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The Uptake of Swainsonine, a Specific Inhibitor of α-D-Mannosidase, Into Normal Human Fibroblasts in Culture

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Swainsonine, an indolizidine alkaloid, found in plants of the genus Swainsona, has been shown to be a strong inhibitor in vitro of the α-D-mannosidase activity in normal human fibroblasts. Therefore, inhibition of α-D-mannosidase activity in extracts of harvested cells grown with swainsonine in the medium has been used to follow the association of the alkaloid with normal human fibroblasts in culture. Swainsonine that could not be removed by extensive washing became associated with the cells within 1 min, and it is concluded that the alkaloid is internalized rapidly by the cells. The amount of swainsonine taken up into the cells depended on the length of time in contact and the concentration of swainsonine in the medium, but at 37°C a plateau of internalized swainsonine occurred after 2 hr with extracellular concentrations of swainsonine of 100 μM or greater. At lower concentrations of swainsonine the rate of uptake was found to be temperature-dependent, increasing greatly at 20°C. The rapidity and temperature sensitivity of the uptake, together with the observation that mannose or mannose-6-phosphate did not prevent the association, suggest that swainsonine enters the cells by permeation rather than by endocytosis. When swainsonine is withdrawn from the culture medium, there is a decrease with time of cell-associated swainsonine. The kinetics of uptake and release of swainsonine and its slightly basic nature make it likely that swainsonine is concentrated initially in the lysosomes. This rapid, but reversible, concentration of swainsonine in lysosomes would be consistent with the observed effects of the toxin in vivo.

Key words: swainsonine, lysosomes, α-D-mannosidase, uptake, human fibroblasts

Lysosomal storage diseases result from a genetic deficiency in a lysosomal hydrolase. Mannosidosis is the lysosomal storage disease resulting from a deficiency of lysosomal acidic α-D-mannosidase (EC 3.2.1.24)[1]. It is characterized by accumulation in the tissues and excretion in the urine of mannose-rich oligosaccharides and has been described in cattle, humans, and a kitten. A phenocopy of the genetic disease can be induced in grazing cattle or laboratory animals by the prolonged

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ingestion of the plant Swainsona canescens [2]. This is due to the presence in the plant of swainsonine, a trihydroxylated indolizidine alkaloid, which is a powerful inhibitor of α-D-mannosidase [3]. Administration of swainsonine can thus be used to induce a deficiency of α-D-mannosidase, providing a model system for the study of the pathogenesis of lysosomal storage diseases. To investigate the mechanism of action of swainsonine at a cellular level, swainsonine has been incorporated into the culture medium in which normal fibroblasts are growing. The mode of association of the inhibitor with the cells in culture and its effect on the fibroblast α-D-mannosidase activities have been investigated.

METHODS

Chemicals

All reagents were of analytical grade and were obtained from BDH Ltd, or Sigma (London) Chemical Co Ltd, both of Poole, Dorset, United Kingdom. Swainsonine was isolated from the plant Swainsona canescens (Benth.) A. Lett by the method of Colegate et al [4].

Assay for α-D-Mannosidase

α-D-mannosidase was assayed routinely at 37°C by using the synthetic fluorigenic substrate, 4-methylumbelliferyl α-D-mannopyranoside (Koch-Light Laboratories, Colnbrook, Bucks, United Kingdom) with a substrate concentration of 5 mM in the reaction mixture [5]. Activity measured at pH 4.0 and pH 5.5 was defined as acidic (lysosomal) and intermediate α-D-mannosidase, respectively [6]. One unit of activity is that amount of enzyme that transforms 1 μmol of substrate/min under the specified conditions. The pH dependence of the α-D-mannosidase activity in extracts of fibroblasts was investigated using the McIlvaine phosphate-citrate buffer system [7]. The value of K_i for the inhibition of α-D-mannosidase by swainsonine was measured at pH 4.0 by including different concentrations of swainsonine (0–1 μM) in the routine assay mixture and analysing the results by the Dixon graphical procedure [8].

