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Correlation of in vitro and in vivo paracetamol availability from layered excipient suppositories

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Abstract

An in vivo investigation of paracetamol availability was carried out on eight healthy volunteers, comparing two paracetamol suppository formulations prepared using two different gliceride bases, a fast drug-releasing one and a slow drug-releasing one, i.e. Witepsol H15 and W35, respectively. The formulations were selected on the basis of a previous in vitro drug release study, which showed that, by superimposing the excipients in two layers within the same suppository, the drug release kinetics could be modulated using different ratios between the two layers. The comparison between the two different formulations in terms of plasma profiles and total amounts of drug excreted in urine revealed an increase in the extent of drug absorption from the layered excipient suppository. As the W35 has a higher monoglyceride content than the H15, this improved paracetamol availability could be ascribed to the absorption-enhancing effect of the monoglycerides. Moreover, the W35 has also a higher viscosity, which could possibly cause the suppository to be retained for a longer time in the lower part of the rectum, where the blood is drained directly to the systemic circulation. It was therefore hypothesized that the enhanced paracetamol availability could be also due to a liver bypass mechanism. For a further examination of the paracetamol absorption kinetics after rectal administration, a one-compartment model was fitted to the drug plasma concentration data. This approach allowed to draw absorption versus time profiles, which showed that a retardation actually occurred in paracetamol absorption when using suppositories containing the slow drug releasing excipient W35. These absorption data were then employed for an A level in vitro–in vivo correlation testing, and a linear relationship was found between in vitro release rate and in vivo absorption rate, both for fast releasing and for the layered excipient suppositories.

1. Introduction

Drug release from suppositories and subsequent absorption through the rectum involves several

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stages, starting from suppository melting or softening at rectal temperature, followed by drug migration through the suppository mass and its transfer from suppository surface to the rectal environment, and finally drug solubilization in rectal fluids and drug permeation across rectal membrane (Realdon et al., 1997a). The rectal absorption occurs mainly by passive diffusion and the drug release from suppository is likely to be an important factor in determining the drug concentration in the rectal fluids and hence its absorption rate. Therefore, both drug solubility and excipient characteristics have a crucial role in the rate of drug absorption. It has been shown that higher drug solubility in the vehicle results in slower drug release and reduced drug absorption from the dosage form. This is attributed to the tendency of the drug to be retained in the base (Shangraw and Walkling, 1971; Pagay et al., 1974). Furthermore, the excipient properties can affect not only the rate, but also the extent of absorption, especially for drugs that undergo saturable presystemic metabolism. For such drugs, the magnitude of the first-pass effect could vary with the drug release rate from the suppositories.

When studying drug availability after rectal administration, the vascularization of the rectal tissue must be considered. The superior and middle rectal veins drain the rectal blood into the hepatoportal system, whereas the inferior rectal vein conveys the blood directly to the inferior vena cava. In contrast to the oral administration, a fraction of the drug entering the systemic circulation could bypass the liver (Donavan and Flanagan, 1996). Therefore, it is reasonable to assume that the fraction of the drug undergoing hepatic first-pass effect could also depend on the placement and the extent of spreading of the suppository within the rectum.

In a previous work Realdon et al. investigated the effect of different base compositions on the in vitro release of paracetamol from suppositories prepared with a series of Witepsols differing from each other mainly in the monoglyceride content (Realdon et al., 1997a). The tested suppositories were made either with a single or with two different excipients. The forms composed of two excipients were prepared by either mixing the excipients and thus obtaining a homogeneous suppository base, or by superimposing the two excipients to obtain a suppository with two distinct layers. Interesting results were obtained with the layered excipient suppositories, as the drug release from this dosage form seemed to be controllable by varying the ratio of the two excipient layers.

The in vivo investigation described in the present paper was undertaken as a subsequent step to the previous in vitro study (Realdon et al., 1997a). By studying the release properties of suppositories in vitro and evaluating their properties in vivo, the biopharmaceutic and pharmacokinetic properties of the newly designed paracetamol suppositories could be better characterized and understood as a basis for the future application in the patients.

2. Materials and methods

2.1. Chemicals

Paracetamol was purchased from Chimifarm, (Verona, Italy). Witepsol H15 and W35 (Hüls, Werk Witten, Germany) were used as excipients for suppository preparation. Some physico-chemical characteristics of these two excipients are reported in Table 1.

Table 1
Physicochemical characteristics of paracetamol suppository excipients

	Composition	Hydroxyl index	Melting point (°C)	Solidification temperature (°C)
Witepsol H15	low mono-di-glyceride content	15 max.	33.5–35.5	32.5–34.5
Witepsol W35	high mono-di-glyceride content	40–50	33.5–35.5	27.0–32.0

For the determination of unchanged paracetamol in plasma and urine, ethylacetate (BDH, Pool, UK) was used for paracetamol extraction and a phosphate buffered mixture of acetonitrile (BDH) and bidistilled water was used as mobile phase. The internal standard was 2-acetamidophenol (Fluka, Buchs, Switzerland).

For the determination of the amount of metabolized paracetamol in urine, acetonitrile (BDH), bidistilled water and sodium laurilsulphate (Fluka) were used as mobile phase components. 2-aminophenol (Fluka) was employed as internal standard.

Methanol, sodium hydroxide and hydrochloric acid (pro analysis grade), were all supplied by BDH.

2.2. Suppository preparation

Three different paracetamol suppository formulations were studied: 1) formulation 1 was prepared with Witepsol H15 as the excipient, 2) formulation 2 was made up of two different layered excipients, Witepsol H15 and W35 (50:50 v/v), 3) formulation 3 was composed of paracetamol in Witepsol W35 alone. All three formulations weighted 3 g and contained 500 mg of paracetamol. They were prepared according to the method of Realdon et al., 1997a.

