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Correlation of in vitro and in vivo acetaminophen availability from albumin microaggregates oral modified release formulations

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Abstract

The aim of this study was to develop albumin microaggregated oral formulations for controlled drug release, and to reveal the possible influence of the release site on drug absorption. Acetaminophen was chosen as the model drug, which is included in the Class 1 group of the Biopharmaceutics Classification System (BCS). Albumin micro aggregates were formulated into tablets to obtain different drug release rates: Immediate Release (IR) tablets, multiparticulate systems with an intermediate release rate, and matrix systems showing slow release rate. The properties of the products were initially tested via dissolution studies, and then via bioavailability studies in healthy volunteers. Controlled release albumin microaggregated acetaminophen formulations for oral administration were obtained. The extent of drug absorption was comparable for all formulations, suggesting that the differences found in saliva concentration and urine cumulative profiles could be attributed merely to differences in drug release kinetics, as confirmed by the in vitro–in vivo correlation study. Therefore, it can be concluded that extended release of acetaminophen does not influence its absorption via intestinal heterogeneity.

Keywords: Acetaminophen; Albumin microaggregates; Site-specific drug delivery

1. Introduction

Microencapsulation using albumin, is a technological strategy used to accomplish modified/controlled release of drugs (Torrado-Durán et al., 1991). Microaggregated albumin particles (MA) formulated using the microencapsulation method previously reported by Torrado-Durán et al., (1995) showed a relative good bioavailability (Torrado-Durán et al., 1991). The use of organic solvents or oils was not necessary to prepare the albumin microaggregated particles (Torrado-Durán et al., 1995).

It is known that, in the vast majority of instances, drug delivery and absorption for Immedi-

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ate Release (IR) products takes place in the upper small bowel, i.e. duodenum and jejunum. However, a Modified Release (MR) product, following faster administration can be expected to reach the colon in a few hours. Thus, in this case it is important to realise that permeability could vary significantly through the gastrointestinal tract (Davis et al., 1986; Gardner et al., 1997; Lennernäs, 1998).

Over the last few years, incorporating the BCS approach, a regulatory guidance for IR products has appeared, having a great importance in bioequivalence studies, with special interest for BCS Class 1 drugs. It will be not long before its extrapolation for the approval of MR forms will be discussed (Wilding, 1999).

In this study, acetaminophen was selected as model drug due to its high solubility and high permeability (Class 1 group) according to the Biopharmaceutics Classification System (BCS) (Amidon et al., 1995) and due to the experience of our investigation group in the process of microencapsulation of the mentioned drug by using albumin. Those facts made acetaminophen suitable to investigate the influence of the drug release site — which is dependent upon formulation — on drug absorption.

Numerous sustained and controlled release formulations of acetaminophen have been reported (Torrado-Durán et al., 1995; Chicco et al., 1999; Gren and Nystrom, 1999).

The aims of this study were first to achieve acetaminophen formulations with different release rates by using MA particles containing acetaminophen and second, to reveal the possible influence of heterogeneity in permeability, lumenal pH, lumenal contents and wall metabolism through the gastrointestinal tract on the absorption of Class 1 drugs.

2. Materials and methods

2.1. Materials

Acetaminophen (Krisch Pharma, Spain) and lyophilised egg albumin (Ovosec, Spain) were used for microaggregates formation. Excipients included in tablet formulation, microcrystalline cellulose (Avicel PH 102, FMC, USA), and sodium starch glycolate (Explotab, USA) were obtained from the indicated sources. Acetaminophen (Sigma, USA) was used for the validation of the HPLC method. Tylenol (Abello Farmacia, Spain) was chosen as the reference for acetaminophen formulation in these studies. All other chemicals used were of analytical grade.

2.2. Preparation of microaggregated egg albumin particles containing acetaminophen

These microaggregated particles were prepared according to the method previously reported by Torrado-Durán et al. (1995). Acetaminophen was added to an aqueous egg albumin solution and energetically stirred to produce a suspension. The system was then heated at 60°C with continuous stirring for 30 min to coagulate the albumin, promoting the formation of microaggregated egg albumin particles. The resulting particles were isolated by decantation and dried for 10–30 mm at 60°C, followed by a first sieving through a (no. 18) sieve (Cisa, Spain). These particles were then dried at 60°C for 24 h and sieved to obtain a particle size < 1 mm. The final acetaminophen content in the microaggregated particles was 70% (w/w), (spectrophotometric measurement at 234 nm).