Assay for β-D-Mannosidase

The β-D-mannosidase in an extract of normal fibroblasts was assayed using 0.67 mM-p-nitrophenyl β-D-mannopyranoside (Koch-Light) in phosphate-citrate buffer, pH 4.0, at 37°C [9].

Protein Determination

Protein was determined by the Folin method using bovine serum albumin as the standard [10].

Culture of Fibroblasts

Normal human fibroblasts and fibroblasts from a patient with mannosidosis were cultured in tissue culture flasks (25 cm^2) in the alpha modification of Eagle's medium (Flow Laboratories Ltd, Irvine, Scotland), HEPES (20 mM), streptomycin (100 μg/ml medium), penicillin (100 U/ml of medium) (all from Sigma), $NaHCO_3$ (0.84 g/liter), newborn calf serum (6.5% v/v) (Flow), and foetal calf serum (3.5% v/v) (Flow) were included in the medium. Cells were brought into suspension for

propagation by trypsinization using trypsin (0.1% w/v) (Difco Laboratories Ltd, West Molesey, Surrey, United Kingdom) in PBS (phosphate-buffered saline; Dulbecco's formula, Flow) containing 15 mM sodium citrate, pH 7.0.

Incubation of Swainsonine With Cultures of Fibroblasts at 37°C

Medium (5 ml) to which had been added 1 ml of PBS containing swainsonine of the appropriate concentration, was added to monolayers (25 cm^2) of fibroblasts, which had been confluent for 3 days. Medium (5 ml) and PBS (1 ml) were added to control flasks. After the chosen length of time, the medium was poured off and the monolayers were washed three times with cold (approximately 4°C) PBS (5 ml) containing 10 mM-mannose. The cells were brought into suspension by trypsinization and the suspension of cells washed four times with cold PBS (5 ml). The cells were then resuspended in 0.5 ml of PBS and sonicated for two 15-sec pulses at 20 kH_z in an MSE ultrasonic disintegrator. The supernatant obtained after centrifugation in an MSE bench centrifuge at 1,000g for 10 min was used for the determination of α-D-mannosidase and protein. To investigate whether mannose or mannose-6-phosphate affected the association of swainsonine with the fibroblasts, 60 mM mannose or 6 mM mannose-6-phosphate was included in the 1 ml of PBS added to the 5 ml of medium.

Incubation of Swainsonine With Cultures at Different Temperatures

Cultures that had been maintained at 37°C were cooled to 4°C in an ice-water mixture prior to addition of medium and PBS containing swainsonine, which had also been cooled to 4°C. Subsequent washings were carried out at 4°C. The same procedure was used at other temperatures, which were maintained by incubation in thermostatically controlled water baths.

RESULTS

Inhibition of Human Fibroblast α-D-Mannosidase by Swainsonine In Vitro

Acidic α-D-mannosidase, with a pH optimum of 4.0, accounts for the majority of activity in normal human fibroblasts (Fig. 1). Intermediate α-D-mannosidase with a pH optimum of 5.5–6.0, and a trace of neutral α-D-mannosidase with a pH optimum of 6.0–6.5 are also present, but their activity is obscured in the activity-pH profile by the preponderant acidic enzyme. Swainsonine inhibited over 99% of the acidic α-D-mannosidase, at pH 4.0, in an extract of normal fibroblasts. It also appeared to inhibit the intermediate and neutral α-D-mannosidases, but to clarify the effect of swainsonine on these forms of the enzyme, the experiment was repeated using an extract of fibroblasts from a patient with the lysosomal storage disease, mannosidosis. Acidic α-D-mannosidase is deficient in this disorder, and the intermediate and neutral α-D-mannosidase activities between 5.0 and 7.0 are evident (Fig. 1). Swainsonine strongly inhibited the residual mutant acidic α-D-mannosidase and the intermediate and neutral α-D-mannosidase in these cells (Fig. 1). However, the highest remaining activity was at pH 5.5, the pH optimum of the intermediate α-D-mannosidase, both in the mannosidosis cells and in the normal cells, where the intermediate activity is lower. this suggests that one of the forms of human intermediate α-D-mannosidase [11] may not be inhibited by swainsonine. It has been shown recently that rat liver Golgi α-D-mannosidase II, but not Ia and Ib, is inhibited by swainsonine [12].

