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Some aspects on the bioavailability of a controlled release clonidine formulation in man

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Summary

A reliable and sensitive gas-liquid chromatographic (GLC) method of analysis has been developed for clonidine. The assay method has been utilised to study the pharmacokinetics of a dosage form containing capsulated controlled release pellets of the drug (Tenso-Timelets) in healthy normotensive subjects in single and multiple dose studies. When compared with a reference dosage solution of clonidine hydrochloride, the controlled release product produced a slower absorption of drug and a broad plasma peak at 6–8 h after administration and then slow declining levels which at 24 h were higher than those achieved by the dosage solution. There was no evidence of drug accumulation and the formulation is a convenient way of administering clonidine hydrochloride on a once per day dosage.

Introduction

Clonidine hydrochloride (2-[2,6-dichlorophenyl-amino]2-imidazoline hydrochloride) is a potent hypotensive agent which appears mainly to act centrally by activating α_2-adrenoceptors in the vasomotor centre in the brain resulting in a diminished sympathetic outflow from the CNS (Isaac, 1980). The substance is rapidly absorbed after oral administration and bioavailability is high. Peak plasma concentrations are observed 1.5–3 h after oral administration, declining with a half-life of 5–13 h (Lowenthal, 1980).

When given orally its effects appear in about 30 min reaching a maximum after two to four hours and lasting about 6–8 h (Martindale, 1982). Plasma concentrations correlate with decrease in blood pressure up to values of 1.5–2.0 ng/ml and maximally effective concentrations in plasma may occur after a dose of 0.3 mg twice daily for the majority of patients (Pettinger, 1980). Initial dose is 0.1 mg given twice daily with increments of 0.1 or 0.2 mg/day until the desired response is achieved. For maintenance, the usual daily dose ranges from 0.2 to 0.8 mg in divided amounts (Am. Med. Assoc. Drug Evaluations, 1986).

The concomitant administration of a diuretic (e.g. chlorthalidone) enhances the antihypertensive efficacy of clonidine and such combinations are available on the market.

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Major adverse effects of clonidine reported in man are dry mouth, drowsiness and sedation, usually diminishing with continued therapy.

Due to lack of reliable analytical methods, insufficient information is available on the pharmacokinetics and bioavailability of clonidine, e.g. of controlled release forms. The reports are contradictory exhibiting different results and consequent interpretations (Lowenthal, 1980; Frisk-Holmberg et al., 1980; Frisk-Holmberg et al., 1981; Velasquez et al., 1983; Arndts et al., 1983; Dollery et al., 1976; Davies et al., 1976; Hogan et al., 1981).

Since the usual therapeutic dose of clonidine is very small, it is important to have sensitive and accurate assays to monitor drug levels in biological fluids. We proposed to develop a reliable and accurate analytical method and apply it to evaluate the bioavailability of a controlled release (c.r.) clonidine hydrochloride formulation in healthy subjects after single and multiple dose and to compare it to that of a reference solution (single dose).

Several gas chromatographic methods with electron capture detection for determination of clonidine in plasma are known (Edlund and Paalzo, 1977; Edlund, 1980; Chu et al., 1979). These methods use various reagents for the derivatization of clonidine (pentafluorobenzylbromide, heptafluorobutyric anhydride) which allow the smallest amount of clonidine to be determined in plasma samples with a confidence level of 0.1–0.2 ng/ml. Application of these assays in our study were unsatisfactory. Therefore, we developed our own gas chromatographic procedure (using electron capture detector and derivatization technique) and used it in the present study.

Subjects and Methods

Subjects

Four healthy normotensive human subjects participated in our studies. All volunteers were non-smoking adults, 3 females and one male, aged 20–22 years, weighing between 52 and 80 kg. A medical examination prior to start of the study showed that all 4 subjects were in good health with no evidence of any illness. Informed consent was obtained from each subject after they were told about the treatments and possible side-effects.

