Biopharmaceutical Studies on the Clinical Inequivalence of Two Carbutamide Tablets

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Biopharmaceutical investigations were made to determine the reason for differences in therapeutic responses to two types of carbutamide tablets with the same carbutamide content. A two-way cross-over study in five healthy adult men on the serum concentration of carbutamide, measured by HPLC, showed differences between the tablets in terms of the mean serum concentration at 2 h (31.48 and 42.89 μg/ml, respectively) and C_{max} (37.30 and 48.83 μg/ml, respectively). These findings were reflected in differences in therapeutic effects. Marked differences were also found between the tablets in *in vitro* tests, *e.g.*, in disintegration time, hardness and dissolution rate. The findings were consistent with clinical findings only when a pre-incubation technique was employed in the *in vitro* dissolution test.

Keywords——carbutamide; bioavailability; dissolution; HPLC; tablet; serum level

Carbutamide is an oral sulfonylurea compound widely used in Tunisia in the management of maturity-onset diabetes mellitus. Two products, designated as tablets A and B, which are both indicated to contain 500 mg of the main drug are used. Patients are adequately controlled by a given daily dose of carbutamide in product B, but not by brand A tablets. Therefore, biopharmaceutical studies were performed to determine the reason for this clinical discrepancy. However, little work has been done on comparative studies of the bioavailabilities of chemically equivalent carbutamide tablets. It is not known which factors predominantly determine the bioavailability. Our approach was therefore to look for some *in vivo–in vitro* correlation.

Experimental

Materials——Two types of commercial tablets, designated as tablets A and B, were purchased from local pharmacies in Tunisia. Each tablet was indicated to contain 500 mg of carbutamide. Carbutamide powder was a commercial product (Ono Pharmaceutical Co. Ltd., Osaka, Japan). All other chemicals were of reagent grade.

Solubility——The solubility profile was determined by measurement of the drug concentration in saturated aqueous solutions of carbutamide shaken at 30 strokes/min at 37°C for 48 and 72 h at pH 1.2 (dilute HCl), 3, 4, 5, 6 and 7.2 (McIlvaine buffer). Samples were taken using a syringe equipped with a membrane filter (1.0 μm), and the absorbances were read after dilution with pH 9.2 phosphate buffer (0.1 M) at 254 nm.

Disintegration Time——Disintegration times were determined in an apparatus conforming to JP IX specifications, using 6 tablets of each product. Tablets were tested in aqueous medium adjusted to pH 1.2 (dilute HCl) and maintained at 37°C.

Carbutamide Content of Tablets——Tablets of both brands were analyzed for carbutamide by grinding 6 tablets and dissolving 10 mg of the resulting powder in ethanol. The suspension was centrifuged and a given volume of the supernatant was diluted with 0.1 M phosphate buffer (pH 9.2). Its absorbance was read at 254 nm.

***In Vitro* Dissolution Test**——a) *In vitro* tests were carried out by the USP XIX paddle method, and the oscillating method with a disk.[2] The dissolution medium consisted of 900 ml of distilled water adjusted to pH 1.2 (dilute HCl), 4 or 7.2 (McIlvaine buffer), and maintained at 37°C. A stream of sample was pumped at 3.0 ml/min through a flow-cell using tubing with a glass filter (3G) at the end, and the absorbance of the medium was measured spectrophotometrically. The rotating speed of the paddle was 120 rpm.

b) Tests of *in vitro* release after pretreatment of the tablets were also performed by the USP XIX paddle method (rotating speed: 120 rpm). The pretreatment consisted of disintegration of the tablets in

5 ml of deionized water with shaking at 90 strokes/min at 37°C for 30 min in a bottle. The resulting suspension was poured into the dissolution medium, and the bottle was washed with 10 ml of deionized water to recover all the suspension.

Hardness——Hardness was determined in a hardness tester (Kiya Seisakusho Ltd., Tokyo, Japan), using 12 tablets of each product.