2.3. Determination of formulation viscosity during drug release

The viscosity of the suppositories was evaluated during drug release testing at 1-h time intervals, as described in a previous article (Realdon et al., 1997b), using a Rotovisco RV 12 viscosimeter (Haake, Karlsruhe, Germany) with a PG 142 programmer and NV measurement equipment. The rheological determinations were carried out after stopping the drug release testing: the suppositories were first cooled down to 5°C and subsequently incubated at 37°C for 20 h, and then tested for viscosity.

2.4. Determination of paracetamol partition coefficient

50 g of paracetamol saturated phosphate buffer solution (pH = 7.4) at 37 ± 0.2°C were poured into a flask containing 50 g of melted Witepsol at 37 ± 0.2°C. The flask was placed in a water bath at 39 ± 0.2°C and whirled for 6 h. The flask was kept in a vertical position for another hour to let the phases separate. The system was then left to cool down at room temperature and after Witepsol solidification the water phase was withdrawn and filtered. The paracetamol concentration in the filtered solution was determined spectrophotometrically at 242 nm (Shimadzu UV A160) (Ibrahim et al., 1980; Pflegel et al., 1993).

The partition coefficient K was calculated using Eq. (1):

$$K = \frac{c_0}{c_a} \quad (1)$$

where c_0 is the concentration of the paracetamol in the lipophilic phase and c_a is the paracetamol concentration in the aqueous phase.

2.5. In vitro drug release study

Paracetamol release from suppositories was determined by a technique described in a previous paper (Realdon et al., 1997a), using a dialysis tube and detecting the paracetamol released in the diffusion medium by spectrophotometric assay.

Paracetamol diffusion through isolated rat rectum after its release from suppositories was also evaluated, following a method already described in a previous work (Realdon et al., 1997c). Wistar male rats weighting about 250 g were anaesthetized and sacrificed. The sigma-rectal tract of the intestine was isolated, washed with isotonic phosphate buffer (pH = 7.4) and stored in the same isotonic buffer solution until assayed. For the assay the isolated rectum was first tied up at 3 cm from the anal sphincter and a 400 mg microsuppository containing 66 mg of paracetamol was then introduced in the rectal cavity. The isolated organ was then fixed on a proper polymetacrilate support and subsequently placed in a 600 ml beaker containing 500 ml of isotonic phosphate

buffer (pH = 7.4), and kept at a constant temperature of 37°C and stirred at 100 rpm. 1 ml samples of the solution were withdrawn every 15 min and the solution volume was restored adding an equivalent amount of buffer solution at 37°C. The paracetamol concentration in the samples was determined spectrophotometrically at 242.0 nm.

2.6. Study protocol for in vivo evaluation of paracetamol rectal availability

This was a randomized cross-over study. Eight healthy volunteers, five males and three females, aged between 24 and 38 years (mean age 30.7 years) and weighting 52–90 kg (mean weight 70.1 kg) participated in this study. Written informed consent was signed by each subject participating in the study.

The volunteers had normal hepatic and renal function, with the exception of one, who was found to have Gilbert's disease (hyperbilirubinemia) and was included in the study.

All subjects were asked not to take any drugs 1 week before and during the study period, and to fast from 12 h before suppository administration until lunch on the treatment day. They were also not allowed to smoke, nor to take coffee or alcoholic beverages 12 h before and 48 h after the study drug administration. The subjects were all given a standard lunch 3.5 h after the dosing, and were allowed to drink water during the treatment period.

The formulations under study were administered to each volunteer with a 1-week interval between the single treatments.

Blood samples (5 ml) were drawn at 0, 0.3, 0.6, 1, 1.5, 2, 3, 4, 6, 8 and 12 h following suppository administration. Each sample was collected in a heparinized tube, and plasma was immediately separated by centrifugation (at 1500 rpm for 10 min) and subsequently frozen.

Urine samples were collected at 2, 4, 6, 8, 10, 12, 24, 30, 36 and 48 h after dosing, and immediately frozen.

The plasma and urine samples were stored at −20°C until assayed.

2.7. Analytical procedure

2.7.1. Determination of unchanged paracetamol in plasma and urine

Unchanged paracetamol in plasma samples was determined following the method described by Ameer et al. (1981), with some modification. 75 μl of a 10% w/v methanol solution of 2-acetamidophenol (used as internal standard) were pipetted in a glass tube and the solvent was removed under reduced pressure in a rotary evaporator. 0.5–1 ml of plasma or urine were subsequently added to the tubes, followed by the addition of 5 ml of ethylacetate for paracetamol extraction. The mixture was vortexed for 2 min and centrifuged at 3000 rpm for 10 min. The organic phase was transferred to a clean glass tube and the solvent removed under reduced pressure. The residuum was then dissolved in 100 μl of methanol and 20 μl of this solution were injected into a reverse phase HPLC C18 column. A mixture of phosphate buffer (pH = 4) and acetonitrile (65:35 v/v) was employed as a mobile phase.

The same procedure was adopted to determine the amount of unchanged paracetamol excreted in the urine, but in this case the residuum obtained at the end of the procedure was dissolved in 2 ml of methanol.