Drug products

The following drug products were used: (a) test formulation c.r. capsules Tenso-Timelets (Batch G 165/34, Temmler Werke, Marburg/Lahn, F.R.G.) containing 0.25 mg clonidine hydrochloride; these contain pellets of the drug from which the rate of release is controlled by diffusion of the drug through membranes in the outer portion of the pellets; (b) reference solution containing 0.25 mg of clonidine hydrochloride in every 30 ml, prepared from powdered clonidine hydrochloride (Temmler Werke, An. no. 820267).

In vitro dissolution tests were performed at pH 1.5, 4.5, 6.9, 7.2 and 7.5 using the 22 h programme of the Bio-Dis apparatus (Graepel et al., 1986) with buffer solutions as dissolution media. Clonidine analysis was carried out in triplicate.

Prior to the clinical tests, the potency of the capsules was determined using the same gas chromatographic analytical method as in the sample measurement and gave 100% from the labeled.

The in vitro release of clonidine from the c.r. capsules is shown in Fig. 1.

Procedures

Single-day study. On the day of the test the subjects appeared at 0.800 h having fasted overnight and having taken no medication for the previous week. No food was allowed for 4 h after administration of the drug formulation after which time a light lunch was served. No xanthine-containing items were taken until the end of the blood-drawing period. Subjects were resting for the first 4 h after the dosage, after which time they were free to move around but avoiding stress and abnormal physical activity.

The appropriate drug formulation was administered with 240 ml of water. The pattern of drug administration was as follows:

(A) Clonidine HCl c.r. formulation – containing 0.25 mg drug at time 0;

(B) 10 ml of reference solution – 0.25 mg/30 ml at times 0, 6 and 12 h, i.e. 0.083 mg per dose;

Week 1 Dosage A (subjects MEA and GV)
Dosage B (subjects REZ and JS)
Week 2 Dosage A (subjects REZ and JS)
Dosage B (subjects MEA and GV)
One week was allowed between each treatment as a "washout period".

Multiple-dose study. Clonidine c.r. capsules containing 0.25 mg drug, i.e. 3.1–4.8 μg/kg, were administered once daily for 5 days at 0.800 h with 240 ml of water. Identical food and activity requirements were observed as in the single dose treatment, but xanthine-containing items were not taken throughout the 6-day period of the study.

Dose schedule was designed according to the following pattern:

(C) Clonidine c.r. capsules – containing 0.25 mg drug at time 0 on days 1–5.

Collection and processing of samples. Blood samples (8 ml), drawn by venipuncture, were collected into disposable, heparinized Vacutainer glass tubes and they were assigned identification numbers unknown to the analytical personnel. Plasma was separated immediately by centrifugation and it was frozen until analysis. Blood samples were taken just before drug administration (time 0) and in single day trials at 1, 2, 4, 6, 8, 12, 24, 30 and 48 h (c.r. capsules) and at 1, 2, 4, 6, 8, 10, 12, 14, 24, 30 and 48 h (solution). In multiple-day trials, blood samples were drawn at 0, 6, 9 and 12 h on days 1, 2, 3 and 4. On day 5, blood was collected at 0, 3, 6, 9, 12 and 14 h. On day 6 (no drug taken) blood samples were taken at 24, 27, 33 and 36 h after the last dose on day 5.

Analytical method. Clonidine in plasma was determined by gas chromatography with electron capture detector using an internal standard.

Materials and reagents

Methyl-*tert*-butyl ether (MTBE) and ethylacetate, employed in the extraction and derivatization procedure, were used as obtained (Fisher Scientific Co., Pittsburgh, PA – HPLC grade purity).

The phosphate buffer, 0.2 M (pH 9.5) was prepared from the monobasic potassium phosphate and it was adjusted to the appropriate pH with potassium hydroxide solution (p.a. grade purity).

Trifluoracetic anhydride (TFAA) was used for derivatization and it was obtained in 1 ml amber glass ampules (Fisher Scientific Co.).

The internal standard (IS) was 2-[2,6-dichloro-4-methyl-phenyl)imino]imidazoline (Boehringer Ingelheim, Ridgefield, CT).

