Use of 1-Methyl-Pyrrolidone as a Solubilizing Agent for Determining the Uptake of Poorly Soluble Drugs

Antje S. Uch,[1] Ulrich Hesse,[1] and Jennifer B. Dressman[1,2]

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INTRODUCTION

Many drugs being developed today are poorly soluble to the extent that dissolution may be at least partly rate-limiting to their oral absorption. In order to get a clear picture of which factors are the greatest limitations to absorption, it is also necessary to evaluate the stability of the compound in the luminal fluids, the permeability of the intestinal mucosa to the compound and any first pass metabolism which may occur in the gut wall and/or the liver (1). To facilitate the determination of the permeability, it is highly desirable to identify a solubilizing agent which is generally applicable but does not enhance or interfere with transport of the test compound across the gut wall.

The solubilizing agent tested in this work was 1-methyl-2-pyrrolidone (NMP) (see Fig. 1), an agent which readily forms complexes with aromatic rings (2) and halogens (3) and has been widely applied to enhance the solubility of therapeutically active agents. We first studied the uptake behavior of two well-characterized compounds, hydrocortisone and mannitol, which are absorbed by transcellular and paracellular passive absorption respectively. The uptake of these compounds was compared in absence and presence of NMP. Then we applied NMP solubilization to facilitate characterization of the uptake of itraconazole, an orally active broad-spectrum triazole antifungal with extremely poor (< 0.1 μg/mL at intestinal pH values) solubility. Its molecular weight is 705 and the log P is 5.66 (4).

EXPERIMENTAL METHODS

Materials

Non-radiolabelled itraconazole and tritiated itraconazole (97–98% label purity) were donated from Janssen Pharmaceutica (Beerse, Belgium). Non-radiolabelled hydrocortisone and mannitol were purchased from E.Merck (Darmstadt, Germany), [1,2,6,7-^{3}H] hydrocortisone and D-[1-^{14}C] mannitol from Amersham Buchler (Braunschweig, Germany). MES (2-morpholino-ethansulfonic acid monohydrate) and 1-methyl-2-pyrrolidone (NMP) were purchased from Fluka Chemie AG (Buchs, Switzerland), sodium taurocholate was purchased from Sigma (St. Louis, USA), and egg-lecithin Lipoid E PC was donated from Lipoid GmbH (Ludwigshafen, Germany).

Incubation Fluids

The modified MES buffer solution was composed of 10 mM MES, 135 mM sodium chloride, 5 mM potassium chloride and 11 mM D-glucose. The pH was adjusted to pH 6.5 by the addition of 1.0 N sodium hydroxide with the aid of a calibrated pH meter (Orion Model 720 A, Orion Research Inc., Boston, USA) and the batch was sterilized in an autoclave (Pacs 2000, Getinge, Germany). Sodium taurocholate (NaTC) 15 mM was dissolved in the buffer solution and lecithin (Lec) 3.75 mM, pre-dissolved in chloroform, was added. The chloroform was then removed from the resultant emulsion by evaporation, leading to formation of a mixed micellar solution.

The final incubation media consisted of cold and hot drug dissolved in modified MES/NaTC/Lec buffer pH 6.5 containing 2% NMP.

Intestinal Uptake Methodology

Fasted male Wistar rats (~300 g) were euthanized using sodium pentobarbital (Nembutal®). Immediately following euthanasia, the jejunum and the colon were excised, washed in ice-cold sodium chloride solution 0.9% and placed into oxygenated modified MES buffer held at 4°C. The intestinal segments were everted, cut into ~2 mm rings and hooked individually over a five-arm anchor (5), which was then lowered into the incubation medium maintained at 37°C. After the designated time interval, the rings were removed, washed briefly with isotonic sodium chloride solution of 4°C, blotted dry and weighed (wet tissue weight). The tissue was then dissolved and the uptake determined by radioactive counting.

