Departments of Pharmaceutical Technology[1] and Physiology[2], Albert Szent-Györgyi Medical University, Szeged, Hungary

Bioavailability of acetaminophen containing suppositories in rats

G. Regdon[1], M. Szikszay[2], Gy. Gebri[1], G. Regdon Jr. and I. Erős[1]

Dedicated to Prof. Dr. G. Zessin, Halle(Saale), on the occasion of his 65th birthday

An attempt was made to optimize the rectal delivery of acetaminophen (paracetamol). One of the aims was to determine which of the various lipophilic and hydrophilic suppository bases proved to be the best with respect to *in vitro* drug liberation. Among several experimental vehicles the Witepsol H 15 base with 10 of Miglyol 812 and the Macrogolum 1540 with 5% of Span 20 proved to be excellent. Furthermore, a well-known pharmacological effect of the paracetamol containing suppositories was evaluated *in vivo* in rats. The antipyretic response to the drug depends on the vehicle administered, showing an excellent correlation between *in vitro* and *in vivo* results.

1. Introduction

Paracetamol (acetaminophen) is currently approved for use in several countries [1–3], it is widely used as an antipyretic and analgesic drug both in the treatment of adults an children [4].

However, a distinction has to be made as the given composition aims to achieve an analgesic [5–6] or an antipyretic effect [7]. There are several examples or rectal administration as reviewed by Thoma [8] and Müller [9]. In the case of suppositories it is important for paracetamol to have a great degree of dispersion [10]. Considerable differences also arise depending on the lipophilic or hydrophilic nature of the suppository base [11–13]. Today various additives are indispensable to modern technology [14], including suppository formulation [15–18]. The bioavailability of drugs with different physical-chemical properties, among them paracetamol, was studied by several authors comparing oral and rectal drug administration [19–23]. In a previous paper, we demonstrated some factors influencing drug liberation from paracetamol containing suppositories *in vitro* [24]. Now, the bioavailability of paracetamol following rectal administration in hydrophilic and lipophilic suppositories was evaluated in rats.

2. Investigations, results and discussion

Under *in vitro* conditions paracetamol was liberated to various extents from different suppository bases (Fig. 1). The diffusion of 300 mg paracetamol powder was regarded as standard. This served as a control for the comparison of drug liberation from drug containing suppositories.

In Table 1 considerable differences can be seen in the *in vitro* relative availability values. Suppositories could be sreened in this way, as diffusion was influenced favourably by Miglyol 812 and unfavourably by Aerosil. The worst results were obtained with suppositories formulated with the conventional lipophilic cocoa butter vehicle.

The vehicles to be used for *in vivo* studies were selected on the basis of the *in vitro* liberation studies. Thus the tested substances included the best hydrophilic composition (code #55), the best lipophilic base (code #56) and cocoa butter (code #53), from which paracetamol is poorly liberated. Nevertheless, it was included in the study on account of its traditional use for more than two centuries. The Witepsol H 15 lipophilic suppository base containing Miglyol 812 (code #54) served as control, because according to the results of previous experiments this composition, which showed very good drug liberation, did not have disturbing effects in the animals used in the experi-

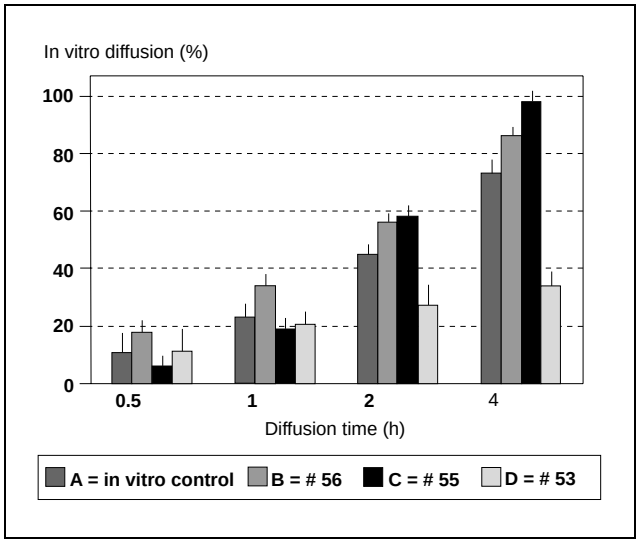

Fig. 1: *In vitro* diffusion from paracetamol containing rectal suppositories (300 mg/2.0 g). A = paracetamol powder (without suppository base).

