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A new approach to the study of serum concentrations of orally administered cephalexin

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Studies of cephalexin in man showed that the conventionally constructed average serum level curves could give misleading results, with the average peak serum level from the curve being lower than the peak titres of the individual curves contributing to it. It was also difficult to determine whether there were significant differences between such curves obtained from different preparations. Smooth curves were constructed from the observed data by computer methods; they were then arranged by the computer to have their peaks coincident in time and then averaged to give a curve much more similar in shape to its components. After the computer had fitted the smooth curve for each individual, it was possible to calculate a number of different parameters for each volunteer. Analysis of variance could then be done on these parameters, and thus significant differences between the results from tests on different preparations could readily be determined.

Cephalexin, a new orally administered cephalosporin antibiotic, is virtually completely absorbed from the upper small intestine, both in experimental animals (O'Callaghan Ryan & others, 1970) and in man (Muggleton, O'Callaghan & others, 1968) and is excreted unchanged in the urine, partly by glomerular filtration and partly by tubular secretion. Some problems posed by the studies of blood levels in men after administration of different doses and different types of preparation have been investigated in detail. The solutions adopted and the conclusions drawn are presented here.

MATERIALS AND METHODS

Cephalexin (7β-(D-α-aminophenylacetamido)-3-methylceph-3-em-4-carboxylic acid) is sparingly soluble in water (about 1·5% at neutral pH). It was given as a 5·25% suspension in 60% sucrose solution, or as two different solid preparations in capsules. All three preparations are commercially available. Of the two types of capsule used, one contained 500 mg cephalexin and virtually no excipient, the other contained 250 mg cephalexin and an approximately equal weight of corn starch. Both products were presented in the same type of size 0 capsule.

Assay of cephalexin

Cephalexin was assayed in serum by the large plate agar diffusion method against *Bacillus subtilis* (Muggleton & others, 1968). A spore suspension of *B. subtilis* ATCC 6633 was inoculated at 0·01% into agar made up as follows: 0·5% peptone, (Oxoid), 0·3% Lab Lemco, 1% sodium citrate and 1·5% agar (Oxoid) at pH 7.

Standard solutions

Standard solutions of cephalexin were prepared at 500 μg/ml in 0·2M phosphate buffer at pH 6 and stored at 4° for up to 4 days. Working standards at the required concentrations were freshly prepared each day by dilution into pooled human serum.

Volunteers

Young, healthy, men were given one dose of cephalexin in the morning about 1 h after a standard light breakfast (2 slices of buttered toast and preserve and 2 cups of tea or coffee), unless otherwise stated. Blood was taken by venepuncture at ½, 1, 1½, 2, 3, 4 and 6 h after the dose, although not all the men were bled at all the times. The serum was separated and its antibiotic content estimated as described above.

RESULTS

Average serum level curves

The magnitude and duration of the serum concentrations of cephalexin were obtained for each individual. The averaged results for each type of preparation and each dose are given in Table 1. Such values have been reported for cephalexin

Table 1. *Average serum concentrations as observed after oral doses of cephalexin*

Dose in mg	Type of preparation	Fed or Fasting	Serum concentration in μg/ml at h ½	1	1½	2	3	4	6
1000	Tight fill capsule	Fasting*	15·5	28·0	NT**	20·2	NT	4·7	<1·6
1000	,, ,,	Fed, 1 dose	1·25	11·6	NT	18·5	NT	9·4	1·8
1000	,, ,,	Fed, 5 doses	2·9	8·0	NT	22·5	NT	11·6	2·3
500	Tight fill capsule	Fasting	4·3	11·5	12·4	11·5	6·0	2·7	<1·9
500	,, ,,	Fed, 1 dose	4·2	13·2	NT	11·5	NT	3·9	<1·25
500	,, ,,	Fed, 5 doses	1·0	11·5	NT	14·4	NT	5·8	NT
500	Loose fill capsule	Fasting	3·0	11·5	12·9	12·5	7·1	2·8	NT
500	Suspension	Fasting	14·8	13·9	NT	8·6	NT	1·3	<1·3
300	Solution	Fasting	14·6	10·1	NT	3·1	2·0	<1·2	NT
250	Tight fill capsule	Fasting	<2·9	5·1	6·4	6·8	NT	1·3	<0·4

* Fasting volunteers had had a minimal breakfast consisting of two small slices of toast and two cups of tea or coffee one hour before the dose.
** NT = Not tested.

