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D-Cycloserine Uses an Active Transport Mechanism in the Human Intestinal Cell Line Caco 2†

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In a previous study we have shown that cultured epithelial cell lines can be used to measure the transepithelial passage of antimicrobial agents across the intestine and to obtain information on the mechanisms of transport utilized and predict the bioavailability of the antimicrobial agents after oral administration. In particular, among the drugs investigated, D-cycloserine had been shown to be transported in a polarized manner only in the intestinal cells. In the present work, further characterization of the transport of D-cycloserine in the human intestinal cell line Caco 2 has shown that this occurs in the apical-to-basolateral direction by an active mechanism which is energy dependent but only partially sodium dependent. Competition studies have also indicated that the transport of D-cycloserine occurs via a carrier for imino acids, amino acids with aliphatic side chains (L-Ala, D-Ala, and βAla), and L-Trp, L-Tyr, L-Cys, and α-amino isobutyric acid. This system may correspond to a proton-dependent system for L-proline and β-alanine recently described for Caco 2 cells. In contrast with the cephalosporins, which are taken up by the Caco 2 cells via a dipeptide carrier, D-cycloserine transport cannot be inhibited by either cephalexin (a member of the class of cephalosporins) or dipeptides.

It has previously been shown by us and by others that intestinal cell lines in culture can constitute useful models for the study of the oral bioavailability of drugs (3, 16, 20, 29, 39). Several studies have determined the transport of drugs across cultured intestinal cells and attempted to relate it to the physicochemical characteristics and in vivo pharmacokinetics of the drugs (3–5, 29). The elucidation of the transport mechanisms increasingly suggests that cell lines could be used to predict potential oral bioavailability and thereby eliminate the need for animal models, at least in preliminary studies, although oral bioavailability will eventually need confirmation in vivo.

Absorption of solutes from the intestinal lumen can occur passively through paracellular or transcellular routes and actively through the cellular pathways (33, 38). The active absorption of hexoses and amino acids has been studied in some detail and shown to exhibit wide species and regional differences (17). Cultured cell lines of specific origins may provide an alternative model for studies of such mechanisms. Recently, human intestinal cell lines capable of differentiating in vitro, e.g., Caco 2 and HT-29 (41), have been used to study the active transport of amino acids (13, 18, 25, 36, 37), dipeptides (34, 35), vitamins (9, 28), and other compounds (2, 14, 30). However, the involvement of these active mechanisms in drug transport has not been thoroughly investigated. Recent studies on the uptake of cephalosporins in Caco 2 cells have demonstrated that these drugs use an energy- and proton-dependent dipeptide carrier for uptake into the cells (7, 8). More recently, the transepithelial passage of these drugs across Caco 2 cell monolayers has shown that the cephalosporins are accumulated in the cells and subsequently transported to the basolateral (BL) side; while the uptake appears to occur via the H^+-dipeptide cotransport system localized in the apical (AP) membrane, another specific transport system may be involved in the efflux across the BL membrane (20).

We have recently shown that differentiated Caco 2 cells grown on permeable supports can be used to study the transcellular passage of drugs or nutrients (29, 32). The use of several different classes of antimicrobial agents and two cell lines of different tissue origin suggested that the transcellular passage could be classified into four different categories. The behavior of D-cycloserine indicated the presence of a specific polarized active transport expressed only in the intestinal cell line (29). In this study, we have further investigated the transport characteristics of this drug and show that D-cycloserine passes through the intestinal epithelial cell line Caco 2 by an active mechanism operating in the AP-to-BL direction. This is a mechanism which requires energy, shows a strong dependency on acidic pH on the AP side, and is only partially sodium dependent. Furthermore, competition experiments with amino acids and dipeptides have shown that this transport has the characteristics of an amino acid carrier system.

MATERIALS AND METHODS

Cell culture. The intestinal Caco 2 cell line (donated by A. Zweibaum, Institut National de la Santé et de la Recherche Médicale, Villejuif, France) and the renal Madin-Darby canine kidney (MDCK) type II cell line (donated by E. Rodriguez-Boulan, Cornell University Medical College, New York, N.Y.) were grown as previously described (29). Briefly, Caco 2 cells were routinely grown in Dulbecco modified minimum essential medium containing 25 mM glucose and 3.7 g of $NaHCO_3$ $liter^{-1}$ and supplemented with 4 mM L-glutamine, 10% fetal calf serum, 1% nonessential amino acids, 100 U of penicillin ml^{-1}, and 100 μg of streptomycin ml^{-1}, while the MDCK cells were grown in the same medium without addition of nonessential amino acids and with 10% donor horse serum in place of fetal calf serum. At confluency, the cells were passaged by detachment with 0.25% trypsin (1:250) and 10 mM EDTA in calcium-free and magnesium-free phosphate-buffered saline.