Table 1: *In vitro* relative availability values of paracetamol-containing (300 mg/2.0 g) suppositories

Suppository vehicles	Solubility properties	*In vitro* relative availability (%)
Control (300 mg of paracetamol powder without vehicle)	1 part of powder dissolved in 60 parts of water	100.0 (corresponds to 73.2 absolute %)
Cocoa Butter	lipophilic	45.5*
Witepsol W 35	lipophilic	62.2
2.5% Aerosil 97.5% Witepsol W 35	lipophilic	33.9
Massa Estarinum 299	lipophilic	65.1
Witepsol H 15	lipophilic	81.0
5% Miglyol 812 95% Witepsol H 15	lipophilic	82.1
10% Miglyol 812 90% Witepsol H 15	lipophilic	113.6*
10% Polysorbatum 20 10% Polysorbatum 61 80% Witepsol W 35	lipohydrophilic	64.3
5% Span 20 95% Macrogolum 1540	hydrophilic	136.2*

Mean values measured after 4 h of diffusion

* The antipyretic effect was measured in animal experiments

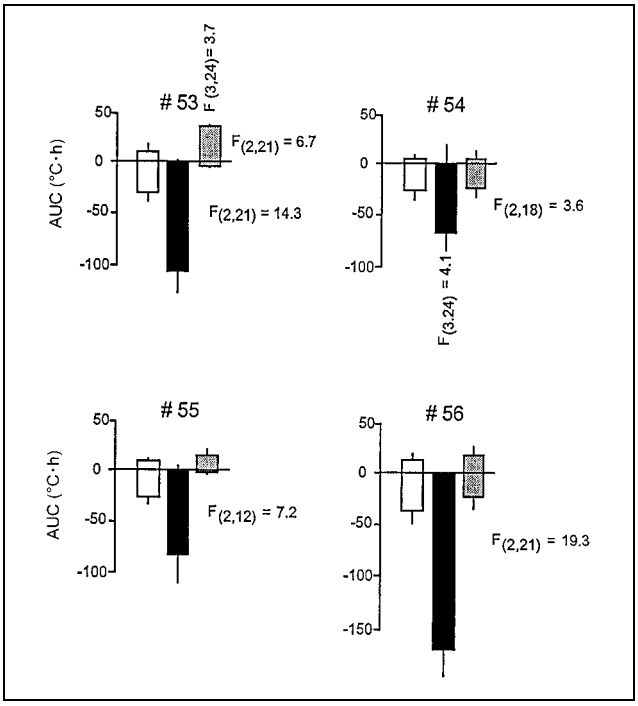

Fig. 2: Area-under the curve values (X ± SEM) after the administration of paracetamol-containing suppositories. Open bar = prior to suppository administration and after 1 h (black bar) and additional 2 h (hatched bar), respectively. The columns show the AUC changes in body temperature/time dependence.

ment (irritation index = 0). Temperature taken 1 h prior to suppositories on fevered animals served as pretreatment control. The febrile response to a yeast suspension showed no significant intergroup difference (Table 2).

Fig. 2 sums up the extent and the time course of the antipyretic effect of the suppositories in rats. The areas under the effect-time curve were analysed. The columns show the integrated values of the area under the temperature-response curve (X ± SEM). Compared to the initial value (baseline temperature) the antipyretic effect and the temperature rise are represented by the areas under the negative and the positive curves, respectively. The number of animals were 6–8 per group. The F-values are the results of one-way ANOVA, where significant differences were found in suppositories. The vertical F-values compare the antipyretic effect of the 4 suppository bases, while the horizontal F-values indicate the significant change within individual suppository bases in time (Fig. 2). The antipyretic effect was found to be significant both statistically and biologically with the use of the lipophilic suppository base of code #56.

Fig. 3 shows the time effects of different suppositories together with the standard error of the mean. Thus the antipyretic effect of paracetamol containing suppositories formulated with various vehicles can be compared well. The antipyretic effect occurred the soonest and lasted for the longest time with suppositories of code #56.

The two-way ANOVA revealed significant differences ($p < 0.001$) in the antipyretic effect of rectal suppositories containing different vehicles. This is indicated by the F-value (175.7) with degrees of freedom being 3 and 1416. The time course of the antipyretic effect was significant from the beginning [$F_{(58.1416)} = 13.2$] at $p < 0.001$. Further statistical analysis definitely showed that the suppository with code #56 was the most efficient even in the third hour after administration [$F_{(3.984)} = 91.6$].

