Acta Poloniae Pharmaceutica – Drug Research, Vol. 71 No. 3 pp. 469–475, 2014 ISSN 0001-6837
Polish Pharmaceutical Society

IN VITRO – IN VIVO EVALUATION OF A NEW ORAL DOSAGE FORM OF TRAMADOL HYDROCHLORIDE – CONTROLLED-RELEASE CAPSULES FILLED WITH COATED PELLETS

DANUTA SZKUTNIK-FIEDLER[1]*, MONIKA BALCERKIEWICZ[1], WIESŁAW SAWICKI[2], TOMASZ GRABOWSKI[3], EDMUND GRZEŚKOWIAK[1], JAROSŁAW MAZGALSKI[4] and HANNA URJASZ[1]

[1]Department of Clinical Pharmacy and Biopharmacy, Poznan University of Medical Sciences, Św. Marii Magdaleny 14, 61-861 Poznań, Poland
[2] Department of Physical Chemistry, Medical University of Gdańsk, J. Hallera 107, 80-416 Gdańsk, Poland
[3]Polpharma Biologics, Trzy Lipy 3, 80-172 Gdańsk, Poland
[4]Polpharma S.A., Pelplińska 19, 83-200 Starogard Gdański, Poland

Abstract: The aim of this study was an *in vitro – in vivo* evaluation of a new oral dosage form of tramadol hydrochloride (TH), controlled-release capsules filled with coated pellets, 100 mg (TC), compared to the sustained release tablets, Tramal Retard®, 100 mg (TR). *In vitro* release study of both formulations showed a similar release profile of TH over 8 h (f2 was 52). *In vivo* study (single oral, 100 mg dose administration in 8 rabbits) showed that the amount of TH absorbed into the systemic circulation after TC and TR administration was also similar (90% CI for AUC_{0-t} and $AUC_{0-\infty}$ were 90–124% and 97–109%, respectively). However, a comparison of AUC_{0-t} of pharmacokinetics of TC and TR indicates significantly prolonged absorption and elimination processes of TH when the drug is given in controlled-release capsules filled with coated pellets. It was manifested by longer: mean absorption time ($p = 0.0016$), mean residence time ($p = 0.0268$), absorption half-life ($p = 0.0016$), elimination half-life ($p = 0.0493$) and lower: absorption rate constant ($p = 0.0016$), elimination rate constant ($p = 0.0148$) and total body clearance Cl/F ($p = 0.0076$). It may be concluded that the new TH formulation could be expected to have a more prolonged analgesic activity than commercial sustained release tablets.

Keywords: tramadol hydrochloride, controlled-release capsules, pellets, rabbits

Tramadol hydrochloride (TH) is a well-tolerated and effective synthetic, centrally acting analgesic used to treat moderate, severe, and chronic pain. It is a widely prescribed analgesic marketed in over 90 countries. The mean absolute bioavailability of TH after oral administration is approximately 70%, irrespectively of concomitant intake of food. TH has a linear pharmacokinetic profile within the therapeutic range (100–300 ng/mL). The short elimination half-life of 6 h necessitates administration of immediate-release (IR) TH preparations to patients every 4–6 h, which may be inconvenient for patients who require long-term treatment (1, 2). High-frequency dosage regimens can result in non-compliance and subsequent inappropriate plasma drug concentrations and inadequate analgesia (3). Pain management guidelines recommend the use of long-acting agents in patients with chronic pain as they provide sustained analgesia for 12 to 24 h (4, 5). Many oral sustained-release (SR) formulations of TH, including those with pellets, have been described (1, 2, 6–10). Compared to the traditional formulations, multiple-unit dosage forms with pellets are characterized by a relatively high surface area of drug release and a short diffusion way, which contributes to a more efficient use of the entire active ingredient. The pellets are less irritating to the mucous membrane of the digestive tract and they are more evenly distributed inside the stomach, which leads to a reduced risk of high local concentration and of adverse effects. What is important, it is the possibility of only partial destruction e.g., when crushing with teeth (11–16).

In order to improve pain therapy, our study proposes an alternative drug delivery system for TH – controlled-release capsules filled with coated pel-

* Corresponding author: e-mail address: d.szkutnik@wp.pl; phone: +48 (61) 6687853; fax: +48 (61) 6687855

lets (TC). The aim of our study was an *in vitro – in vivo* evaluation of this new oral dosage form of TH, developed at the Department of Pharmaceutical Technology, Medical University of Gdańsk, Poland, compared to the 100 mg SR tablets – Tramal Retard® (TR).

