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Problems of pharmacokinetic studies on alpha-difluoromethylornithine in mice*

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Summary. The pharmacokinetics of α-difluoromethylornithine (DFMO), an irreversible inhibitor of polyamine biosynthesis, were investigated in BALB/c (nude) mice after i.p. injection and after oral administration of radiolabeled drug. After i.p. injection the compound was rapidly cleared from the serum ($t\frac{1}{2}\alpha = 14$ min; $t\frac{1}{2}\beta = 2.1$ h) and from tissues such as muscle, liver and kidney ($t\frac{1}{2}\alpha = 30–60$ min; $t\frac{1}{2}\beta = 2.1$ h). DFMO concentrations were proportional to the administered dose (10–2000 mg/kg) in both serum and tissues.

Oral administration of DFMO was carried out by dissolving the compound in drinking water at a concentration of 20 g/l. Studies on the distribution showed that DFMO did not accumulate preferentially in any particular tissue. An extremely wide variation in the dose actually achieved in different animals was observed; this ranged from 350 to 2800 mg/kg for a 14-h treatment period. A significant correlation ($r = 0.83–0.92$) between the dose of DFMO, calculated from the consumption of drinking water for each individual animal, and the DFMO concentrations in serum, muscle, spleen, liver and kidney was found. Similarly, it was shown that oral administration of DFMO during the daytime resulted in 10- to 15-fold lower levels than administration during the night. After discontinuation of treatment DFMO levels in serum and tissues decreased by 50% in approximately 6 h.

From these results it is concluded that the optimal treatment schedule of mice with DFMO (or other drugs with similar pharmacodynamic properties) consists in a combination of oral administration via the drinking water and additional i.p. injection (during the daytime). Furthermore, the drug intake of the individual animals should be monitored to check whether the experimental requirements are actually fulfilled.

Introduction

DL-α-Difluoromethylornithine (DFMO) is an irreversible inhibitor of ornithine decarboxylase (ODC) [6], the rate-limiting enzyme in polyamine biosynthesis [3, 7]. It exerts growth-inhibitory effects in many experimental tumor systems in vitro and in vivo [4, 9]. In studies with tumor-bearing rodents the drug is commonly administered by the oral route, a 2%–3% solution being given in the drinking fluid.

Optimization of treatment procedures requires knowledge of the pharmacokinetic properties of the drugs involved. With DFMO the continuous presence of relatively high concentrations is necessary, since its target, ODC, is a rapidly inducible enzyme. In this paper the pharmacokinetics of DFMO in mice after administration of the drug by i.p. injection or by the oral route are described.

Materials and methods

Animals and tumors. Either homozygous athymic nude (nu/nu) mice or their heterozygous littermates (nu/+) were used. The animals were of BALB/c background and were kept in controlled environmental conditions [8]. Acidified drinking water (pH 2.9) was supplied ad libidum.

The human prostatic cancer cell line PC-93 [8] and the human renal carcinoma line RC-21 [5] were grown in nude mice as described earlier.

Administration of drug. DFMO was generously supplied by the Merrell Dow Research Institute (Strasbourg, France). Radiolabeled drug, DL-α-difluoromethyl-[5-^{14}C]ornithine (specific activity 60 mCi/mmol) was purchased from Amersham (Bucks., England). The drug was dissolved either in the drinking fluid at a concentration of 2% (w/v) for oral administration or in 0.9% saline at various concentrations for injection. Labeled DFMO was added to give a final specific activity in drinking fluid of 12 μCi/mmol; for i.p. administration approximately 1 μCi in a volume of 0.15–0.25 ml was injected per animal.

Measurement of drug concentrations. The animals were sacrificed at various times after the administration of radiolabeled drug. Serum was obtained after bleeding from the orbital sinus. A variety of tissues, including tumors, were dissected and appropriate aliquots (up to 300 mg) were weighed and solubilized in glass scintillation vials with 1 ml Soluene-350 (obtained from Packard, Brussels, Belgium) at a temperature of 50 °C. After the addition of Instagel scintillation fluid (Packard) supplemented with 10 ml acetic acid and 1 g butylated hydroxytoluene per l, radioactivity was counted in a liquid scintillation spec-

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Abbreviations used: DFMO, α-difluoromethylornithine; ODC, ornithine decarboxylase

trometer (Searle, Isocap-300). Corrections for quenching were made using the external standard channels ratio. DFMO concentrations were calculated from the known specific activity (dpm/nmol) and expressed as nanomoles per gram of tissue or per milliliter of serum.

Thin-layer chromatography. In order to verify the purity of the compound used and to check for possible metabolic conversion, thin-layer chromatography was performed on F1500 Silica Gel plates (Schleicher & Schull, Dassel, FRG). ^{14}C-labeled and unlabeled L-ornithine and DFMO were used as reference standards. The plates were developed twice using the solvent system ethanol/ammonia/water (80/4/16, v/v/v). Spots were visualized with ninhydrin reagent (0.1%). The localization of radioactivity was determined using a Panax TLC scanning apparatus.