Smith and Hamburger [25] introduced the administration of a yeast suspension to produce fever in rats. Yeast fever

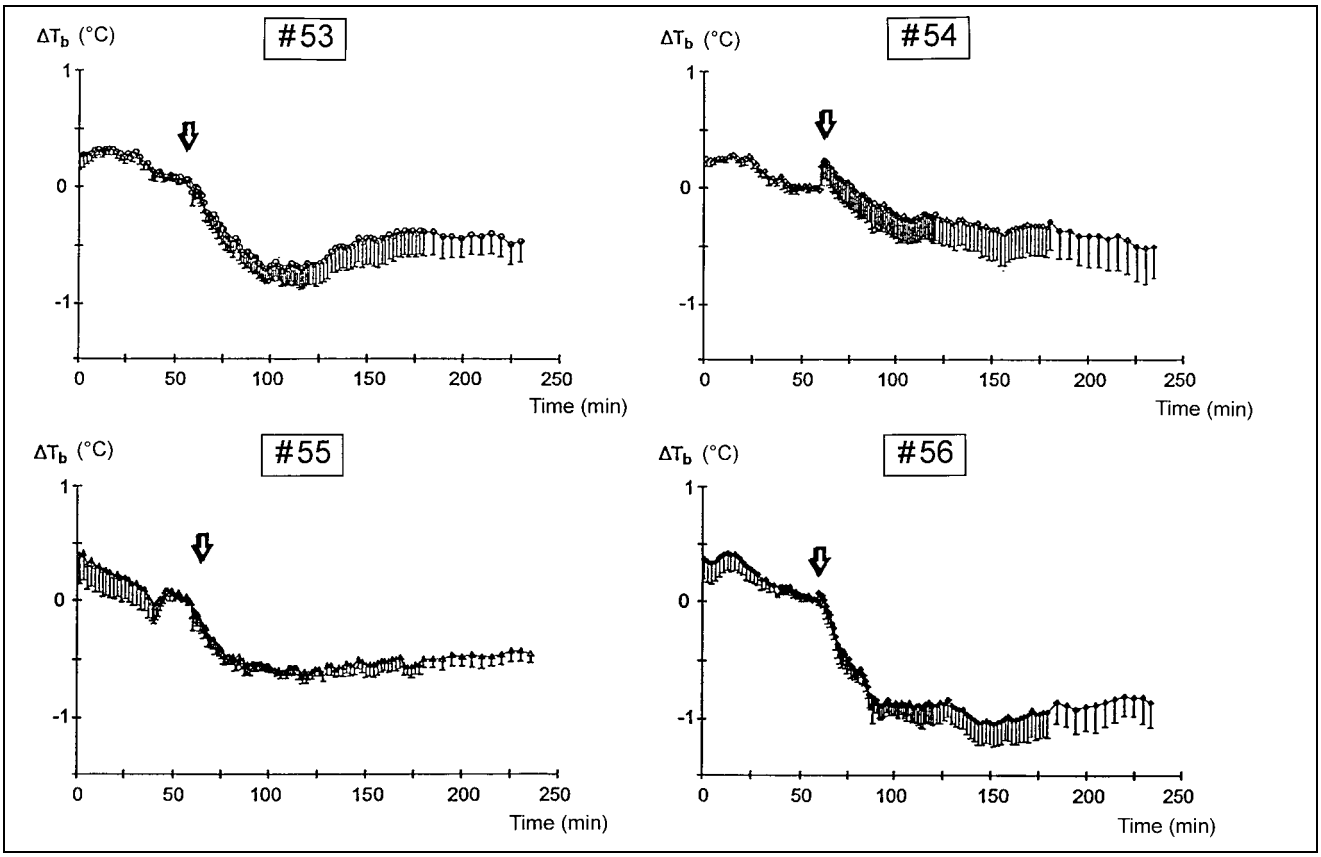

Fig. 3: Time course of the antipyretic effects of rectal paracetamol using different bases. Arrows indicate the time of application of suppositories. The ordinate shows the relative change in body temperature with respect to the initial value. The vertical lines represent the standard error of the mean (X ± SEM).

