In Vivo and *In Vitro* Diclofenac Sodium Evaluation After Rectal Application of Soft Gelatine Capsules Enabling Application Induced Transformation (AIT) into a Semisolid System of Liquid Crystals (SSLC) for Controlled Release

Axel Schneeweis[1] and Christel C. Müller-Goymann[1,2]

Received July 7, 1997; accepted September 18, 1997

Purpose. Reverse micellar solutions of diclofenac sodium were encapsulated in soft gelatine capsules. On contact with aqueous media they exhibited an application induced transformation (AIT) into a semisolid system of liquid crystals (SSLC) which slows down drug release. The aim of the present paper was to study *in vitro* and *in vivo* drug release from these systems after rectal application.

Methods. *In vitro* drug release was determined in a self-constructed dissolution apparatus to simulate rectal application. For *in vivo* bioavailability studies rabbits were used as animal models. *In vitro* release and *in vivo* bioavailability of the capsules was compared to Voltaren® suppositories.

Results. The release profiles of the *in vitro* experiments show zero-order kinetics. The *in vivo* bioavailability studies show bioequivalence in terms of AUC for both formulations (capsules and Voltaren® suppositories). The mean residence time (parameter of sustained release) of the capsules is three time longer in comparison to Voltaren® suppositories.

Conclusions. Rectal administration of capsules provides an appropiate route for controlled release via AIT-SSLC which could be clearly verified in rabbits.

KEY WORDS: diclofenac sodium; lecithin; liquid crystals; reverse micellar solution; controlled release; rectal application.

INTRODUCTION

Nonsteroidal antiinflammatory drugs are widely marketed active substances. To relieve chronic pain, an almost constant plasma concentration of the specific drug is desired between administration intervals.

The present paper deals with the nonsteroidal antiinflammatory drug diclofenac sodium encapsulated in soft gelatine capsules as a reverse micellar solution in order to study controlled release after rectal application *in vivo* and *in vitro*.

Usually an oily solution or suspension is encapsulated in soft gelatine capsules. In a previous paper an oily reverse micellar solution (RMS) was reported to be appropriate for soft gelatine encapsulation (1–3). The structure of the RMS has been characterised by photon correlation spectroscopy (PCS) and small angle X-ray scattering. The results of the investigation show an oblate shape of the micelles with an average micellar diameter of about 10 nm (1). This RMS exhibits an application induced transformation into a semisolid system of liquid crystalline microstructure (AIT-SSLC) on contact with aqueous media (4). The growth of the liquid crystalline microstructure at the surface of the RMS can be described mathematically as a Verhulst process (5). The structure of the liquid crystal has been identified by polarized light microscopy as a lamellar mesophase (1). In this mesophase the apparent diffusion coefficient has decreased in comparison with an oily or aqueous solution (4). Thus AIT-SSLC may enable controlled drug release via zero-order kinetics which could be mathematically calculated for a period of up to 30 hours (6).

From an *in vivo* study after peroral application, however, a controlled release could not be proven due to interindividual variations (3). The presence of endogeneous substances in the upper intestine (for example bile salts and lipase) disturb the integrity of the formulation, which could also be demonstrated in an *in vitro* release experiment in presence of bile salts and lipase (2).

The present paper deals with an application route without the disturbance of the endogeneous substances in the upper intestine: the rectal application. The aim is to study whether or not controlled release is obtained in rabbits via rectal application of the drug formulation.

MATERIAL AND METHODS

Chemicals

Diclofenac sodium (DS) was supplied by Ciba-Geigy (CH-Basel); isopropylmyristate (IPM) was purchased from Henkel (D-Düsseldorf). The lecithin used was Phospholipon 90 G® (Rhone-Poulenc Rorer, D-Köln), which consisted of pure soya lecithin with a content of at least 90% phosphatidylcholine. Propyleneglycole was purchased from Hüls (D-Marl). Taurocholic acid T-0750 was purchased from Sigma (D-Deisenhofen). Lipase type II 37, 306-0 was purchased from Aldrich (D-Steinheim). The applied buffers were an isotonic phosphate buffer of pH 7.4 according to German Pharmacopoeia and an 0.03 molar phosphate buffer of pH 6.5. Voltaren® 25 mg suppositories for comparative purposes was purchased in a retail pharmacy.

Preparation of Reverse Micellar Solution and Encapsulation (7)

While being stirred with a teflon coated magnet, lecithin was dissolved in IPM at a temperature of 60° C. A yellowish solution was obtained. Diclofenac sodium and propyleneglycole were solubilised in the RMS by being stirred with a teflon coated magnet at room temperature for about 2 hours.