Results

DFMO concentrations in serum and tissues of mice were determined at various times after i.p. injection. After a single injection of labeled DFMO at a dose of 1000 mg/kg body weight, large amounts of radioactivity appeared in the serum and in various tissues within 15 min. The concentration of DFMO thus achieved in mouse serum 15 min after injection was approximately 8.5 m*M*. Figure 1 shows that the concentration of the drug in serum decreased rapidly. The apparent initial half-life ($t_{1/2}\alpha$) of DFMO in serum was calculated to be 14 min, while the initial half-lives in muscle, kidney, and liver were 31, 35, and 62 min, respectively, as determined from the semilogarithmic plot shown in Fig. 1. The eleimination half-life ($t_{1/2}\beta$) was calculated to be approximately 2.1 h. Similar kinetics were observed after i.p. administration of DFMO at a dose of 200 mg/kg or 2000 mg/kg. DFMO concentrations measured in serum and tissues (kidney, liver, spleen, and muscle) 2 h after i.p. injection showed a linear relationship with the administered dose over the whole range studied (up to 2000 mg/kg body weight).

When urine was collected 4 h after i.p. injection of labeled DFMO, variable but high concentrations of radioactive material were found to be present. The chromatographic behavior of the compound recovered in the urine was similar to that of the original compound dissolved in urine from control mice, but clearly different from that of, for instance, L-ornithine, which showed a much lower mobility in the thin-layer system employed. Chromatographic analysis of labeled compound(s) in tissue homogenates could not be performed because concentrations of radioactivity were too low to allow such an analysis. However, on the basis of the result obtained with urine samples it was assumed that DFMO was not metabolized to any considerable extent in mice in the conditions used and that DFMO concentrations were thus accurately reflected in the measured levels of radioactivity.

As an alternative to i.p. injection, DFMO was administered p.o. dissolved in drinking fluid. The distribution in various tissues in mice, measured after a period of 14 h, is shown in Fig. 2. To reduce the wide variation observed in the absolute values, DFMO concentrations were expressed relative to the concentration in muscle for each individual animal. The highest concentrations were present, as expected, in the organs which are part of the uptake and elimination pathway (intestine, liver, and kidney). In none of the other tissues was preferential accumulation of DFMO observed (Fig. 2). In this respect, no differences were observed between 'normal' (immunocompetent) and nude mice. Drug levels were also only 1.5-fold higher in xenografts of the human tumor lines PC-93 and RC-21 than in muscular tissue.

To investigate whether the variation in the DFMO concentrations from one mouse to another was related to differences in the intake of the drug, the consumption of drinking fluid was monitored for each individual animal. Considerable variations of the intake of drinking fluid

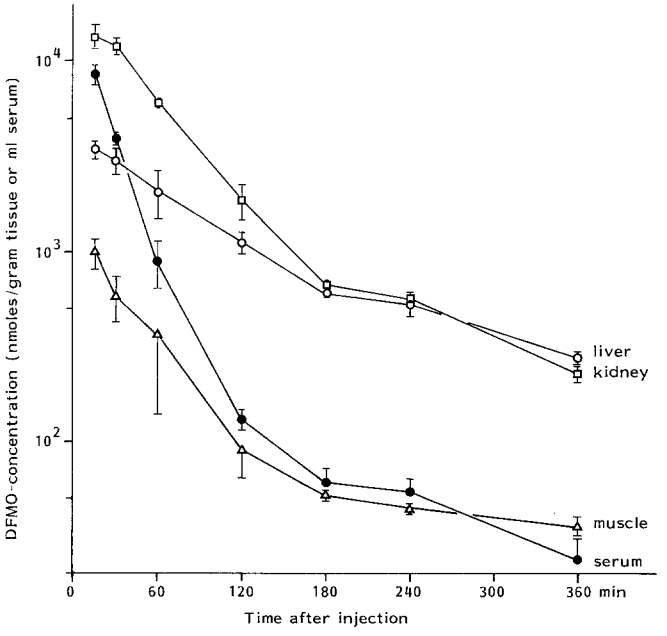

Fig. 1. Pharmacokinetics of DFMO in nu/+ mice after i.p. injection at a dose of 1000 mg/kg. *Points,* means; *bars,* SD ($n=4$)