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All cell culture reagents were from Flow Laboratories International (Opera, Milan, Italy). The fluorescent dye bisbenzimide (H 33258; Boehringer Mannheim, Milan, Italy) was routinely used to screen cells for mycoplasma contamination (6).

For drug transport experiments, the cells were seeded on polycarbonate filter cell culture chamber inserts (Transwell, 24-mm diameter, 4.7-cm^2 area, 0.45-μm pore diameter; Costar Europe, Badhoevedorp, The Netherlands) at a density of 2×10^6 cells per filter; the high seeding density allows confluency to be reached within 48 h. Caco 2 cells were allowed to differentiate at confluency for 14 to 16 days, while MDCK cells were used 6 to 8 days after seeding; the medium was regularly changed three times a week.

Drug transport experiments. The intactness of the tight junctions between the cells was monitored in filter-grown cell monolayers by determining the transepithelial passage of the radiolabelled extracellular marker D-1[^{3}H(N)]-mannitol (specific activity, 706.7 GBq/mmol) (NEN Research Products, Florence, Italy). Briefly, the radioactive compound in complete growth medium was added to the AP compartment, and after 2 h of incubation at 37°C the radioactivity in the BL medium was measured in a liquid scintillation counter (LS 1801; Beckman Instruments Inc., Irvine, Calif.). As an additional control of tight-junction integrity, the transepithelial electrical resistance (TEER) of filter-grown monolayers was measured with the Millicell ERS apparatus (Millipore Co., Bedford, Mass.) according to the manufacturer's instructions.

Transport experiments were carried out essentially as described previously (29), using phosphate-buffered saline containing 1 mM $MgCl_2$ and 1 mM $CaCl_2$ and supplemented with 5.5 mM glucose (PBS^+) as the transport buffer. D-Cycloserine and cephalexin were directly dissolved to the required concentration in PBS^+ before addition to the donor compartment; fresh PBS^+ was added to the acceptor compartment at the start of the transport experiment. Except where otherwise stated, the donor compartment was adjusted to pH 6.0 and the acceptor compartment was adjusted to pH 7.5, in analogy with the pH conditions of the small intestinal lumen and of the submucosal compartment (40). To determine the effects of pH on the transport of D-cycloserine, the pH of the AP medium was varied between pH 4.5 and 7.5, while the BL medium was maintained at pH 7.5. Experiments at 4°C were conducted with the multiwells containing the filter-grown Caco 2 cells kept on ice and with all solutions used at the same temperature.

Prior to drug transport experiments, the cells were preequilibrated for 10 min in the presence of the drug, after which the donor and acceptor solutions were replaced. In order to avoid drug backflow, the acceptor medium was changed after each time point with fresh prewarmed medium. The initial rates were calculated under "sink" conditions (3) from the linear portion of the drug appearance curve (i.e., before >10% of the drug had been transported).

To determine sodium dependency of transport, the NaCl in PBS^+ was replaced with equimolar amounts of either KCl, LiCl, or choline chloride and sodium salts were replaced with their potassium equivalents. To adjust the pH of PBS^+, 10 mM buffers were added as follows: between pH 4.5 and 5.0, citric acid; between pH 5.5 and 6.5, MES (morpholineethanesulfonic acid); and between pH 7.0 and 7.5, HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid).

At the end of the experiment, the total protein of each filter was assayed by the method of Lowry et al. (21) after the cells were dissolved in 1 M NaOH.

Assay for D-cycloserine. The D-cycloserine concentration was routinely measured spectrofluorimetrically as described elsewhere (10). This method may be subject to interferences when performed in the presence of other molecules, e.g., amino acids or dipeptides. Furthermore, D-cycloserine may be unstable or inactivated during the passage through the cells, and consequently it is important that the amount of active drug be determined by a microbiological assay. For competition studies, we have therefore used both the fluorimetric assay, as it is highly sensitive, and a microbiological assay, to eliminate possible interferences and to assess the amount of active drug passing through the cells.