Validation for the analysis of paracetamol in plasma and urine was carried out. The limit of quantification was approximately 0.1 μg of paracetamol per ml of plasma or urine. The coefficient of variation determined on six identical samples ranging from 0.2 to 8 μg/ml did not exceed 5%. The precision of the assay method was calculated by determining the relative standard deviations of peak height ratios obtained from six replicate assays within a concentration interval of 0.15–10 μg/ml. The relative standard deviation for intraday analysis ranged from 2.6 to 6.3%, and for interday analysis ranged from 3.2 to 7.6%. The absolute recoveries of paracetamol and internal standard in plasma and urine were determined by comparing the slopes of the processed human plasma and human urine standard curves to standard curves prepared in methanol. The recovery of paracetamol and internal standard in plasma was 91 ± 5% and 92 ± 6% respectively, whereas in

urine the recovery was slightly lower (82 ± 4 and $84 \pm 6\%$ respectively).

2.7.2. Determination of the total amount of paracetamol excreted in urine (unchanged and conjugated)

The total amount of paracetamol (unchanged and conjugated) excreted in urine was determined in terms of amount of 4-aminophenol released by hydrolysis with HCl from unchanged paracetamol and its glucuronide, sulphate, cystein and mercapturic acid conjugates.

4-ml sample of urine was mixed with 4 ml of distilled water and an appropriate quantity of 2-aminophenol was added as internal standard. The mixture was then hydrolyzed by adding 16 ml of a 4 M HCl solution and heated under reflux for 90 min. Thereafter, the mixture was neutralized with a 8 M NaOH solution and lyophilized. The lyophilized product was suspended in 50 ml of anhydrous ethanol and stirred for 45 min. After centrifugation the organic phase was withdrawn and the solvent removed in a rotary evaporator. The residuum was dissolved in ethanol and 20 μl of this solution were injected into the column. The mobile phase was prepared with water and acetonitrile (60:40 v/v), and sodium laurilsulphate was added as a counterion (1.6 g/l). Orthophosphoric acid was ultimately added in order to adjust pH to 3.

The absolute recovery of paracetamol was determined by comparing the slope of processed human urine standard curve to standard curve of 4-aminophenol prepared in methanol. Recovery was $82 \pm 13.2\%$. The recovery of internal standard was determined with the same procedure analyzing various concentrations of 2-aminophenol in blank urine. The recovery was $85 \pm 3\%$.

2.8. Data analysis and determination of pharmacokinetic parameters

Plasma concentration–time data for paracetamol were analyzed using non-compartmental and compartmental pharmacokinetic methods.

Paracetamol concentration–time data were plotted on a semilogarithmic scale and the terminal log–linear phase was identified by inspection. The elimination rate constant (β) was determined as the slope of the linear regression for the terminal log–linear portion of the concentration–time curve. A terminal half-life value was calculated as 0.693 divided by β. Maximum plasma concentration (C_{max}) and the corresponding sampling time (t_{max}) for paracetamol were recorded as observed. Area under the plasma concentration versus time curve ($AUC_{(0\rightarrow T)}$) was calculated by the trapezoidal method and extrapolated to infinite time as:

$$AUC_{(0\rightarrow\infty)} = AUC_{(0\rightarrow T)} + \frac{C_T}{\beta} \quad (2)$$

where C_T is the paracetamol concentration at the last sampling time.

Based on these $AUC_{(0\rightarrow\infty)}$ values, a model independent estimation of the systemic plasma clearance (CL_p) was performed using Eq. (3).

$$\frac{CL_p}{F} = \frac{D}{AUC_{(0\rightarrow\infty)}} \quad (3)$$

where D is the administered dose of paracetamol and F is systemic availability representing the net fraction of the dose reaching systemic blood or plasma circulation following possible losses from incomplete release from the dosage form, destruction in the gastrointestinal tract, and first-pass metabolism.

Non-compartmental pharmacokinetic calculations were performed using MS EXCEL software.

The paracetamol pharmacokinetics after rectal administration was also evaluated using a model-dependent approach. Two-compartment models are commonly used to describe paracetamol pharmacokinetics (Albert et al., 1974; Liedtke et al., 1979; Forrest et al., 1982; Eandi et al., 1984; Pedraz et al., 1988; Studenberg and Brouwer, 1993), but one-compartment approaches can be also found in literature concerning paracetamol pharmacokinetic studies (Degen and Maier-Lenz, 1984; Anderson and Holford, 1997). As explained later in the paper, in this study plasma data were fitted both with one- and two-compartment models using ADAPT software (D'Argenio and Schumitzky, 1997). This program gave an estimation of the absorption and elimination rate constants (k_{01} and k_{10} respectively) and volume of the cen-

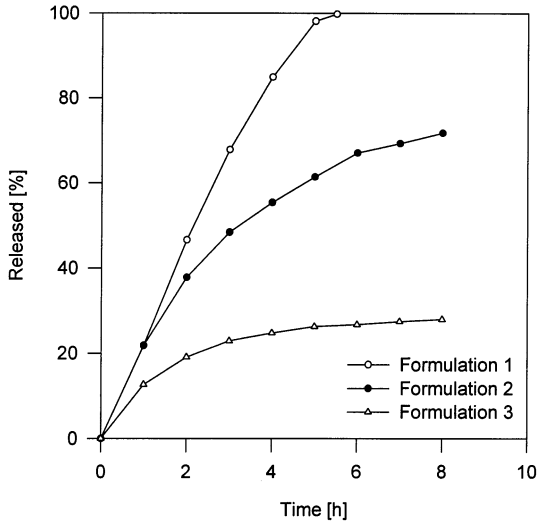

Fig. 1. In vitro release profiles of paracetamol from various formulations.

tral compartment (in terms of V_d/F, where V_d is the distribution volume). These parameters were then used for further evaluation of paracetamol pharmacokinetics after rectal administration. A model-dependent estimation of the clearance (Eq. (4)) was performed in order to compare this parameter with the one obtained by the model-independent approach (Eq. (3)) and thus to have a further confirmation of the adequacy of the chosen model.

