bischoff J, Kornfeld R (1984) Biochem Biophys Res Commun 125:324-31.
12 Elbein AD, Legler G, Tlusty A, McDowell W, Schwarz R (1984) Arch Biochem Biophys 235:579-88.
13 Furhmann U, Bause E, Legler G, Ploegh G (1984) Nature 307:755-58.
14 Fleet GWJ, Smith PW, Evans SV, Fellows LE (1984) J Chem Soc Chem Commun 1240-41.
15 Bashyal BP, Fleet GWJ, Gough MJ, Smith PW (1987) Tetrahedron 43:3083-93.
16 Palamarczyk G, Mitchell M, Smith PW, Fleet GWJ, Elbein AD (1985) Arch Biochem Biophys 243:35-45.
17 Daniel PF (1987) Methods Enzymol 138:94-116.
18 Daniel PF, Newburg DS, O'Neil NE, Smith PW, Fleet GWJ (1987) Proc 9th Int Symp Glycoconjugates, eds. Montreuil J, Verbert A, Spik G, Fournet B, Secretariat, Lille, p D7.
19 Newburg DS, Yatziv S, McCluer RH, Raghavan SS (1986) Biochim Biophys Acta 877:121-26.
20 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) J Biol Chem 193:265-75.
21 Fleet GWJ, Gough MJ, Smith PW (1984) Tetrahedron Lett 25:1853-56.
22 Nordén NE, Lundblad A, Svensson S, Autio S (1974) Biochemistry 13:871-74.
23 Warren CD, Daniel PF, Bugge B, Evans JE, James LF, Jeanloz RW (1988) J Biol Chem 263:15041-49.

24 Aronson NN, Jr (1987) Proc 9th Int Symp Glycoconjugates, eds Montreuil J, Verbert A, Spik G, Fournet B, Secretariat, Lille, p D3.
25 Daniel PF, Warren CD, James LF (1984) Biochem J 221:601-7.
26 Chotai J, Jennings C, Winchester B, Dorling P (1983) J Cell Biochem 21:107-17.
27 Cenci di Bello I, Fleet GWJ, Namgoong SK, Tadano K-I, Winchester B (1989) Biochem J 259:855-61.
28 Daniel PF, Warren CD, James LF, Jolly RD (1985) in Plant Toxicology, eds Seawright AA, Hegarty MP, James LF, Keeler RF, Queensland Poisonous Plants Committee, Yeerongpilly, Australia, p 290-300.
29 Song W, Li S-C, Li Y-T (1987) Biochem J 248:145-49.
30 Tabas I, Kornfeld S (1979) J Biol Chem 254:11655-63.
31 Tulsiani DRP, Hubbard SC, Robbins PW, Touster O (1982) J Biol Chem 257:3660-68.
32 Bischoff J, Liscum L, Kornfeld R (1986) J Biol Chem 261:4766-74.
33 Schweden J, Legler G, Bause E (1986) Eur J Biochem 157:563-70.
34 Shoup VA, Touster O (1976) J Biol Chem 251:3845-52.
35 Bischoff J, Kornfeld R (1986) J Biol Chem 261:4758-65.
36 Lubas WA, Spiro RG (1987) J Biol Chem 262:3775-81.