KINETIC STUDY OF ACAMPROSATE ABSORPTION IN RAT SMALL INTESTINE

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Abstract — Acamprosate (calcium bis acetyl-homotaurine), a homotaurine derivative, a structural analogue of γ-aminobutyric acid (GABA) and an upper homologue of taurine, is a relatively new drug used to prevent relapse in weaned alcoholics. When administered orally as enteric-coated tablets at relatively high doses, this drug has a bioavailability of about 11%; however, the intestinal absorption mechanism has not been studied in depth. The present study was therefore planned to characterize the intestinal transport of acamprosate in the rat and the effect of chronic alcohol treatment on this process, quantifying its kinetic parameters and investigating possible inhibitors. Using an in vitro technique, acamprosate absorption was measured in the rat intestine from three different groups: alcohol group [fed a liquid diet containing 5% (w/v) ethanol for 4 weeks], isocaloric pair-fed control, and a solid diet group. Intestinal acamprosate absorption was found to occur mainly by passive diffusion with a diffusive permeability of 0.213 ± 0.004 cm/h in control pair-fed animals, 0.206 ± 0.001 cm/h in animals receiving chronic alcohol treatment, and 0.193 ± 0.001 cm/h in the solid diet group. Inhibition studies showed that at a 10^{-6} M acamprosate concentration, some compounds such as GABA, taurine, proline, and glycine at 40 mM each did not affect acamprosate transport. Nevertheless, when a lower concentration of the drug (10^{-4} M) was assayed, a significant reduction of acamprosate transport in the presence of taurine or GABA 40 mM was found. These results suggest that acamprosate in the rat jejunum, could be transported, in part, by a carrier system. Further experiments using different concentrations of taurine (10, 20, and 80 mM) showed that the maximum inhibition (32%) is achieved at 20 mM of taurine. These latter results suggest that acamprosate and taurine share, at least, an intestinal carrier system in rat jejunum. From the above results, it can be concluded that there are probably two pathways involved in the intestinal absorption of acamprosate: passive diffusion and mediated transport, with the former being predominant. Moreover, neither chronic ethanol intake nor the type of diet seems to alter the intestinal absorption of the drug.

INTRODUCTION

Acamprosate (calcium bis acetyl-homotaurine), a synthetic compound with a chemical structure similar to that of γ-aminobutyric acid (GABA) and an upper homologue of taurine, is used as an adjunct for treatment of alcohol dependence due to its anticraving and relapse-reducing properties. After oral administration, few side-effects and adverse reactions have been observed, with nausea and diarrhoea being the most frequent. Tolerance is usually good. However, due to the limited absorption of acamprosate, the drug is administered at relatively high doses (1998 mg/day if body weight is ≥60 kg or 1332 mg/day if body weight is <60 kg). Some dose-dependent adverse effects have been observed (Withworth et al., 1996). Some of these effects seem to be related to the calcium moiety of the molecule (Soyka, 1997). When the drug is administered as enteric-coated tablets, the mean absolute oral bioavailability in humans is 11 ± 1% (Saivin et al., 1998). Acamprosate is neither protein bound nor metabolized. Moreover, degradation into the intestinal lumen or by intestinal cells does not appear to contribute to the relatively low bioavailability (Saivin et al., 1998).

In recent years, research into the mechanism of acamprosate action has flourished, although it is as yet unclear. On the contrary, few studies have focused on the intestinal absorption mechanisms of the drug. Chabenat et al. (1988) concluded that, due to its physicochemical properties, the drug presumably crosses the biological barriers with the help of a transporter. However, recently some authors have postulated that the acamprosate absorption pathway is predominantly by the paracellular route (Saivin et al., 1998). Additionally, the kinetic parameters of drug transport are not known. Related compounds, such as GABA or taurine, can permeate the intestinal membrane via a mediated transport system plus passive diffusion in the rat (Munck et al., 1994; Nächer et al., 1994; Munck and Munck, 1994).