Authentic standard solutions in MTBE : ethylacetate (80 : 20) containing 0.1 mg/ml. They were

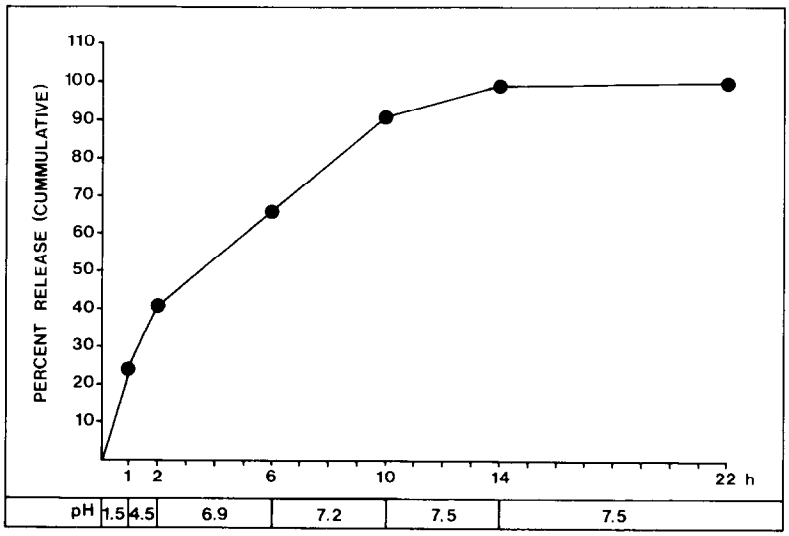

Fig. 1. Cumulative release of clonidine in vitro dissolution performed at gradient pH.

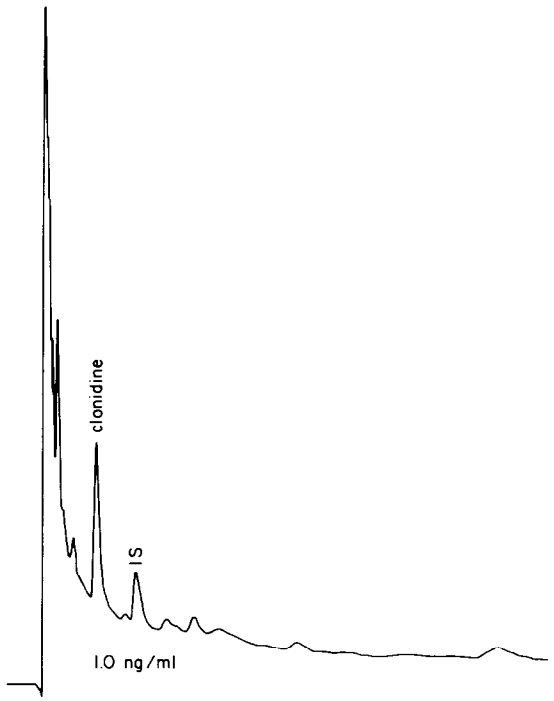

Fig. 2. Peaks observed when scanning clonidine and IS with GLC.

used for construction of the standard curve by plotting area ratios of clonidine over IS vs clonidine concentrations (ng/ml).

The glassware was not silanized since the extraction of the drug from plasma samples was not improved with silanization.

Apparatus

The instrument was a gas chromatograph (Model 3700, Varian Instrument Division, Palo Alto, CA) fitted with a 63Ni electron capture detector. A 3% OV-17 column on 80–100 mesh Gas Chrom Q was used. Nitrogen was the carrier gas.

Conditions of the instrument were: column temperature 170 °C, injector temperature 220 °C, detector temperature 250 °C, gas flow was adjusted to 30 ml/min. The retention times for the derivatized clonidine and IS were 4.0 and 6.0 min (Fig. 2).