In Vivo **Study**——Studies were made on five male volunteers, weighing 60 to 78 kg and aged 22 to 26 years. A dose of 250 mg of carbutamide (half a tablet) was administered to each subject with 200 ml of water by a two-way cross-over design with a 3-week washout period between administations. All subjects fasted from 8 h before to 4 h after drug administration. Blood samples (6 ml) were taken at 1, 2, 3, 4, 6, 8, 24, 32 and 49 h after drug administration, and the serum was stored in a freezer ($-15°C$) until assayed.

Determination of Carbutamide in Serum by HPLC——A stainless steel column ($4\,mm \times 150\,mm$) of styrene-divinylbenzene copolymer (ammonium substituted, Hitachi Gel 3011-N) was used. The chromatographic solvent was as follows: 0.1 N NaOH: 0.1 M NaCl: 65% methanol (v/v). The temperature was ambient, and the flow rate was 1.1 ml/min. The detector (254 nm) was set at a sensitivity of 0.02 absorbance unit full-scale, and the chart speed was 2 mm/min. Double extraction was carried out using chloroform and phosphate buffer. Carbutamide was extracted from serum (1.0 ml) with chloroform (6 ml) at pH 4 (2 ml of phosphate buffer, 0.1 M), and then the sample was re-extracted from the organic phase (4 ml) with 0.5 ml of 0.1 M phosphate buffer (pH 9.2) containing sulfamonomethoxine (10 µg/ml) as an external standard. The concentration of carbutamide in a 20 µl sample was finally determined by HPLC at 254 nm. The retention times of carbutamide and sulfamonomethoxine were 5.6 min and 8.8 min, respectively.

Results and Discussion

Solubility

Carbutamide has two pK_a values (1.6 and 5.75).[3] The solubility profile in Fig. 1 shows a minimum at pH 4 (solubility: 0.52 mg/ml).

In Vitro Experiments

The contents of carbutamide, disintegration times and hardnesses of both tablets are listed in Table I. Tablet B showed a very large time of disintegration, but the means of hardness for the two products did not differ significantly. Tests of dissolution of the tablets by the paddle method showed that the dissolution rate of tablet A, which is supposed to have
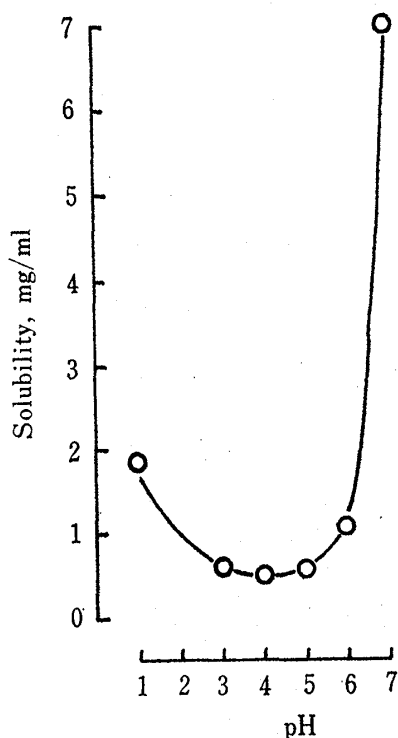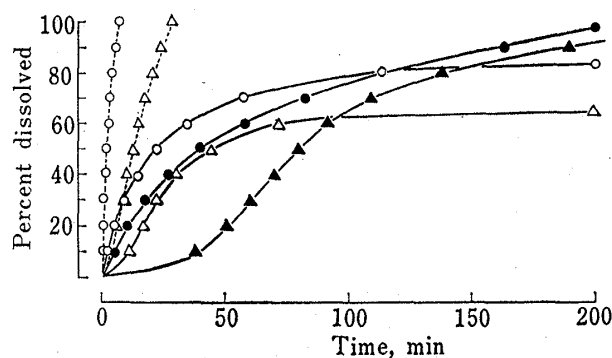