The composition of the RMS was: DS 4.75%, lecithin 27.075%, IPM 63.175%, propyleneglycole 5% (w/w).

The encapsulation of the RMS in soft gelatine capsules containing 25 mg diclofenac sodium was performed by Scherer Ltd. Company (D-Eberbach).

In Vitro Drug Release

The drug release of the capsules was compared to Voltaren® 25 mg suppositories. The dissolution apparatus consisted

[1] Institut für Pharmazeutische Technologie, TU Braunschweig, Mendelssohnstr. 1 D-38106 Braunschweig.
[2] To whom correspondence should be addressed.

0724-8741/97/1200-1726$12.50/0

of a waterbath (37° C) containing a beaker. The acceptor medium in the beaker (800 ml of isotonic phosphate buffer adjusted to a pH of 7.4) was stirred by a KMO2 magnetic stirrer (Janke und Kunkel, D-Staufen) at 750 rpm. Either a capsule or a suppository was inserted together with 1.0 ml isotonic buffer into a dialysis membrane tubing of 5.2 cm length (Spectra Por® Membrane (MWCO 6–8,000), Spectrum Medical Industries Inc., USA-Los Angeles), siliconized by a 2% (w/w) solution of silicon oil in diethylether to make the membrane hydrophobic. After closing in airfree packaging with clips, the tube was placed into the beaker.

Aliquots of 5 ml were removed from the acceptor medium during six hours and replaced by fresh buffer.

Drug concentration was measured by UV detection using a photometer (Shimadzu, D-Duisburg) at a wavelength of 280 nm.

In Vivo Investigation

The bioavailability after rectal application was tested according to German Animal Protection Law in female chinchilla bastard rabbits of an average weight of 4.5 kg. They were provided with water and food ad libitum. A capsule or a Voltaren® suppository 25 mg was applied respectively. The rabbit was chosen as a model because its rectum is histologically comparable to the human rectum (8). To guarantee the contact between the formulation and the rectal mucosa during the whole absorption time, the anus was manually kept shut over this period which was assumed to take 2 hours for the suppositories and 6 hours for the capsules, respectively.

Five capsules and five suppositories were tested in seven rabbits, that means three out of seven animals received both the capsules and the suppositories in a cross over study.

After drug application, blood samples (2.0 ml) were taken from marginal ear vein catheter (9) at 10, 20, 30, 60, 90, 120, 180, 240, 300, 360 min (capsules) or 5, 10, 15, 20, 25, 30, 60, 90, 180, 240 min (suppositories), respectively. The specimens were centrifuged immediately at 2000 rpm with an UJ3s centrifuge (Heraeus, D-Osterode) for 30 min. The plasma was transferred into polyethylene tubes and stored at −18°C prior to analysis.

Drug Analysis in Plasma

Sample preparation

The plasma was prepared using the method of Wiese and Hermanson (10). Plasma (0.5 ml) was mixed with acetonitrile (0.5 ml). The samples were shaken at 400 motions/min with an IKA-VIBRAX-VXR (Janke & Kunkel GmbH, D-Staufen). After centrifugation with an UJ 15 centrifuge (Heraeus Christ GmbH, D-Osterode) for 15 min at 12000 rpm, the supernatants were injected into the HPLC.

HPLC analysis

Chromatographic analysis was carried out on a Beckman Gold-System (Beckman, D-München) consisting of a programmable solvent module 126 and a programmable detector module 166. Samples of 20 µl were injected by a Promis II (Spark, NL-Emmen) autoinjector with a 100 µl filling loop on a Hypersil ODS reversed-phase column (5 µm, 125 × 4mm ID, Grom, D-Herrenberg-Kayh) equipped with a guard column. The UV absorbance of diclofenac sodium was monitored at λ = 261 nm. Data analysis and calculation were performed with system gold software (Beckman, D-München).

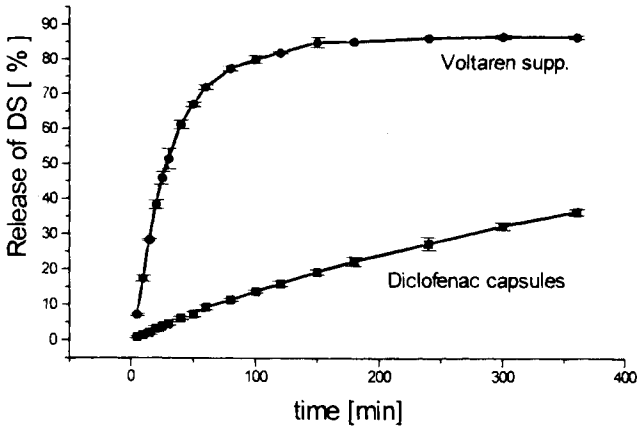

Fig. 1. Released amount of DS from capsules and Voltaren® suppositories *in vitro* including standard deviation. Acceptor solvent: isotonic phosphate buffer of pH 7.4. Three investigatated samples per formulation.