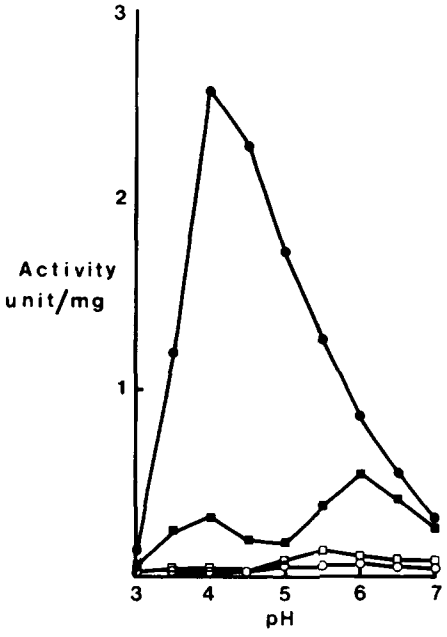

Fig. 1. pH dependence of α-D-mannosidase in normal and mannosidosis fibroblasts in the presence and absence of swainsonine. Extracts of fibroblasts were assayed for α-D-mannosidase activity at a series of pH values in the presence and absence of 1 mM swainsonine. ●, normal fibroblasts, no swainsonine; ○, normal fibroblasts with swainsonine; ■, mannosidosis fibroblasts, no swainsonine; □, mannosidosis fibroblasts with swainsonine.

Variation of the concentration of swainsonine showed that over 99% of the acidic α-D-mannosidase was inhibited at concentrations of 10 μM or greater. Analysis of the inhibition of acidic α-D-mannosidase at different concentrations of swainsonine and substrate by the Dixon plot indicated that the toxin was a reversible competitive inhibitor at concentrations of less than 1μM with a $K_i$value of 80 nM (Fig. 2). The β-D-mannosidase activity at pH 4.0 in an extract of normal human fibroblasts was not inhibited by 1 mM swainsonine.

Effect of Swainsonine on Acidic α-D-Mannosidase in Cultures of Fibroblasts

Preliminary experiments indicated that addition of swainsonine to the culture medium in which normal human fibroblasts were growing at 37°C led to rapid inhibition of the intracellular acidic α-D-mannosidase. When monolayers of cells were placed in contact with medium containing 1 mM swainsonine for less than 1 min, approximately 60% of the intracellular acidic α-D-mannosidase was inhibited. To ensure that this inhibition was due to swainsonine associated with the cells, and not to swainsonine carried over from the medium and exposed to the enzyme on lysis of the cells, the cells were washed thoroughly and the presence of swainsonine in the washings was investigated. Monolayers (25 cm^2) that had been in contact with swainsonine were washed three times with 5 ml of phosphate-buffered saline (PBS), pH 7.4, before harvesting by trypsinization. The suspension of cells was then washed with a further three volumes of PBS. The presence of swainsonine in the PBS

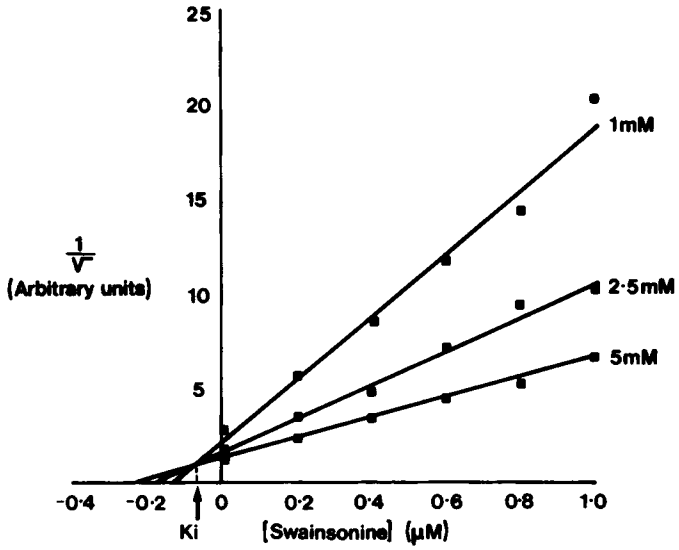

Fig. 2. Inhibition of human fibroblast acidic α-D-mannosidase at different concentrations of swainsonine. Different concentrations of swainsonine (0–1 μM) were included in assay mixtures containing 1, 2.5, and 5 mM substrate. The nature of the inhibition and the value of K_i were determined graphically by the Dixon procedure.