The fluorimetric assay (10) is based on the reaction of D-cycloserine in aqueous solution at pH 8.2 with *p*-benzoquinone, which leads to the formation of a fluorescent compound with an excitation maximum at 381 nm and an emission maximum at 502 nm. To compensate for any variability of the fluorescence yield on different days and in order to determine possible interferences with the fluorescence of the D-cycloserine-benzoquinone complex, standard curves were run in each experiment at both pH 6.0 and 7.5 and included all chemicals present in the transport buffer. The fluorescence intensity of the complex was linearly related to concentration between 3 and 50 μM. The coefficients of variation of the assay performed on the same day were 2% at 3 μM, 2.6% at 25 μM, and 5.1% at 50 μM. Fluorescence was measured in a spectrofluorometer (LS 4; Perkin Elmer, Beaconsfield, Buckinghamshire, England).

D-Cycloserine and cephalexin activities were determined microbiologically with *Bacillus subtilis* and *Staphylococcus aureus* as the test organisms in an agar plate diffusion assay. *B. subtilis* ATCC 6633 and *S. aureus* ATCC 6538 were grown overnight at 30°C, the former in Davis minimal medium and the latter in Iso-Sensitest medium (both media were from Difco Laboratories, Detroit, Mich.). The lower limit of detection of D-cycloserine on *B. subtilis* was 8 μM, and those of cephalexin on *B. subtilis* and on *S. aureus* were 10 and 20 μM, respectively. In competition studies, D-cycloserine activity was determined after degradation of cephalexin by incubation for 1 h with 50 μg of penicillinase IV (Sigma Chemical Co., St. Louis, Mo.) per ml. D-Cycloserine passage in the absence of penicillinase was 4.6 nmol min^{-1} mg of $protein^{-1}$ and that in the presence of penicillinase was 2.9 nmol min^{-1} mg of $protein^{-1}$, as D-cycloserine activity is somewhat reduced in the presence of protein. After treatment with penicillinase IV, there was no activity against *S. aureus*, indicating that no residual cephalexin was present. Since D-cycloserine exhibits no activity against *S. aureus* even at high concentrations under the test conditions used, this organism was used to assay cephalexin activity.

Statistical analysis. Kinetic analysis of D-cycloserine passage was performed by fitting the data to theoretical equations by computer analysis (Sigma Plot; Jandel Scientific, Corte Madera, Calif.), using the Marquardt-Levenberg algorithm (24).

RESULTS

The TEER (15) and passage of ^{3}H-mannitol (29) were used to monitor the establishment of a continuous cell monolayer with well-formed tight junctions. The TEER and percent mannitol passage of Caco 2 cell monolayers during in vitro differentiation are shown in Fig. 1. Although the data presented refer to the mannitol passage in the AP-to-BL direction, a similar amount of passage in the opposite direction was observed (data not shown). Approximately 12 days after seeding, both parameters reached a plateau corresponding to a TEER of 700 to 800 $\Omega \cdot cm^2$ and a ^{3}H-mannitol passage of less than 1% per h (Fig. 1). Transport experiments were therefore

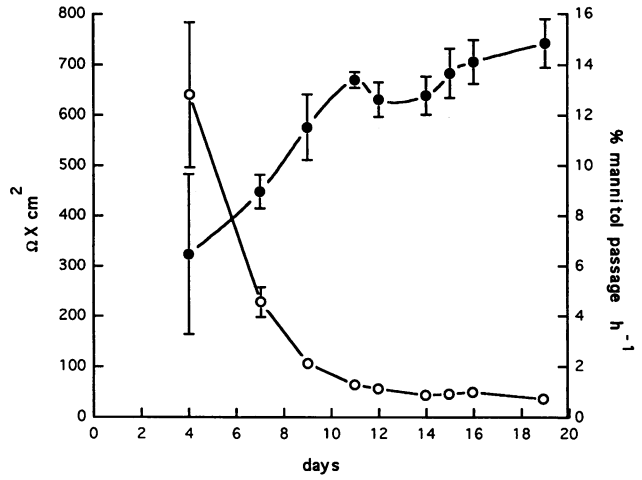

FIG. 1. Development of the filter-grown Caco 2 cell monolayers during differentiation. The integrity of the tight junctions was measured by TEER (●) and ^{3}H-mannitol passage per hour (○) from days 4 to 19 after seeding. Data represent means ± SD for six filters from two different experiments.

performed in the second week (14 to 18 days) after seeding. To ensure that the transepithelial passage of D-cycloserine was not a consequence of perturbation of tight-junction permeability, these parameters were also measured before and after each experiment.