Fig. 1. Solubility Profile of Carbuta-mide at 37°C

Fig. 2. Effect of pH Value of the Dissolution Medium on the Dissolution Rates of Carbutamide Tablets, measured by the Paddle Method (USP XIX)

A	B	
—●—	—▲—	pH 1.2
—○—	—△—	pH 4
—○—	--△--	pH 7.2

TABLE I. Some Pharmaceutical Properties of Carbutamide Tablets used for the Tests

Tablet	Content mg (%)	Disintegration time (S.D.) min	Hardness (S.D.) kg
A	506.4(101.3)	3.53(0.74)	13.37(9.14)
B	486.9 (97.4)	45.94(8.64)	12.29(3.30)

less clinical efficiency, was faster at all pH values tested though tablet A showed an incomplete dissolution at pH 1.2 and 4 (Fig. 2). In the oscillating basket method, which has a greater mechanical destructive force,[2] the dissolution rate of tablet B was enhanced, especially at pH 1.2. However, the dissolution from tablet A at pH 4 was only 70% after 200 min, probably due to the weak agitating intensity of the method.[2] Thus the poor disintegrating efficiency of the paddle apparatus was probably mainly responsible for the slow dissolution of tablet B, and the higher mechanical destructive efficiency of the oscillating basket apparatus improved the dissolution process. The differences in dissolution rates of the tablets may be attributed to the large differences of disintegration times of these tablets (Table I). On dissolution of already disintegrated tablets, the dissolution rates of both tablets, especially of tablet B, were higher at all pH values tested (Fig. 4). In the pretreatment method, tablet B showed faster dissolution than tablet A.
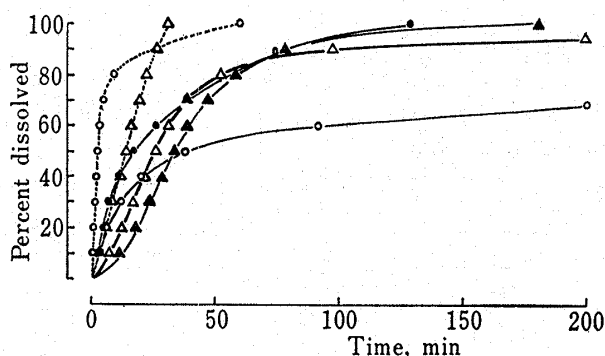
Fig. 3. Effect of pH Value of the Medium on the Dissolution Rates of Carbutamide Tablets, measured by the Oscillating Basket Method as modified by Ogata et al.[2]
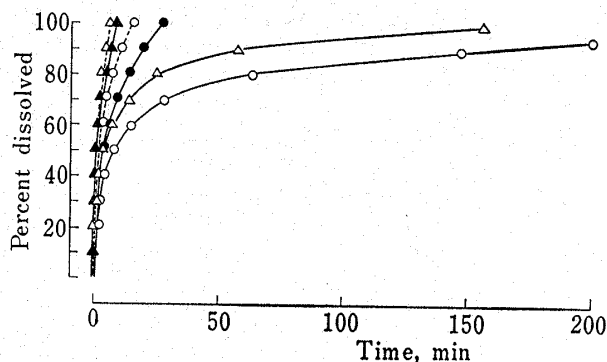
A	B	
—●—	—▲—	pH 1.2
—○—	—△—	pH 4
--○--	--△--	pH 7.2

Fig. 4. Dissolution Rates of Pretreated Carbutamide Tablets, by the Paddle Method