washings was detected by measuring the ability of a sample (50 μl) to inhibit the assay of the acidic α-D-mannosidase in an extract of fibroblasts that had not been in contact with swainsonine. Although the first two washings produced considerable inhibition, the third and subsequent washings produced less than 1% inhibition. Addition of 50 μl of the original medium produced 97% inhibition. It was concluded that the inhibition of the intracellular α-D-mannosidase found after harvesting the cells was due to swainsonine taken up into the cells. The degree of inhibition was assumed to be a measure of the amount of swainsonine taken up into the cells.

Effect of Mannose and Mannose-6-Phosphate on Rapid Internalization of Swainsonine

As the inhibitory action of swainsonine is attributed to its partial structural resemblance to mannose [3], it is possible that cellular receptors recognising mannose are involved in the association of swainsonine with fibroblasts. Therefore, 10 mM mannose was added to medium containing 100 μM swainsonine to see if this would prevent the uptake of swainsonine into the cells. However, the degree of inhibition was the same in the presence or absence of the mannose after contact for 1 min or 4 hr with swainsonine. Ten mM mannose was also added to the PBS to wash cells that had been in contact with swainsonine to see whether bound swainsonine could be displaced by the mannose. Again, the presence of mannose did not alter the inhibition. However, as a general precaution against any endogenous inhibitors in the medium, 10 mM mannose was incorporated routinely in the PBS used to wash the monolayers of cells prior to typsinization.

Receptors that recognise mannose-6-phosphate are involved in the intracellular transport and endocytosis of lysosome enzymes [13]. To ascertain whether these receptors are involved in the rapid uptake of swainsonine into fibroblasts, monolayers of cells were placed in contact for 1 min with medium containing 100 μM swainsonine, with and without 1 mM mannose-6-phosphate. The presence of mannose-6-phosphate did not prevent inhibition by the swainsonine but caused a slight (approximately 10%) increase in the inhibition. Mannose-6-phosphate (1 mM) alone in the medium did not inhibit the intracellular α-D-mannosidase.

These observations suggest that the rapid uptake of swainsonine into fibroblasts is not mediated by a process involving recognition of mannose like structural features in swainsonine. As the length of time in contact, and the concentration of swainsonine appeared to affect the degree of inhibition of intracellular α-D-mannosidase by swainsonine, these factors were investigated in more detail.

Contact Time

The rapid uptake of swainsonine by fibroblasts in culture, as shown by inhibition of the intracellular α-D-mannosidase, was studied at 37°C as a function of time at two concentrations of swainsonine, 1 μM and 100 μM, which produce incomplete and complete inhibition respectively in vitro (Fig. 3). It can be seen that the inhibition is essentially instantaneous and increases with the concentration of swainsonine. There is a subsequent slow increase in inhibition to a plateau level, which is dependent on the concentraation of the swainsonine. Although 100 μM swainsonine completely inhibited acidic α-D-mannosidase in vitro, it did not produce this effect in culture, even after contact for 24 hr. There was no measurable depletion of the swainsonine from the culture medium over 24 hr, as determined by the inhibitory capacity of an aliquot from the medium. Thus, the limit in inhibition is not due to destruction of the swainsonine in the medium.

The inhibition of the intermediate, or Golgi-associated, α-D-mannosidase, assayed at pH 5.5, also increased over a 24-hr period.