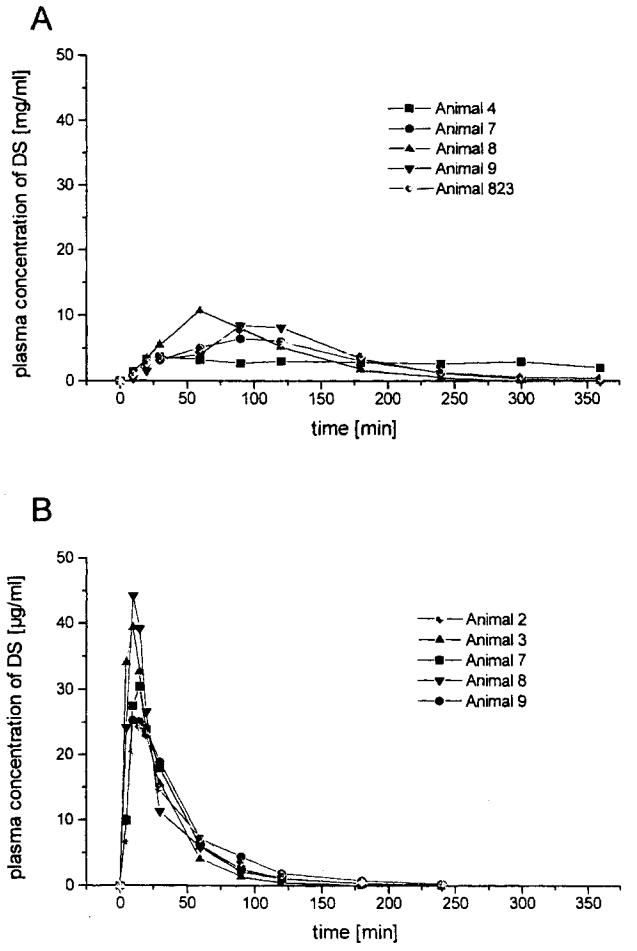

Fig. 2. Plasma concentration—time curves of DS after rectal application of capsules (A) and Voltaren® suppositories (B), both containing 25 mg DS.

Elution was performed at a flow rate of 1.6 ml/min. The composition of the flow medium was acetonitrile 40 parts, bidistilled water 60 parts, acetic acid 2 parts (v/v).

Under these conditions, the retention time of diclofenac sodium was 8.5 min. The limit of detection was 50 ng/ml.

Data Analysis of Bioequivalence Parameters

The parameters used in the bioequivalence studies were c-max (maximum plasma concentration), t-max (time of maximum plasma concentration), AUC (area under the curve) and MRT (mean residence time). The values of c-max and t-max were taken from the plasma concentration time curves. The AUC (the area under the plasma concentration time curve) was calculated via trapezoidal method.

The MRT (quotient of ABC and AUC) (11) was used as a parameter of sustained release. The ABC is the area between the AUC time curve and the asymptote of this curve (12). The value of the ABC was calculated via trapezoidal method.

RESULTS AND DISCUSSION

The growth of the semisolid liquid crystalline system at the surface of the RMS on contact with water which is assumed to follow a Verhulst process (4) leads to a decrease of the diffusion coefficient thus causing sustained release of the drug from the capsules.

In vitro and in vivo investigations after peroral application (3) have shown that the presence of bile salts and lipase led to a distinctive emulsification of the filling. The system was rapidly disintegrated into small droplets with a large specific surface. The growing surface compensated for the decrease of the diffusion coefficient and thus caused the failure of controlled release after peroral application.

Table I. Individual, Mean, Standard Deviation (sd) and Relative Standard Deviation (rel sd) Values of c-max, t-max, AUC, ABC and MRT after Rectal Application of Capsules (A) and Voltaren® Suppositories (B), Respectively

