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Absorption Study with a Dissolution Independent Drug - Acetaminophen

OLIVER YOA-PU HU[1],SHANG-TAI HO[2],
SHU-FEI CHAN[3], JIN-SHING LAI[3], AND PING-HONG CHUNG[3]

1. Pharmaceutical Research Institute, National Defense Medical Center, Taipei, Taiwan, Republic of China
2. Department of Anesthesiology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, Republic of China
3. Veterans Pharmaceutical Plant, Taipei, Taiwan, Republic of China

ABSTRACT

Absorption of acetaminophen has been shown to be dissolution independent in human. Both extent and rate of absorption are important variables in the treatment of pain. The absorption of acetaminophen was studied by using two different acetaminophen tablets Depyretin® manufactured by Veterans Pharmaceutical Plant, Taiwan, and Scanol®, manufactured by Scanpharm, Denmark. Both Scanol and Depyretin, each of 500 mg single dose, were administered orally to sixteen healthy young Chinese males with an one-week washout period in a randomized crossover design. Plasma samples used for acetaminophen measurement were taken prior to drug administration and at various times up to 12 hr subsequent to administration. The plasma samples were measured for acetaminophen concentration by an HPLC method. Values for areas under plasma concentration-time curve (extent of absorption), peak plasma concentration, time to peak concentration (rate of absorption), elimination rate constant, elimination half-life and oral clearance were compared for each preparation. Statistical analyses including ANOVA, power analysis and 90% confidence intervals after logarithmic transformation, showed no significant difference in the rate and extent of acetaminophen absorption between Scanol and Depyretin. Furthermore, *in vivo* absorption did not significantly correlate with *in vitro* dissolution performance in water. However, differences between these two formulations both in *in vitro* dissolution and *in vivo* absorption were not seen.

Key words : Acetaminophen, absorption, natural logarithmic transformation, pharmacokinetics.

INTRODUCTION

Acetaminophen has been shown to be an effective analgesic and antipyretic agent used for releaving mild to moderate pain[1-3]. Acetaminophen is marketed in the United States under approximately 50 brand names and in almost 200 proprietary combinations with other drugs [4]. There are at least 36 brand names in the Republic of China.

Studies in healthy volunteers have shown that oral administration of acetaminophen is always associated with rapid absorption. Mattok

Correspondence to : Oliver Yoa-Pu Hu
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[5] reported that the absorption of acetaminophen *in vivo* is not dissolution dependent. Therefore, acetaminophen becomes a commonly used drug in the evaluation of gastric emptying[6-11]. Approximately 2% of acetaminophen is excreted unchanged in urine after administered. Glucuronide and sulfate conjugates are nontoxic and account for approximately 95 % of the drug, while a much smaller amount, estimated to be 3%, is oxidized to a chemically reactive intermediate via the hepatic cytochrome p-450 system which combines with liver glutathione to form a nontoxic substance. However, after massive single doses of acetaminophen the supply of liver glutathione is exhausted and the excess reactive arylating intermediate binds covalently to vital hepatocellular macromolecules, leading to necrosis. The best indicator of potential liver injury from acetaminophen is elimination half-life of acetaminophen. A half-life greater than 4 hours is uniformly associated with liver injury. Also, plasma levels greater than 300 μg/ml at 4 hours post-ingestion are consistant with liver injury, whereas levels less than 120 μg/ml at 4 hours post-ingestion are usually not[12].

With the increased general prescription of acetaminophen and because many pharmacists are now able to engage in drug product selection, bioequivalence studies are warranted in order to ensure the equity of therapeutic value regardless of its source of manufacture.

The primary purpose of this research was to study the comparative rate and extent of acetaminophen dissolution *in vitro* and absorption in human subjects using Scanol® and Depyretin ®. The results could be applied to ensured the same therapeutic effect of the locally made acetaminophen with the proprietary product. Secondly, to study the possible *in vitro* and *in vivo* relationship.