As we have, however, demonstrated previously, chronic ethanol intake can modify intestinal drug absorption depending on the mechanism involved. Using seven compounds belonging to a homologous series (ciprofloxacin derivatives), we have shown that the effect of chronic alcohol treatment on passive diffusion depends on the physicochemical properties of the drug. Accordingly, chronic ethanol intake modifies only the absorption rate of highly and markedly hydrophilic homologues (Merino et al., 1997). If mediated transport is involved in the absorption, chronic ethanol treatment can decrease the absorption rate, as in the case of methionine (Polache et al., 1996). When both mechanisms are operating, the global effect of ethanol will depend on how much each mechanism participates as well as on the physicochemical characteristics of the drug. When we performed experiments with taurine, a homologue of acamprosate that can permeate the intestinal membrane by both mediated transport and passive diffusion, the results showed that chronic ethanol intake enhances the influx of taurine across the brush border membrane. In fact, taurine is a hydrophilic compound and its passive diffusion is more important than mediated transport (Martín-Algarra et al., 1998).

In order to gain the advantage of maximal oral drug bioavailability, it is first necessary to identify the barriers to oral absorption and so investigate the intrinsic absorption mechanisms of acamprosate. The pharmacotherapy of alcohol dependence with acamprosate could therefore be improved if we had a better understanding of the intestinal absorption
mechanisms of this drug and knew more about its kinetic parameters and possible interactions with different compounds present in the intestinal lumen (other drugs or food components). The main aim of this work was therefore to gain an insight into the intestinal absorption mechanism of acamprosate, using an in vitro technique and characterizing its kinetic parameters, as well as the possible influence of chronic ethanol intake on the process. The second aim was to investigate whether the presence of some structurally related compounds in the intestinal lumen inhibit the transport of the drug.

MATERIALS AND METHODS

Animals and treatments

Male albino Wistar rats weighing 230–275 g were used. The animals were maintained under controlled conditions of light and dark (12:12 h), temperature (23°C), and humidity (60%). Rats were fed the Lieber–DeCarli diet containing either 5% (w/v) (36% of calories) ethanol or an isocalorically (60%). Rats were fed the Lieber–DeCarli diet containing either 5% (w/v) (36% of calories) ethanol or an isocalorically balanced supplement with dextrose-maltose for pair-fed controls (Lieber and DeCarli, 1982), for 4 and 2 weeks respectively. Another group of animals were fed a standard rat chow solid diet and allowed free access to food and water before the experiment.

The animals were distributed randomly into three groups: liquid pair-fed control (control group), alcohol (alcohol group), and solid control (solid group).

After treatment, rats were anaesthetized with ether, the abdomen was opened and the mid-small intestine was removed and rinsed briefly in ice-cold buffer solution. Only the central segment of the small intestine (mid-jejunum) (20 cm) was used for monitoring in the influx chamber.

Acamprosate absorption studies

To characterize acamprosate absorption we used an in vitro technique (Schultz et al., 1967) which allows the unidirectional influx of acamprosate across the brush border membrane of the rat mid-jejunum ($J^{\text{Aca}}$) to be measured. Acamprosate influx rate was measured using $[^{14}\text{C}]$acamprosate from LIPHA (Lyon, France) and $[^{3}\text{H}]$polyethylene glycol (M = 4000; $[^{3}\text{H}]$PEG-4000) from American Radiolabeled Chemicals (ARC; St Louis, MO, USA). The structure of $[^{14}\text{C}]$acamprosate is: \[ \text{CH}_3-\text{CO}--\text{NH}--\text{CO}--\text{CH}_3. \]

In all experiments, we maintained an osmotic balance by adding mannitol to keep the total concentration of acamprosate and mannitol constant (Munck et al., 1994). All solutions were made with a HEPES buffer (pH 7.4) with the following composition (mM): 140 Na+, 8 K+, 2.6 Ca2+, 1 Mg2+, 140 Cl−, 16 HEPES, 1 SO42−, 5 D-glucose. Eleven solutions of acamprosate, at different concentrations, were used in the experiments with the control pair-fed group: $10^{-5}$, $10^{-4}$, $6 \times 10^{-4}$, $10^{-3}$, $2.5 \times 10^{-3}$, $5 \times 10^{-3}$, $10^{-2}$, $1.5 \times 10^{-2}$, $2.5 \times 10^{-2}$, $4 \times 10^{-2}$, and $8 \times 10^{-2}$ M; 10 different concentrations of acamprosate in the alcohol group: $10^{-3}$, $10^{-2}$, $10^{-1}$, $2.5 \times 10^{-2}$, $5 \times 10^{-2}$, $10^{-3}$, $1.5 \times 10^{-2}$, $2.5 \times 10^{-2}$, $4 \times 10^{-2}$, and $8 \times 10^{-2}$ M; and 11 concentrations in the solid group: $10^{-3}$, $2.5 \times 10^{-3}$, $10^{-2}$, $6 \times 10^{-2}$, $2.5 \times 10^{-3}$, $5 \times 10^{-3}$, $10^{-2}$, $1.5 \times 10^{-2}$, $2.5 \times 10^{-2}$, $4 \times 10^{-2}$ and $8 \times 10^{-2}$ M. If a mediated transport system is involved in acamprosate absorption, it would be important to examine a possible sodium-dependent transport in order to characterize the carrier. For that reason, additional experiments, using acamprosate at a concentration of $10^{-4}$ M, were carried out, in the control pair-fed and alcohol groups, in these sodium chloride was substituted with choline chloride in order to maintain the osmotic balance. Thus, it is possible to calculate drug influx in the presence and in the absence of Na+.