Table 2: Description of experimental groups

Code #	Suppository bases used in the experiment	Baseline temperature (°C)
53	Cocoa butter	38.64 ± 0.27
54	Control: 10% Miglyol 812 90% Witepsol H 15	38.29 ± 0.29
55	5% Span 20 95% Macrogolum 1540	38.38 ± 0.21
56	10% Miglyol 812 90% Witepsol H 15	38.65 ± 0.25

(Paracetamol dose = 150 mg/1.0 suppository/rat)
The animal studies were approved of by the Institutional Review Board of the Medical University

has been used to investigate the effect of antipyretic drugs [26]. Since rats require higher doses of pyrogens than rabbits, they probably would not be as sensitive as test objects for the assay of pyrogens in pharmaceutical solutions as the latter species [27]. However, our results demonstrate that yeast-treated rats respond well to the antipyretic drug, and can be used for the assay of such compounds. Our results demonstrate that under proper conditions rats give a reasonably uniform and predictable hyperthermic response to yeast suspension, and that this response is easily adapted to the assay of antipyretic drugs.

The *in vitro* studies proved to be useful and could be applied for *in vivo* animal experiments. It was confirmed that the lipoid solubility of the pharmacon and the lipophilic/hydrophilic nature of the vehicle are of great importance in influencing drug liberation and rectal absorption. From the experiments it is obvious that in the case of suppositories prepared with cocoa butter (#53) a close correlation can be found between the *in vitro* and *in vivo* results, although liberation was poor and the antipyretic effect was insufficient. The results obtained *in vivo* agreed well with those of the *in vitro* study. Therefore a Witepsol H 15 lipophilic suppository base containing 10 Miglyol 812 is recommended for therapeutic use.

Finally, it should be noted that the hydrophilic Macrogol mixture vehicle (code #55) used in our experiments is also capable of exerting the required antipyretic effect.

3. Experimental

The studied suppository bases were predominantly of lipophilic nature. They included cocoa butter, Witepsol and Estarinum bases. In addition, hydrophilic macrogol bases were also studied (Chemische Werke, Hüls AG, Troisdorf, Germany).

Additives were tested for various purposes, for example colloidal silicium dioxide (Aerosil) to increase viscoxity, neutral oil (Miglyol 812) to soften consistency, polysorbatum 20 and 61 (Tween 20 and 61) for moistening and sorbitatum monolaurinicum (Span 20) to facilitate diffusion (Th. Goldschmidt, AG, Essen, Germany).

3.1. In vitro examination

Drug liberation from the suppositories was measured based on 5 parallel measurements in each series with a vibrostat at 37 ± 0.2 °C, on the basis of the principle of dynamic diffusion. A kidney dialysing membrane with a surface of 18 cm^2 and a pore diameter of 2–8 nm was used (Union Carbide Co. Chicago, USA) (Table 1).

3.2. Fever induction and body temperature measurement

In the *in vivo* experiments male Wistar rats were used (body weight: 250–300 g). The study was approved by the Institutional Review Board of the Medical University.

For the test period the animals were housed individually at an ambient temperature of 23 ± 1 °C. A light-dark schedule of 12–12 h was maintained. The animals were fasted for 24 h before and during experiment. The suppository was inserted to a depth of 2 cm from the anus, and the anus was gently closed with a wood-device for 15 min.

Yeast aqueous suspension (12%) was administered subcutaneously in the neck 24 h before the temperature in a total volume of 30 ml kg^{-1} body weight [28]. The thermoregulatory reaction was tested in 4 groups of rats (Table 2) which were put together according to the vehicle. The different groups were tested in random order, and the observer was unaware of the content of suppositories. Control rats were treated with vehicle-containing suppositories without paracetamol. Each rat was tested only once.

Body temperature (Tb) was taken by a thermometer probe inserted 6.5 cm deep into the colon. The restrained rats were kept in special wire-mash cages which prevented them from turning around. They had been restrained for 1 h before the experiment to avoid the well-known emotional hyperthermia [29]. Tb was taken repeatedly as indicated in Fig. 3 for 3 h. To minimize the confounding effects of rats' circadian rhythm, all experiments were performed between 11:00–16:00 h [30].

3.3. Statistical analysis

The area under the time-response curve ($AuC_{0-180\,min}$) was calculated for each rat. The data are expressed as mean areas ±SEM. The statistical significance was carried out by one-way analysis of variance (ANOVA). In the Results section F-values are given, with degrees of freedom in parantheses.

The statistical significance of different suppositories on the antipyretic response was assessed over time by two-way ANOVA. The main effects tested were vehicle difference (group) and time. Post hoc comparison was made with the Dunnett test at a significance level of 0.05.