MATERIALS AND METHODS

I .*Drug*

Scanol (Batch No. B8779), produced by Scanpharm Pharmaceutical Plant, Denmark and Depyretin (Batch No. T-7202), produced by Veterans Pharmaceutical Plant, Taiwan, were obtained locally. According to the pharmacopoeia method of USP XXII, Scanol and Depyretin contained 99.1 $\pm$ 0.2% and 100.4 $\pm$ 0.3% of the labeled amount of acetaminophen respectively. These results were within the acceptable range, 95%～105%, as recorded in USP XXII. The content uniformity of these two products also met the USP XXII requirement. The bioavailability parameters were not normalized by the slightly different content.

II.*Apparatus*

The HPLC was equipped with a Waters 510 pump, Waters 712 WISP autosampler, Waters 441 variable wavelength Ultraviolet detector and a 740 integrator. Separations were performed at 40°C controlled by a cp-1100 W Thermo Watch on a C-18 Shodex pak column.

III.*Dissolution Test*

The dissolution test of the acetaminophen tablet was determined according to the general method of USP XXII. Apparatus 2, paddle stirring method (50 rpm; replication = 3), was used. Amount of dissolved durg at two-minute intervals was determined spectrophotometrically (λmax=280 nm).

IV.*Subject*

The subjects involved in this study were 16 healthy Chinese males (Table 1) whose body weights ranged from 60 to 85 kg (mean $\pm$ S. D.:67.1 $\pm$ 6.7kg), were within 10% of their ideal body weight, and whose ages ranged from 20 to 29 years (mean $\pm$ S.D.: 23.3 $\pm$ 2.2 years). All subjects were in good physical condition as determined by complete physical and clinical examinations such as complete blood count (hemoglobin, hematocrit, red blood cell count, red cell indices and white blood cell count with dif-

Table 1. Sex, age, weight and height for each subject

Subject	Sex	Age (years)	Weight (kg)	Height (cm)
1	M	29	60.0	170.0
2	M	22	65.0	171.0
3	M	25	61.0	178.0
4	M	23	77.0	182.0
5	M	22	63.0	175.0
6	M	23	65.0	180.0
7	M	22	63.5	170.0
8	M	23	68.0	170.0
9	M	23	64.0	174.0
10	M	22	66.0	181.0
11	M	21	60.0	169.5
12	M	20	68.0	174.0
13	M	24	63.5	166.5
14	M	26	73.0	182.5
15	M	22	71.5	174.0
16	M	25	85.0	174.5
Mean		23.3	67.1	174.5
S.D.		2.2	6.7	5.0

ferential); platelet count, blood urea nitrogen, serum creatinine, serum glutaminic-oxaloacetic transaminase, blood sugar, etc. before the study. The subjects were instructed to abstain from any drugs for at least 2 weeks prior to or during the study. No vitamin supplements were permitted 48 hours prior to each day's dosing. Subjects with a history of drug or alcohol abuse or drug sensitivity were excluded. To each subject the study was explained, and informed consent was obtained from each volunteer.

V. *Study Design*

A randomized double-blind cross-over design with a seven day washout period was used. The subjects were randomly divided into two groups(8/group). Each subject fasted from 10 PM of the night before each experiment. A single 500 mg acetaminophen tablet administered with 200 ml water was given between 7:30 and 9:30 a.m. on the following day. No solid or liquid, except water, were permitted until four hours subsequent to dosing. A 3 ml blood sample was obtained from the left forearm vein without anticoagulant just before and at 5, 10, 20, 30, 45, 60 minute intervals and 1.5, 2, 3, 4, 5, 6, 8 and 12 hours after dosing. The blood samples were centrifuged immediately to express plasma. All plasma samples were stored at -50°C until subsequent assays. Subjects remained under observation for 12 hours and maintained a low level of physical activity. Blood pressure, heart rate and any possible side effects were monitored closely throughout the study. The collected samples were assayed for acetaminophen by a self-developed, specific and accurate HPLC method.