MATERIALS AND METHODS

Capsules filled with coated pellets

Pellet cores were prepared by wet granulation of powder mixture followed by spheronization of the extruded mass. On the basis of the initial experiments, the composition of pellet cores was determined as follows: TH 60.0%, microcrystalline cellulose**,** PH101 35.0% and glyceryl behenate 5.0%. A detailed process of preparing coated pellets with TH was described in our previous work (15).

Eighty pellets of 0.6–1.0 mm grain size with ethylcellulose film were enclosed in white hard gelatine capsules no. 2. Average mass of single capsule was 225 mg ± 1.7% and the contents of TH was 101.2 mg ± 1.3%.

In vitro release study

In vitro release study was performed using an automated Hansson Research Sr8+ basket apparatus dissolution tester (Hansson Research, Chatsworth, CA, USA) with an on-line UV/VIS spectrophotometer (Agilent 8453, Wilmington, USA; the wavelength – 272 nm, medium – 1000 mL of water at 37 ± 0.5°C, the concentrations of TH in the samples analyzed at 1, 2, 3, 4, 5, 6, 7 and 8 h; reference product SR tablets (Tramal Retard®, 100 mg) No. AN043 (Grünenthal, Aachen, Germany), all dissolution profiles – the mean of 12 dissolution tests performed under sink conditions.

Similarity of dissolution profile of the formulations was compared using model-independent method by linear regression at specified time points, and calculating a similarity factor f_2:

$$f_2 = 50\times\log\{[1 + (1/n)\sum_{t=1}^{n}(R_t - T_t)^2] - 0.5\times100\}$$

where: f_2 = the similarity factor, n = the number of time points, R_t = the mean percent drug dissolved of e.g., a reference product, and T_t = the mean percent drug dissolved of e.g., a test product (17). An f_2 value between 50 and 100 suggests that two dissolution profiles are similar.

In vivo study

Animals

The study was performed using a rabbit model: eight adult healthy New Zealand white rabbits (mean weight ± SD, 3.3 ± 0.2 kg). Animals fasted for 12 h prior to drug administration. During this time, free access to fresh water was provided. Twelve hours after drug administration, the animals were allowed access to the feed. The study was performed according to a protocol approved by the Local Ethical Committee at the University of Life Sciences in Poznan (agreement No. 71/2008), and was in accordance with the rules and guidelines concerning the care and the use for laboratory animal experiments (18).

Experimental design

Controlled-release capsules filled with coated pellets, 100 mg (TC) prepared in the Department of Pharmaceutical Technology, Medical University of Gdańsk, Gdańsk, Poland, and SR tablets Tramal Retard®, 100 mg (TR), (batch No. 292L01, Grünenthal, Aachen, Germany) were used for oral administration. A two-treatment, two-period, two-sequence, single-oral dose, randomized, crossover design was performed. The washout period was 14 days. All animals received *per os* one capsule or tablet (100 mg of TH, mean dose 30.32 ± 0.16 mg/kg) of each formulation. To ensure that the capsule or tablet was swallowed and entered the stomach, 20 mL of water were given to the rabbits at the same time as the capsule/tablet was administered.

To calculate absolute bioavailability (F) and mean absorption time (MAT) of TH two weeks after oral administration, all animals received TH intravenously (10 mg/kg, Poltram 100 mg/2 mL, batch no. 510804; Polpharma, Poland).

All TH formulations were administered between 8 a.m. and 9 a.m. Blood samples (1.5 mL) were obtained from the catheter remaining in the ear vein, prior to TH administration (sample 0) and 15, 30, 45, 60, 120 min and 4, 8, 24, 30 h following oral administration, or 1, 5, 10, 15, 30, 45, 60, 120 min and 4, 6, 8 h following intravenous administration. Blood samples were transferred into collection tubes containing lithium heparin, immediately centrifuged at 2880 × g for 10 min, then the plasma was frozen at –30°C until the time of analysis.

Drug analysis

Chemicals and reagents

Tramadol hydrochloride, $C_{16}H_{25}O_2N \times HCl$, CAS: 27203-92-5, phenacetin (internal standard), CAS: 62-44-2 and triethylamine (HPLC grade) were from Sigma-Aldrich (Steinheim, Germany). Acetonitrile, n-hexane, methanol, ethyl acetate (HPLC grade) were purchased from Merck (Darmstadt, Germany). Sodium hydroxide, monopotassium phosphate, anhydrous potassium hydrogen phos-

phate (analytical grade, pure for analysis) were from POCh (Gliwice, Poland).