The time course of the transcellular passage of D-cycloserine in both the AP-to-BL and the BL-to-AP directions in the Caco 2 cell line is shown in Fig. 2A. The passage of 1 mM D-cycloserine increased linearly with time in both directions; AP-to-BL passage remained approximately 10-fold higher than that in the opposite direction. The same passage was also determined in the renal MDCK cell line. MDCK cells formed cell monolayers joined by intact tight junctions 4 to 5 days after seeding and were therefore used after this time, as previously described (29). As shown in Fig. 2B, there was minimal passage of the drug in the MDCK cell line in both directions, similar to the small amount of passage observed in the BL-to-AP direction in the Caco 2 cells. The TEER and ^{3}H-mannitol passage were unaffected in these experiments, suggesting that the large amount of AP-to-BL passage in the Caco 2 cell line may be due to some specific transport mechanism present only in this cell line. Reduction of the temperature to 4°C had a much more marked effect on the AP-to-BL passage than on the BL-to-AP passage (decreased by 10-fold versus fourfold), suggesting the involvement of an active mechanism. The passage of D-cycloserine in the BL-to-AP direction was similar to the passage of ^{3}H-mannitol in both directions (approximately 1% per h) and probably represents the passive diffusion of the drug through the cell monolayer; a similar rate of passage of the drug was also observed in the MDCK cell line in both directions (Fig. 2B).

The drug passage was also determined at several different concentrations ranging from 0.25 to 10 mM. The initial rates of passage were determined from the linear portion of the curves and plotted as a function of drug concentration (Fig. 3). The initial rate of passage increased with increasing concentration and tended towards a plateau at high (7 to 10 mM) concentrations. The specific AP-to-BL passage was determined by subtracting the BL-to-AP component, which, as noted above, may represent passive diffusion. Only a single saturable com-

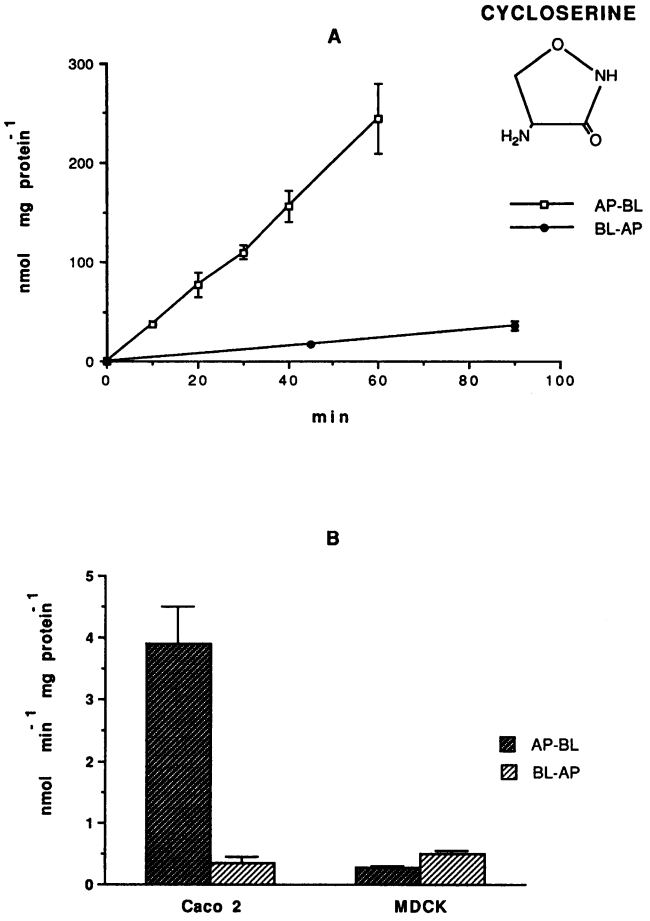

FIG. 2. (A) Time course of the AP-to-BL and BL-to-AP passage of D-cycloserine across monolayers of Caco 2 cells. The drug was applied to the donor compartment at an initial concentration of 1 mM. The transport rates were calculated between 0 and 60 min and between 0 and 90 min for AP-to-BL and BL-to-AP transport, respectively. Each point represents the mean ± SD of five experiments performed in duplicate. The structural formula of cycloserine is shown. (B) Comparison of the rates of passage of D-cycloserine in the AP-to-BL and BL-to-AP directions in Caco 2 and MDCK cell monolayers.