A	B	
—●—	—▲—	pH 1.2
—○—	—△—	pH 4
--○--	--△--	pH 7.2

Human Study

Table II shows the average serum concentrations at sampling times, the peak serum levels (C_{max}), the peak times (t_{max}), and the areas under serum level–time curves from 0 to 49 h ($AUC_{0-49 h}$). The results of ANOVA are also shown. Figure 5 is a plot of the serum levels of carbutamide for 49 h after administration of tablets. As can be seen, tablet A had somewhat lower values for most parameters than tablet B, and especially for the serum levels at 2 and 3 h and the peak serum level. In preliminary experiments using tablet B, we had obtained a half-life of about 24 h in the terminal phase, and we designed a protocol such that the blood was withdrawn during 49 h (2 half-life times). However, tablet A showed a longer half-life (about 40 h) between 8 and 49 h probably because of the very slow absorption of the drug from tablet A. The serum levels after administration of tablet A did not reach the post-absorptive phase till 49 h. Thus, we can not use $AUC_{0-49 h}$ or $AUC_{0-\infty}$ values for evaluation of the extent of the bioavailabilities. We can therefore discuss the bioavailabilities only in

terms of C_{max}, which represents the rate of bioavailability. If statistical evaluation shows significant differences between the tablets in terms of serum levels, it can be expected that very slow absorption is primarily responsible for the clinical inequivalence of the two products. However, the ANOVA shows that there was no statistical difference between the selected parameters for the two products. The two tablets do differ in clinical efficiency, but the data obtained in the present study seem to be insufficient to substantiate the lack of bioequivalence. Therefore, we consider that observed differences between the products, even though not statisticaly significant, are likely to be real from the clinical standpoint. Thus, the power of analysis of variance in our study has to be reconsidered.

The power of analysis, $1-\beta$, is directly related to the ϕ value, which is described by the following equation[4]

$$\phi^2 = \frac{[\sum_{i=1}^{k} x_i^2 + (\sum_{i=1}^{k} x_i)^2/k]N}{ks^2}$$

where

β : type II error

x_i: mean of the "i" treatment

k : number of treatments

s^2: residual mean square

N: total number of subjects used in the cross-over design

TABLE II. Mean Serum Concentrations and Related Parameters of Carbutamide after Oral Administration of a 250 mg Carbutamide Tablet to Humans

Time (h)	Mean serum concentration (μg/ml)				Result of ANOVA
	Treatment A	C.V. (%)[a]	Treatment B	C.V. (%)	
1.0	29.00	32.7	27.40	52.4	N.S.[c]
2.0	31.48	38.3	42.98	14.8	N.S.
3.0	34.04	32.2	39.09	26.1	N.S.
4.0	34.73	25.5	44.52	16.8	N.S.
6.0	32.14	27.7	42.43	18.5	N.S.
8.0	32.09	20.2	40.66	14.4	N.S.
24.0	25.65	14.8	31.61	21.4	N.S.
32.0	20.95	28.6	25.95	23.4	N.S.
49.0	16.25	14.3	15.64	18.2	N.S.
C_{max}, μg/ml	37.30	22.0	48.83	11.2	N.S.
t_{max}, h	3.2	40.6	2.6	50.0	N.S.
AUC_{0-49h}, μg/ml/h[b]	1210.18	18.2	1480.20	16.8	N.S.

a) Coefficient of variation.
b) Calculated by trapezoidal rule.
c) Not significant.

TABLE III. ϕ Value of the Bioavailability Test used in This Paper and Estimated Number of Subjects required to achieve $1-\beta\geq0.6$ or 0.8, assuming a 20% Difference between the Tablets

	x_1[a]	s^2	ϕ[b]	Estimated number of subjects[c]	
				$1-\beta\geq0.6$	$1-\beta\geq0.8$
C_{max}	48.83	90.927	1.145	12	18

a) The larger mean value in the treatment.
b) Calculated using $N=5$, $a=0.05$ and Δ(difference between the treatments)$=0.2$.
c) Number of subjects required to achieve $1-\beta\geq0.6$ or 0.8, assuming a 20% difference in the selected bioavailability parameter between the tablets with $a=0.05$.