Study Design

The transport of mannitol (paracellular) and hydrocortisone (transcellular) into the intestinal tissue was first investigated to determine whether the presence of NMP in the incubation medium would have any effect on their permeability. Following these studies, timed uptake studies covering a time range between 15 and 600 seconds were conducted to characterize the uptake profile of itraconazole. In particular, the adsorption of itraconazole to the tissue of the jejunum and colon, the rate of itraconazole uptake and the equilibrium uptake were determined, as well as the appropriate incubation time for subsequent shaking rate and concentration dependency studies. In order to determine the shaking rate necessary to minimize aqueous resistance, jejunal and colonic uptake was studied as a function of shaking rate at the appropriate incubation time. Concentration dependent uptake studies were conducted to characterize the uptake mechanism and the uptake rate (UR) of itraconazole by the jejunal and colonic mucosa.

Incubation Conditions

Validation experiments with 2% NMP in the incubation medium were conducted at a concentration of 0.3 mM (mannitol and hydrocortisone) and an incubation time of 60 seconds,

[1] Johann Wolfgang Goethe University, Department of Pharmaceutical Technology, Biocenter, 60439 Frankfurt/Main, Germany.

[2] To whom correspondence should be addressed. (e-mail: dressman@em.uni-frankfurt.de)

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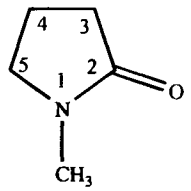

Fig. 1. Chemical formula of 1-methyl-2-pyrrolidone.

experimental conditions that had been previously optimized for these two compounds (5). Timed uptake studies with itraconazole were conducted at 0.75 μM for incubations of up to 600 seconds. The effect of shaking rate on uptake was determined at 0.75 μM, an incubation time of 60 seconds and using shaking rates of 40, 60 and 75 reciprocations per minute (rpm). Concentration dependency studies were conducted at predetermined incubation times of 60 seconds and 90 seconds for the jejunum and colon, respectively. The shaking rate was 60 rpm and the concentration range studied was 0.1 to 4.5 μM. Fifteen rings (5 rings from each of 3 rats) were studied at each time point/shaking rate/concentration.

Statistical Evaluations

The results obtained were evaluated using Analysis of Variance and t-test with the aid of SigmaStat® 2.0 (Jandel Scientific Software, Erkrath, Germany). Comparison of the two (jejunal and colonic) slopes followed the equation (6):

$$t = \frac{\text{Difference of the regression slopes}}{\text{Standard error of the difference of the regression slopes}}$$

The resulting t value is compared with the critical value of t corresponding to $v = n_1 + n_2 - 4$ degrees of freedom. In all cases significance was considered at the $p = 0.05$ level.

RESULTS

Hydrocortisone

Uptake studies in intestinal rings from jejunal and colonic tissues of rats using 2% NMP in the incubation medium showed no influence of NMP on the uptake of hydrocortisone ($P_{jejunum}$ = 0.6, P_{colon} = 0.2). Mean values of the pooled data for treatments with and without NMP were 44.6 ± 10 nmoles/g tissue and 46.4 ± 10 nmoles/g tissue, respectively, for the jejunum, and 44.7 ± 8 nmoles/g tissue and 49.0 ± 9 nmoles/g tissue, respectively, for the colon (Fig. 2).

Mannitol

Uptake studies in intestinal rings from both tissues using 2% NMP in the incubation medium showed, similarly to hydrocortisone, no influence of NMP on the uptake of mannitol ($P_{jejunum}$ = 0.7, P_{colon} = 0.8). Mean values of the pooled data for treatments with and without NMP were 39.4 ± 8 nmoles/g tissue and 38.0 ± 11 nmoles/g tissue, respectively, for the jejunum, and 29.3 ± 7 nmoles/g tissue and 29.9 ± 7 nmoles/g tissue, respectively, for the colon (Fig. 2).

Validation of Itraconazole Solubilization

Itraconazole was first dissolved in neat 1-methyl-2-pyrrolidone and this solution was added to the aqueous modified MES buffer solution to yield a final NMP concentration of 2%. This procedure enabled a dramatic increase in dissolved itraconazole in aqueous solution (up to 8 μg/mL compared with the aqueous solubility of <0.1 μg/mL at intestinal pH values), with no precipitation occurring for at least 2 hours as determined by HPLC analysis (data not shown).