ponent was observed for the specific transport when analyzed by fitting to the equation $v = V_{max}[S]/(K_m + [S])$ where v is the velocity of passage, $[S]$ is the substrate concentration, V_{max} is the maximal velocity of passage, and K_m is the substrate concentration at which the velocity is half maximal. Conversely, the BL-to-AP passage was linear with concentration. Figure 3 shows the AP-to-BL passage, the corrected specific passage (AP-to-BL − BL-to-AP), and the BL-to-AP passage. The apparent kinetic parameters calculated for the specific AP-to-BL passage were a K_m of 4.2 ± 0.3 mM and a V_{max} of 21.1 ± 0.8 nmol min^{-1} mg of protein^{-1}. The calculated diffusion coefficient (K_d) for the BL-to-AP passage was 0.29 ± 0.026 nmol min^{-1} mg of protein^{-1} mM^{-1}.

The pH dependence of the AP-to-BL transport of D-cycloserine was also examined. As shown in Fig. 4, the passage was maximal between pH 4.5 and 5.5, decreased by about 35% at pH 6.0, and was barely detectable at pH 7.0 to 7.5 (<10%). However, since D-cycloserine is unstable at low pH (31) and the average luminal pH in the small intestine is 6.0 (40), all

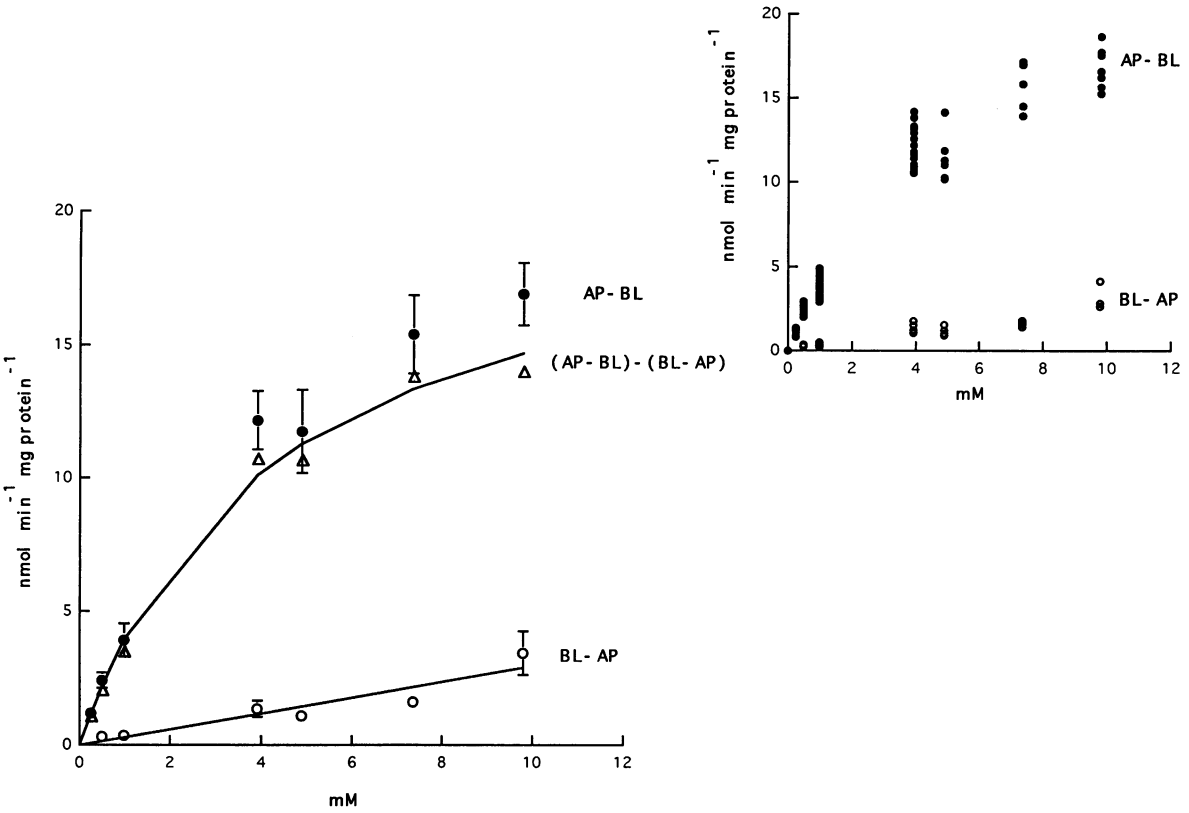

FIG. 3. Rates of AP-to-BL and BL-to-AP passage of D-cycloserine across Caco 2 cell monolayers versus initial drug concentration. Each point represents linear velocities calculated from the slope of the drug appearance curve at every concentration; normally, the transport rates were calculated between 0 and 60 min and between 0 and 60 or 90 min for AP-to-BL and BL-to-AP transport, respectively (Fig. 2A). The means ± SD for the AP-to-BL and the BL-to-AP passages are shown (each point represents the mean of two or three experiments, performed in duplicate); in addition, the