VI. *Assay Method*

50 μl (100μg/ml) of internal standard acetanilid, 5 ml 1 M phosphate buffer and 10 cc ethyl acetate were added to 1 ml of subject plasma. The mixture was rotated for 15 minutes and centrifuged at 2100 rpm for 12 minutes to pellet the precipitated proteins. The upper organic layer was collected by disposable pipette and evaporated to dryness. Residue was reconstituted with 300 μl methanol, then centrifuged at 3000 rpm for 12 minutes before HPLC injection. The injected volume was 100 μl.

The assay for acetaminophen and acetanilid was performed at 40°C and controlled by Thermo Watch cp-1100 W on a C-18 Shodex pak column. The mobile phase consisted of 15 % acetonitrile and 85 % water. All water was Milli -Q grade. A 1.8 ml/min flow rate was employed and the eluent was monitored by a Waters 441 UV detector at 254 nm under an attenuation of 128. Under these conditions, the retention times of acetaminophen and acetanilid were 5 and 15 minutes respectively.

In order to validate the precision of sample preparation and HPLC analysis, within-day and between-day standard curves were run. In addi-

tion, along with each subject's sample analysis, a standard curve was constructed from each subject's blank plasma.

VII. *Data Analysis*

Area under plasma concentration-time curve from time zero to infinity (AUC∞) was calculated by:

$$AUC\infty = AUC_{0\text{-}t} + Cp/\beta$$

where $AUC_{0\text{-}t}$ is the area under the plasma concentration-time curve estimated from time 0 to t by using trapezoidal rule and t is the last sampling time. Cp is the last measured concentration-time point. The apparent elimination rate constant, β, was estimated from the slope of the terminal log-linear phase of the semilog plot of concentration versus time. The maximum plasma concentration achieved (Cmax) and the time to maximum plasma concentration (Tmax) were observed from the measured plasma concentrations following drug administration.

Pharmacokinetic parameters (presented as mean $\pm$ s.d.) such as elimination half-life, apparent volume of distribution, and total plasma oral clearance were calculated from each individual subject according to the standard formulae (Gibaldi & Perrier, 1982)[13].

Analysis of variance (ANOVA), power analysis and 90% confidence intervals of AUC∞, Cmax, LnAUC∞ and LnCmax were used to statistically evaluate of the data and for the assessment of bioequivalence.

The 90% confidence interval (C.I.) for AUC∞ and Cmax can be obtained as follows:

$$90\%\ C.I. = [\ \text{Mean (test)} - \text{Mean (reference)}\ ] \pm t\ (\alpha/2,\ n_1+n_2-2)\sqrt{0.5 \cdot MSE(1/n_1+1/n_2)}$$

where n_1 and n_2 are the subject number in group 1 and group 2 respectively. While $n_1=n_2$, the above equation can be reduced as follows:

$$90\%\ C.I. = [\ \text{Mean (test)} - \text{Mean (reference)}\ ] \pm t\ (\alpha/2,N\text{-}2)\sqrt{2 \cdot MSE/N}$$

where N is the total number of subject, $N = n_1 + n_2$.

After log transformation, aside from a 90% C.I. range change to 0.8-1.25, an anti-log was taken to obtain the ratio of the test and reference products instead of the difference.

The *in vivo* percent of absorption for Scanol and Depyretin at any given time was also determined by using Exact Loo-Riegelman method[14]. The following equation was used to calculate the extent of acetaminophen at any given time:

$$\%\ \text{absorbed} = 100\% \cdot \frac{A_T}{V} = C_T + k\int_0^T Cdt$$

$$\frac{A_{max}}{V} = \frac{1}{n}\sum_{i=1}^{n}\left\{C_T + k\int_0^T Cdt\right\} = k\int_0^{Inf} Cdt$$

where A_T and Amax are amount of drug absorbed or which reaches the central compartment in time T and infinity respectively; V: volume of central compartment; C_T : concentration of drug in the central compartment at time T; k: first order elimination rate constant; n is the number of C.

