BIOAVAILABILITY OF DALARGIN AND ITS METABOLISM WHEN ADMINISTERED INTRANASALLY TO RATS

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UDC 615.31:[547.95:547.943].032.21.033

KEY WORDS: dalargin; opioids; metabolism; bioavailability.

Intensive research has been conducted in recent years into new therapeutic substances based on endogenous regulatory peptides. The original peptide preparation dalargin, a synthetic analog of the N-terminal fragment of dynorphin, with the structure Tyr-D-Ala-Gly-Phe-Leu-Arg, developed in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, has been introduced into clinical practice in gastroenterology. In its antiulcerative action dalargin is not inferior to H_2-histamine receptor blockers [2]. However, its widespread use in clinical practice (especially in out-patients) has been delayed because of the necessity of injecting the substance parenterally, for if given perorally it is broken down by enzymes of the gastrointestinal tract. Meanwhile there is evidence that peptides can reach the systemic circulation and exert a biological effect if administered by the intranasal route [5-7].

The aim of the present investigation was to study the bioavailability of dalargin when administered by the intranasal and intramuscular routes.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 300-400 g. Under pentobarbital anesthesia (50 mg/kg, intraperitoneally) tracheostomy was performed through a midline incision in the neck [4]. The esophagus was exteriorized into the wound, ligated, and divided and a polyethylene catheter was introduced through it into the nasal cavity. The carotid artery was isolated and catheterized. The palatonasal aperture was closed with "Furaplast" glue. ^{3}H-Dalargin (12 Ci/mmole) was administered intramuscularly and intranasally (through the catheter in the nasal cavity) into three animals in a dose of 300 μg/kg in 80 μl of physiological saline. The intramuscular injection was given 1 h after the intranasal. Another three animals received ^{3}H-dalargin (150 μg/kg in 0.5 ml of physiological saline) intravenously, into the caudal vein, in the course of 10 sec. Blood samples in all cases were taken through the catheter in the carotid artery.

Dalargin is known to undergo rapid enzymic degradation in blood serum [1]. The samples were therefore taken in the medium suggested by Bennett and coworkers for extraction of peptides from tissues [3], which inactivates the enzymes. Blood (150 μl) was added to 300 μl of medium, the mixture was centrifuged for 30 min at 4000 rpm, and the supernatant was then withdrawn and kept at −20°C until analysis. Analysis was carried out on an Altex Model 342 liquid chromatograph with Uvicord SD optical density detector at 226 nm. An Si 100: Polyol RP_{18} column, 46 × 250 mm, with particle size of 5 μ, was used. The elution profile was from 0 to 16% of solvent A for 10 min, then from 16 to 58% of solvent B for 11 min (solvents A and B consist of 5 and 95% aqueous solutions of acetonitrile respectively with 0.1% trifluoroacetic acid). The rate of flow of the eluent was 1.5 ml/min. Minute fractions of eluate were collected in a flask containing Bray's scintillator and their radioactivity was determined on a Beckman scintillation counter (Sweden).

Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 7, pp. 48-50, July, 1988. Original article submitted June 29, 1987.

 0007-4888/88/0007-0968$12.50

TABLE 1. Pharmacokinetic Characteristics of Dalargin Administered by Different Routes

Mode of injection	Dose, μg/kg	k_{el}, min^{-1}	$t_{1/2}$, min	$AUC_{0\to\infty}$, ng·min·ml^{-1}	Cl_t, liters(min·kg)$^{-1}$	$F_{R/iv}$, %	$F_{R/im}$, %
Intravenous	150	0,042	16,4	816,4	0,2	100	
Intramuscular	300	0,030	23,2	248,5	1,3	15	
Intranasal	300	0,033	21,3	133,1	2,5	8	53,5

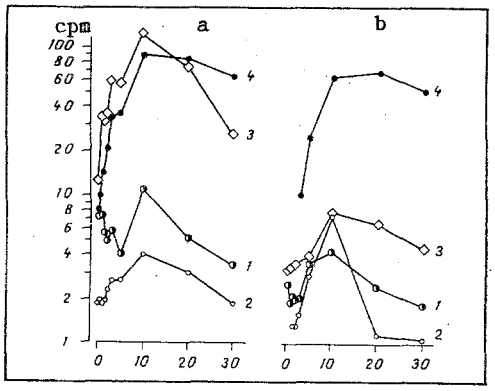

Fig. 1

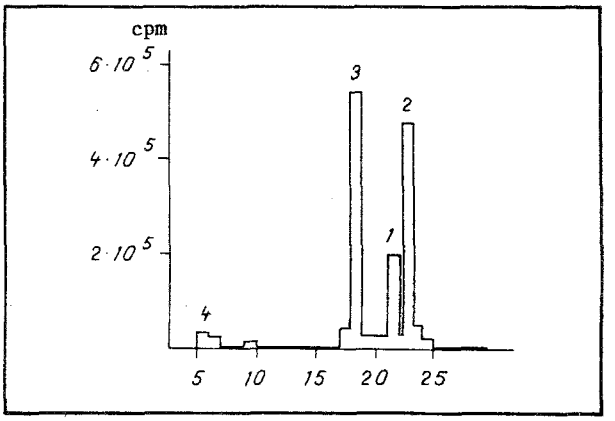

Fig. 2

Fig. 1. Dependence of concentration of dalargin and its tyrosine-containing metabolites (in cpm/30 μl blood, in %) on time for intramuscular (a) and intranasal (b) injection. Abscissa, time after injection (in min). 1) ^{3}H-Dalargin, 2) ^{3}H-Tyr-D-Ala-Gly-Phe-Leu, 3) ^{3}H-Tyr-D-Ala-Gly-Phe, 4) ^{3}H-tyrosine.