curves fitted to the specific transport (AP-to-BL) − (BL-to-AP) and to the BL-to-AP passage are shown, constructed by nonlinear regression analysis and curve fitting to equations for a saturable component and a nonsaturable diffusional component, respectively. The apparent kinetic parameters calculated for the specific AP-to-BL D-cycloserine passage were a K_m of 4.2 mM and a V_{max} of 21.1 nmol min^{-1} mg of protein^{-1}, and for the BL-to-AP passage a K_d of 0.29 nmol min^{-1} mg of protein^{-1} mM^{-1} was calculated. The raw data for AP-to-BL and BL-to-AP passage rates are shown in the inset (each point represents a single filter).

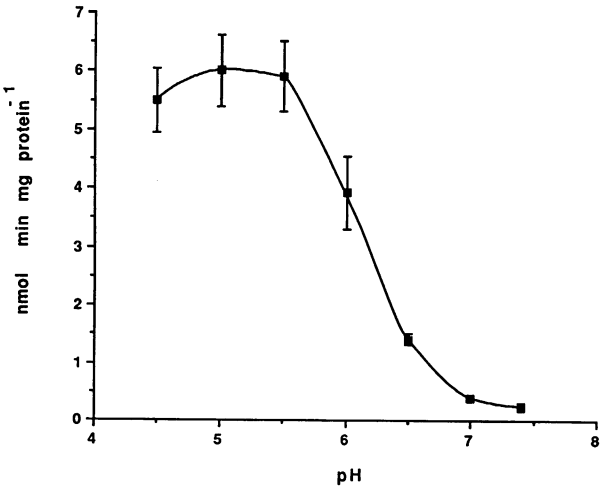

FIG. 4. pH dependence of the AP-to-BL transport of 1 mM D-cycloserine. Each point represents the mean of linear velocities calculated for triplicate filters ± standard deviation.

further experiments were performed with the AP pH maintained at 6.0 and the BL pH maintained at 7.5.

The energy and sodium dependence of D-cycloserine transport were examined in triplicate experiments. The addition of 1 mM NaN_3 and 50 mM 2-deoxy-D-glucose, metabolic inhibitors which dissipate energy, led to a decrease in the passage of D-cycloserine of 53.7% ± 3.6% (mean ± standard deviation [SD]) compared with the control (PBS^+ containing 137 mM NaCl and 9.6 mM Na_2HPO_4). The sodium dependence of the transport was examined by substituting for NaCl in the AP and the BL compartments either KCl, LiCl, or choline chloride. LiCl and choline chloride resulted in reductions of 32.7% ± 2.9% and 32.0% ± 2.8%, respectively, in the passage compared with the passage in PBS^+ containing NaCl. Substitution of NaCl by KCl produced a more marked reduction (58.6% ± 3.5%) in the passage.