Analytical method

The TH in rabbit plasma was determined using high-performance liquid chromatography with UV detection (HPLC Waters 2695 Separations Module with autosampler, Waters 2487 Dual l Absorbance Detector) according to methods described by Gan et al. (19) and Szkutnik-Fiedler et al. (20). The conditions were as follows: the wavelength 218 nm, LiChrosorb RP-18, 250 × 4.6 mm, 5 µm column from Waters, mobile phase: acetonitrile – 0.01 M phosphate buffer (30:70, v/v) with an addition of 0.05% triethylamine (0.5 mL) to achieve pH of mobile phase = 3.0, flow rate of mobile phase 1.0 mL/min, volume of each injection 100 µL, retention time of TH and phenacetin: 5.64 and 8.19 min, respectively, total analysis time 12.0 min. Data collection and processing were carried out using Empower Pro software, v. 1154. This HPLC method was adapted to the conditions of our lab and fully validated in accordance with the published EMA guidelines (21). The lower limit of quantification (LLOQ) and limit of detection (LOD) of TH were 10 ng/mL and 5 ng/mL, respectively. The calibration for TH was linear in the range of 10–1000 ng/mL. Intra- and inter-day coefficients of variation were less than 10%. TH in rabbit plasma samples was stable during the storage, freeze-thaw cycles, processing and analysis.

Pharmacokinetic and statistical analysis

Pharmacokinetic parameters of TH: the elimination rate constant (k_{el}), elimination half-life ($t_{1/2kel}$), area under the plasma curve from zero to the last measurable concentration (AUC_{0-t}), area under the plasma curve from zero to infinity ($AUC_{0-\infty}$), area under the first moment curve from zero to infinity ($AUMC_{0-\infty}$), total body clearance (Cl/F) and mean residence time (MRT) were calculated using the non-compartmental methods with validated software WinNonlin® 5.3 Professional (Pharsight, Corp., USA). The maximum drug plasma concentration (C_{max}) and the time at which C_{max} was achieved (t_{max}) were determined directly from the concentration *vs*. time curve. The absolute bioavailability (F) of TH was calculated from the AUC ratio obtained following *p.o.* and *i.v.* administration, indexed to their respective dose: $F(\%) = [AUC_{0-\infty p.o.} \times D_{i.v.}/AUC_{0-\infty i.v.} \times D_{p.o.}] \times 100$. Mean absorption time ($MAT$), absorption rate constant (k_a) and absorption half-life ($t_{1/2k_a}$) were determined according to the following equations: $MAT = MRT_{p.o.} - MRT_{i.v.}$, where MRT is the mean residence time after *p.o.* and *i.v.* administration, respectively, $k_a = 1/MAT$, $t_{1/2k_a} = 0.693 \times MAT$.

As TH was given to the rabbits in different doses (intravenous administration: 10 mg/kg; oral administration: mean dose of 30.32 ± 0.16 mg/kg) AUC and $AUMC_{0-\infty}$ values were dose normalized.

The statistical calculations were performed using Statistica PL 10 software (StatSoft, Inc.).

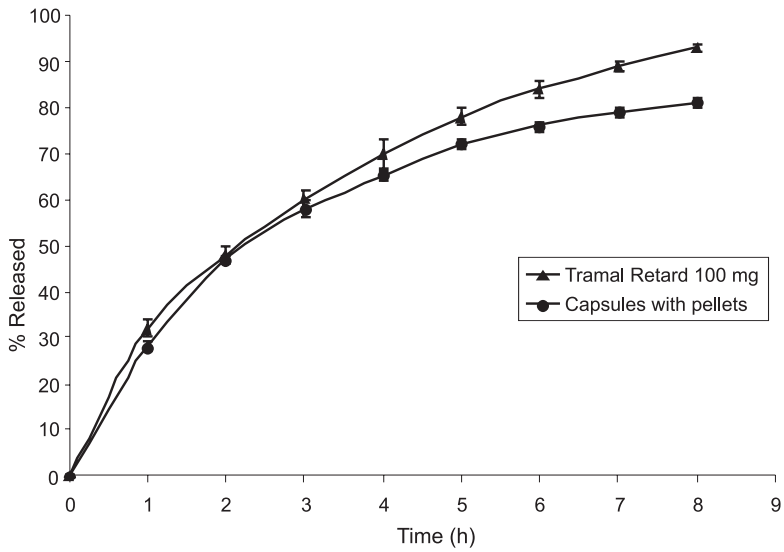

Figure 1. *In vitro* tramadol hydrochloride release (the mean ± RSD) from capsules filled with coated pellets compared to Tramal Retard® tablets (n = 12)

