Transport of Harman Alkaloids across Caco-2 Cell Monolayers

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This study examined the intestinal transport of five harman alkaloids using the Caco-2 cell monolayer as a model of the human intestinal mucosa. Transport parameters, permeability coefficients and percent transports, were calculated and compared under identical conditions with atenolol. Permeability coefficients were also compared with the reported values for model compounds like mannitol, propranolol and glucose. Sodium fluorescein was used as the marker for paracellular leakage. These alkaloids, in the concentration range of 250—500 μM, demonstrated substantial transport across the monolayer with moderate to high efflux rates and permeability coefficients. The transport was linear with time and was concentration dependent.

Key words harman alkaloid; passiflora; intestinal transport; bio-availability; Caco-2 monolayer

The harman alkaloids are a group of beta-carboline compounds that occur in a number of medicinal plants. *Passiflora edulis* (passion fruit), *Passiflora incarnata* (passion flower), *Peganum harmala* (syrial rue) and *Bansteriopsis caapi* (yage) are the good examples. The seeds of *P. harmala* constitute the most concentrated natural source of harmaline and harmane and this plant was used in traditional medicine to treat asthma, jaundice, lumbago, and other human ailments.[1,2] Harman alkaloids are also found in common plant derived foods (*e.g.* wheat, rice, corn, barley, soybeans, rye, grapes, mushrooms and vinegar) and plant derived beverages (*e.g.* grape juice, wine, beer, whisky, brandy). A number of pharmacological effects have been attributed to such alkaloids related to the central nervous systems such as tremorogenesis,[3] hypothermia,[4] hallucinogenesis,[5] monoamine oxidase inhibition.[6,7] They are also known to bind to receptors like 5-HT receptors and the benzodiazepine binding site of GABA receptors.[8] The spasmolytic effects of harman, harmine and harmaline have also been reported on guinea-pig isolated trachea.[9] In addition, these compounds possess antioxidative and radical scavenging properties,[10,11] inhibition of platelet aggregation,[12] and cytotoxic effects on cancer cell lines and inhibition of DNA topoisomerase I.[13] Reports are also available on cardiovascular actions of these alkaloids[14,15] and the vasorelaxant effects of harman, harmine, harmaline and harmalol seem to contribute to their hypotensive effects.[15] Anthelmintic and antiprotozoal activities have also been attributed to harman alkaloids.[16] In view of the pharmacological effects of harman alkaloids and their presence in orally administered herbal preparations, for which no bioavailability studies have been conducted, it was interesting to examine the intestinal absorption of these compounds (alone or in combination) in an *in vitro* model to predict their bioavailability. We have utilized Caco-2 cell monolayers that represent the intestinal mucosa.[17,18]

An HPLC method, previously developed in our lab.[19] was used for quantitation. In addition their uptake by the cells was also examined.

Experimental

Materials Harmane, harmaline, harmine, harmol, harmalol and atenolol were obtained from Sigma Chemical Co. Caco-2 cells were obtained from American Tissue Culture Collection (ATCC, Manassas, VA, U.S.A.). Cell culture medium and reagents were from Gibco Laboratories. Transwell™ plates were from Corning Costar. All chemicals used were of analytical grade and solvents used were of HPLC grade.

Cell Culture and Preparation of Caco-2 Monolayers Caco-2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum, 1% nonessential amino acids, 1% L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin in 75 cm^2 culture flasks, at 37 °C in an environment of 90% humidity and 5% CO_2. For the transport experiments, cells were seeded onto Transwell® inserts (4.7 cm^2 growth area, 0.4 μm pore size) at a density of 5×10^5 cells/insert. They were grown for 21 d and the integrity of monolayer was determined by measuring TEER (transepithelial electric resistance) routinely during the 21 d culture as described earlier.[18]