Extraction procedure

To 2 ml of plasma samples in 15 ml screw-cap centrifuge tubes were added 0.2 ml of IS solution and 0.5 ml of phosphate buffer to provide a pH of 9.5. The samples were shaken with 4 ml of MTBE : ethylacetate (80 : 20) on a multipurpose rotator Model 150-V (Scientific Industries, Springfield, MA) for 10 min. The tubes were centrifuged for another 10 min at 1300 *g* and the organic phase was separated and evaporated using a stream of nitrogen at 55 °C (N-EVAP Model No. 112, Organomation Assoc., Shrewsbury, MA). One ml of 1% TFAA in ethylacetate was added to the residue, the tubes were stoppered and the mixture was refluxed for 45 min at 90 °C. The ethylacetate mixture was evaporated under nitrogen (55 °C) to dryness and the residue was dissolved in 3 ml of MTBE : ethylacetate (80 : 20) and 0.25 ml of phosphate buffer. The tubes were shaken for 2–3 min and centrifuged. The organic layer was separated and evaporated to dryness (nitrogen, 55 °C). The residue, consisting of derivatized clonidine and IS was dissolved in 0.1 ml of MTBE : ethylacetate mixture and 10–15 μl was injected into the gas chromatograph.

Recovery

The recovery of clonidine was determined by adding varying known amounts of the drug (0.25–2.0 ng) and constant amounts of IS (4.0 ng) to human plasma. A blank plasma sample with no clonidine or IS was carried through the assay, also. These plasma samples were extracted, derivatized and injected into the instrument as described above. The ratios of peak areas of the drug and the IS were compared with peak area ratios obtained with respective authentic standards injected direct into the gas chromatograph.

Calculations

The standard curve for clonidine (Fig. 3) shows a linear relationship between the area ratios and clonidine concentrations from 0.25 to 2.0 ng/ml.

Recoveries of clonidine from spiked plasma samples were 82.0–95.5% (Table 1).

Evaluation of the bioavailability

The bioavailability of clonidine in the c.r. capsules was determined from the corresponding areas

TABLE 1

Recovery of clonidine from spiked human plasma (ng/ml)

Standard deviations are shown in parentheses

Clonidine added (ng/ml)	Number of samples	Clonidine recovered (ng/ml)	Percent recovery
0.25	4	0.21 (0.017)	84.0 (6.83)
0.50	4	0.43 (0.034)	86.0 (6.81)
0.75	4	0.68 (0.073)	90.7 (6.06)
1.00	4	0.82 (0.055)	82.0 (5.48)
1.50	4	1.39 (0.075)	92.7 (5.08)
2.00	4	1.91 (0.035)	95.5 (1.74)

TABLE 2

Mean plasma clonidine levels for each treatment in single-day study

Standard deviations are shown in parentheses

Sample	Time (h)	Plasma level (ng/ml)	
		c.r. capsule	Solution
1	0	0	0
2	1	0.10 (0.080)	0.16 (0.053)
3	2	0.21 (0.085)	0.37 (0.054)
4	4	0.35 (0.070)	0.24 (0.062)
5	6	0.47 (0.045)	0.18 (0.036)
6	8	0.44 (0.054)	0.45 (0.046)
7	10	–	0.27 (0.062)
8	12	0.33 (0.079)	0.20 (0.045)
9	14	–	0.51 (0.095)
10	24	0.19 (0.044)	0.10 (0.042)
11	30	0.08 (0.024)	0.02 (0.035)
12	48	0	0

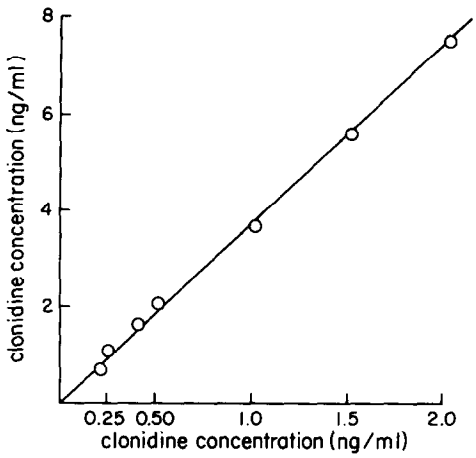

Fig. 3. Clonidine standard curve in MTBE:ethylacetate (80:20).

under the plasma concentration curves (AUC) after oral administration in the single-day and multiple-day treatments by comparison with those produced by clonidine from the solution in the single-day treatment.