A	c-max [μg/ml]	t-max [min]	AUC [μg/ml*min]	ABC [μg/ml*min²]	MRT [min]
animal 4	3.65	30	986.90	173992	176.3
animal 7	6.39	90	1008.00	128353	127.33
animal 8	10.66	60	1081.40	104693	96.81
animal 9	8.38	90	1106.80	140065	126.56
animal 823	7.60	90	1123.50	148656	132.32
mean	7.34	72	1061.32	139152	131.86
sd	2.58	26.83	60.66	25539	28.51
rel sd [%]	35.21	37.27	5.72	18.35	21.62
B	c-max [μg/ml]	t-max [min]	AUC [μg/ml*min]	ABC [μg/ml*min²]	MRT [min]
animal 2	24.22	15	1158.10	58457	50.48
animal 3	39.38	10	1183.80	36089	30.49
animal 7	30.38	15	1203.60	50558	42.00
animal 8	39.18	15	1269.47	45678	35.98
animal 9	25.26	10	1343.35	68544	51.02
mean	31.68	13	1231.66	51865	41.99
sd	7.32	2.74	74.82	12354	8.97
rel sd [%]	23.09	21.07	6.07	23.82	21.37

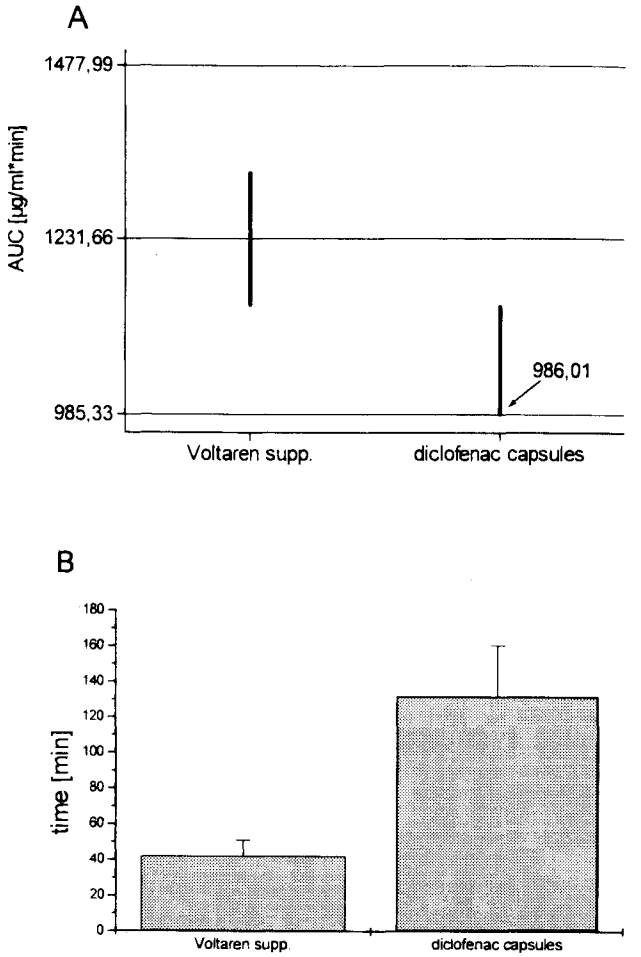

Fig. 3. (A) 95% confidence level of the AUC of capsules and Voltaren® suppositories after rectal application. The confidence level of capsules is located in the 80–120% Westlake limits referring to Voltaren® suppositories. (B) Mean MRT of capsules and Voltaren® suppositories including positive standard deviation (n=5).

The in vivo investigations after peroral application have shown large interindividual variations between the plasma concentration versus time profiles, because variations of the endogeneous substances caused differences in the release behaviour and thus in the bioavailability of the respective animals.

Fig. 1 shows the amounts of DS released versus time for the in vitro simulation of rectal application. The release of DS from the capsules is slower than that from Voltaren® suppositories. The release from the gelatine capsules follows nearly zero-order kinetics. 37.3% of DS are released from the capsules during 6 hours. Voltaren® suppositories release 38.3% of the drug within 20 min.

The sustained release from the capsules is not hindered by any system emulsifying properties. Since the water availability in the rectum is limited to about 1 ml (13), the latter volume was also chosen for the release experiment. 1 ml of water is sufficient to dissolve the gelatine shell.

The in vivo drug release profiles were evaluated in animal investigations. Fig. 2 shows the plasma concentration versus time curves for the DS release from capsules (Fig. 2A) and Voltaren® suppositories (Fig. 2B) after rectal application in rabbits.

Differences in terms of c-max (maximal plasma concentration) and t-max (time of maximal plasma concentration) between capsules and suppositories may be determined. C-max of the capsules is lower than c-max of the suppositories (capsules: 7.34 μg/ml/suppositories: 31,68 μg/ml (n = 5)) and the t-max time is longer (capsules: 72 min/suppositories: 13 min) (Tab. I). The interindividual differences of these parameters of bioavailability are smaller after rectal application in comparison to peroral application (compare (3)).