Transport Experiment Transport experiments were performed as described earlier using Krebs–Ringer buffer supplemented with 40 mM glucose, pH 7.4 as the transport medium.[18] A 10 mM stock solution of test compounds (harmane, harmine, harmaline, harmol, harmalol or atenolol) was prepared in DMSO and diluted in transport medium to the appropriate concentration prior to the experiment. Transport experiment was initiated by replacing the transport medium with the diluted compound in the donor side (apical side, for apical to basolateral transport). Thereafter, samples (200 μl) were taken out from the receiver side (basolateral) every hour for 7 h. The volume of the receiver side was kept constant by adding similar volume of fresh transport medium. At the end of the experiment an aliquot was also taken from the donor side and analyzed by HPLC to determine the stability of compound during the experiment. The TEER value was measured at the end of the experiment to check the integrity of monolayers. The final concentration of DMSO (5% maximum) did not affect the cell monolayers during the transport experiment. Sodium fluorescein was used as the paracellular leakage marker. For examining the uptake by the cells, at the end of the transport experiment, cells were washed with ice cold PBS, extracted with methanol and analyzed for the presence of the test compounds.[18]

Permeability coefficient (Pc) was calculated as follows:

$$Pc = dQ/dt \times 1/60 \times 1/A \times 1/C_o$$

where Pc = permeability coefficient (cm/s); dQ/dt = rate of appearance of the drug on the receiver side (μg/min); C_o = initial drug concentration on the donor side (μg/ml); and A = surface area of the monolayer (cm^2).

Percent transport (% T) was calculated as the ratio of cumulative concentration in the receiver chamber to the concentration in the donor chamber $\times 100$.

All experiments were conducted at least in triplicate and the data are presented as mean ± S.E.M. Statistical analysis was performed by Students *t*-test using GraphPad Prism.

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Analytical Methods Quantitation of harman alkaloids was performed by an HPLC method developed in our lab as reported previously.[19] Atenolol was also measured by HPLC according to the previously reported method.[20]

Results and Discussion

In this study we have utilized the Caco-2 cell monolayer as a model of intestinal absorption. The drug permeability to Caco-2 monolayers is expected to correlate well with that of intestinal membrane *in vivo*.[17,21] In cell culture, Caco-2 cells spontaneously differentiate to mature cells to form intact monolayers. They acquire many features of absorptive intestinal cells during culture such as microvillus structure, expression of hydrolytic enzymes and carrier-mediated transport systems for sugars, amino acids and several drugs. Thus, they show morphological and biochemical similarity to normal intestinal enterocytes.[17] The adjacent cells adhere through tight junctions formed at the apical side of the monolayer, allowing discrimination of the transcellular and paracellular transport of drugs across the epithelial layer.[21] The present study was undertaken to determine the bioavailability and intestinal transport of the harman alkaloids: harmane, harmaline, harmine, harmol and harmalol (Fig. 1) using Caco-2 cell monolayer. The two transport parameters, Pc and %T, for these compounds were determined in the apical to basolateral (absorptive), as well as in the basolateral to apical (secretive) direction of the monolayer. Transport was monitored for a period of 7 h. The cumulative amount transported with respect to time is shown in Fig. 2. The transport