Unidirectional influx across the brush border membrane of the rat jejunum was measured essentially as described for the rabbit and rat intestines (Munck, 1985, Munck et al., 1994). For each rat, the mid 20 cm of the total small intestine were excised, opened along the mesenteric attachment, cut in half, and rinsed in ice-cold buffer. Each segment was mounted on a Lucite plate with the mucosal surface facing upwards and a Lucite block was clamped on top of the plate. In this way, four mucosal areas each of 0.35 cm$^2$ were exposed at the bottom of the wells, where the solution was oxygenated and stirred by high rates of O$_2$ flow. By using two blocks, eight measurements were possible for each rat.

The tissues were incubated for 15 min with an acamprosate-free solution containing 5 mM glucose, which was then withdrawn. The well and mucosal surface were gently wiped with soft paper to remove the adhered incubation fluid before the test solution was injected. The 0.5-min incubation period was terminated by aspiration of the incubation fluid and flushing of the well with an ice-cold 300 mM mannitol solution. The exposed tissues were then punched out, briefly rinsed in ice-cold mannitol solution, blotted on hard filter paper, and extracted for 18 h in 0.1 M HNO$_3$. Analysis by liquid scintillation spectrometry was performed with equal quench in all samples. The amount of $[^{3}\text{H}]$PEG-4000 was used to correct for extra-cellular contamination assuming that this compound cannot cross the intestinal membrane and remains extracellularly, so that it is possible to quantify exactly the percentage of extra-cellular contamination and to transform it for $[^{14}\text{C}]$acamprosate; thus corrected, the $[^{14}\text{C}]$-labelled acamprosate activity was used to calculate the rate of influx across the brush border membrane.

Inhibition studies

Several inhibition studies were carried out to check whether mediated transport across biological membranes took place, as suggested by some authors (Chabnet et al., 1988). Three series of experiments were performed using several compounds as inhibitors. In the first series, paired measurement of the flux across the brush border membrane, $J^{\text{Aca}}$, were made at $10^{-3}$ M acamprosate with 40 mM glycine, proline, GABA or taurine, in both the control and alcohol groups. Additionally, in a second series of assays, paired measurements of $J^{\text{Aca}}$ were performed at $10^{-4}$ M acamprosate with 40 mM GABA or taurine, in both the control and alcohol groups. Finally, a third series of studies were performed in the control group, measuring $J^{\text{Aca}}$ at $10^{-4}$ M acamprosate in the presence of 0, 10, 20 or 80 mM taurine, or a mixture of 40 mM GABA and 40 mM taurine.

Mathematical and statistical methods

Given that no saturation was detected in the absorption process, we assumed that the unidirectional influx of acamprosate could be described as a passive diffusion [equation (1)]
or as the sum of a saturable process and a passive diffusion [equation (2)].

\[ J^{\text{Aca}} = J_p \]  
\[ J^{\text{Aca}} = J_s + J_p \]  

where \( J_p \) is the flux caused by a passive process and \( J_s \) is the transport by a saturable process. Substituting these fluxes by the corresponding equations, we have:

\[ J^{\text{Aca}} = P \times A \]  
\[ J^{\text{Aca}} = \frac{J_{\text{max}} \times A}{K_m + A} + P \times A \]  

where \( J^{\text{Aca}} \) and its maximum rate, \( J_{\text{max}} \), are given in nmol/cm²/h (serosal area), \( K_m \) is the Michaelis–Menten constant in µM, \( P \) is the diffusive permeability in cm/h and \( A \) is the concentration of acamprosate at the absorption site.