RESULT

Both Scanol and Depyretin tablets had 85 percent of amount dissolved in water at 37^{0}C within 13 minutes. The sixteen volunteers were in good physical conditions as determined by complete physical and clinical examinations. None experienced any adverse effects during or after the experiment. Each subject's sex, age, weight and height are shown in Table 1 and all are within normal ranges.

Under the described assay conditions, linearity was observed in plasma standard curve over a range of 0.2 - 30 μg/ml (Fig. 1). Plasma within-day and between-day standard curves had a correlation coefficient, r, greater than 0.998. Although correlation between *in vitro* dissolution and *in vivo* absorption of acetaminophen was not seen, both Scanol and Depyretin had a similar profile of percent dissolved in water and percent absorbed *in vivo* (Fig. 3). Both exhibited 85% of tablets dissolved in water within 13 minutes and over 90% dissolved within 30 minu-

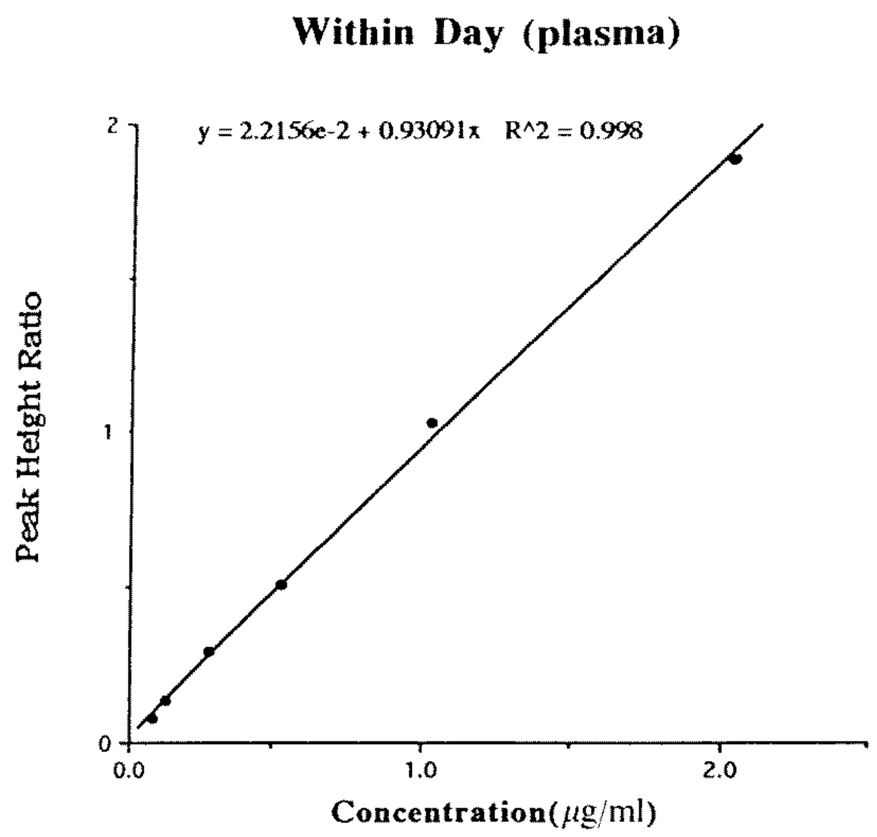

Figure 1. Acetaminophen within-day and between-day standard curves in plasma (Mean ± S.D., n = 3).

tes. Over 80% of acetaminophen was absorbed within 45 minutes after the oral dosing.