The bioavailability was investigated further by comparing the AUC's (area under the curve). Bioequivalence of the capsules exists, if the 95% confidence level of the AUC is located in the 80–120% Westlake limits (14) referring to Voltaren® suppositories. Fig 3 shows that the 95% interval of the capsules lies within the Westlake limits and this is an evidence for the bioequivalence of the two formulations in terms of AUC.

Next the MRT (mean residence time) was determined for each application (capsules and Voltaren® suppositories, respectively) as a parameter for sustained release. The MRT is the quotient of the ABC (area between the curves) and the AUC. Fig. 3 shows the mean values of the MRT of both the capsules and the suppositories. The MRT of the capsules is significantly different from that of the suppositories. The MRT of Voltaren® suppositories is 41.99 min ±21.62% (sd rel) (n = 5). The capsules had a three times higher MRT (132.0 min ±21.37% (sd rel) (n = 5)). It is obvious that the relative standard deviations of both formulations are nearly the same.

A significant elongation of the MRT demonstrates the appropriateness of the drug formulation under investigation in terms of sustained release after rectal application. Even controlled release is possible, because variations in the composition of the encapsulated reverse micellar solution result in a modification of the liquid crystal growth at the interface between the RMS and the surrounding aqueous medium (4). This modification of the liquid crystal growth coincides with a modified diffusion coefficient and thus with a different release profile. As long as the drug release is the rate limiting step for the absorption process, the resulting plasma concentration versus time curve reveals the control of drug release via AIT-SSLC. In order to minimize animal experiments, however, we stopped after studying different formulations *in vivo*.

CONCLUSION

The investigation shows that an encapsulated reverse micellar solution, which exhibits an application induced transformation into a semisolid system of liquid crystalline microstructure on contact with aqueous media, controls drug release after rectal application in rabbits. As a consequence, the formulation presented may be considered a rectal therapeutic system.

Variations of excipients (in order to incorporate a higher content of drug into the formulation) as well as variations of drug (in order to broaden the indication area) are in progress.

ACKNOWLEDGMENTS

We thank Dr. Thomas Vieregge, ZET der TU Braunschweig for support in organization and performance of the *in vivo* testing in rabbits and Scherer ltd. for the encapsulation of the AIT-SSLC formulation. We acknowledge Rhone-Poulenc Rorer, Henkel and Ciba-Geigy for the support with substances.

REFERENCES

1. I. Papantoniou and C. C. Mueller-Goymann. *Pharm. Pharmacol. Lett.* **5**:28–31 (1995).
2. I. Papantoniou and C. C. Mueller-Goymann. *Pharm. Pharmacol. Lett.* **5**:49–52 (1995).
3. A. Schneeweis, I. Papantoniou, and C. C. Mueller-Goymann. *Pharm. Pharmacol. Lett.* **7**:42–44 (1997).
4. C. C. Mueller-Goymann and H. J. Hamman. *J. Controlled Release* **23**:165–174 (1993).
5. R. Hirsch and C. C. Mueller-Goymann. *Int. J. Pharm.* **120**:229–234 (1995).
6. R. Hirsch. *Diffusion aus einer Arzneiform mit autogener Diffusionsbarriere.* Thesis TU Braunschweig, 1996.
7. I. Papantoniou. *Invers mizellare Lösungen mit modifizierter Wirkstoffreigabe durch applikationsinduzierte Transformation in lyotrope Mesophasen,* Thesis TU Braunschweig, 1995.
8. C. De Muynck, C. Cuvelier, D. Van Steekiste, L. Bonnarens, and J. P. Remon. *Pharm. Res.* **8**:945–950 (1991).
9. C. Mignat. *Analyse der Kathecholaminkonzentration im Plasma und in der Cerebrospinalflüssigkeit des Kaninchens unter der Einwirkung von Temperaturbelastung, Pyrogen und der antipyretisch wirksamen Substanz Naproxen,* Thesis TU Braunschweig, 1991.
10. B. Wiese and J. Hermansson. *J. Chrom.* **567**:175–183 (1991).
11. K. Yamaoka, T. Nakagawa, and T. Uno. *J. Pharmacokin. Biopharm.* **6**:547–558 (1978).
12. P. Guitard. Perorale Retardformen. In H. Sucker, O. Fuchs, and P. Speiser (editors). *Pharmazeutische Technologie.* Georg Thieme Verlag Stuttgart, New York, 1991, pp. 383–386.
13. B. W. Mueller (editor). *Suppositorien.* Wissenschaftliche Verlagsgesellschaft, 1986, p. 45.
14. W. J. Westlake. *J. Pharm. Sci.* **62**:1579–1589 (1973).