Cephalexin, an orally bioavailable antimicrobial agent, has been reported to use the dipeptide carrier for uptake into Caco 2 cells (7, 20). In order to determine if D-cycloserine and cephalexin share a similar carrier system, we have performed competition studies. The passage of D-cycloserine was unaffected by the presence of a 10- to 35-fold excess of cephalexin in the AP compartment, suggesting that the two drugs do not share the same transport mechanism (Table 1). Several dipep-

TABLE 1. Comparison of D-cycloserine and cephalexin transport across Caco 2 cell monolayers[a]

Antimicrobial agent(s)	Cephalexin transport for *S. aureus* without penicillinase	D-Cycloserine transport for *B. subtilis* with penicillinase
D-Cycloserine alone	0	2.9
D-Cycloserine + 10 mM cephalexin	5.8	2.6
D-Cycloserine + 35 mM cephalexin	13.5	3.2

[a] The AP-to-BL transport of 1 mM D-cycloserine, alone or in the presence of a 10- to 35-fold excess of cephalexin, was assessed by measuring the antimicrobial activities of the two drugs in the BL medium as described in Materials and Methods. D-Cycloserine activity measured on *B. subtilis* with penicillinase is somewhat reduced by the presence of protein in the assay, while in the absence of penicillinase it is similar to that measured in previous experiments. However, it is clear that the rate of passage of D-cycloserine is unaffected by the presence of cephalexin. All values are expressed as nanomoles per minute per milligram of protein.

tides were also used in competition studies with D-cycloserine, and only carnosine (βAla–L-His) competed for the transepithelial passage (79.0% ± 3.2% inhibition of transport compared with the control). L-Phe–Gly and L-Ala–L-Ala produced only minimal decreases in D-cycloserine passage (34.3% ± 2.7% and 20.2% ± 2.1% inhibition, respectively), while other dipeptides (L-Ala–L-Pro and L-Pro–Gly) had no effect (14.0% ± 1.4% and 0% inhibition, respectively). Inhibition by Gly–L-Pro and Gly-Gly could not be determined fluorimetrically because of interference with the assay, and no inhibition by these dipeptides was detected microbiologically. These data suggest that the transport carrier used by D-cycloserine is distinct from the dipeptide transport carrier used by orally absorbable cephalosporins.

The chemical structure of D-cycloserine has features in common with dipeptides and amino acids (Fig. 2A); in fact, D-cycloserine is a modified amino acid. In bacterial cells, D-cycloserine uptake is known to be mediated by an amino acid permease (11, 22). Since absorptive enterocytes express several amino acid carriers with overlapping specificities (17, 33), the effects of amino acids on the transport of D-cycloserine were examined in competition experiments. As shown in Table 2, strong inhibition was observed with imino acids, amino acids with aliphatic side chains (L-Ala, D-Ala and βAla), and L-Trp, L-Tyr, L-Cys, and α-amino isobutyric acid. Other amino acids had little effect (<40% inhibition) or failed to compete for D-cycloserine passage.

TABLE 2. Inhibition of AP-to-BL D-cycloserine transport by amino acids[a]

Side chain type and amino acid or analog	Inhibition of transport in: Fluorimetric assay (% ± SD)	Microbiological assay
Acidic		
L-Asp		No
L-Glu		No
Aliphatic		
L-Ala	58.4 ± 2.8	Yes
βAla	65.7 ± 2.8	Yes
D-Ala	40.2 ± 3.2	Yes
Gly	39.2 ± 1.4	Yes
Aliphatic hydroxy amide		
L-Ser		No
L-Thr	20.4 ± 1.8	Yes
L-Asn		No
L-Gln		No
Aromatic		
L-Phe		No
L-Trp	60.4 ± 2.0	Yes
L-Tyr	ND	Yes
Basic		
L-Lys		No
L-Arg		No
L-His		No
Branched aliphatic		
L-Leu		No
L-Ile		No
L-Val		No
Imino		
L-Pro	72.2 ± 2.1	Yes
OH-L-Pro	40.9 ± 3.4	Yes
D-Pro	66.6 ± 1.5	Yes
Sulfur containing		
L-Cys	ND	Yes
L-Met		No
L-Tau	13.3 ± 1.6	
Synthetic analogs		
αAIB	61.6 ± 7.6	Yes
αMeAIB[b]		No

[a] Inhibition of 1 mM D-cycloserine AP-to-BL passage across Caco 2 cell monolayers was measured in the presence of a 100-fold excess of competing amino acids in the AP medium. The drug in the BL medium was measured fluorimetrically except when the amino acids produced interference with the assay (ND, not detectable); in these cases the microbiological assay was employed. Values are expressed as percentages of the control value and are means ± SD of triplicate experiments performed in duplicate.

[b] αMeAIB, α-methylamino isobutyric acid.