The mean plasma concentration-time data is listed in Table 2 and shown in Figure 2. The time to maximum plasma concentration(Tmax), areas under the plasma concentration-time curve from time 0 to infinity(AUC∞), peak plasma concentration(Cmax) and the values of their natural logarithmic transformation and elimination half-life, elimination rate constant, oral clearance and apparent volume of distribution were all compared between the two brands and

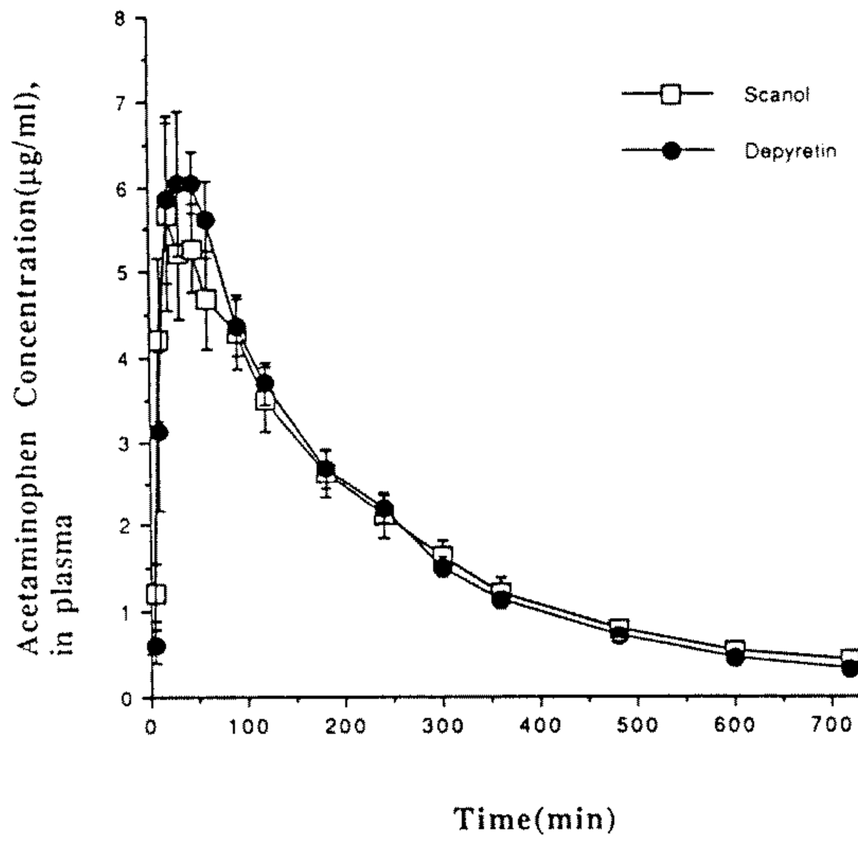

Figure 2. Acetaminophen plasma concentration-time profile after oral administration of 500 mg acetaminophen tablets in 16 normal healthy volunteers. (Mean ± S.E.)

shown in Table 2 and 3 respectively. Statistical analysis was determined by analysis of variance (ANOVA) and two one-sided tests. Mean peak plasma concentration(Cmax) had a wider 90% confidence intervals range than the range of 0.8-1.2 and a less power value than 0.8 indicating that it has no significant effect in this study. However, by applying the natural logarithmic transformation method after considering the additivity (linearity) property of the ANOVA with general linear model and the normality of the parameters, such as AUC∞ and Cmax, it showed no significant difference in the two preparations. In other words, the rate and extent of acetaminophen absorbed did not differ significantly between these two brands. Both LnAUC∞ and LnCmax had 90% confidence intervals between 0.8 and 1.2 with very high statistical power. By comparing the overall oral clearance of these two preparations with those of each subject, intrasubject variation can be tolerable.