Results

Single-day study

Complete data for analysis were obtained from the 4 subjects and none of the subjects experienced any side-effects during the course of the study.

TABLE 3

Some pharmacokinetic parameters in single-day study of clonidine

Subject numbers	Controlled release capsules			Solution			Relative bioavailability
	C_{max} (ng/ml)	T_{max} (h)	$AUC_{0\text{-}24}$ (ng · h/ml)	C_{max} (ng/ml)	T_{max} (h)	$AUC_{0\text{-}24}$ (ng · h/ml)	
1	0.42	6.0	6.480	0.50	2.0	6.485	0.999
2	0.44	8.0	7.560	0.58	2.0	7.110	1.063
3	0.50	6.0	7.520	0.48	2.0	6.485	1.160
4	0.50	8.0	7.735	0.48	2.0	6.490	1.192
n	4	4	4	4	4	4	4
Median	0.48	7.0	7.540	0.49	2.0	6.485	1.112
Min	0.42	6.0	6.885	0.48	2.0	6.640	1.024
Max	0.50	8.0	9.470	0.58	2.0	7.890	1.359

The mean plasma clonidine concentrations observed after administration of each product are presented in Table 2. Significant differences were seen in the character of the plasma concentration–time curves produced by each product. As was expected when a standard conventional preparation (solution) is compared with a c.r. product (capsules), the latter produced lower peak concentrations than the solution (Table 3). After an initial lag time of 0.6 h clonidine from c.r. capsules was rapidly absorbed with an absorption half-life of 2.6 h, and peak plasma concentration plateau was attained between 6 and 8 h. The mean peak plasma clonidine level registered after solution administration was 0.49 ng/ml and that seen after the c.r. pellets was 0.48 ng/ml. After solution administration, peak plasma clonidine levels were achieved at 2 h.

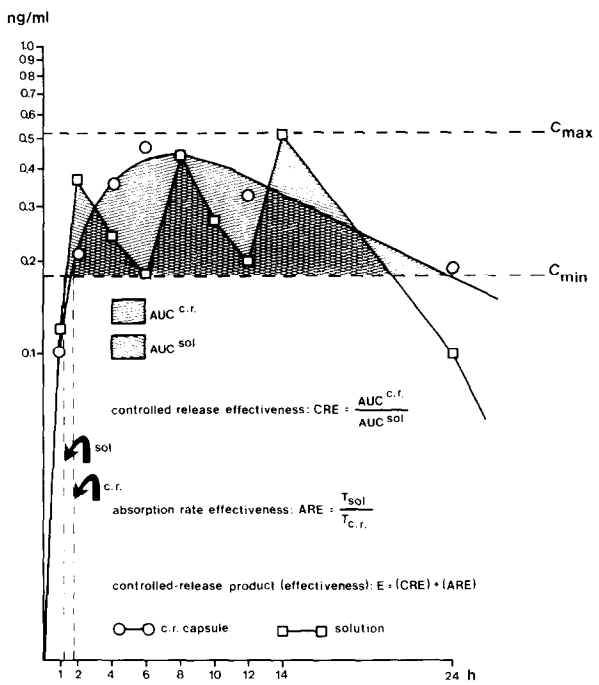

Fig. 4. Mean plasma clonidine levels in single-day study. Comparative curves for clonidine solution (administered at 0, 6 and 12 h) and c.r. capsules (administered at 0 h) showing the respective AUCs above and below C_{min} and C_{max}, respectively. C_{min} and C_{max} are obtained from the solution administered 3 times. Also the time the two preparations reach C_{min} are shown.