2.3. Tableting

Microaggregated egg albumin particles containing acetaminophen (1:3 w/w) alone or in combination with different excipients (Avicel PH 102, Sodium starch glycolate) were formulated into tablets at different pressures, using an eccentric tablet press (Bonals, model B-40, Spain). Flat-faced tablets, with a diameter of 11 mm were obtained. A mechanical strength tester (Pharma Test, model PTB 311, Spain) was used to determine tablet hardness. A dose of 250 mg of acetaminophen was used for tablet formulation in order to avoid high sized tablets when adding different excipients. The different formulations obtained are listed in Table 1.

2.4. In vitro drug release

In vitro study of drug release from microaggregated tablet formulations and reference formulation was performed according to the US Pharmacopeia 24 (2000), using a Hanson Research SR8, Venkel VK 700 apparatus (USA). As dissolution medium, phosphate buffer (pH 5.8) in a volume of 900 ml at 37°C was used. The speed of rotation was 50 rpm. Samples of 5 ml were collected at the established sampling points for each study. UV spectrophotometry was the analytical method used for acetaminophen determination. Absorbances of the dissolution medium at 234 nm were recorded in a Beckman DU 6 spectrophotometer (Beckman, USA). The studies were repeated three times to obtain a mean value.

2.5. Bioavailability studies

Five healthy volunteers of both sexes aged 22–35 and weighting 50–80 kg participated in this randomised cross-over, and single dose bioavailability study. The volunteers were informed about the possible risks and adverse effects relating to the drug, and written consent was obtained. The experiments were performed at the Department of Pharmaceutical Technology, Faculty of Pharmacy, Alcala de Henares University (Madrid, Spain).

Each subject was given a single oral dose (equivalent to 500 mg of acetaminophen) of one formulation each time after an overnight fast. A commercial oral acetaminophen (500 mg) formulation (Tylenol, Abello Farmacia, Spain) was used as reference. In all the experiments, a standard lunch was provided 2 h after drug administration. A washout period of one week was allowed between two administrations. Saliva and urine samples were collected at predetermined intervals and stored frozen at -20°C until analysis.

2.6. Preparation of test samples for the saliva assay

To 400 μl of centrifuged (4000 rpm, 20 min) saliva, 1 ml of a 3 M solution of barium hydroxide was added. After vortexing for 5 min at 2000 rpm, 1 ml of 1% (w/v) zinc sulphate was added and vortexed again at 4000 rpm for 20 min. The supernatant was collected and filtered through a 0.45 μm HPLV nylon filter (Millipore, USA).

2.7. Preparation of test samples for the urine assay

To a 0.5 ml urine sample, 1.5 ml of methanol was added, followed by vortexing for 5 min at 2000 rpm. Afterwards, centrifugation was carried out at 4000 rpm (Hucoa-Erlös-Spain). The supernatant was filtered through a 0.45 μm pore size nylon HPLV filter, and collected for HPLC analysis.

Table 1
Characteristics of acetaminophen formulations

Formulation	Composition (%)		Dosage form	Hardness (Kp)
	MA	Avicel		
F-1 [a]	80.35	16.39	IR	6.11
F-2	90.00	10.00	MR	11.04
F-3	81.60	18.40	MR	9.50
F-4	70.00	30.00	MR	9.10
F-5	100.00	–	Matrix	6.10
F-6	100.00	–	Matrix	9.30
F-7	100.00	–	Matrix	>15

[a] F-1 contains 3.25% of sodium starch glycolate.