Table 3 summarizes pharmacokinetic parameters obtained from the sixteen subjects following an oral 500 mg dose of acetaminophen. With analysis of variance, Scanol possesses a

Table 2. Bioavailability parameters for acetaminophen

Parameters	Scanol		Depyretin		Statistics (ANOVA)
	MEAN	STD	MEAN	STD	(90% CI; Power)
mean peak time (min)	42.50	32.86	36.88	20.73	not significant
mean peak plasma conc. (μg/ml)	8.23	3.45	8.24	3.07	not significant (0.67-1.33; 0.45)
mean AUC∞ (μg*min/ml)	1413.09	587.37	1401.00	308.54	not significant (0.80-1.18; 0.88)
Ln mean peak plasma conc. (μg/ml)	2.02	0.43	2.05	0.35	not significant (0.86-1.17; 0.97)
Ln AUC∞ (μg*min/ml)	7.16	0.45	7.22	0.22	not significant (0.97-1.03; 1.00)

Table 3. The pharmacokinetic parameters in 16 healthy volunteers following 500mg of oral dose of acetaminophen

Parameters	Scanol	STD	Depyretin	STD	Overall	STD	Statistics (ANOVA)
t1/2(β) (hr)	3.34	0.94	3.43	1.29	3.38	1.12	n.s.
k (min^{-1})	0.004	0.001	0.004	0.001	0.004	0.001	n.s.
Cl/F (ml/min)	428.4	214.1	373.2	81.1	400.8	161.71	n.s.
Vd/F (L)	118.66	66.39	112.36	56.69	115.51	60.81	n.s.

t1/2(β) : elimination half-life

k : elimination rate constant

Cl/F : apparent oral clearance

Vd/F : apparent volume of distribution

n.s. : not significant

shorter mean elimination half-life, the same mean elimination rate constant, but not a significantly larger means of oral clearance or volume of distribution compared to Depyretin.

DISCUSSION

No significant difference was exhibited in age, body weight and height between the two groups involved in this study. Since a double-blind randomized cross-over design was used, intersubject variation was therefore minimized. From the pharmacokinetic data including oral clearance, half-life, apparent volume of distribution between proprietary and generic products, intrasubject variation can be acceptable. Factors including individual physiological variation, emotional responses, G-I evacuation[6,15], extent of physical activities, and others may contribute to this intrasubject variation.

Both Scanol and Depyretin exhibited very similar profiles in all *in vitro* percent dissolved and *in vivo* absorption. Both exhibited 85% of tablets dissolved in water within 13 minutes and over 90% dissolved within 30 minutes. Over 80% of acetaminophen was absorbed within 45 minutes after the oral dosing. Depyretin appeared to have an insignificantly greater percentage and faster of drug absorption. Analysis of variance (ANOVA) confirmed the insignificant