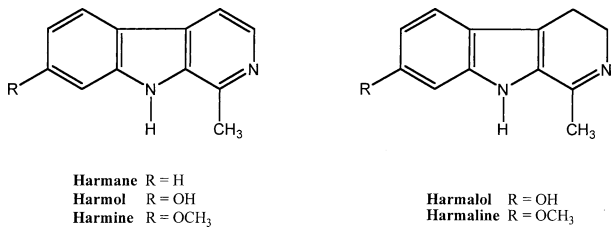

Fig. 1. Chemical Structures of Harman Alkaloids

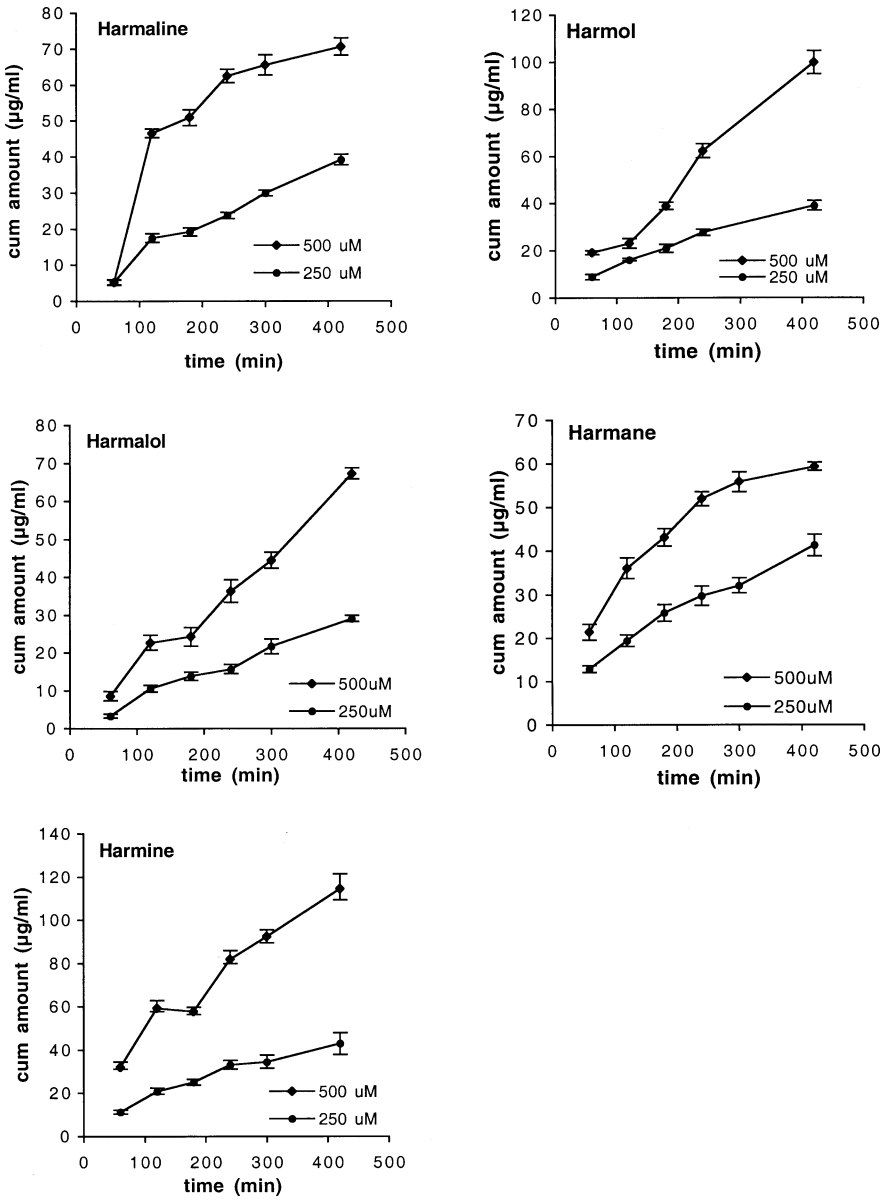

Fig. 2. Cumulative Amount Transported across the Caco-2 Monolayer in the Apical to Basolateral Direction

Table 1. Transport of Harman Alkaloids across Caco-2 Monolayer

A. Apical to Basolateral

Compounds	Permeability coefficient ($cm/s \times 10^{-6}$)		Percent transport (%T)	
	Single	Mixture	Single	Mixture
Harmaline	6.76±0.26	4.75±0.31[a)]	28.77±1.57	18.79±0.51[a)]
Harmol	4.96±0.22	4.15±0.62	19.50±0.60	16.22±0.92
Harmalol	3.80±0.40	4.50±0.09	15.40±0.82	18.18±1.10
Harmane	5.98±0.51	4.51±0.28	32.51±0.91	17.89±0.82[a)]
Harmine	6.07±0.82	4.34±0.51	29.62±0.81	16.93±1.35[a)]