Concentration Dependence of the Rapid Uptake of Swainsonine

The previous experiment had shown that the initial rapid internalization of swainsonine and consequent inhibition had depended on the concentration of swainsonine in the medium. To investigate this concentration dependence, monolayers of fibroblasts were washed at 37°C with medium containing different concentrations of swainsonine (ie, 1 min in contact) and the inhibition of the intracellular acidic α-D-mannosidase measured (Fig. 4). The effect of the same concentrations of swainsonine on an extract of the same number of cells in vitro is also shown. The results confirm that the initial rapid uptake is concentration-dependent and reaches a limiting value which is less than that obtained in vitro. It can be seen that the uptake of swainsonine into fibroblasts in cultures, as assessed by the inhibition of intracellular acidic α-D-mannosidase, reaches a limit, or saturation level, both with respect to time of contact and the concentration of the swainsonine.

Effect of Temperature on Uptake of Swainsonine Into Fibroblasts

Internalization of molecules by endocytosis is markedly decreased at lower temperatures [14]. Therefore, the experiments carried out to investigate the effects of time and concentration on the uptake of swainsonine into fibroblasts were repeated at

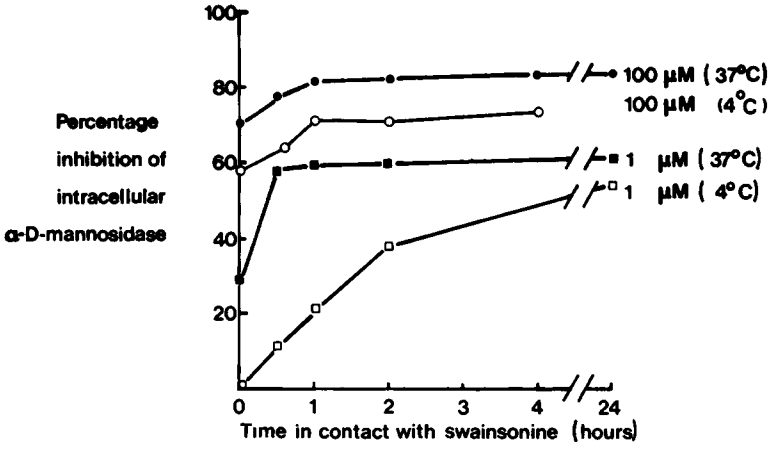

Fig. 3. Effect of time in contact on the uptake of swainsonine into fibroblasts in culture. Swainsonine (1 μM or 100 μM) was incorporated in the medium in which normal human fibroblasts were growing at 37°C or 4°C. At suitable intervals of time, the cells were harvested and the amount of swainsonine associated with the cells was determined as the percentage inhibition of the acidic α-D-mannosidase activity in the swainsonine-treated cells compared with that in controls not exposed to swainsonine. ●, 100 μM swainsonine at 37°C; ○, 100 μM swainsonine at 4°C; ■, 1 μM swainsonine at 37°C; □, 1μM swainsonine at 4°C.