Fig. 2. Elution profile of radioactivity of washings from rat nasal cavity 15 min after injection of ^{3}H-dalargin. Abscissa, elution time (in min); ordinate, radioactivity of minute fractions of eluate. Identity of peaks the same as in Fig. 1.

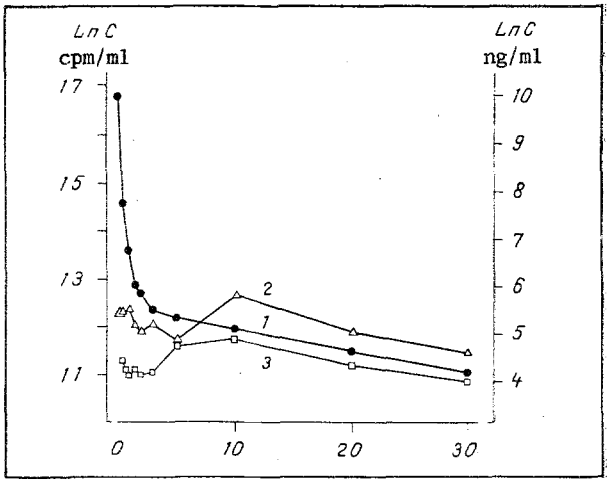

Fig. 3. Time course of changes in dalargin concentration after intravenous (1), intramuscular (2), and intranasal (3) injection into rats.

The bioavailability of the preparation was calculated by the equation:

$$F = \frac{AUC_R^{0 \to \infty} \cdot D_{iv}}{AUC_{iv}^{0 \to \infty} \cdot D_R},$$

where $AUC_R^{0 \to \infty}$ represents the area beneath the kinetic curve in the case of extravascular administration and $AUC_{iv}^{0 \to \infty}$ the area beneath the kinetic curve in the case of intravenous injection; D_{iv} is the dose of the preparation injected intravenously, D_R the dose administered intranasally. The areas beneath the concentration–time curves ($AUC^{0 \to \infty}$) were determined planimetrically, using the trapezium rule, with interpolation to infinity. The total clearance was found by the equation:

$$Cl_t = \frac{D}{AUC \cdot G},$$

where G is the animal's body weight and D the dose of the preparation. The elimination rate constant (k_{el}) was obtained from the linear regression equation deduced by the method of least squares. The half-elimination time was found by the equation:

$$T_{1/2} = \frac{0.693}{k_{el}}.$$

EXPERIMENTAL RESULTS

Chromatographic analysis revealed changes in the concentration of ^{3}H-dalargin and its principal metabolites and enabled their time course to be monitored. Curves showing dependence of blood levels of dalargin and its metabolites on time after intramuscular and intranasal injection (for a single animal) are given in Fig. 1. The highest blood level of dalargin was observed in both cases 10 min after injection. The principal metabolite is an N-terminal tetrapeptide. The absence of ^{3}H-tyrosine from the blood during the first 3 min after intranasal injection will be noted. Aminopeptidase activity against the Tyr^1-D-Ala^2 bond is evidently not exhibited during absorption of dalargin from the nasal cavity. Analysis of the nasal washings (Fig. 2) showed that the dalargin in them is degraded by enzymes of the mucosa with the formation of N-terminal tetra- and pentapeptides and no significant quantities of ^{3}H-tyrosine were present. The appearance of ^{3}H-tyrosine in the blood 3 min after intranasal injection of dalargin was thus due mainly to hydrolysis of the hexapeptide already absorbed and its fragments in the blood stream.

As a result of absorption and degradation of dalargin, its content 15 min after injection amounted to 5% of the dose. The time course of changes in the dalargin concentration after intravenous, intramuscular, and intranasal modes of injection is illustrated in Fig. 3. The terminal regions of the three kinetic curves are virtually parallel, evidence that the elimination rate constant is independent of the route of injection of the preparation, so that the rule of corresponding areas can be used to calculate bioavailability.

Values of the elimination rate constant, the half-elimination time, the area beneath the concentration–time curve, total clearance, and bioavailability of dalargin when administered by different routes are given in Table 1. The absolute bioavailability of dalargin when injected intramuscularly and intranasally was low (Table 1). Nevertheless, intramuscular injection of dalargin under clinical conditions demonstrates its high efficacy in peptic ulcer. The relative bioavailability of the preparation by the intranasal route (54% of the intramuscular value) is therefore sufficiently high to warrant the intranasal route as a possible mode of its administration.

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