TABLE 4

Mean plasma clonidine levels in c.r. capsules multiple-dose study

	h	Plasma levels (ng/ml) median (and range, $n = 4$)
Day 1	0	0
	6	0.38 (0.28–0.54)
	9	0.34 (0.30–0.37)
	12	0.28 (0.20–0.35)
Day 2	0	0.18 (0.11–0.22)
	6	0.64 (0.36–0.70)
	9	0.55 (0.30–0.74)
	12	0.36 (0.29–0.46)
Day 3	0	0.23 (0.12–0.29)
	6	0.71 (0.38–0.73)
	9	0.73 (0.39–0.81)
	12	0.50 (0.40–0.63)
Day 4	0	0.27 (0.20–0.35)
	6	0.77 (0.42–0.81)
	9	0.76 (0.46–0.86)
	12	0.62 (0.42–0.65)
Day 5	0	0.28 (0.24–0.34)
	3	0.51 (0.50–0.53)
	6	0.79 (0.54–0.84)
	9	0.80 (0.49–0.89)
	12	0.65 (0.44–0.68)
	14	0.60 (0.38–0.65)
Day 6	24	0.29 (0.26–0.30)
	27	0.24 (0.14–0.27)
	33	0.19 (0.08–0.25)
	36	0.11 (0–0.20)

The mean values of the area under the plasma concentration–time curves (AUC) for each treatment are also shown in Table 3. All AUC values were calculated by the trapezoidal rule (Gibaldi and Perrier, 1975). The $AUC_{0\text{-}24}$ (ng/h/ml) values were 7.540 (c.r. capsules) and 6.485 (solution) giving relative bioavailability (F) of capsules vs solution of 1.112.

For evaluating the c.r. product the controlled-release product effectiveness (E) was also calculated according to Vallner et al. (1983) with the following results: $CRE = 1.33$; $ARE = 0.67$ and $E = 1.00$. This may be considered proof that Tenso-Timelets is an effective c.r. product (Fig. 4).

TABLE 5

Individual and mean plasma clonidine levels in c.r. capsules multiple-day treatment at 24 h after 1st, 2nd, 3rd, 4th and 5th dosing period

Subject numbers	Plasma levels (ng/ml) at 24 h				
	Dosing period				
	1st	2nd	3rd	4th	5th
1	0.12	0.18	0.26	0.29	0.26
2	0.21	0.20	0.28	0.25	0.30
3	0.30	0.31	0.26	0.30	0.18
4	0.15	0.23	0.31	0.30	0.29
n	4	4	4	4	4
Mean	0.195	0.230	0.278	0.285	0.258
S.D.	0.079	0.055	0.024	0.024	0.094
Min	0.12	0.18	0.26	0.25	0.18
Max	0.30	0.31	0.31	0.30	0.30

Multiple-day study

Individual and mean plasma concentrations of clonidine obtained after the administration of c.r. capsules appear in Table 4.

Table 5 demonstrates that at 24 h after the administration of the c.r. capsules the mean plasma levels were 0.195 ng/ml after the first dosing period and 0.208 ng/ml after the last dosing period. The plasma concentration peaks of clonidine from the product increased after day 1 and the highest individual concentrations were found on days 4 and 5 with a range from 0.46 to 0.89 ng/ml (Table 6) and individual degrees of fluctuation ($C_{max} - C_{min}/C_{avg}$) ranging from 0.8 to 1.6. Plasma level curves after the c.r. formulation had virtually identical shape throughout the dosing period (Fig. 5).

The steady-state minimum plasma concentration was reached after 3 days after c.r. formulation: 0.278 ng/ml and it remained constant during days 4, 5, and 6 (Table 5).

Some individual and mean pharmacokinetic parameters and areas under the curve (AUC) for the c.r. capsules after 0–24 h, and at steady-state 96–120 h, are presented in Table 6. The mean $AUC_{0\text{-}24}$ (ng · h/ml) was 5.78 and the mean $AUC_{96\text{-}120}$ (ng · h/ml) was 13.49.