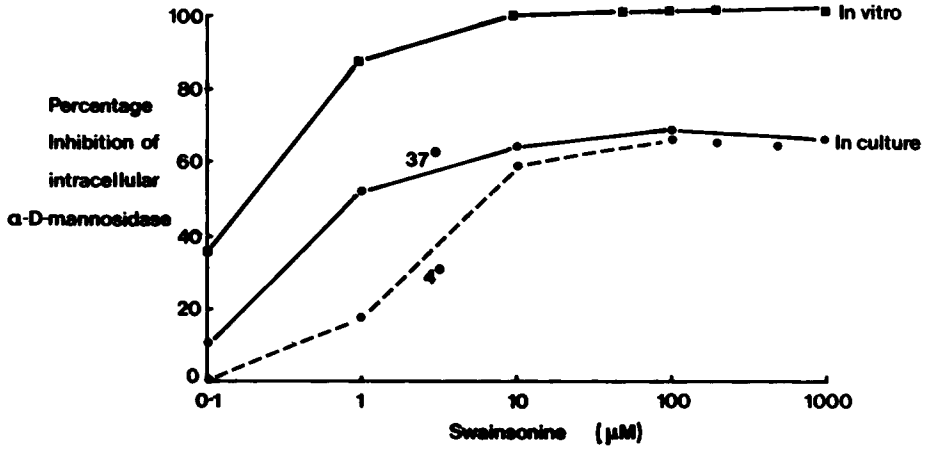

Fig. 4. Effect of the concentration of swainsonine in the medium on the rapid uptake of swainsonine into fibroblasts in culture. Confluent monolayers of normal fibroblasts were exposed to medium containing different concentrations of swainsonine (0–1 mM) for 1 min at 4°C and 37°C. After washing off the swainsonine-containing medium the cells were harvested and the acidic α-D-mannosidase activity was measured and compared with the activity in control cells not exposed to swainsonine: ;●—●, 37°C; ●--●, 4°C. The inhibition of the activity in the same number of cells was also measured when the same concentration of swainsonine was added after homogenization (■).

4°C, at which temperature endocytosis is negligible. Although the rate of inhibition was slower at 4°C than at 37°C, the time courses in the presence of 1 and 100 μM swainsonine both showed the saturation phenomenon (Fig. 3), suggesting that endocytosis was not the mechanism of uptake of swainsonine into the cells.

When fibroblasts were briefly exposed to medium containing different concentrations of swainsonine, the inhibition was lower at 4°C than 37°C for concentrations of swainsonine less than 100 μM (Fig. 4). Thus, temperature affects the uptake of the toxin into the cells at lower or nonsaturating concentrations of swainsonine, but not at saturating concentrations. This observation was investigated more fully by measuring the inhibition produced by a nonsaturating concentration of swainsonine (1 μM) at different temperatures (Fig. 5). It can be seen that at about 20°C the inhibition rises sharply, suggesting that a physical change in the plasma or another membrane has increased the accessibility of the swainsonine to an intracellular compartment.

Retention of Swainsonine in Normal Human Fibroblasts After Removal of Toxin From Medium

Confluent layers of fibroblasts were maintained in medium, with and without 1 mM swainsonine, for 4 hr at 37°C. After removal of the medium the monolayers were washed, and fresh serum-free medium without swainsonine was introduced. The acidic α-D-mannosidase in the cells and medium was measured at different times up to 3 days. The specific activity of the acidic α-D-mannosidase in the cells pulsed with swainsonine was initially only 6% of that in the controls, but it increased to 40% within 4 hr and remained at that level up to 3 days. There was an approximately 25% increase in the specific activity of the control cells over this period. This suggests that the amount of swainsonine in the cells decreases after it is removed from the medium. This decrease in swainsonine in the cells could be due to its metabolism to an inactive derivative or to its release into the medium. In support of the latter is the observation that the acidic α-D-mannosidase activity measured in the medium of the cells pulsed with swainsonine was very low, being only 5–13% of that in the controls. To confirm that swainsonine was being released into the medium, samples (50 μl) of the medium conditioned by cells pulsed with swainsonine were added to the assay of α-D-mannosidase in an extract of normal fibroblasts that had not been in contact with swainsonine. All the samples of medium from the pulsed cells resulted in inhibition of the assay, whereas samples of medium from nonpulsed cells did not. The degree of inhibition reached a constant value with the sample taken after 4 hr and did not increase with samples taken after a longer period. These results suggest that swainsonine is released rapidly from an intracellular compartment, probably the lysosomes, and that a new equilibrium is set up between the intracellular and extracellular compartments in this culture. An alternative explanation for the decrease in α-D-mannosidase activity inside and outside the cells is that the swainsonine has affected the de novo synthesis or processing of the α-D-mannosidase, perhaps by inhibiting the Golgi-associated α-D-mannosidase.

DISCUSSION

Swainsonine has been shown to be a very effective inhibitor of human fibroblast α-D-mannosidase activity in vitro. Therefore inhibition of α-D-mannosidase is a very sensitive way of detecting the presence of swainsonine, and this has been used to