2.8. Acetaminophen determination

Acetaminophen concentration in saliva and urine samples was determined by means of HPLC, using the method previously described by Miners et al. (1984) with slight modifications. The HPLC system was equipped with two Gilson 305 and 306 pumps, a Gilson 231 XL automatic sampler, attached to an injection valve (20 μl sample loop or 100 μl sample loop for urine and saliva samples, respectively). A 20×0.46 cm Kromasil C_{18} column, with a particle size of 5 μm, maintained at room temperature was used. The mobile phase consisted of ortophosphoric acid 0.137% (v/v), pH 4.7 and potassium chloride (1 M)–acetonitrile (97.5:2.5). The mobile phase was filtered through a 0.45 μm pore size Millipore HVLP filter, prior to use. The flow was maintained at 1 ml/min. A Gilson 116 variable wavelength UV detector at 244 nm was used for detection. Data were recorded on a Spectra-Physics SP integrator. The standard curve was found to be linear ($r^2 = 0.9996$) over the concentration range of 0–100 μg/ml. The analytical method was validated in relation to accuracy, precision, limit of quantification, specificity and reproducibility.

2.9. Pharmacokinetic parameters

The pharmacokinetic parameters assessed were the following: maximum plasma concentration (C_{max}), time to peak concentration (t_{max}), area under the time–concentration curve, extrapolated to infinity ($AUC_{0\tau\infty}$), area under the "first moment time–concentration curve", extrapolated to infinity ($AUMC_{0\tau\infty}$), cumulative amount excreted at 24 h (X_{cu}^{24}), and mean residence time (MRT).

Acetaminophen concentration data obtained from saliva samples can be considered equivalent to those in plasma, since the concentrations of acetaminophen in saliva and plasma were confirmed to be linearly proportional (Shim et al., 1990).

As reported by Goicoechea and Vila-Jato (1998), there is no saturation of presystemic biotransformation at the acetaminophen doses administered in this bioavailability study. Therefore, urine data can be employed to confirm saliva parameters. C_{max} and t_{max} were determined from saliva data. $AUC_{0\tau\infty}$, as well as $AUMC_{0\tau\infty}$ values were calculated according to the trapezoidal method, and allowed further determination of MRT. Data obtained from urine assays allowed the obtainment of cumulative curves and the determination of X_{cu}^{24}. Statistical analyses were carried out using the STATGRAPHICS 4.0 program, by the one-way analysis of variance (ANOVA).

Finally, an in vitro dissolution–in vivo bioavailability correlation study was undertaken. Mean dissolution time (MDT) and MRT were the chosen parameters to perform the in vitro-in vivo correlation of acetaminophen formulations, following USP 24 criteria.

3. Results and discussion

3.1. Dissolution tests

Drug release profiles of test and reference tablet formulations are shown in Fig. la–c.

As can be seen in Fig. 1a, following the USP 24 requirements for conventional acetaminophen tablets, reference and F-1 formulations showed percentages dissolved higher than 95% at 30 min. The F-1 drug release profile is similar to that of reference formulation. However, a short lag time of drug release of F-1 formulation compared to reference formulation must be remarked (F-1 showed a drug release of 70.2% at 5 min versus the 87.8% showed by the commercial reference formulation). The short delay in drug release from F-1 formulation as compared to reference formulation results in partially masking the bitter taste of acetaminophen.

For formulations referred to as MR formulations (see Fig. lb), disintegrant quantity included in the formulation had a marked effect on release rate. Dissolution rate decrease as the quantity of disintegrant was reduced. Among these MR formulations, significant differences ($p < 0.001$) were found in their dissolution percentages at 30 min, as well as in their $t_{50\%}$ and $t_{80\%}$