$$\frac{CL_p}{F} = \frac{V_d k_{10}}{F} \tag{4}$$

The paracetamol absorption kinetics was investigated on the basis of absorption versus time profiles, which were obtained according to the Wagner–Nelson method for a one-compartment model (Wagner, 1993) and using the following equation:

$$\frac{A_t}{A_{total}} = \frac{C_t + k_{10}\int_0^t C_t dt}{k_{10}\int_0^\infty C_t dt} \tag{5}$$

where A_t is the amount of drug absorbed up to time t, A_{total} is the total amount of drug absorbed, and C_t is the paracetamol plasma concentration at time t. A_t/A_{total} can be viewed as the fraction of the drug absorbed up to time t (FA) and it was used to estimate the absorption rate constant k_a, by fitting the following expression (Wagner, 1993):

$$FA = 1 - e^{-k_a t} \tag{6}$$

FA was utilized for testing a level A in vitro–in vivo correlation, by plotting the FA estimates and in vitro drug release at common time points and subsequently processing the plotted data by linear regression (Young et al., 1997).

For further evaluation of in vivo data, the $AUC_{(0\rightarrow\infty)}$ values obtained by testing different formulations, were compared in relation to the total amount of drug that entered the body after its release from the suppository. The ratio between the $AUC_{(0\rightarrow\infty)}$ and the total amount of paracetamol excreted in the urine up to 48 h after dosing ($U_{total(0\rightarrow\infty)}$) and expressed as the parent compound and metabolites, was calculated according to the following equation:

$$\frac{AUC_{(0\rightarrow\infty)}}{U_{total(0\rightarrow\infty)}} = \frac{F_h}{CL_p} \tag{7}$$

where F_h is the contribution to the F term related to first pass hepatic metabolism.

Statistical evaluations of the differences in pharmacokinetic parameters obtained with the tested formulations were carried out by Student's t-test ($\alpha = 0.05$) for paired data, using MS EXCEL software.

3. Results

3.1. In vitro study

The in vitro profiles of paracetamol release from the three suppository bases, previously reported by Realdon et al. (1997a), are shown in Fig. 1. The release profile obtained with the formulation 2 (two layered excipients) represents the sum of two independent release processes originating from the two separate excipient layers.

The results indicate that the highest paracetamol release rate is obtained with excipient H15, whereas excipient W35 produces a slower release of the drug, which can be linked to the different

viscosity behaviour of the two excipients during drug release (Fig. 2).

The paracetamol diffusion through the isolated rat rectum, following the release from two different excipients, is depicted in Fig. 3. In contrast to the in vitro release results, the two profiles are essentially superimposed and suggest that in addition to the physico-chemical properties governing the in vitro release of the drug, other factors may play a role in diffusion processes through a biologic membrane.

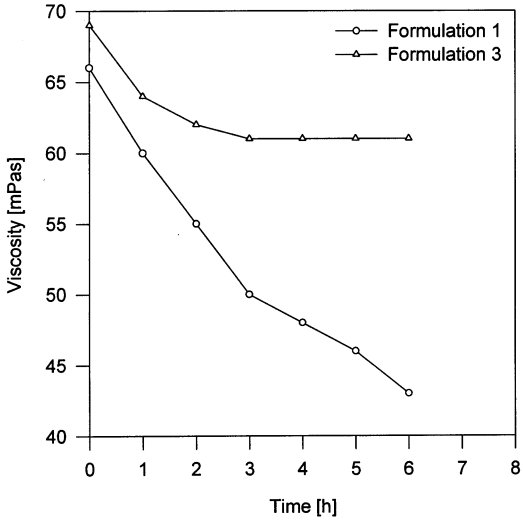

Fig. 2. Viscosity of the formulations during drug release testing.

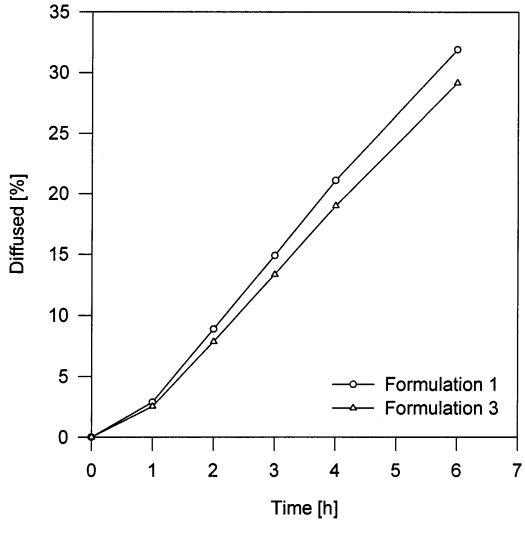

Fig. 3. Paracetamol diffusion through isolated rat rectum.

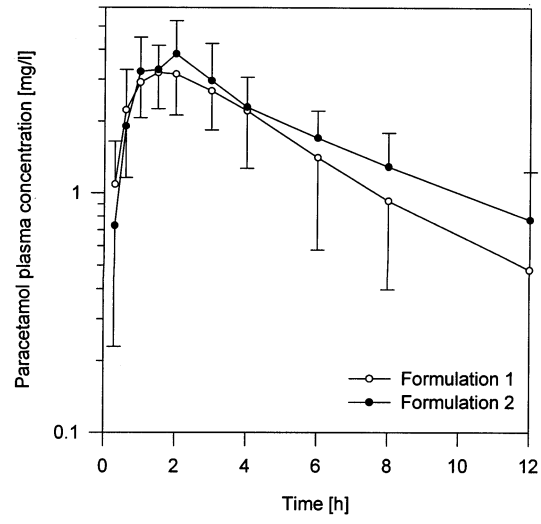

Fig. 4. Paracetamol plasma concentration profiles following rectal administration (mean ± S.D.).