(Gower & Dash, 1969; Griffith & Black, 1968; Kind & Kestle & others, 1968; Perkins, Carlisle & Saslaw, 1968). There were wide variations in the time and magnitude of the peak titre similar to those shown by Clark & Turck (1968). When the results from the individuals in any one experiment were compared with the average serum levels the peak serum level of the average was lower than the peak serum level of every individual contributing to it (Table 2). When serum concentration was plotted against time, the curve from the average was much flatter and wider than the curves from the individual values (Fig. 1). It was concluded that no meaningful statistical comparison could be made between the types of preparation given, nor could the effect of the different doses be estimated.

Mathematical expression of the cephalexin serum level curve

Examination of the individual serum level curves obtained from 95 volunteers showed that in most cases the serum level curve could be expressed by the formula:—

$$y = A(e^{-p(t-t_0)} - e^{-q(t-t_0)})$$

Table 2. *Individual serum concentrations obtained after one dose of two loose fill low density 250 mg capsules and the average calculated from them*

Volunteer	Serum concentration in μg/ml at h					
	½	1	1½	2	3	4
1	0·7	17·1*	16·4	9·7	4·9	2·2
2	0·5	12·0	13·8	13·9*	5·2	2·2
3	9·4	18·1*	9·7	5·1	2·2	1·1
4	0·7	7·2	9·2	14·2*	11·7	7·7
5	<0·5	4·3	13·3	19·5*	6·0	3·2
6	2·9	6·5	11·0	15·7*	5·7	3·3
7	12·0	23·7*	13·2	4·1	2·3	1·3
8	<0·5	3·3	16·7	17·0*	4·3	2·1
Average	3·4	11·5	12·9*	12·4	5·3	2·9

* Peak titres.

in which y is the serum level at time t after administration, and the constant t_0 is the time taken for the dose administered to reach the site of absorption. The constants p and q are respectively related to the rate constant concerned with the speed of absorption and the rate constant concerned with the speed of excretion, there being no loss due to destructive metabolism. The constant A, given the values of p and q, and t_0 determines the area under the serum level curve. The same model was used by Mueller & Lieberman (1970) for salicylate blood levels.

The rationale behind this simple equation is that cephalexin, once it has reached the site of absorption, is absorbed so rapidly that it behaves as if it were an injectable

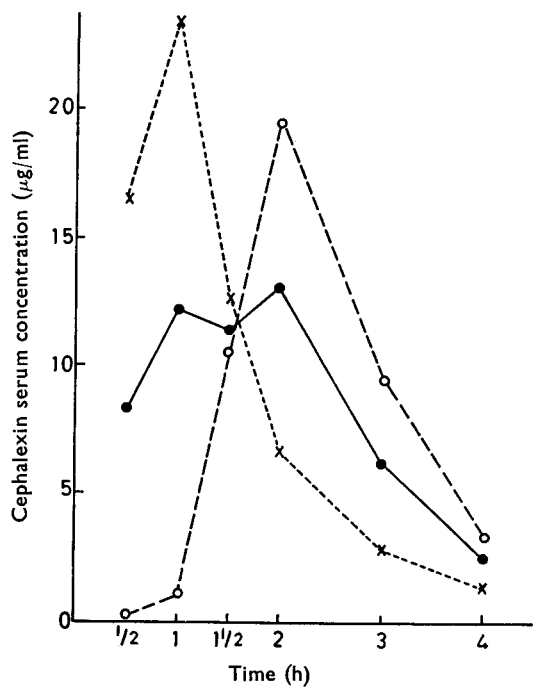

FIG. 1. Serum level curves from 2 volunteers after 1 oral dose of 500 mg cephalexin (observed values) and the conventional average curve constructed from them.

antibiotic. The chemically related injectable antibiotic cephaloridine is known to comply with an equation of this form but with $t_0 = 0$.