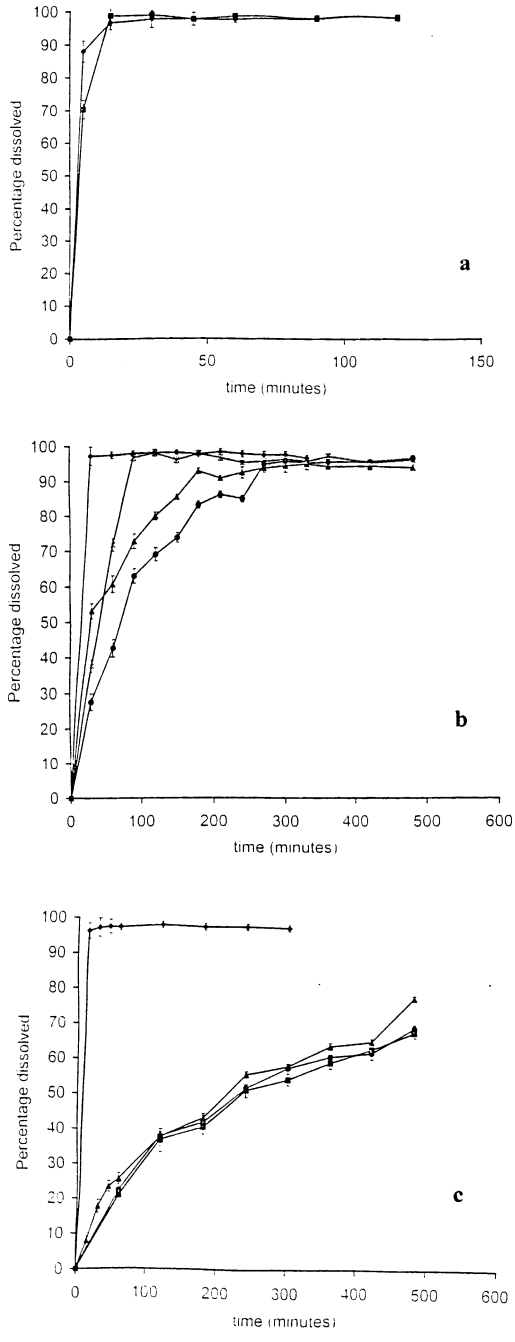

Fig. 1. (a) In vitro mean dissolution profiles of acetaminophen (■) IR F-1 and (◆) reference formulations. (b) In vitro mean dissolution profiles of acetaminophen MR (●) F-2; (▲) F-3; (*) F-4; and (◆) reference formulations. (c) In vitro mean dissolution profiles of acetaminophen matrix (▲) F-5; (●) F-6; (■) F-7; and (◆) reference formulations.

(data not shown). The percentages dissolved at 30 min found for these formulations were 26.8 (F-2), 42.2 (F-3), and 70.1% (F-4). All these values are out of the USP 24 limits for IR acetaminophen tablet formulations.

For matrix formulations, as can be seen in Fig. 1c, the absence of disintegrant leads to great delays in dissolution rates, showing significant differences ($p < 0.001$) in their $t_{50\%}$ and $t_{80\%}$ (data not shown), compared to those for IR and MR formulations. Percentages dissolved at 30 min for matrix formulations were $< 25\%$.

Considering these results, formulations selected for the bioavailability studies were: reference formulation (Tylenol); an IR formulation (F-1); a MR formulation showing an intermediate drug release rate (F-3); and a matrix formulation (F-5).

3.2. Bioavailability studies

All the formulations selected for bioavailability studies showed different in vitro behaviours.

As deduced from $AUC_{0\tau\infty}$ and X_{cu}^{24} values shown in Table 2, bioavailability in extent was the same for all types of formulations (see Figs. 2 and 3).

As can be seen in Fig. 2, formulations exhibiting high dissolution rates (reference and F-1), did not show delay in acetaminophen absorption. No statistical difference was found in all the parameters studied (t_{max}, C_{max}, $AUC_{0\tau\infty}$, and X_{cu}^{24}). These results, which are in accordance with those previously reported (Galia, et al., 1998) for a BCS Class 1 drug, are consistent with those found in dissolution assays in vitro. F-1 formulation can be considered an IR acetaminophen formulation, being bioequivalent to reference formulation and, in addition, having the advantage of partially masking the bitter taste of acetaminophen.

On the other hand, MR formulation (F-3) with an in vitro percentage dissolved at 30 min out of the USP 24 specified limits for IR acetaminophen tablets, exhibited a similar in vivo absorption pattern as the reference and F-1 formulations (see Fig. 2). t_{max}, and C_{max} parameters for this formulation did not show statistical

Table 2
Pharmacokinetic parameters of acetaminophen IR (F-1), MR (F-3) Matrix (F-5) and reference formulations (mean $\pm$SD, $n=5$)

Parameter	Reference	F-1	F-3	F-5	Statistics
AUC ($\mu g\,ml^{-1}\,h$)	15.4 ± 0.9	14.7 ± 1.3	15.5 ± 3.6	14.0 ± 1.2	$p>0.05$
C_{max} ($\mu g\,ml^{-1}$)	4.2 ± 0.4	4.1 ± 0.5	3.2 ± 0.3	2.0 ± 0.1	$p<0.05$ $R/F-$
t_{max} (h)	1.2 ± 0.2	1.2 ± 0.5	1.4 ± 0.2	3.6 ± 0.4	$p<0.05$ $R/F-$
X_{cu}^{24}	24.4 ± 1.6	25.1 ± 1.6	23.1 ± 1.4	23.5 ± 1.6	$p>0.05$

significance with those of the reference formulation, as can be seen in Table 2. However, a trend in the values of the parameters can be observed, and significance would be predicted if the number of volunteers was increased.