For the uptake studies, itraconazole was therefore first dissolved in neat NMP and added to the incubation medium to yield a final NMP concentration of 2%.

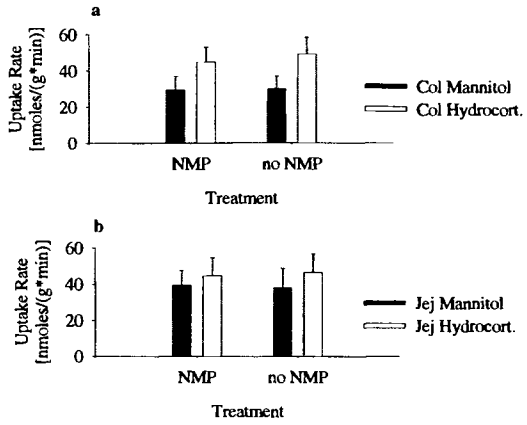

Fig. 2. Uptake of the reference substances mannitol and hydrocortisone in the presence and absence of NMP using jejunal and colonic tissue of rats expressed as mean uptake (5 rings from each of 3 rats, n = 15).

Timed Uptake Studies

Itraconazole uptake was linear for at least 120 seconds in the jejunum (R^2 = 0.95) and for at least 240 seconds in the colon (R^2 = 0.95) (Fig. 3), after which a plateau was observed. In comparison to jejunal uptake, colonic uptake was slightly lower on a per gram wet tissue basis. Extrapolation of the linear segment to the zero time point gave the extent of non-specific intestinal tissue binding, which was minimal for both tissues (2.6–2.9 pmoles/g). Subsequent concentration dependency studies were conducted using incubation times of 90 seconds for the colon and 60 seconds for the jejunum.

Shaking Rate Dependency Uptake Studies

Shaking rate dependency uptake studies showed that increase of agitation did not lead to any significant alteration of itraconazole uptake in either jejunum (p = 0.416) or colon

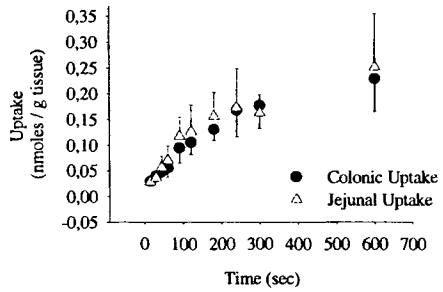

Fig. 3. Jejunal (Δ) and colonic (●) uptake of itraconazole as a function of time at a concentration of 0.75 μM, at pH 6.5 and 37°C, expressed as mean uptake (5 rings from each of 3 rats, n = 15) ± SD.

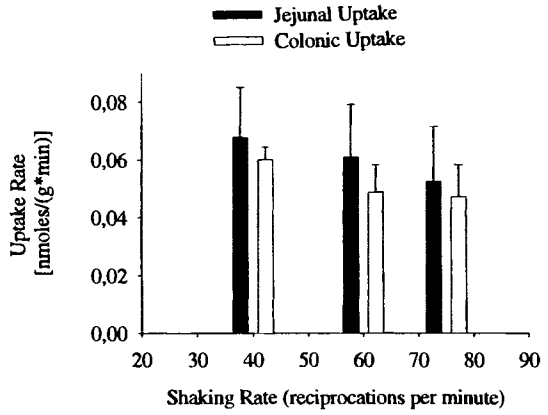

Fig. 4. Jejunal and colonic uptake rate of itraconazole as a function of shaking rate at a concentration of 0.75 μM, at pH 6.5 and 37°C, expressed as mean uptake (5 rings[4] from each of 3 rats, n = 15) ± SD.

(p = 0.09) (Fig. 4) even though a trend to a decrease in uptake at higher shaking rates was apparent in both tissues.