The constants A, p, q and t_0 are estimated from the observations by the method of least squares. However, the usual methods do not generally converge and it was necessary to use a descent method derived from that of Fletcher & Powell (1963). Even this method can lead to overflow in the computer unless special precautions are taken when p and q happen to be approximately equal.

A serum level curve, obtained from concentrations measured at arbitrary times, may miss the peak concentration actually achieved. When the formula was fitted to the data, however, the rate constant for absorption, the rate constant for excretion, the delay before any cephalexin was detected in the serum and the area under the curve, could all be calculated. Using these constants, fitted values were interpolated at close time intervals and, in this way, fitted curves were drawn. One of these, as drawn by the computer, is shown in Fig. 2; it has a sharp peak and illustrates that the highest observed serum concentration may not be the actual peak titre.

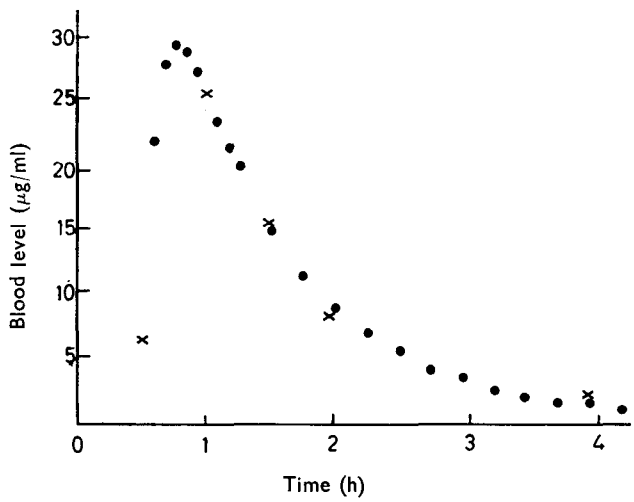

FIG. 2. An example of a computer fitted curve for the observed serum levels from one volunteer after 1 oral dose at 500 mg cephalexin. X Observed points. ● Computer plotted points.

With some volunteers or preparations, the serum concentration measured at one or more times was zero; if this meant that there were then less than 4 actual values measured, the calculation could not be applied. It was also not possible to calculate the constants if the earliest or the latest value obtained was the highest. The fitted curves were drawn for 95 of the 124 sets of serum levels actually measured, i.e., 77% overall. In the later experiments, the times chosen for bleeding were grouped more closely around the expected peak time, and over the last 30 volunteers the number where computer calculation was possible increased to 90%.

Construction of average serum level curves

The fitted curves for all the individual volunteers in any one experiment were, in a sense, moved along the time scale by the computer so that each peak occurred at the

origin of the time scale. Average curves were then computed and were found to be very much more like their individual component curves; they avoided the anomaly of having an average peak value markedly lower than all the values contributing to it.

Parameters calculated from the fitted curves

The calculation whereby the fitted curves were obtained also gave the following information for each individual:

1. Time of first blood level. 2. Time of peak serum level. 3. Time interval from first blood level to peak. 4. Time interval from peak to half peak concentration. 5. Ultimate half life, as determined from the rate of loss of cephalexin from the serum when absorption had ceased. 6. Peak serum level. 7. Area under the curve. 8. Length of time for which a nominated serum concentration would be exceeded. Calculations were made for 6·25, 8 and 12·5 μg/ml for all volunteers.

The average values for (2), (6) and (8) are given in Table 3, and the remainder in Table 4.

Table 3. *Average peak serum concentrations, time of peak concentration and time in excess of nominated serum levels after oral doses of cephalexin*

Dose in mg	Type of preparation	Fed or fasting	Peak level in μg/ml	Time of peak (min after dose)	No of min concentration exceeded (in μg/ml) 6·25	8	12·5
1000	Tight fill capsule	Fasting	40·8	54·4	189·6	169·8	132·5
1000	,, ,,	Fed, 1 dose	18·6	112·1	245·7	212·1	137·5
1000	,, ,,	Fed, 5 doses	22·5	113·5	245·2	217·1	158·6
500	Tight fill capsule	Fasting	19·5	75·7	126·0	104·8	53·8*
500	,, ,,	Fed, 1 dose	15·6	84·4	148·7	117·3	41·8*
500	,, ,,	Fed, 5 doses	16·7	62·8	174·9	140·4	72·7
500	Loose fill capsule	Fasting	17·3	85·5	122·1	101·3	50·1*
500	Suspension	Fasting	15·7	53·8	131·7	108·2	48·2
300	Solution	Fasting	18·4	26·0	71·0	58·0	29·2*
250	Tight fill capsule	Fasting	9·9	84·6	55·1*	32·7*	3·0*

* not all the volunteers reached the nominated level.