DISCUSSION

We have previously shown that the use of two cell lines of different tissue origin (Caco 2 and MDCK) can be employed to discriminate the mechanism of transcellular passage of different antimicrobial agents (29). This earlier study suggested that D-cycloserine may use an active transport mechanism. We now show that D-cycloserine is actively transported and that passive diffusion plays only a minor role in its passage across intestinal epithelial cells.

The specific transport of D-cycloserine in the AP-to-BL direction measured in the Caco 2 cells kinetically behaves as a single saturable component, although the presence of more components with similar kinetic parameters cannot be excluded. This transport is energy dependent, as it is markedly reduced (approximately 54%) in the presence of metabolic inhibitors. The real energy dependence may be higher but could not be determined since the "gate" function of tight junctions is itself ATP dependent and would have been affected if stronger energy depletion had been used (reference 23 and unpublished observations). The observation that at 4°C the passage of D-cycloserine was markedly reduced further confirms its active nature; however, the BL-to-AP passage was also reduced (by about fourfold), presumably due to changes in membrane fluidity at 4°C.

The amino acid transporters are operationally defined in terms of sodium dependence, kinetic criteria of saturation, and competition with amino acids (17). The sodium dependence of D-cycloserine passage is only about 30%. Total transport would then result from a sodium-dependent component (30%), a sodium-independent component (60%), which may not be kinetically distinguishable, and the passive diffusion component (10%), derived from the BL-to-AP passage. Competition studies with different amino acids show that the transport of D-cycloserine is markedly inhibited by imino acids (L-Pro,

D-Pro, and OH-L-Pro) and by β-alanine and L-alanine. It has recently been shown that the transport of both L-proline and β-alanine in Caco 2 cells exhibits a strong proton dependency, while it is not affected by sodium ions (36, 37). In addition, although the apparent kinetic parameters calculated for these two transports are similar and although β-alanine transport is inhibited by L-proline and L-alanine, the presence of one or more transporters with similar characteristics remains to be determined (37).

Since D-cycloserine is highly ionized at the pH utilized in this study, it is unlikely that the pH dependency of the transport is due to the charge status of the molecule. In fact, calculations of the extent of ionization of each ionizable group of D-cycloserine showed that the acidic hydroxy group was almost 100% ionized at pH 7.5 and that the basic amino group was almost 100% ionized at pH 4.5; at pH 6.0, each group was approximately 97% ionized (1). Therefore, the pH effect exhibited by the D-cycloserine transport is most probably due to proton dependency, and the carrier may well correspond with the one described for L-proline and β-alanine.

Another proton-dependent system, identified both in the small intestine in vivo and in the Caco 2 cells, is the transporter for dipeptides and tripeptides (12, 34, 35). This carrier also serves for the uptake of the orally absorbed cephalosporins in Caco 2 cells (7, 8, 20) and in vivo (19, 26, 27). However, D-cycloserine transport across Caco 2 cells does not appear to utilize the dipeptide carrier, as indicated by the observations that (i) a large excess of cephalexin does not affect D-cycloserine passage (Table 1), (ii) dipeptides which inhibit cephalexin uptake have little or no effect on D-cycloserine passage (this study and reference 7), and (iii) amino acids that inhibit D-cycloserine passage have no effect on cephalexin uptake (Table 2 and reference 7).

Surprisingly, carnosine strongly inhibits both D-cycloserine passage and cephalexin uptake, although this probably occurs by two different mechanisms. Carnosine (βAla-L-His), a nonhydrolyzable peptide, is in fact a good substrate for the intestinal dipeptide carrier used by cephalexin (7). It is possible that the βAla moiety of the intact dipeptide may block the amino acid transporter for D-cycloserine. Alternatively, this dipeptide may interfere with the proton pump which is responsible for the gradient presumably driving D-cycloserine transport, and not necessarily by directly interacting with the carrier. It has in fact been suggested that competition experiments on proton-coupled transport in intact cell systems are not very conclusive, as the inhibitory action may arise as a consequence of two substrates competing directly for transport or by interactions with the proton electrochemical gradient, the driving force for transport (37).

In conclusion, we have shown that D-cycloserine is transported across the intestinal Caco 2 cell line by an active mechanism, operating in the AP-to-BL direction, which has the characteristics of a proton-dependent amino acid carrier and may correspond to the system responsible for the uptake of L-proline and β-alanine recently described for this cell line (36, 37). From the results obtained in the present study, it appears that this system may have a much broader specificity than previously described and may also be responsible for the high degree of bioavailability of D-cycloserine after oral administration.

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