3.2. In vivo study

Based on the in vitro results, two formulations were selected for further in vivo evaluation: formulation 1 (H15) as the fast-releasing suppository, which was thought to give the highest plasma levels, and formulation 2 (H15/W35 = 50/50), which was expected to lead to a prolonged paracetamol release.

The mean paracetamol plasma concentration time profiles obtained after administration of formulations 1 and 2 are shown in Fig. 4. From four of the eight volunteers urine excretion data were also available and the cumulative amounts of unchanged and total paracetamol excreted within 48 h from dosing are represented in Fig. 5. The paracetamol urine recovery, i.e. the percentage of the dose that was found in the urine (both as unchanged and metabolized paracetamol) was 70.5 ± 4.7 for formulation 1 and 75.2 ± 14.4 for formulation 2.

The non-compartmental pharmacokinetic parameters (C_{max}, t_{max} and $AUC_{(0\rightarrow\infty)}$) are summarized in Table 2. The absorption kinetics of

Table 2
Paracetamol pharmacokinetic parameters based on plasma concentration data following rectal administration of the formulations under the study

Subject/Formula	C_{max} (mg/l)		t_{max} (h)		β (h^{-1})		$AUC_{(0\rightarrow\infty)}$ (mgh/l)		k_{10} (h^{-1})		k_{01} (h^{-1})		k_a (h^{-1})		CL_p/F (l/h)			
															$D/AUC_{(0\rightarrow\infty)}$		$(V_c k_{10})/F$	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
A	2.78	3.59	1.5	1	0.15	0.09	21.3	35.0	0.91	1.82	0.25	0.13	0.16	0.09	23.5	14.3	29.3	16.9
B	3.17	3.32	1.5	1.5	0.24	0.09	17.8	40.5	1.45	1.44	0.26	0.10	0.26	0.10	28.1	12.3	27.9	12.3
C	4.04	2.83	1	1.5	0.35	0.15	15.9	22.1	0.66	1.26	0.66	0.18	0.49	0.16	31.4	22.6	32.4	23.7
D	2.85	5.13	1	1	0.28	0.21	12.8	20.8	1.62	1.51	0.28	0.40	0.31	0.21	39.2	24.1	37.4	28.2
E	5.44	6.75	2	2	0.26	0.25	30.9	33.4	0.95	0.45	0.28	0.45	0.27	0.28	16.2	15.0	16.5	15.5
F	3.4	2.92	1	1	0.16	0.13	14.7	17.2	1.49	1.20	0.31	0.32	0.18	0.14	34.1	29.0	41.6	40.3
G	3.53	3.77	1.5	2	0.15	0.04	20.9	61.5	0.96	0.30	0.30	0.30	0.17	0.05	23.9	8.1	29.5	22.3
H	3.47	5.13	3	2	0.09	0.27	38.9	25.2	0.29	0.47	0.30	0.47	0.16	0.34	12.8	19.9	16.2	20.4
Mean	3.59	4.18	1.6	1.5	0.21	0.15	21.7	32.0	1.04	1.06	0.33	0.29	0.25	0.17	26.2	18.2	28.9	22.5
S.D.	0.85	1.37	0.7	0.5	0.09	0.08	8.9	14.4	0.45	0.57	0.13	0.14	0.11	0.10	8.9	6.9	9.0	8.8

paracetamol was evaluated by fitting a pharmacokinetic model to the plasma concentration data. The graphical and statistical analysis of the results, along with the Aikake and Schwartz criteria (D'Argenio and Schumitzky, 1997) confirmed that the one-compartment model best described the data. The choice of a one-compartmental model was also corroborated by the relatively good linear fitting of the data in the $\ln(1-FA)$ versus time plot to determine k_a (Eq. (6)) and the R^2 of all the trendlines were above 0.96. The pharmacokinetic rate constants k_{01} and k_{10} and the parameters related to the distribution volume and to the clearance are listed in Table 2. From the table it can be seen that there is a quite good agreement between the pharmacokinetic parameters determined with the non-compartmental method and those obtained by the modeling, this being an evidence of the adequacy of the one compartment model for the description of the paracetamol pharmacokinetics after administration of the studied formulations.