Table 4. *Average time of first serum level, serum half life and area under the curve after oral doses of cephalexin*

Dose in mg	Type of preparation	Fed or fasting	Time of first serum level (min)	Time from peak to half peak (min)	Ultimate half life (min)	Area under curve
1000	Tight fill capsule	Fasting	23·5	72·9	50·1	74·0
1000	,, ,,	Fed, 1 dose	32·5	133·8	56·0	65·6
1000	,, ,,	Fed, 5 doses	41·6	120·7	50·3	72·7
500	Tight fill capsule	Fasting	38·4	73·1	42·7	34·2
500	,, ,,	Fed, 1 dose	30·2	97·6	49·2	38·0
500	,, ,,	Fed, 5 doses	27·8	112·1	82·6	47·9
500	Loose fill capsule	Fasting	46·8	72·8	39·2	31·8
500	Suspension	Fasting	9·1	81·3	40·8	32·7
300	Solution	Fasting	9·9	41·4	33·2	19·8
250	Tight fill capsule	Fasting	50·8	65·5	37·5	16·5

Statistical analysis of the computer fitted serum level curves

The parameters calculated were examined for each preparation and any significant differences were noted. The comparisons made and the results obtained were as follows.

The relation between the dose given, the peak serum concentrations, the area under the curve and the time of the peak. Comparisons were made between volunteers who received single doses of 1000, 500 and 250 mg, presented virtually without excipient in hard gelatin capsules. After a single dose of 1000 mg, the average peak titre in 10 volunteers, as calculated by the computer, was 40·8 (±4·6) μg/ml at 54·4 min after dosing. This was almost exactly double the value for the 20 volunteers who received 500 mg; they had a peak titre of 19·5 (±1·9) μg/ml, at 75·7 min after dosing. Seven volunteers who had 250 mg gave an average of 9·9 (±0·8) μg/ml at 84·6 min after the dose. Thus, there was an almost exact arithmetic relation between the magnitude of the peak titre and the dose given, the titre doubling as the dose was doubled. The area under the curve also doubled as the dose doubled, doses of 1000, 500 and 250 mg giving respective areas of 74·0 (±4·3), 34·2 (±1·4) and 16·5 (±1·1), and the time taken to achieve peak titre diminished as the dose increased. The average serum levels following these doses are given in Fig. 3.