B. Basolateral to Apical

Compounds	Permeability coefficient ($cm/s \times 10^{-6}$)		Percent transport (%T)	
	Single	Mixture	Single	Mixture
Harmaline	4.29±0.47[b)]	3.60±0.45	17.00±0.60[b)]	15.87±0.14
Harmol	3.10±0.60	2.80±0.15	12.80±0.70[b)]	12.17±0.20
Harmalol	3.29±0.30	1.50±0.08[a)]	11.30±0.52	5.50±0.30[a)]
Harmane	7.50±0.42	4.70±1.20	32.17±0.92	25.30±1.11[a)]
Harmine	3.10±0.15	3.90±0.20	20.00±0.60[b)]	21.24±0.71

a) Significantly different than the value for single compound ($p<0.05$). *b*) Significantly different than apical to basolateral value ($p<0.05$).

was linear with time and was concentration dependent for all the compounds. The Pc for harmine, harmane, harmaline, harmol and harmalol ranged from 3.8×10^{-6} to 6.76×10^{-6} cm/s and the %T ranged from 15 to 33% in apical to basolateral direction (Table 1A). This observation confirmed a significant transport of these alkaloids in this direction. A mixture of all five harman alkaloids was also applied to the donor side and the transport was monitored in apical to basolateral direction. Generally, lower permeability values were observed when the compounds were applied as a mixture. However these differences in Pc values were not significant, except for harmaline that showed a significantly lower value of Pc in the mixture compared to the single compound (Table 1A). The %T for each compound in the mixture ranged from 16—19% and was also lower for all the compounds except harmalol. This difference was significant for harmaline, harmane and harmine ($p<0.05$).

Atenolol, a model compound known for passive transport, was included in the study. It demonstrated a Pc value of 4.5×10^{-6} cm/s and a %T of 17.0% in the apical to basolateral direction at a concentration of 100 μM. These values were comparable to those reported previously.[22)]

The apical to basolateral Pc values of these compounds (ranging from 3.8×10^{-6} for harmalol to 6.76×10^{-6} for harmaline) are much higher (7.6 fold to 13.5 fold) than the reported Pc value of mannitol (0.5×10^{-6} cm/s), which is a paracellular transport marker.[22,23)] Propranolol is a transcellular marker which is highly lipophilic and has a high Pc value of 23×10^{-6}.[22,23)] Glucose follows an active transport mechanism and the Pc is reported to be 36.8×10^{-6} in the apical to basolateral direction.[23)] The Pc values of these harman alkaloids are much lower than the values for propranolol and glucose. Based on these observations, the transport of harman alkaloids seems to follow a concentration dependent passive diffusion mechanism.

When the compounds were loaded onto the opposite side (basolateral side), basolateral to apical flux was also observed demonstrating a lack of directional preference. Although the Pc tended to be lower for all the compounds in this direction (Table 1B), the differences were not statistically significant except for harmaline ($p<0.05$). The %T for harmaline, harmol and harmine were significantly lower in this direction compared to apical to basolateral direction (Table 1B). This bidirectional transepithelial transport also suggests a passive diffusion mechanism for their transport across the Caco-2 monolayer. Transport was linear with time in this direction also (data not shown). This observation is similar to the transport of genistein, an isoflavone found in natural product dietary supplements, that has shown a bi-directional transport in a similar Caco-2 cell model and was reported to follow a passive diffusion mechanism.[23)] The transcellular flux rate of propranolol has also been reported to be similar in both directions but with much higher flux than these alkaloids in our study.[23)] The data in Table 1 also show that the two most polar of the five alkaloids, harmol and hamalol, had lower Pc and %T values than the less polar ones. This was apparent in both directions of transport and indicates that the more lipophilic alkaloids may possess higher bioavailability.

We also examined the uptake of the harman alkaloids by Caco-2 cells during the transport experiment. No significant amount was detected in the cells indicating that there was no uptake of compounds by the cells during the experiment. The recovery of all the compounds was >90% in the overall experiments, again, confirming that there was no degradation, metabolism or binding to the cells.

In conclusion this study demonstrates a substantial transport of harman alkaloids through Caco-2 cell monolayer. The transport is linear with time and concentration dependent and seems to follow a passive diffusion mechanism. The potential bioavailability of these compounds supports their possible use as biomarkers, which can be useful in clinical studies utilizing herbal products containing some or all of these alkaloids.

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