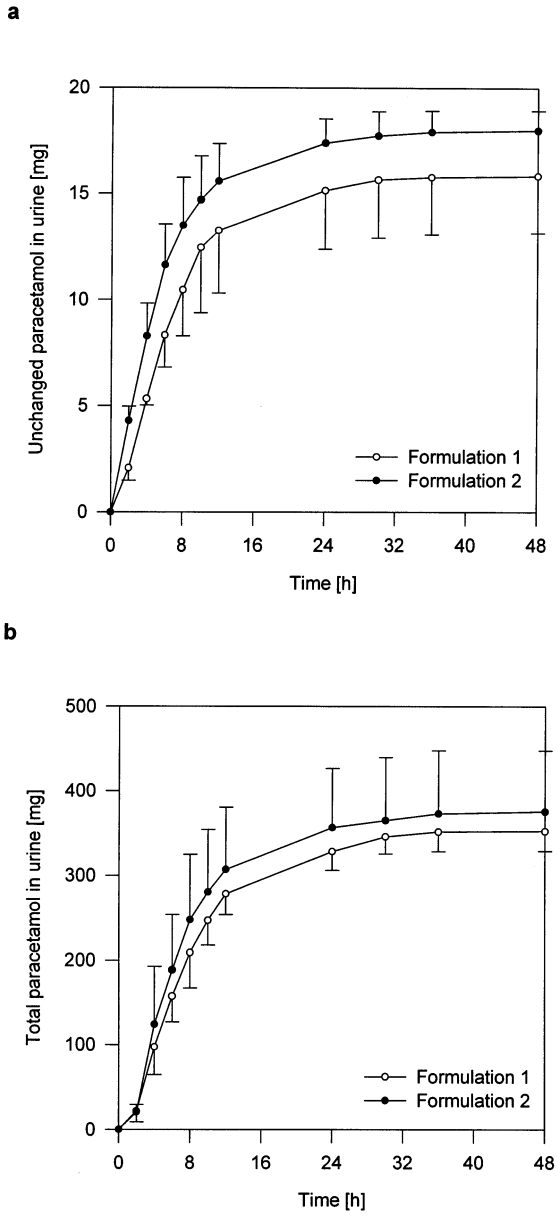

Fig. 5. Paracetamol urinary excretion profiles (mean ± S.D.) (a) unchanged paracetamol excreted in urine (b) total paracetamol (unchanged and metabolites) recovered in urine.

C_{max} and t_{max} obtained with the suppository 1 were not significantly different from those obtained with suppository 2 ($P = 0.19$ and $P = 0.73$ respectively). The $AUC_{(0\rightarrow\infty)}$ values show that there was no statistically significant difference in the extent of paracetamol absorption from the two formulations ($P = 0.11$). This observation is supported by the urinary data, indicating that in the majority of subjects there is little difference between the profiles of cumulative urinary excretion of paracetamol following the administration of two distinct dosage forms (Fig. 5). However, both plasma and urine data show a certain trend apparently related to the presence of W35 in the formulation: $AUC_{(0\rightarrow\infty)}$ values obtained after administration of suppository 2 are all higher than those obtained with suppository 1 (except $AUC_{(0\rightarrow\infty)}$ for subject H). Correspondingly, the amounts of both unchanged and conjugated paracetamol excreted in urine were higher for formulation 2 compared to those obtained with formulation 1. Therefore, formulation 3 (paracetamol in W35 alone) was administered to one of the eight volunteers (subject F) as a 'proof of concept'. With this formulation a further increase in $AUC_{(0\rightarrow\infty)}$ was achieved ($AUC_{(0\rightarrow\infty)} = 23.2$ mgh/l) and a slightly higher amount of total paracetamol was found in urine (Table 3).

As to β and k_a values (Table 2), no significant difference was found between the two formulations ($P = 0.21$ and $P = 0.17$ respectively), but

Table 3
Paracetamol pharmacokinetic parameters based on urine excretion data following rectal administration of the formulations under the study

Subject/Formula	$AUC_{(0\rightarrow\infty)}/U_{total(0\rightarrow\infty)}$ (*h*/l)			$U_{unchanged(0\rightarrow\infty)}/D$ (%)			$U_{total(0\rightarrow\infty)}/D$ (%)		
	1	2	3	1	2	3	1	2	3
E	0.082	0.074		3.17	3.64		75.04	91.40	
F	0.036	0.054	0.068	3.31	3.42	2.42	64.90	65.69	68.10
G	0.067	0.33		2.45	3.49		68.50	60.69	
H	0.080	0.063		3.74	3.84		73.76	82.88	
Mean	0.066	0.130		3.17	3.60		70.55	75.17	
S.D.	0.021	0.133		0.54	0.19		4.71	14.40	

again, like for the parameters described above, the same trend can be observed, that is, k_a is lower for retard formulation. Moreover, the profiles in Fig. 6 representing the time-course fraction of drug absorbed estimated for each subject by the Wagner–Nelson method (Eq. (5)) are another evidence of a possible retardation in the paracetamol absorption from formulation 2 (except for subjects E and H).

Considering that for each subject the absorption rate constant is substantially smaller than the elimination rate constant, a flip–flop model can be hypothesized, and therefore the terminal phase slope β should actually represent the absorption constant. In fact, a fairly good agreement can be found between the β values, k_{01} and the k_a obtained by Eq. (6).

3.3. In vitro–in vivo correlation

For a quantitative evaluation of in vitro–in vivo correlation, a level A correlation was performed by graphical representation of the in vitro release data and absorption estimates at common time points. As depicted in Fig. 7, a good correlation was achieved between the in vitro release and in vivo absorption kinetics for the two-paracetamol suppositories.

4. Discussion

Drug release rate from a suppository is known to be dependent upon several excipient properties (Bornschein et al., 1976; Realdon et al., 1997a). The excipients used in this study differ from each other mainly in their mono- and diglycerides content and hence in their hydrophylicity, as well as in the viscosity decrease during drug release. Considering the drug release mechanism from suppositories, a correlation can be established between the differences in the physico-chemical parameters of the two excipients and their in vitro release profiles. Indeed, before dissolving in the aqueous rectal secretion and thus becoming available for absorption, the drug particles, which are suspended in the excipient, must first migrate through the molten base mass in order to reach the suppository surface (Realdon et al., 1997a).

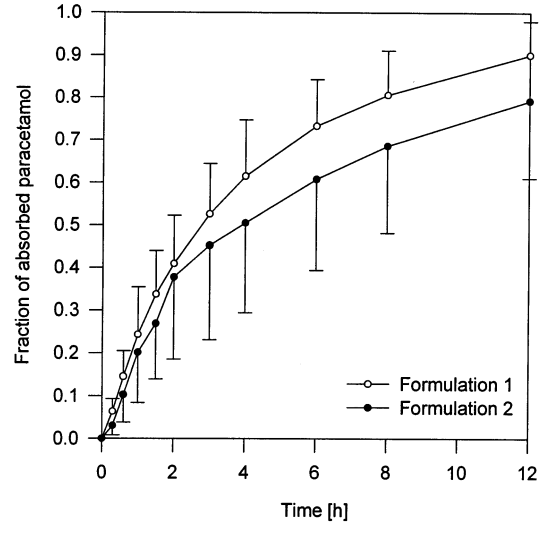

Fig. 6. Paracetamol absorption profiles (mean ± S.D.).