It is clear from this equation that for given values of the residual mean square (s^2) and number of treatments (k), ϕ is directly proportional to $\sqrt{N}$, where N is equal to the total number of subjects involved in the study. Accordingly we estimated the number of subjects, N, required to achieve $1-\beta \geq 0.6$ or 0.8, assuming 20% difference in C_{max} at the 5% level of α. Table III shows that for statistical evaluation of C_{max}, 12 subjects are needed to achieve a power of 0.6. Thus, if the present study was repeated using 12 subjects in one group, we should be able to detect a 20% difference in the C_{max} value between treatments with a power of 0.6 and $\alpha = 0.05$.
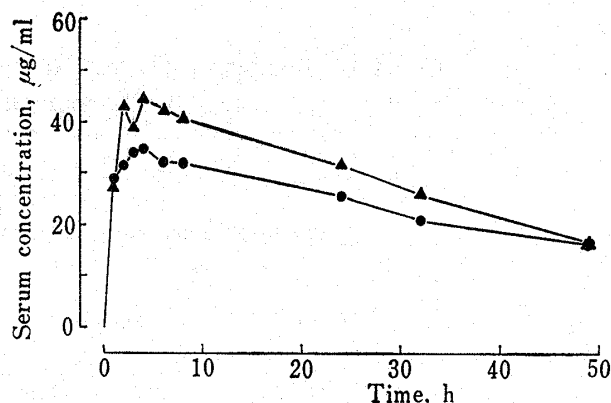

Fig. 5. Mean Serum Levels of Carbutamide after Oral Administration of 250 mg of Carbutamide as Half a Tablet of A (●) or B (▲)

Conclusion

In the present study, we attempted to determine the biopharmaceutical reasons for the discrepancy in therapeutic effectiveness of two carbutamide tablets. The main findings were that tablet B, which is supposed to be more effective therapeutically, had a longer *in vitro* disintegration time (45.9 ± 8.6 min) than tablet A (3.5 ± 0.7 min), but that the absorption rate from tablet B was greater than that from tablet A. The absorption rates probably account for the clinical differences between the tablets, though significant differences could not be found statistically due to the small number of subjects used in the bioavailability test.

It is very interesting that tablet B showed better bioavailability than tablet A in spite of the very long disintegration time. Ritschel pointed out that particle size could be of paramount importance in the bioavailability of drugs having low solubility in water or biological fluids, especially if the solubility is less than 0.3%.[5] This is the case with carbutamide at low pH values. For drugs such as carbutamide that dissolve and are absorbed mainly in the small intestine, the dissolution rate, subsequent to disintegration, is a rate-limiting step in absorption. However, in dissolution tests for such drugs we can not cancel out the effect of disintegration on dissolution if the test is carried out at a fixed pH without any pretreatment. Ogata *et al.* suggested from experiments with sulfonamides that a drug having a solubility larger than 0.3% in 0.1 N HCl at 37°C can reasonably be tested for dissolution rate in an acidic medium, while a drug having a solubility less than 0.3% should be tested under neutral conditions after pretreatment in acidic conditions.[6] The carbutamide study reported in this paper seems to support their suggestion.

References and Notes

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2) H. Ogata, T. Shibazaki, T. Inoue, and A. Ejima, *J. Pharm. Sci.*, **68**, 708 (1979).
3) R. Alric and R. Peuch, *J. Pharmacol.* (Paris), **2**, 141 (1971).
4) J.G. Wagner, "Fundamentals of Clinical Pharmacokinetics," Drug Intelligence Publication, Illinois, 1975, Chap. 8.
5) W.A. Ritschel, "Problems of Clinical Pharmacology in Therapeutic Research: Phase I," ed. by H.P. Kuemmerle, T.K. Shibuya, and E. Kimura, Urban and Schwarzenberg, München, 1977, pp. 116—168.
6) H. Ogata, T. Shibazaki, T. Inoue, and A. Ejima, *Chem. Pharm. Bull.*, **27**, 1281 (1979).