Concentration Dependency Uptake Studies

After correction for non-specific tissue binding, itraconazole uptake was linear in the jejunum (UR = 0.009 + (0.121*conc); R^2 = 0.96) and colon (UR = 0.0162 + (0.133*conc); R^2 = 0.96) (Fig. 5). In contrast to jejunal results, colonic results indicate that at concentrations greater than 3 μM the variability of uptake increases (regression not shown).

The normalized uptake rates were calculated according to the equations given above to be 121 ± 5.9 nmoles/(g*min*mM) for the jejunum and 133 ± 5.6 nmoles/(g*min*mM) for the colon, suggesting that colonic uptake may occur to a slightly greater extent. This difference of the slopes from the jejunum and colon was, however, not statistically significant.

DISCUSSION

As a weakly basic drug with very poor aqueous solubility and high lipophilicity, itraconazole should be rapidly transported into the intestinal mucosa, provided it can be dissolved. In the present study, the effect of addition of 1-methyl-2-pyrrolidone (NMP) as a solubilizing agent was evaluated for the determination of the permeability of itraconazole. NMP was shown to improve itraconazole solubility in aqueous media and to keep itraconazole in solution over the experimental time period. In the case of itraconazole, a 2% solution was necessary to ensure that the drug stayed in solution for the duration of the experiment (with a 1% solution, less than 95% remained in solution after two hours). The concentration used for other substances will depend on their solubilities and how well they are solubilized by NMP.

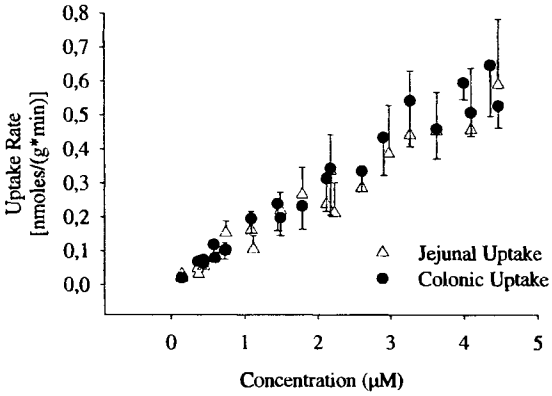

Fig. 5. Jejunal (Δ) and colonic (•) uptake rate of itraconazole as a function of concentration, at pH 6.5 and 37°C, expressed as mean uptake (5 rings from each of 3 rats, n = 15) ± SD.

In the presence of NMP, itraconazole is easily taken up by the jejunal and colonic tissue of rats. The uptake was shown to be linear and occurred via passive, probably transcellular, diffusion; no active transport mechanism could be detected applying an Eadie-Scatchard plot. The normalized jejunal uptake rate was calculated to be 121 ± 5.9 nmoles/(g*min*mM) and colonic uptake rate to be 133 ± 5.6 nmoles/(g*min*mM), which was slightly lower than hydrocortisone[3] (normalized jejunal and colonic uptake rate 169 nmoles/(g*min*mM) and 151 nmoles/(g*min*mM), respectively (7)), but higher than mannitol[3] (106 nmoles/(g*min*mM) and 89 nmoles/(g*min*mM), respectively). Itraconazole colonic uptake was not significantly different from jejunal uptake, which indicates that uptake is not site specific, even though there was a trend for the normalized uptake rate to be higher in the colon. Uptake studies in intestinal rings from jejunal and colonic tissues of rats using 2% NMP in the incubation medium showed no influence of NMP addition on the uptake of either mannitol or hydrocortisone. This indicates that an NMP concentration of 2% in the incubation medium does not increase transport of passively transported drugs due to, for example, increased tight junction permeability in the case of mannitol.

To conclude, 2% NMP appears to be a promising solubilizing agent for the determination of uptake of compounds with very poor water solubility, as demonstrated in these studies with itraconazole. Its use enabled us to show that itraconazole is readily taken up by a passive transport mechanism in both jejunum and colon of rats. Low solubility together with high rate of uptake suggest that itraconazole belongs to Class II of the Biopharmaceutics Classification System (8).

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[3] Values corrected for non-specific tissue binding.
[4] 10 rings at shaking rate 60 rpm.

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