RADIOIMMUNE AND RADIORECEPTOR STUDY OF THE PHARMACOKINETICS OF THE ENKEPHALIN ANALOG DALARGIN IN MAN

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UDC 615.243:[547.95:547.943].033.07

KEY WORDS: dalargin; pharmacokinetics; enkephalins.

The discovery of the endogenous opioid peptides — enkephalins and endorphins — led to the synthesis of their numerous structural analogs, differing in their biological activity and showing promise as therapeutic preparations [2]. On the basis of experimental studies, one of these enkephalin analogs, having the structure Tyr-D-Ala-Gly-Phe-Leu-Arg, and given the name dalargin, was suggested as a remedy accelerating the healing of duodenal ulcers.

The aim of this investigation was to study the pharmacokinetics of dalargin in man by combined use of radioimmune and radioreceptor methods.

METHODS

The pharmacokinetics of dalargin was studied in seven men aged from 23 to 46 years, treated with the peptide for duodenal ulcer. Dalargin was dissolved in isotonic NaCl solution to a concentration of 1 mg/ml and injected intravenously for 1 min: into two patients in a dose of 1 mg, into four in a dose of 5 mg, and into one in a dose of 10 mg. Venous blood was taken before and 2, 4, 6, 8, 10, 15, 30, 60, and 120 min after injection of dalargin into cooled test tubes with heparin (for radioreceptor analysis) or into tubes with EDTA (0.7 mg/ml blood) and trasylol (500 units/ml blood) for analysis by the radioimmune method. The plasma thus obtained was kept at −40°C until analysis.

To obtain antiserum, dalargin was conjugated with bovine serum albumin with the aid of bis-diazotized benzidine [3]. The conjugate (200-500 µl) was emulsified by ultrasound in 1 ml ofphysiological saline and 1 ml of Freund's complete adjuvant and injected in equal portions into Chinchilla rabbits subcutaneously at 18-20 points in the dorsal region. For reimmunization the same doses of conjugate were injected every 2 weeks, but with Freund's incomplete adjuvant. Antibodies were found after six or seven immunization cycles. To study the pharmacokinetics of dalargin, an antiserum not cross-reacting with enkephalins or with various fragments and analogs of dalargin, was used. Dalargin was iodinated with the aid of chloramine, and the labeled peptide was separated from free iodine by gel-filtration with the use of Bio-Gel P-2.

Components of the incubation mixture (labeled dalargin, antibodies to dalargin in a dilution of 1:200, and blood plasma from the patients investigated) were diluted in 0.1 M phosphate buffer, pH 7.4.

To construct a calibration curve, each sample was treated with 100 µl of plasma, pooled from 10 donors. After incubation of the mixture for 24 h at 4°C, 100 µl of nonimmune rabbit serum (diluted 1:20), 100 µl of donkey antiserum to rabbit γ-globulin, and polyethylene-glycol (mol. wt. 6000 daltons) in a final concentration of 8 vols. % were added to the samples. After incubation for 30 min at 4-6°C the samples were centrifuged and radioactivity of the residues was determined with the RackGamma-2 automatic scintillation counter (from LKB, Sweden).

The sensitivity of the radioimmune method of assay of dalargin was 0.5 ng peptide/ml blood plasma. Different fragments of dalargin (with absence of the N-terminal tyrosine, C-terminal arginine, and the Leu-Arg dipeptide), and also endogenous Leu- and Met-enkephalins

Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 101, No. 6, pp. 702-704, June, 1986. Original article submitted February 11, 1985.

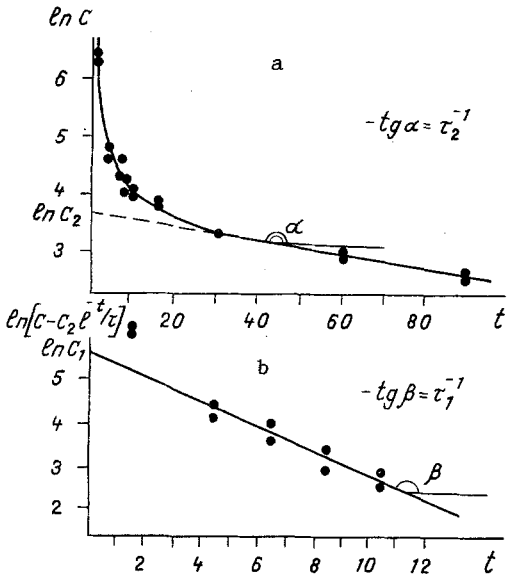

Fig. 1. Changes in opioid activity of dalargin, measured by the radioreceptor method. Abscissa, time (in min); a) determination of τ_2, C_2; b) determination of τ_1, C_1.

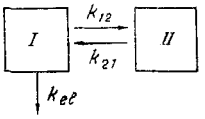

Fig. 2. Pharmacokinetic model of change in opioid activity of dalargin based on results of radioreceptor analysis.

and α-, γ-, and β-endorphins did not compete with dalargin for binding with antibodies in concentrations up to 1 mg/ml.