References

1 DAB 10. Band 3. Amtliche Ausgabe, Deutscher Apotheker Verlag Stuttgart, Govi-Verlag GmbH Frankfurt, 1991
2 USP 23/NF 18. United States Pharmacopoeial Conv. Inc. Rockville. 16–28
3 Euopäisches Arzneibuch 3. Ausgabe, p. 1441. Dtsch. Apoth. Verlag. Stuttgart, Govi Verlag Eschborn, 1997
4 Insel, P. A.; In: Goodman and Gilman's. (eds.): The Pharmacological Basis of Therapeutics, Ed. 9. Chapt. 27. Mac Millan, New York, 1996. pp. 631–633
5 Romsing, J.: J. Clin. Pharm. Ther. **21**, 159 (1996)
6 Anderson, B., Kanagasundarum, S.; Woollard, G.: Anaesth. Intensive Care **24**, 669 (1996)
7 Hopkins, C. S.; Underhill, S.; Booker, P. D.: Arch. Dis. Child. **65**, 971 (1990)
8 Thoma, K.: Arzneiformen zur rektalen und vaginalen Applikation. p. 33, Dtsch. Apoth. Verlag, Stuttgart 1980
9 Müller, B. W.: Suppositorien. Pharmakologie, Biopharmazie und Galenik rektal and vaginal anzuwendender Arzneiformen. p. 223, Wissenschaftl. Verlags GmbH., Stuttgart, 1986
10 Moolenaar, F.: Pharm. Weekbl. **115**, 477 (1980)
11 Dibbern, H. W.; Wirbitzki, E.: Pharm. Ind. **45**, 985 (1983)
12 Raclot, G.; Minazzi, H.: Gastroenterol. Clin. Biol. **17**, 872 (1993)
13 Warth, H.; Astfalk, W.; Walz, G. K. Anaestesiol. Intensivmed. Notfallmed. Schmerzther. **29**, 90 (1994)
14 Fiedler, H. P.: Lexikon der Hilfsstoffe für Pharmazie, Kosmetik und angrenzende Gebiete, Ed. Cantor Aulendorf i. Württ. 1994
15 Pflegel, P.; Klaus, T.; Schöbel, H.; Gruno, H.: Pharmazie **48**, 741 (1993)
16 Regdon, G.; Dorogi-Jakab, I.; Bándi, D.; Várföldi, T.; Regdon, G. jun.; Selmeczi, B.: Eur. J. Pharm. Biopharm. **38**, 150 (1992)
17 Regdon, G.; Berényi, M.; Gombkötő, S.; Selmeczi, B.: Pharmazie **50**, 152, (1995)
18 Realdon, N.; Ragazzi, E.; Dal Zotto, M.; Dalla Fini, G.: Int. J. Pharm. **148**, 155 (1997)
19 Pfeifer, S.; Pflegel, P.; Borchert, H. H.: Biopharmazie. p. 165, Ullstein Mosby GmbH, Berlin 1995
20 Gruno, M.; Pflegel, P.: Pharmazie **48**, 907 (1993)
21 Loth, H.; Bosche, P.: Pharmazie **51**, 571 (1996)
22 Blume, H.; Ali, S. L.; Else, M.; Krämer, J.; Scholz, M. E.: Arzneim.-Forsch. **46**, 975 (1996)
23 Kolloffel, W. J.; Driessen, F. G.; Goldhoorn, P. B.: Pharm. World Sci. **18**, 26 (1996)
24 Regdon, G.; Gebri, Gy.; Regdon, E.; Selmeczi, B.: Pharm. Ind. **53**, 296 (1991)
25 Smith, P. K.; Hambourger, W. E.: J. Pharm. Exp. Ther. **54**, 346 (1935)
26 Bavin, E. M.; Macrae, F. J.; Seymour, D. E.; Waterhouse, P. D.: J. Pharm. Pharmacol. **4**, 872 (1952)
27 Ott, W. H.: J. Am. Pharm. Assos. Sci. Ed. **38**, 179 (1949)
28 Loux, J. J.; DePalma, P. D.; Yankell, S. L.: Toxicol. Appl. Pharmacol. **22**, 672 (1972)
29 Bläsig, J.; Höllt, V.; Bauerle, U.; Herz, A.: Life Sci. **23**, 2525 (1978)
30 Scales, W. E.; Kluger, M. J.: Am. J. Physiol. **253**, 306 (1987)

Received July 6, 1998
Accepted September 28, 1998

Prof. Dr. I. Erős D. Sc.
Dept. of Pharmaceutical Technology
Albert Szent-Györgyi Medical University
P.O. Box 121
6701 Szeged
Hungary