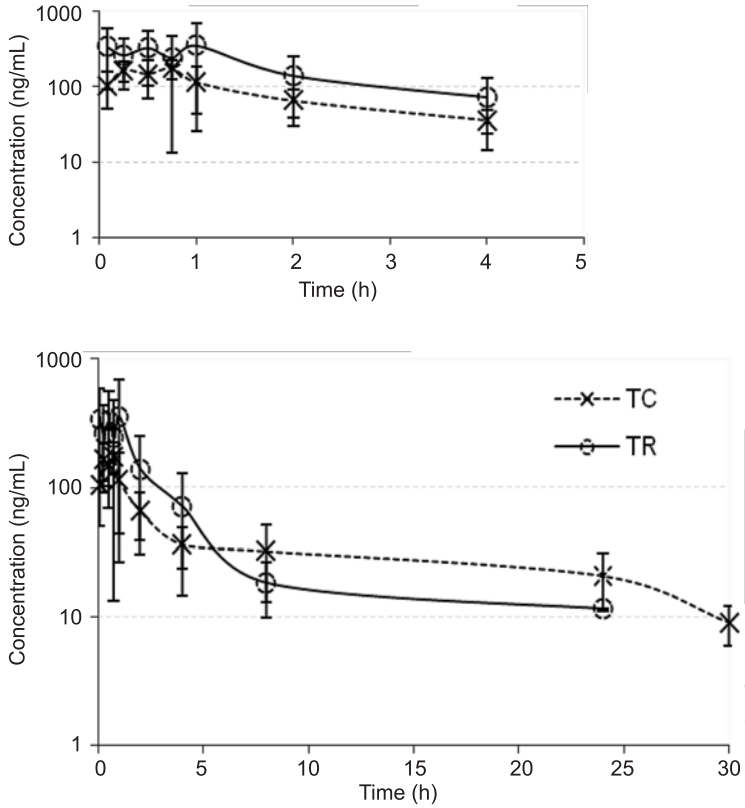

Figure 2. Plasma concentration-time profiles (the mean ± SD) of tramadol hydrochloride after oral administration of capsules filled with coated pellets (TC) and Tramal Retard® tablets (TR) in rabbits (n = 8). Significant differences (paired *t*-test) were observed for the following time points: 0.08 h (p = 0.0089), 0.25 h (p = 0.0185), 0.5 h (p = 0.0029), 1 h (p = 0.0002), and 2 h (p = 0.0147)

Paired *t*-test or Wilcoxon test (data with non-normal distribution) were used to compare plasma concentrations and pharmacokinetic parameters of TH. The results were presented as the mean ± SD (standard deviation) or median (range) (t_{max} and t_{last}). Differences resulting in a p value of less than 0.05 were considered statistically significant. Statistical analysis of variance (ANOVA) was used to compare the bioequivalence of these two formulations. The standard 90% confidence intervals (90% CIs) of the geometric mean ratios TC/TR with logarithm (ln)-transformed $AUC_{0-t,}$ $AUC_{0-\infty}$ and C_{max} were calculated. The bioequivalence acceptance criteria required that the 90% CI be within the range of 80–125%.

RESULTS

In vitro release study

Similarity factor f_2 for the TH release profiles between TC and TR formulations was 52 which suggests that the two dissolution profiles are similar (Fig. 1).

In vivo study – pharmacokinetic analysis

No adverse effects were observed after oral (100 mg, mean dose 30.3 ± 0.16 mg/kg) and intravenous (10 mg/kg) TH administration in rabbits. Mean plasma concentration-time profiles of TH after oral administration of TC and TR are shown in Figure 2. The statistical evaluation of plasma TH concentrations after TC and TR administration showed significant differences for the following time points: 0.08 h (p = 0.0089), 0.25 h (p = 0.0185), 0.5 h (p = 0.0029), 1 h (p = 0.0002), and 2 h (p = 0.0147) after administration. After 4 h, plasma concentrations of TH were similar (Fig. 2). The pharmacokinetic parameters of TH and their statistical evaluation are summarized in Tables 1 and 2. The profile of plasma TH concentration *vs*. time showed that the elimination of TH from TC formulation is longer than from TR tablets after single dose administration in rabbits. The time for the last observed concentrations (t_{last}) (median (range)) was: 30 (24–30) h and 8 (4–24) h for TC and TR, respectively (Table 2). In the group of TC-receiving rab-