The fits of equations to the experimental data have been performed using a non-linear least-square regression (Sigmaplot 4.0) or a linear least-squares procedure (Statgraphics 7.0).

Statistical comparisons among \( P \)-values were carried out using the ANOVA test for linear regression analysis. When significant differences were found, Dunnet’s multiple range test was applied in order to detect significantly different means. A probability level of less than 0.05 was considered to be statistically significant.

RESULTS

Acamprosate transport across the brush border membrane

In order to investigate absorption mechanisms of acamprosate transport, drug influx across the brush border membrane was studied in the three experimental groups (control, alcohol, and solid) over a wide concentration range (\( 10^{-5} - 8 \times 10^{-2} \) M) that included concentrations probably reached in the intestinal lumen after oral administration of the drug in humans. The experiments were performed in the mid-jejunum of the rat, because in the case of mediated transport of amino-acidic substances the carriers are located in this part of the gut (Munck et al., 1994). The results are shown in Fig. 1. This figure allows comparison of the data obtained and shows that the absorption profile and magnitude are similar in the three groups studied, suggesting that there are no differences in acamprosate influx.

After fitting equation (3) to the experimental data, we calculated the diffusive permeability value (\( P \)) for the three experimental groups from equation (3). The results of the kinetic analysis are summarized in Fig. 2 and Table 1. The statistical analysis of these data demonstrates that acamprosate influx across the brush border membrane is significantly different in the three groups studied, although the diffusive permeability \( P \), is quite similar in the three groups and can be considered as identical for practical purposes.

Inhibition studies

According to our results, passive diffusion is the main mechanism implicated in acamprosate absorption. However,
in order to detect the possible coexistence of a mediated transport system, we performed inhibition studies with several compounds. Figure 3 shows that, using a concentration of acamprosate of $10^{-3}$ M and 40 mM glycine, proline, GABA or taurine, no significant differences were found in the influx of acamprosate across the brush border membrane in the presence of inhibitors. However, since in these experiments we used a relatively high acamprosate concentration and under these conditions the drug is probably transported mainly by diffusion, we decided to test the effect of the inhibitors using a lower concentration of acamprosate ($10^{-4}$ M). Figure 4 shows that, under these conditions, the influx of acamprosate was significantly reduced in the presence of 40 mM taurine or GABA ($P < 0.005$ in all the cases). The reduction was 32% for taurine in control and alcohol groups and 22 and 40% for GABA in control and alcohol groups respectively, suggesting that an imino-acid carrier could be involved in the acamprosate specialized transport system in the rat intestine. To further investigate this possibility, inhibition experiments were performed using $10^{-4}$ M acamprosate and several concentrations of taurine, and a mixture of taurine and GABA in order to detect a possible additive effect. As shown in Fig. 5, a statistically significant decrease in the influx of acamprosate was observed when taurine or a mixture of taurine and GABA was present, suggesting that acamprosate is transported by the intestinal imino-acid carrier, as GABA and taurine are.

**DISCUSSION**

As we have pointed out previously, there is little information on acamprosate absorption in the literature, and existing
information is contradictory. While some authors propose that a carrier system is involved in acamprosate transport across biological membranes (Chabenat et al., 1988), others suggest paracellular transport (Saivin et al., 1998). In order to gain an insight into the process of acamprosate absorption, a kinetic study was developed in three experimental groups using a wide concentration range. The results of drug influx in the three conditions studied (Fig. 1) show that the magnitude of acamprosate transport and even the profile are very similar in all cases.

The results of the kinetic analysis of $J_{\text{Aca}}$ data clearly indicate that passive diffusion [equation (3)] is the main mechanism involved in acamprosate intestinal absorption. This conclusion was drawn because it was impossible to reach satis-

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**Fig. 3.** Inhibitory effect of glycine, proline, GABA, and taurine on $J_{\text{Aca}}$ of rat jejunum. Bars represent means ± SD of five to seven observations of a series of paired measurements of acamprosate transport at $10^{-3}$ M alone or with four different amino acids each at 40 mM in both the control pair-fed and alcohol groups.

**Fig. 4.** Inhibitory effect of GABA and taurine on $J_{\text{Aca}}$ of rat jejunum. Bars represent means ± SD of five to ten observations of a series of paired measurements of acamprosate transport at $10^{-4}$ M alone or with the amino acids tested at 40 mM, in both the control pair-fed and alcohol groups.