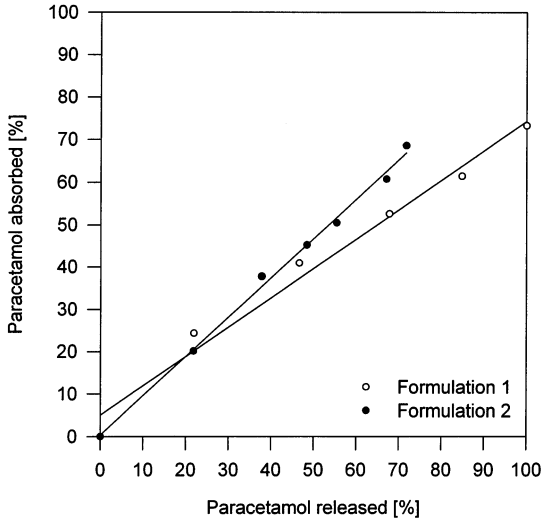

Fig. 7. In vitro in vivo correlations.

Thus, the migration of a drug particle having certain dimensions will be facilitated as the viscosity of the medium decreases. Since the viscosity of excipient H15 decreases more rapidly than that of excipient W35, the drug particle migration through the suppository mass is facilitated, leading to a faster drug release.

An additional factor that could also contribute to the difference in the release rate from the two excipients is the partition coefficient (K). The K values of Witepsol H15/Phosphate buffer and Witepsol W35/Phosphate buffer, 0.23 and 0.30 respectively, reveal a slightly higher affinity of paracetamol for W35, as can be also inferred from the higher hydroxyl index of this excipient. Witepsol W35 is therefore supposed to retain the paracetamol more than the H15 base, producing a slower drug release.

The results of the diffusion test through the isolated rat rectum, as well as those obtained from the in vivo study, were not exactly what it was expected from the in vitro outcome. Obviously, factors other than physico-chemical properties of the excipients are involved when the formulation enters a biological environment, the first evidence being the results of the paracetamol diffusion testing through isolated rat rectum after its release from H15 and W35 suppository base. In fact, the difference between the profiles obtained with the two distinct excipients is much lower than what would be expected on the basis of in vitro release profiles (Figs. 1 and 3). The reason for this relatively high diffusion of paracetamol from W35 excipient could lie in the different monoglyceride content of the two excipients. Monoglycerides are known to act as absorption enhancers by disordering the hydrophobic region of the membrane interior and interacting with the polar groups of phospholipids (De Muynck et al., 1994; de Boer and Breimer, 1997). As the monoglyceride content of W35 is substantially higher compared to H15, their effect on the rectal membrane could compensate for the slower release rate of the paracetamol from W35 suppository, leading to a diffusion profile that is comparable to the one obtained with H15 suppository.

The in vivo investigation revealed a remarkable inter-individual variability in plasma profiles and $AUC_{(0\rightarrow\infty)}$ values. Especially data collected from subject G may appear anomalous. Such peculiar behaviour was probably due to the fact that this individual suffers from hyperbilirubinemia, a condition known as Gilbert's syndrome and caused by a deficiency in bilirubin glucuronidation. It has been observed that paracetamol glucuronidation is as well impaired in such subjects (Patel et al., 1992), with the consequence of a lower plasma paracetamol clearance (Forrest et al., 1982). Since only plasma concentration data obtained after application of formulation 2 to this subject differed substantially he was nevertheless included in the statistical analysis.

Although the results of in vivo study did not show significant differences between the two tested formulations, a trend can be observed in the values of some pharmacokinetic parameters, as previously mentioned. In particular, there are two distinct aspects concerning the possible difference between the two formulations, namely the extent of absorption, expressed both by $AUC_{(0\rightarrow\infty)}$ and the fraction of the dose excreted in urine, as well as the rate of absorption, represented by k_a and, since this is a flip–flop case, also by β.

Even if the presence of a flip–flop phenomenon can lead to a loss of reliability of $AUC_{(0\rightarrow\infty)}$ estimation performed by Eq. (2), the trend

showed by this parameter is supported by urine excretion data.

As to the extent of absorption, the monoglyceride effect could not be the only reason for the $AUC_{(0\rightarrow\infty)}$ augmentation observed after administration of formulation 2 and 3, and therefore further considerations are needed.

In general, when dealing with different formulations of a given drug, $AUC_{(0\rightarrow\infty)}$ variations may be a consequence of a modification in availability or a change in the clearance. Assuming that the formulation excipients do not affect the clearance, this parameter is supposed to be the same in a single subject when the same dose of a drug is administered, regardless of the formulation. Therefore, in this study, the differences in $AUC_{(0\rightarrow\infty)}$ observed with the tested formulations were attributed to a modification in paracetamol availability (F). This is confirmed by the CL_p/F values obtained both with a model-independent and model-dependent approach (Table 2). In contrast to many other pharmacokinetic parameters considered above, these values are found statistically different for the two suppositories ($P = 0.027$ and $P = 0.028$) for non-compartmental and compartmental approach respectively. It can be concluded that the paracetamol availability from suppository 2 is probably higher than the one from the formulation 1.