bits, total elimination of the drug was noted in six out of eight animals as late as after 30 h, and in the TR-receiving group elimination in two of eight animals was complete after 24 h, in five animals after 8 h and in one as soon as after 4 h. TH concentration was not determined in any of the TR group animals after 30 h. Although all of the rabbits had similar body weight (3.3 ± 0.2 kg; range from 3.0 to 3.5 kg) and fasted 12 h prior to drug administration and 12 h after administration to minimize the variability caused by food, differences in gastrointestinal transit time, disintegration of the oral dose form and absorption rate can affect C_{max} and t_{max}. Coefficients of variation (CV) for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values were smaller after TC administration compared to the TR formulation (Table 1). Generally, TC exhibited a significantly lower C_{max} appearing at a similar time compared with SR conventional tablet Tramal Retard® (p = 0.0003, paired *t*-test).

Ninety percent CI values for C_{max} were not within the range of 80–125% of the statistical interval proposed by EMA (17) and were 42% to 72%. TC and TR, however, led to equivalent systemic exposure to the drug (90% CI for AUC_{0-t} and $AUC_{0-\infty}$ were 90–124% and 97–109%, respectively) (Table 1). Compared to TR, TC had significantly prolonged absorption and elimination of TH, as evidenced: longer: mean absorption time MAT (p = 0.0016), mean residence time MRT (p = 0.0268), absorption half-life $t_{1/2ka}$ (p = 0.0016), elimination half-life $t_{1/2kel}$ (p = 0.0493); lower: absorption rate constant k_a (p = 0.0016), elimination rate constant k_{el} (p = 0.0148) and total body clearance Cl/F (p = 0.0076) (Table 2).

DISCUSSION

In our study, a new controlled release formulation of TH capsules filled with coated pellets was evaluated. Long-acting analgesics are often proposed for chronic pain management as they provide more consistent plasma drug concentrations (7). The efficacy of slow-release TH in the treatment of chronic pain with better patient compliance during treatment has been confirmed in several studies (7–9). It is known that technological processes and differences between the oral formulations might represent the most important factor responsible for the differences in both rate and extent of absorption of the drug, reflected in the pharmacokinetic parameters, and thus an *in vitro* and *in vivo* evaluation of any new formulation is necessary (22, 23).

In vitro tests have shown that the rate and degree of TH release were similar to those of the reference formulation – Tramal Retard® tablets. In the *in vivo* study, large inter-subject differences in the pharmacokinetic parameters of TH, both after TC and TR administration, were observed. This may be related to the differences in absorption rate, metabolism or gastrointestinal transit time. Inter-animal variability, however, is very common in pharmacokinetic studies using animal model (24–30). Despite intra-individual differences, the amounts of TH absorbed into the systemic circulation after administration of TC and TR in rabbits were similar (90% CI values for AUC_{0-t} and $AUC_{0-\infty}$ were within 80–125%). Nevertheless, TC exhibited a significantly lower maximum plasma concentration and a comparison of other pharmacokinetic parameters of

Table 1. Statistical results (ANOVA) of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} for tramadol hydrochloride after oral administration of capsules filled with coated pellets, 100 mg (TC) and Tramal Retard® tablets, 100 mg (TR) in rabbits (n = 8).

Parameter (unit)	TC	TR	Geometric mean ratio TC/TR (90% CI for the lntransformed data)
AUC_{0-t} (ng h/mL) CV (%)	970.29 ± 307.16 (31.66)	960.45 ± 665.03 (69.24)	107.3 (90 – 124)
$AUC_{0-\infty}$ (ng h/mL) CV (%)	1199.92 ± 287.33 (23.94)	1092.09 ± 658.76 (60.32)	102.8 (97 – 109)
C_{max} (ng/mL) CV (%)	217.6 ± 32.7 (15.02)	459.1 ± 257.2 (56.02)	52.2 (42 – 72)

Data are presented as the mean ± SD. Statistical analysis of the data was performed using the one-way analysis of variance (ANOVA). Abbreviations: C_{max} – maximum plasma concentration, AUC_{0-t} – area under the plasma curve from zero to the last measurable concentration, $AUC_{0-\infty}$ – area under the plasma curve from zero to infinity, CV – coefficient of variation defined as the ratio of the SD to the mean.