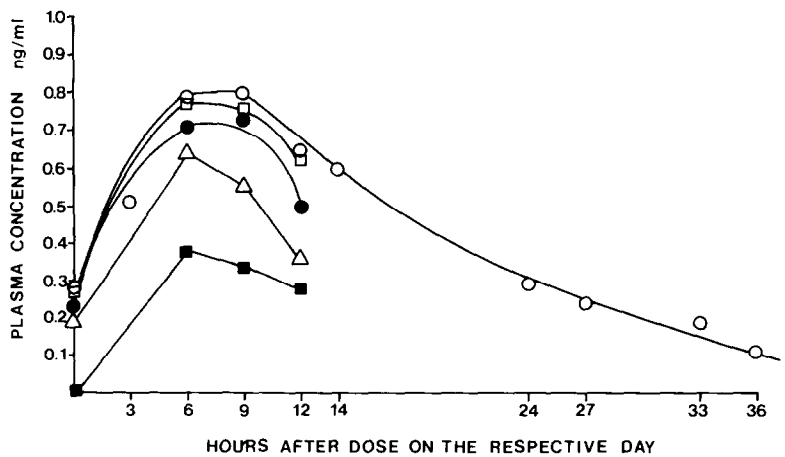

Fig. 5. Mean plasma clonidine levels vs time values in multiple-day study with c.r. capsules. ■———■, day 1; △———△, day 2; ●———●, day 3; □———□, day 4; ○———○, day 5.

TABLE 6

Mean values for AUCs, C_{max} and T_{max} in clonidine c.r. capsules multiple-dose study

		Median (and range, $n = 4$)	
Day 1	AUC_{0-24}	5.78 (5.34–6.66)	ng·h/ml
	C_{max}	0.39 (0.35–0.54)	ng/ml
	T_{max}	7.5 (6–12)	h
Day 2	AUC_{24-48}	9.73 (5.75–10.33)	ng·h/ml
	C_{max}	0.64 (0.36–0.74)	ng/ml
	T_{max}	6.0 (6–9)	h
Day 3	AUC_{48-72}	12.02 (7.45–12.20)	ng·h/ml
	C_{max}	0.73 (0.40–0.81)	ng/ml
	T_{max}	9.0 (9–12)	h
Day 4	AUC_{72-96}	13.10 (8.46–13.68)	ng·h/ml
	C_{max}	0.78 (0.46–0.86)	ng/ml
	T_{max}	7.5 (6–9)	h
Day 5	AUC_{96-120}	13.49 (9.64–14.19)	ng·h/ml
	C_{max}	0.80 (0.49–0.89)	ng/ml
	T_{max}	9.0 (9)	h

No side-effects were noticed in any of the subjects being treated with the controlled release clonidine formulation in the multiple-day study.

Discussion

A reliable and sensitive GLC method of analysis has been developed which gives good recovery of drug from spiked human plasma (Fig. 2 and Table 1) and good calibration curves (Fig. 3).

The application of this method to the analysis of plasma of volunteers receiving 0.083 mg clonidine hydrochloride at 0, 6 and 12 h in solution and on a separate occasion 0.25 mg of clonidine hydrochloride (i.e. 3×0.083 mg) in the form of controlled release pellets in the capsules indicated the suitability of the method for pharmacokinetic studies in man (Fig. 4).

The following conclusions can be drawn from the one day study.

(a) As expected, the absorption of the drug from solution is rapid to give sharp plasma levels and a quick decline after the peak, with the peak level progressively increasing from the first to the third dose (Fig. 4).

(b) The controlled release product c.r. capsules produces a slower absorption of drug and a broad plasma peak 6–8 h after administration and then slow declining levels which at 24 h are higher than those after the final dose of drug in solution (Fig. 4).

(c) The sharp peaks and troughs of drug levels after dosing with solution are replaced by a smooth plasma profile after dosing with c.r. capsules, in which the broad plateau is lower than that of the peak after 3rd dose of the drug in solution and the *AUC* after giving the pellets is not less than after the third dose of drug in solution form.

(d) The c.r. capsules are especially suitable for once per 24 h administration to improve the 3 doses of drug in solution (or in tablets) given at 6 hourly intervals when the total amount of drug administered for 24 h is the same.

The c.r. capsules performed well in the multiple-dose study (Fig. 5) giving a broad plasma peak between 6 and 9 h which increases in height upon daily dosing as does the trough level at 24 h after dosing. The plateaus are only about 3 times the concentration levels of the troughs on this repeated doses of product.