As can be seen in Fig. 2, acetaminophen absorption was clearly delayed in matrix formulation (F-5), for which the smallest percentage dissolved in vitro was found. t_{max} and C_{max} values, shown in Table 2, indicate the slow rate of acetaminophen absorption. Significant differences ($p < 0.05$) were found in these parameters between F-5 and reference formulation.

Based on the observation of the in vitro dissolution and, in vivo absorption, significant differences in t_{max} and C_{max} can be viewed as a result of the delayed release. This suggestion was confirmed by the in vitro–in vivo correlation study, where a correlation coefficient of 0.995 was found between the MDT and MRT values of the studied formulations (see Fig. 4).

The current study demonstrates that the acetaminophen absorption rate can be modified through its formulation within MA particles. In vivo data reveal the possibility of achieving controlled release by using a protein (ovoalbumin) as a matrix system for oral administration.

Assuming the BCS approach, Class 1 drugs absorption is not influenced by the intestinal site where release and subsequent absorption take place. This statement is consistent with the results obtained in this study, where the extent of acetaminophen absorption is comparable for all formulations, even though they showed significant differences in release rates, which implies that acetaminophen has been released in different intestinal sites, exhibiting no change in permeability.

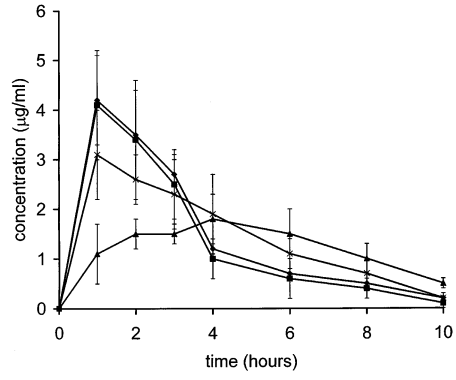

Fig. 2. Mean profiles of acetaminophen saliva concentrations in function of time: (◆) reference; (■) IR formulation; (*) MR formulation; (▲) matrix formulation. Mean $\pm$ SD, $n=5$.

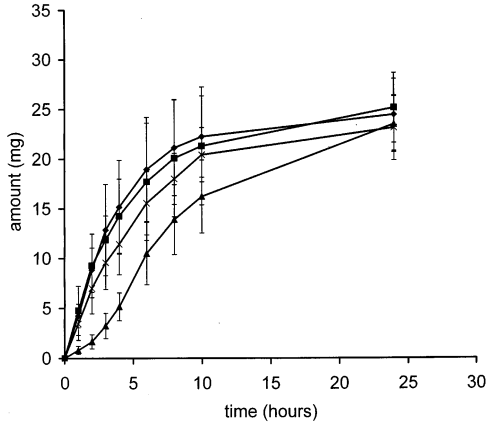

Fig. 3. Mean cumulative curves of dose excreted vs time for acetaminophen after oral administration of the four formulations to five healthy volunteers: (◆) reference; (■) IR formulation; (*) MR formulation; (▲) matrix formulation. Means $\pm$S.D., $n=5$.

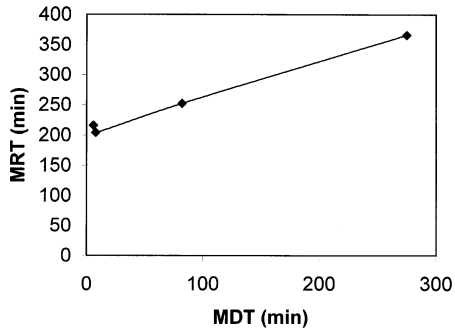

Fig. 4. Linear regression plot of MRT versus MDT values for acetaminophen reference, F-1, F-3 and F-5 formulations.

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