Table 2. The pharmacokinetic parameters of tramadol hydrochloride and their statistical evaluation after oral administration of capsules filled with coated pellets, 100 mg (TC) and Tramal Retard® tablets, 100 mg (TR) in rabbits (n = 8).

Parameter (unit)	TC	TR	TC *vs*. TR
t_{max} (h)	0.75 (0.25 – 1.00)	0.50 (0.08 – 1.00)	NS
t_{last} (h)	30 (24 – 30)	8 (4 – 24)	IS p = 0.0277
$AUMC_{0-\infty}$ (ng h²/mL)	22533.11 ± 14596.89	5158.99 ± 3461.08	IS p = 0.0183
k_{el} (h^{-1})	.06 ± 0.02	0.19 ± 0.12	IS p = 0.0148
Cl/F (mL/min)	1003.99 ± 655.65	1854.62 ± 684.45	IS p = 0.0076
MRT (h)	18.32 ± 11.18	4.79 ± 2.39	IS p = 0.0268
$t_{1/2kel}$ (h)	14.96 ± 8.12	5.12 ± 2.63	IS p = 0.0493
MAT (h)	14.63 ± 11.48	1.86 ± 1.59	IS p = 0.0016
k_a (h^{-1})	0.09 ± 0.06	0.81 ± 0.47	IS p = 0.0016
$t_{1/2ka}$ (h)	10.14 ± 7.96	1.29 ± 1.10	IS p = 0.0016
F (%)	26.95 ± 8.07	24.68 ± 15.68	NS
RB (%)	109.19		

Data are presented as the mean ± SD or median (range) (t_{max} and t_{last}). Statistical analysis of the data was performed using the paired *t*-test or Wilcoxon test (t_{max} and t_{last}). Abbreviations: IS – statistically significant difference ($p < 0.05$), NS – statistically non-significant difference ($p > 0.05$), t_{max} – time to reach maximum plasma concentration, t_{last} – time of the last observed concentration, – area under the first moment curve from zero to infinity, k_{el} – elimination rate constant, Cl/F – total body clearance, MRT – mean residence time, $t_{1/2kel}$ – elimination half-life, MAT – mean absorption time, k_a – absorption rate constant, $t_{1/2ka}$ – absorption half-life, F – absolute bioavailability, RB – relative bioavailability.

TH indicates prolonged absorption and elimination processes, when the drug is given in controlled-release capsules filled with coated pellets.

The mean absolute bioavailability of TH after TC and TR administration was 26.95 ± 8.07% and 24.68 ± 15.68%, respectively. Such a small value could be caused by a higher metabolism of TH in rabbits compared to the humans. For financial reasons, the active metabolites of TH were not determined, which is a true limitation of our study. It has been confirmed that controlled release of the drug in the stomach does not always contribute to its increased bioavailability. Moreover, biological availability of TH in animals after administration of SR tablets is usually lower than after IR tablets (13). Systemic bioavailability of TH reported by Giorgi et al. (24, 25) after oral administration of SR tablets at a dose of 100 mg was only 11% in dogs (24) and 10.5% in horses (25) (SR tablet, dose of 5 mg/kg). Similar results were observed in goats (26) (F = 30%) and horses (27) (F = 3%) administered 2 mg/kg orally; meanwhile, horses treated with 5 mg/kg immediate release capsules (25) had a bioavailability of 64%. In humans, TH bioavailability is 70% after a single oral administration, which is similar to e.g., 65% in dogs (28) but is lower than 93% in cats (29). Sustained-release tablets have a bioavailability of 87–95% compared to the capsules (13). In our study, the relative bioavailability (RB, %) of controlled-release capsules filled with coated pellets with reference to TR tablets was 109.19%.

CONCLUSION

Both TR formulations have a similar *in vitro* release profile and led to an equivalent systemic exposure to the drug. However, prolonged absorption and elimination processes of TH, which have

been achieved in rabbits after administration of controlled-release capsules filled with coated pellets, might suggest that the new formulation could be expected to have a more prolonged analgesic effect in humans than the commercial sustained release tablets. It can be concluded that our new form of TH may be an alternative to the other controlled-release preparations.

Declaration of interest

The authors report no declarations of interest.

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Received: 30. 07. 2013