Radioreceptor analysis was carried out with a preparation of lyophilized rat brain membranes containing opiate receptors [1]. A dried preparation of membranes was resuspended in buffer (10 mM HEPES, pH 7.4), containing $3 \cdot 10^{-5}$ M bacitracin; the protein concentration was about 2 mg/ml. To 300 µl of membrane suspension were added 200 µl of blood plasma, diluted 1:6 to 1:12, 50 µl of buffer (or unlabeled dalargin in the case of construction of calibration curves), 50 µl of ^{3}H-D-Ala^2, and D-Leu^5-enkephalin (concentration in each sample 0.6-1 nM). The samples were incubated for 30 min at 25°C, after which the membrane-bound labeled dalargin was separated by vacuum filtration on Whatman GF/B filters. Activity was determined on a Mark 3 (USA) liquid scintillation counter, using phenoxol as the scintillator. Individual calibration curves for each patient studied were plotted by adding different quantities of dalargin to the blood plasma, taken before injection of the peptide. The sensitivity of the method was 0.5 ng peptide in 1 ml plasma.

RESULTS

Injection of dalargin in doses of 1 and 5 mg was accompanied by a brief sensation of heat in the region of the face, heaviness in the occipital region, and a lump in the throat. These sensations were mild in character and disappeared completely 1-2 min after injection of the peptide. Intravenous injection of dalargin in a dose of 10 mg gave rise, besides to the sensations mentioned above, to a significant fall of arterial pressure (from 130/85 to 100/65 mm Hg) in one subject, 10 min after injection, followed by normalization of the pressure after 25 min.

No significant quantities of dalargin could be detected by the radioimmune method 2 min after its injection in doses of 1 and 5 mg. The concentration of dalargin 2 min after injection of 10 mg of the peptide was 50 ng/ml, falling to 2 ng/ml after 4 min, and none could be detected in the blood 6 min or later after its injection. Hence, after intravenous injection of dalargin, its blood level fell below 0.5 ng/ml by the 2nd to 6th minute, and the limit of sensitivity of the method of radioimmunoassay used. These data correlate well with disappearance of subjective sensations in the course of the same short time interval.

The results of the study of the pharmacokinetics of dalargin by radioreceptor analysis were rather different (Fig. 1). The curve shows that the pharmacokinetics of dalargin is described by two exponential terms:

$$C = C_1 e^{-t/\tau_1} + C_2 e^{-t/\tau_2}, \quad (1)$$

where τ_1 and τ_2 are values of the characteristic time of the process. This biexponential character of the change in activity suggests that a two-part model with direct introduction of the substance into the test chamber (Fig. 2), which k_{12} and k_{21} denote first-order velocity constants of the "transfer" of the substance from the first (test) chamber into the second, and K_{el} is the constant of elimination of the preparation from the body or of its decomposition to a receptor-inactive form, be used to describe the process.

Analysis of the results obtained by calculation with the experimental data leads to the conclusion that the principal phase of change of radioreceptor activity (more than 90%) takes place rapidly, with a characteristic time of 1.5-5 min, and that under 10% of the total initial radioreceptor activity is eliminated relatively slowly with a time of 85-200 min.

Incidentally, the radioimmune and radioreceptor methods of assessment of the pharmacokinetics of dalargin which were used were equal in sensitivity but different in specificity: By means of the radioimmune method only the hexapeptide itself was determined, whereas by means of the receptor method all substances capable of interacting with opiate receptors were determined. In other words, not only dalargin itself, but also its breakdown products, could bind with the membrane receptors, and endogenous opioid substances, whose concentration in the plasma may increase after injection of dalargin, could also interact with them.

The second possibility can be eliminated on the basis of formalization of the results of radioreceptor analysis, because the character of the curves obtained might be explained by release of the endogenous substances only if a considerable quantity of opioids were excreted before the first taking of blood, i.e., 30-60 sec after injection of dalargin, which is extremely unlikely. A more realistic explanation is that degradation products of dalargin, circulating in the blood stream for a long time, are determined by the radioreceptor method. On the basis of general views regarding the character of hydrolysis of opioids in the body [5] and of information on minimal structures capable of binding with opiate receptors [4], it can be tentatively suggested that of all the breakdown products of dalargin, the N-terminal penta- or tetrapeptide can interact with membrane receptors. These substances may be more stable in the blood, and may have a different receptor specificity and different binding characteristics. To shed light on this process experiments must be undertaken with parallel assessment of the pharmacokinetics both of dalargin itself and of its breakdown products.

LITERATURE CITED

1. S. V. Zaitsev, I. N. Kurochkin, and M. G. Sergeeva, Dokl. Akad. Nauk SSSR, 272, 982 (1983).
2. E. I. Chazov, M. I. Titov, and V. A. Vinogradov, Vopr. Med. Khim., No. 3, 47 (1984).
3. R. M. Bassere and R. D. Uteger, Endocrinology, 90, 722 (1972).
4. K. Q. Do, J.-L. Fauchree, and R. Schwyzer, Hoppe-Seyler's Z. Physiol. Chem., 362, 601 (1981).
5. C. Gorenstein and S. H. Snyder, Proc. Roy. Soc. London, B, 210, 123 (1980).