Elimination half-life remained essentially the same in chronic treatment, so that there was no evidence of any drug accumulation.

The c.r. capsules are thus a convenient way of administering clonidine hydrochloride on a once per day basis.

References

AMA Drug Evaluations 6th ed. Lampe, K.F., McVeigh, S. and Rodgers, B.J., (Eds.), *American Medical Association*, Chicago, 1986, pp. 516–517.

Arndts, D., Doevendans, J., Kirsten, R. and Heintz, B., New aspects of the pharmacokinetics and pharmacodynamics of clonidine in man. *J. Clin. Pharmacol.*, 24 (1983) 21–30.

Chu, L.C., Bayne, W.F., Tao, F.T., Schmitt, L.G. and Shaw, J.E., Determination of submicrogram quantities of clonidine in biological fluids. *J. Pharm. Sci.*, 68 (1979) 72–74.

Davies, D.S., Wing, L.M.H., Reid, J.L., Neill, E., Tippett, P. and Dollery, M.B., Pharmacokinetics and concentration–effect relationships of intravenous and oral clonidine. *Clin. Pharmacol. Ther.*, 21 (1976) 593–601.

Dollery, C.T., Davies, D.S., Draffan, G.H., Dargie, H.J., Dean, C.R., Reid, J.L., Clare, P.A. and Murray, S., Clinical pharmacology and pharmacokinetics of clonidine. *Clin. Pharmacol. Ther.*, 10 (1976) 11–17.

Edlund, P.O., Determination of clonidine in human plasma by glass capillary gas chromatography with electron-capture detection. *J. Chromatogr.*, 187 (1979) 161–169.

Edlund, P.O. and Paalzow, L.K., Quantitative gas liquid chromatographic determination of clonidine in plasma. *Acta Pharmacol. Toxicol.*, 40 (1977) 145–152.

Frisk-Holmberg, M., The disposition and pharmacokinetics of clonidine in patients practical consequences. *Uppsala J. Med. Sci.*, Suppl. 31 (1980) 13–15.

Frisk-Holmberg, M., Paalzow, L.K. and Edlund, P.O., Clonidine kinetics in man – evidence for dose dependency and changed pharmacokinetics during chronic therapy. *Br. J. Clin. Pharmacol.*, 12 (1981) 653–658.

Gibaldi, M. and Perrier, D., *Pharmacokinetics*, Marcel Dekker, New York, 1975, pp. 293–296.

Graepel, P.H., Momberger, H. and Kuhn, D., Bio-Dis apparatus for determination of in vitro dissolution of sustained release formulations. *Pharm. Zt.*, 131 (1986) 1820–1823.

Hogan, M.J., Wallin, J.D. and Chu, L.C., Plasma clonidine concentration and pharmacologic effect. *Clin. Pharmacol. Ther.*, 40 (1981) 729–733.

Isaac, L., Clonidine in the central nervous system: site and mechanism of hypotensive action. *J. Cardiovasc. Pharmacol.*, 2 (Suppl. 1) (1980) S5–S19.

Lowenthal, D.T., Pharmacokinetics of clonidine. *J. Cardiovasc. Pharmacol.*, 2 (Suppl. 1) (1980) S29–S37.

Martindale The Extra Pharmacopoeia, 28th edn., Wade, A. (Ed.), Pharmaceutical Press, London, 1982, pp. 139–141.

Pettinger, W.A., Pharmacology of clonidine. *J. Cardiovasc. Pharmacol.*, 2 (Suppl. 1) (1980) S21–S28.

Vallner, J.J., Honigberg, I.L., Kotzan, J.A. and Stewart, J.T., A proposed general protocol for testing bioequivalence of controlled release drug products. *Int. J. Pharm.* 16 (1983) 47–55.

Velasquez, M.T., Rho, J., Maronde, R.F. and Barr, J., Plasma clonidine levels in hypertension. *Clin. Pharmacol. Ther.*, 34 (1983) 341–346.