* Statistically significant differences ($P < 0.05$).
factory convergence during the fitting procedures of equation (4) to the data. Therefore, the diffusive permeability (P) value was calculated as 0.213 ± 0.003 cm/h for the control pair-fed group and 0.206 ± 0.001 for the alcohol group. The statistical comparison shows that they are significantly different although can be considered as identical for practical purposes. From these data, we can conclude that chronic alcohol intake does not alter the absorption of acamprosate across the rat jejunum. This result is supported by our previous studies (Merino et al., 1997; Martín-Algarra et al., 1998), which demonstrated that ethanol treatment only affects the passive intestinal transport of very hydrophilic compounds, such as taurine. Accordingly, acamprosate, a homologue of taurine with an acetyl group, is expected to be more lipophilic than taurine and therefore its transport is unlikely to be influenced by ethanol treatment. Our data are in accord with those obtained by Saivin et al. (1998), who have also demonstrated that acamprosate pharmacokinetics after oral administration are not influenced by alcohol.

However, we can also conclude that the diet type (liquid vs solid) does not modify acamprosate absorption, so in this type of experiment it is possible to use animals fed a solid diet as a control group.

It is noteworthy that the P-value obtained for acamprosate is not as low as we expected considering the low bioavailability of the drug in humans. In fact, this value is similar to that obtained for antipyrine, a compound with good bioavailability (Torres-Molina et al., 1992), using the same technique (Polache et al., 1998). Thus, on the basis of these results we cannot conclude that the magnitude of acamprosate absorption is low. This discordance between our results and those in humans could be explained by the fact that we used an in vitro technique with intact intestine and that acamprosate influx was measured for only 30 s. Under these conditions, it is difficult to detect some processes that could occur in vivo and could markedly reduce the extent of drug absorption (e.g. intestinal secretory transport). The intestinal epithelial membrane has some specialized transport systems which can secrete drugs from the serosal side to the mucosal side, functioning as a barrier to absorption and being an unrecognized cause of low bioavailability (Arimori and Nakano, 1998). A typical secretory system is P-glycoprotein (P-gp) (Lennermä and Regardh, 1993; Arimori and Nakano, 1998). P-gp functions as an efflux transport pump and can be a factor that limits bioavailability of some hydrophobic drugs and peptides (Gramatte et al., 1996). Moreover, it has recently been reported that DMP728, a cyclic peptide fibrinogen antagonist, has an oral absorption limited by its hydrophilicity and by the dominance of secretory transport, probably by P-gp (Aungst and Saitoh, 1996). Acamprosate may also be a substrate for an intestinal secretory system. This possibility has yet to be investigated.

Although our results suggest strongly that passive diffusion is the main mechanism involved in acamprosate intestinal transport, we cannot discard the existence of a simultaneous minor saturable process. To test this hypothesis, several inhibition studies were developed. The first (Fig. 3) showed that 40 mM of glycine, proline, GABA or taurine does not inhibit acamprosate influx at 10^{-3} M. The possible explanation of this result could be the high concentration of acamprosate used. At this concentration the drug is probably transported mainly by diffusion, being unaffected by the compounds tested. When a second study was carried out using a lower acamprosate concentration (10^{-4} M), the drug influx was significantly reduced in the presence of GABA and taurine (Fig. 4). These results suggest that the carrier of imino acids could be involved in the acamprosate specialized transport in the rat intestine. To study this possibility, a third assay was performed in the control group. The results (Fig. 5) showed that acamprosate transport was significantly inhibited by taurine and a mixture of taurine and GABA. Maximum inhibition was reached at 20 mM
such as chitosans can be utilized as enhancers since they are implici-
tations of acamprosate absorption inhibition by taurine, 
Aungst, B. J. and Saitoh, H. (1996) Intestinal absorption barrier and 
effectiveness in increasing membrane permeability (Shipper 
patient by reducing side-effects, but also lead to a saving in 
extraction mechanism which could be responsible for its low 
oral bioavailability. It will be of clinical interest to investigate 
whether, in acamprosate absorption, a specialized secretion 
transport such as P-gp is involved and whether this transport 
could be inhibited to enhance acamprosate absorption. Enhancing acamprosate absorption would not only benefit the 
patient by reducing side-effects, but also lead to a saving in 
drug use effects.

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