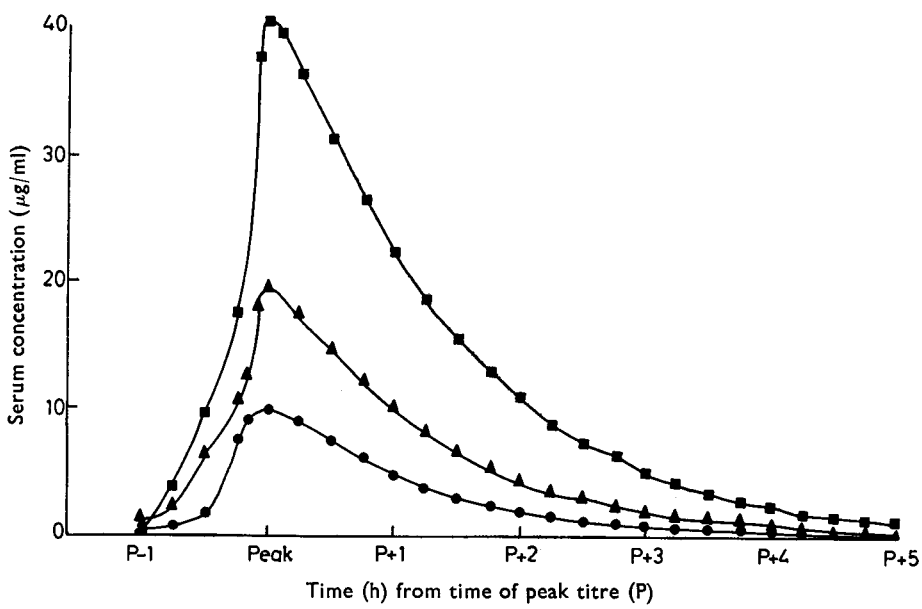

FIG. 3. Average serum levels of cephalexin as calculated by the computer after 1 oral dose of 250 mg (●—8 volunteers), 500 mg (▲—20 volunteers) and 1000 mg (■—10 volunteers). The origin of the time axis is peak time (P).

Comparison of serum concentrations following administration of cephalexin in capsules with and without excipient. The comparison was made between serum levels obtained after administration of one capsule containing 500 mg cephalexin with no excipient (20 volunteers) and those obtained after administration of two capsules, each containing 250 mg cephalexin loosely packed with approximately the same weight of corn starch (14 volunteers). The calculated average peak serum levels of 19·5

(±1·9) and 17·3 (±1·4) μg/ml respectively, occurring at 75·7 and 85·5 min after dosing, were not significantly different. The average serum level curves from the two preparations were virtually identical, none of the calculated parameters differing significantly from one preparation to the other.

The effect of giving the dose with a meal. Single doses of 1000 mg (2 × 500 mg capsules, no excipient) were given to volunteers immediately after they had eaten a large meal. This significantly reduced the peak titre and delayed the time at which it occurred. It also significantly increased the length of time that the titre exceeded 8 μg/ml and the length of time taken for the peak concentration to fall to half. When a dose of 500 mg was given after a large meal, the differences seen were less extreme than with the 1000 mg dose. The rate of fall from peak titre to half peak titre in the volunteers who had had the large meal was significantly slower but none of the other parameters was significantly affected.

Comparison of the serum levels obtained after one dose and after 5 doses. Doses of 1000 mg were given at 6-hourly intervals, the first and fifth being given immediately following a large meal. Between the 1st and the 5th doses the volunteers ate at normal meal times. The peak titre and the length of time the serum levels exceeded 12·5 μg/ml were significantly increased after the fifth dose, which suggested that at certain amount of accumulation was taking place, although not very rapidly.

When 5 doses of 500 mg were given in the same manner, there was no significant increase in any of the parameters after the fifth dose. This suggests that the initial dose of 500 mg was completely excreted in 6 h, whilst the initial dose of 1000 mg was not.

The effect on serum levels of giving the antibiotic in suspension or solution. After a dose of 500 mg given as a 5% suspension in a flavoured paediatric syrup, cephalexin appeared in the serum in 9 min, compared with the 38 min taken when the antibiotic was given as a capsule. The 29 min difference presumably represents the opening time of the capsule. A dose of 300 mg, given entirely in solution, gave cephalexin in the serum in 9·9 min and the peak time at 26 min after the dose was significantly earlier than the peak times after capsules had been taken.

CONCLUSIONS

Several conclusions can be drawn about factors which would have an influence on the size and duration of cephalexin serum concentrations. These are:

The average levels likely to be found after a given dose cannot be predicted with any accuracy from averages arrived at in the conventional way after trials in several persons. The variation in the time taken to absorb an oral dose is sufficiently large, from one person to another, both to make the conventional average misleading and to markedly reduce the apparent average peak level. More meaningful averages are found by calculating the serum level curves on a time scale with its origin at peak titre and then averaging the curve.

A heavy meal delays and also reduces the peak titre obtained after a single oral dose. It lengthens the time that an arbitrary level of 8 μg/ml is exceeded and also lengthens the period between peak and half peak levels. There could be an initial gradual increase in the peak titre after a series of doses of 1000 mg, but this was not found by us with doses of 500 mg.

The serum level curve can be affected by gross differences in the presentation. If,

for example, the cephalexin is partly or wholly in solution, it appears much more rapidly in the serum than when it is given as a solid in a capsule.

The presence of excipient makes no difference to the serum levels attained. After administration of cephalexin in capsules with no excipient or in capsules containing an equal amount of starch, the average serum level curves are indistinguishable.

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