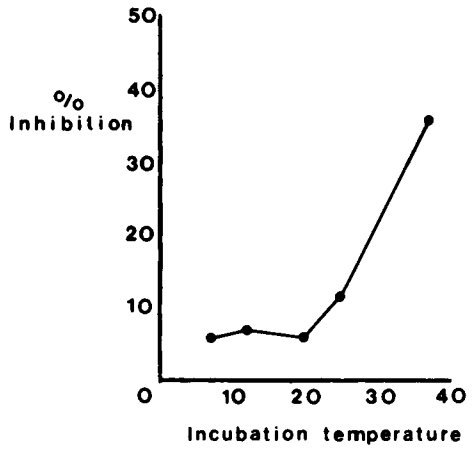

Fig. 5. Effect of temperature on the rapid uptake of swainsonine into fibroblasts in culture. Confluent monolayers of fibroblasts maintained at a series of temperatures were exposed to medium containing 1 μM-swainsonine for 1 min prior to harvesting. The percentage of the acidic α-D-mannosidase inhibited in the swainsonine-treated cells was calculated by comparison with the activity in cells not exposed to swainsonine.

follow the association of swainsonine with normal human fibroblasts in culture. As ingestion of swainsonine has been shown to induce a deficiency of lysosomal (or acidic) α-D-mannosidase activity in vivo, the inhibition of acidic α-D-mannosidase has been measured.

Inclusion of swainsonine in the culture medium leads to a very rapid association of the alkaloid with normal human fibroblasts in culture, as judged by the inhibition of the acidic α-D-mannosidase in extracts of harvested cells. When monolayers and suspensions of cells that had been in brief contact with swainsonine were washed extensively with PBS, mannose, or mannose-6-phosphate, inhibition still occurred. It is concluded that the swainsonine is taken up into the cells. That mannose and mannose-6-phosphate did not prevent the association or displace any bound swainsonine suggests that recognition of mannose-like structural features in swainsonine is not involved. The inhibitory power of swainsonine has been attributed to its resemblance to the postulated transition state in the hydrolysis of the α-mannosidic linkage [3]. Dean (personal communication) has observed that mannose analogues do not affect the uptake of swainsonine into mouse peritoneal macrophages.

The amount of swainsonine taken up into fibroblasts in culture was found to depend on the length of time in contact with the swainsonine (Fig. 3), and the concentration of the swainsonine in the medium (Fig. 4). At 37°C there was an initial very rapid increase in cell-associated swainsonine, followed by a slower increase over about 1 hr to a limiting level which depended on the concentration of the swainsonine in the medium. This rapid association suggests that swainsonine can permeate the plasma membrane. Similar kinetics have been observed for the uptake of several weak bases into rat fibroblasts and mouse macrophages [15, 16]. With a swainsonine concentration of 1 μM the rate of uptake of swainsonine was very much slower at 4°C than at 37°C, but at about 20°C there was a sharp increase in the rate of uptake

(Fig. 5). A similar transition at 20°C, not necessarily attributable to endocytosis per se, has been observed in the uptake of asialo-orosomucoid into isolated rat hepatocytes [17]. It is suggested that permeation, rather than endocytosis, is the major mechanism of uptake of swainsonine into cells.

It is probable, because of the pathological consequences in vivo and its pKa (7.4) [3], that swainsonine is lysosomotropic and accumulates in the lysosomes of the fibroblasts [18]. The rate of internalization and accumulation in lysosomes will depend on the extracellular concentration of swainsonine and the pH of the lysosomes and the medium. Figure 4 shows that the accumulation of swainsonine in the cells reaches a plateau at a concentration of swainsonine in the medium of approximately 100 μM. The accumulation of chloroquine and other weak bases also reaches a limiting value in other mammalian cells [15, 16]. The intralysosomal concentration of swainsonine could be 100–1,000 times greater than the concentration in the medium, depending on the intralysosomal pH [16, 19]. However, when the fibroblasts are homogenized, the swainsonine accumulated in the lysosomes will be dispersed among the total cell contents. Further dilution of the swainsonine occurs in the assay mixture so that the final concentration assayed is much lower than the intralysosomal concentration and lower than in the medium. This explains why the inhibition measured in cells in culture is always less than that found in vitro for the same concentration of swainsonine and number of cells (Fig. 4). It is possible to estimate the final concentration in the assay mixture by making assumptions about the volumes of the lysosomes and cells and the lysosomal pH [18]. Thus, the estimated concentration in the assay mixture due to a 1 μM concentration of swainsonine in the medium would be 0.2–0.5 μM, the concentration observed to give approximately the same inhibition in vitro (Fig. 4).