The overall availability of the drug (F) can be affected by the processes such as incomplete release from the formulation (F_r), reduced absorption due to the drug loss in the gut (F_a), and first pass hepatic metabolism (F_h), as shown in Eq. (8).

$$F = F_r F_a F_h \tag{8}$$

Furthermore, assuming that only hepatic presystemic and systemic metabolites are excreted in urine, one can conclude that fraction of the dose recovered in urine is described by Eq. (9):

$$U_{total} = F_r F_a D \tag{9}$$

Substituting F in Eq. (3) by Eq. (8) and D by Eq. (9) yields an equation (Eq. (7)) which permits the estimation of F_h considering that systemic clearance remains constant.

In the present investigation the formulation that gave the highest $AUC_{(0\rightarrow\infty)}$ is the one that has the highest affinity for the drug, so the contribution of the F_r term to the differences in F is not pronounced, as for this formulation F_r is expected to be lower and the $AUC_{(0\rightarrow\infty)}$ consequently smaller.

On the contrary, F_a is likely to play a more important role in paracetamol availability from the different suppositories under study, since it can be related to the monoglyceride content of the formulation. According to the results, F_a is greater when a higher percentage of monoglycerides is present in the formulation, and thus a better paracetamol availability is achieved. For a further evaluation of the effect of these absorption enhancers and in order to understand if this is really the main reason for the differences in AUC, Eq. (7) was introduced and the values of the ratio between $AUC_{(0\rightarrow\infty)}$ and the total amount of drug excreted in urine were calculated (Table 3). For subjects E and H it can be concluded that there is no substantial difference between the two formulations in terms of $AUC_{(0\rightarrow\infty)}$ versus amount excreted, leading to the conclusion that the improved availability is essentially due to an enhanced absorption (F_a). This is also supported by the percentage of unchanged paracetamol found in urine (Table 3). According to these data, the absorption from formulation 2 seems to be augmented by 15% in subject E and 3% in subject H.

Subject F, who was treated with all three formulations, shows a certain trend in the values of the ratio between $AUC_{(0\rightarrow\infty)}$ and the total amount of drug excreted in urine. This finding reveals the possibility that the paracetamol released from different formulations underwent hepatic metabolism to a different extent, i.e. the ratio between pre-systemic and systemic metabolism changed according to the formulation administered. In particular, the increase in $AUC_{(0\rightarrow\infty)}$ with respect to the increase in the total amount of paracetamol excreted in urine observed with formulation 2 and 3 could be partly due to a smaller first pass effect. This could be related to the formulation viscosity as follows. Since the blood supply from the inferior hemorroidal vein drains directly into the inferior vena cava thus bypassing the hepatoportal system, the lower the

position of the suppository in the rectum the higher the fraction of drug that will enter the systemic circulation avoiding the first pass hepatic metabolism. So, a higher viscosity should prevent, to a certain extent, the formulation 2 from spreading towards the upper part of the rectum, though on the other side it retards the drug diffusion within the formulation. It seems reasonable to hypothesize that the retardation of the drug release from suppository due to the augmented viscosity could be compensated both by the absorption-enhancing effect of the monoglycerides and the liver bypass mechanism.

This last hypothesis is not supported by urine data. In fact, if the ratio between pre-systemic and systemic metabolism changed using a different formulation, there should be a corresponding variation of the ratio between metabolized and unchanged paracetamol excreted in urine. This variation was indeed very small in each tested subject, but since the urine data were available only from four of the eight volunteers, no reliable conclusion can be drawn on the basis of urine data regarding the possibility of an augmented liver bypass using formulation 2.

As already mentioned, the absorption rate is another crucial factor which contributes to the differences between the studied formulations. Although not statistically significant, the absorption rate constants (k_a) based on the plots representing the fraction of the drug absorbed versus time showed a retardation of paracetamol absorption when using suppository 2. At first sight, the drug absorption retardation obtained with this formulation is somehow in contradiction with the observations about C_{max} and t_{max}, which would be expected to be different for the two formulations. A possible explanation for this result is that the higher F of formulation 2 compensates for the slower drug release, making the C_{max} and t_{max} very similar for the two forms, but producing at the same time a larger $AUC_{(0\rightarrow\infty)}$ because of a minor terminal phase slope, as dictated by the smaller β (k_a).

The trend in the absorption rate constant k_a corresponds to in vitro findings, which did reveal a possible retardation in paracetamol absorption as a consequence of its slowed release from the suppository. In fact, a correlation was actually found between in vitro release rate and in vivo absorption rate (Fig. 7).

5. Conclusion

The in vitro studies carried out on layered excipient suppositories prepared with Witepsol H15 and W35 showed that a proper combination of fast paracetamol releasing excipient H15 and slow releasing excipient W35 could give an optimized plasma profile. Based on these in vitro results, two formulations were selected for the in vivo study described in this paper, namely the suppository made of Witepsol H15 and the layered excipient suppository, composed of two superimposed layers of Witepsol H15 and W35. Since the in vivo outcome was not really as it could be expected from the in vitro study, the formulation made of W35 alone was tested on one subject, and the paracetamol absorption turned out to be even higher when compared with the other two forms. Different release and absorption mechanisms were postulated in order to give a possible explanation for the observations and results obtained during the study. The effect of monoglycerides along with the possible decrease of first pass metabolism are the two main factors that could account for the tendency towards a superior paracetamol availability observed when using suppositories containing Witepsol W35. Further studies are needed for a better understanding of paracetamol kinetics after rectal administration. In order to explore in more details the possible mechanisms underlying the absorption and disposition of paracetamol after rectal administration, a physiological model to describe the pharmacokinetic processes is currently being developed. The ultimate goal is to improve and further refine the in vitro release methodology, which would lead to better predictions of in vivo events.

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