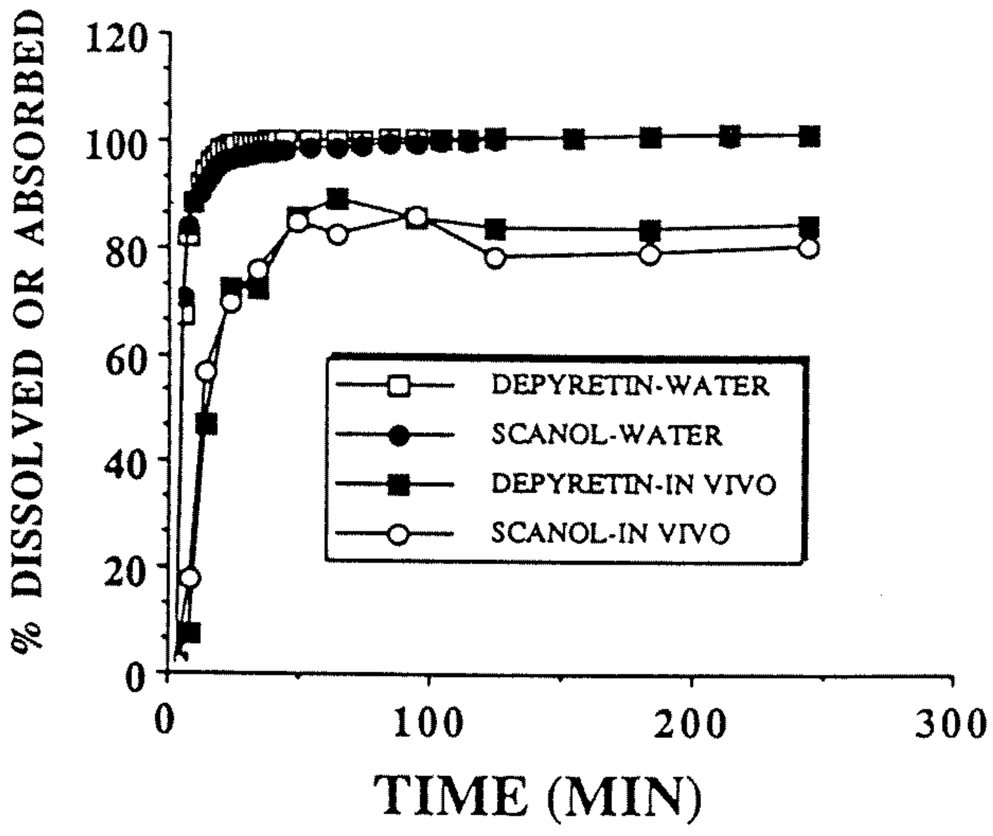

Figure 3. The *in vitro* percent dissolved-time plot and *in vivo* percent absorbed-time plot of Scanol and Depyretin.

difference of each acetaminophen plasma concentration at each given time and acetaminophen's parameters including mean peak time, AUC∞ and the values of its natural logarithmic transformation. Mean peak plasma concentration showed a 90% confidence intervals range (0.67-1.33) which is wider than 0.8-1.2. However, by applying logarithmic transformation method with consideration of the additivity (linearity) property of the ANOVA with general linear model, the rate and extent of absorption of acetaminophen with the two brands was found the same. This also verified by 90% confidence intervals and by power analysis. These phenomena suggest that the rate and extent of absorption between the two acetaminophen tablets produced by different brands had no significant differences. Therefore, we can conclude that these two formulations are bioequivalent.

This study not only reveals the independence between dissolution and absorption but also ensures the same therapeutic effect of the locally made acetaminophen with the proprietary product. Such information can promote the trust and the effectiveness of Taiwan pharmaceutical technology which will naturally widen locally made products' trading channels allowing them to compete in competitive international markets.

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體內吸收和溶離度無關之 acetaminophen的生體相等性研究

胡幼圃[1]何善台[2]
詹素妃[3]賴金星[3]鍾柄泓[3]

國防醫學院藥學系[1]　三軍總醫院麻醉科[2]　榮民製藥廠[3]

摘　要

Acetaminophen經研究認爲其體內吸收和體外溶解度不相關。本研究報告Acetaminophen國人之藥動力學性質與相對生體可用率之研究報告。以十六個自願健康年輕中國男性爲研究對象，經隨意雙盲交叉實驗設計，二次給藥（Scanpharm藥廠及榮民製藥廠之acetaminophen）間隔時間至少相隔一週。給藥500mg後，定時定次採血至12小時。血漿acetaminophen濃度自行改良發展以acetanilid爲內標準品，可精確，準確之高壓液相層析定量。Acetaminophen之血漿濃度曲線下面積(AUC∞)，最高血漿中濃度(Cmax)，到達最高血漿濃度時間(Tmax)，排除速率常數，半衰期，口服清除率，均經計算及互相比較。結果顯示二種錠劑無統計上明顯差異。經對數轉換後90%可信區間及power可推論二藥具生體相等性。而比較其在體內吸收及體外溶離度之關係，本研究再次顯示二者無相關性。

關鍵字：Acetaminophen錠劑，相對生體可用率，藥物動力學。