When swainsonine is withdrawn from the medium, there is a subsequent loss of swainsonine from the cells, again indicating that the membranes are permeable to the alkaloid. Swainsonine is also lost from macrophages but at a faster rate, whereas the uptake into macrophages is slower than into fibroblasts (Dean, personal communication).

Swainsonine has also been shown to inhibit the intermediate, or Golgi-associated, α-D-mannosidase in human fibroblasts (Fig. 1). Prolonged exposure of the cells to swainsonine might therefore disrupt the processing of glycoproteins, which is the proposed function of these forms of α-D-mannosidase [20]. The processing of glycoproteins in the presence of swainsonine has been investigated in both cell-free systems and cultured cells [12, 21, 22]. A decrease in the complex type of asparagine-linked glycan and an increase in the high-mannose types were observed. If this were to occur in vivo, the increase in the high-mannose glycoproteins would exacerbate the accumulation of mannose-rich oligosaccharides owing to the inhibition of the lysosomal α-D-mannosidase.

The results presented in this paper show that swainsonine is taken up rapidly by fibroblasts in culture, probably into the lysosomes, in a reversible manner. These findings are consistent with the action of the toxin in vivo, particularly with the observation that the lysosomal hypertrophy resulting from inhibition of lysosomal α-D-mannosidase is reversible in the early stages of the disease, if ingestion of swainsonine is stopped [1]. Thus, cultivation of cells in the presence of swainsonine is a useful model for studying the consequences to cells of chemically-induced deficiencies of the lysosomal α-D-mannosidase and the Golgi-associated α-D-mannosidases, which are also affected by swainsonine.

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REFERENCES

1. Jolly RD, Winchester BG, Gehler J, Dorling PR, Dawson G: J Appl Biochem 3:273-291, 1981.
2. Dorling, PR, Huxtable CR, Vogel P: Neuropathol Appl Neurobiol 4:285-296, 1978.
3. Dorling PR, Huxtable CR, Colegate SM: Biochem J 191: 649-651, 1980.
4. Colegate SM, Dorling PR, Huxtable CR: Aust J Chem 32:2265, 1979.
5. Burditt LJ, Chotai KA, Winchester BG: FEBS Lett 91:186-189, 1978.
6. Burditt L, Chotai K, Halley D, Winchester B: Clin Chim Acta 104:201-209, 1980.
7. McIlvaine TC: J Biol Chem 49:183-186, 1921.
8. Dixon M: Biochem J 55:170-171, 1953.
9. Phillips N, Robinson D, Winchester B: Biochem J 151:469-475, 1975.
10. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: J Biol Chem 193:265-275, 1951.
11. Hirani S, Winchester B: Biochem J 179:583-592, 1979.
12. Tulsiani DRP, Harris TM, Touster O: J Biol Chem 257:7936-7939, 1982.
13. Kaplan A, Achord DT, Sly WS: Proc Natl Acad Sci USA 74:2026-2030, 1977.
14. Silverstein SC, Steinman RM, Cohn ZA: Ann Rev Biochem 46:669-772, 1977.
15. Wibo M, Poole B: J Cell Biol 63:430-440, 1974.
16. Okhuma S, Poole B: Proc Natl Acad Sci USA 75:3327-3331, 1978.
17. Weigel PH, Oka JA: J Biol Chem 256:2615-2617, 1981.
18. De Duve C, De Barsy T, Poole B, Trouet A, Tulkens P, Van Hoof F: Biochem Pharmacol 23:2495-2531, 1974.
19. Reijngoud D-J, Tager JM: Biochim Biophys Acta 472:419-449, 1977.
20. Tulsiani DRP, Hubbard SC, Robbins PW, Touster O: J Biol Chem 257:3660-3668, 1982.
21. Elbein AD, Solf R, Dorling PR, Vosbeck K: Proc Natl Acad Sci USA 78:7393-7397, 1981.
22. Elbein AD, Dorling PR, Vosbeck K, Horisberger M: J Biol Chem 257:1573-1576, 1982.