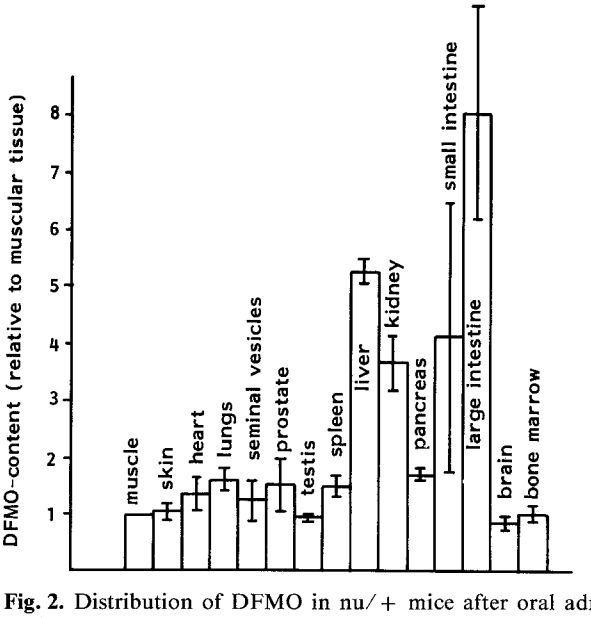

Fig. 2. Distribution of DFMO in nu/+ mice after oral administration. DFMO was given as a 2% solution in drinking water overnight for 14 h. DFMO concentrations, calculated in nanomoles per gram of tissue, are expressed relative to the concentration in muscular tissue for each animal ($n=5$)

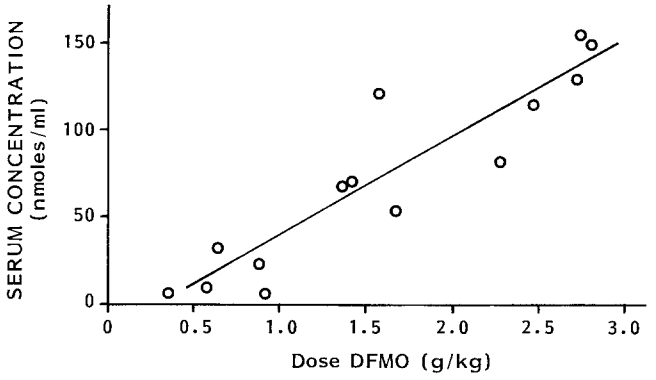

Fig. 3. Dose-dependence of DFMO concentrations in serum of nu/+ mice after oral administration. The dose of DFMO was calculated from the consumption of drinking fluid, which was recorded individually for each animal. The animals were treated with DFMO for 14 h

were observed. However, it appeared that the variations between different mice on one day exceeded the daily variations observed with the same animal, indicating that some animals consistently consumed more drinking fluid than others. The daily consumption of drinking fluid, monitored in 10 mice on 5 subsequent days, showed an average coefficient of variation of 16% (range: 8–32); the average coefficient of variation for the water consumption by the same animal on 5 subsequent days was 10% (range: 3–19; $n=10$ mice). After replacement of the regular drinking water with DFMO solution the inter-mouse variations increased even further, to 23% (range: 7–46). This effect, which was most pronounced during the first 2 days of DFMO administration, was probably caused by the fact that the mice (and some animals in particular) found the DFMO solution unpleasant. In preference tests (in which DFMO and normal drinking water were available simultaneously) the animals consumed the regular drinking fluid at an 8-fold higher rate on average (range: 4–12) than the DFMO solution. Figures 3 and 4 show a (non-random) selection of 15 animals, all kept under the same environmental conditions, for which the intake of (labeled) DFMO varied from 0.35 to 2.8 g/kg body weight (as indicated by the horizontal distribution of the values). There was significant correlation between DFMO dose and DFMO concentration in the serum (Fig. 3; $r=0.92$), in liver (Fig. 4; $r=0.87$), kidney ($r=0.85$), spleen ($r=0.87$), and muscle ($r=0.83$).

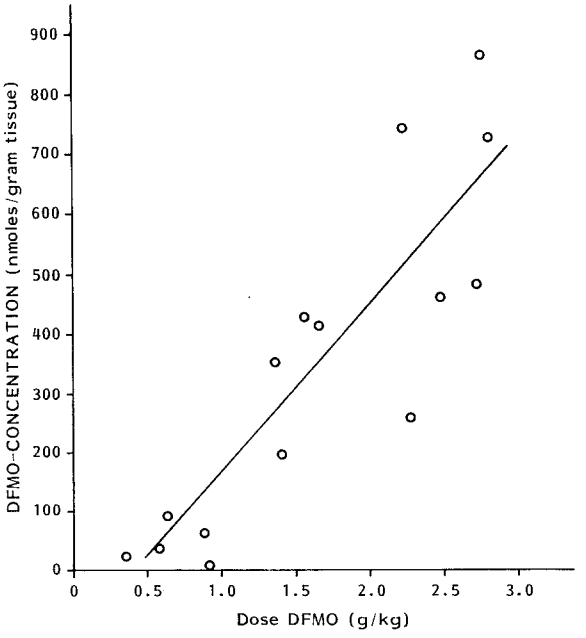

Fig. 4. Dose-dependence of DFMO concentrations in mouse liver tissue after oral administration. Same animals as in Fig. 3

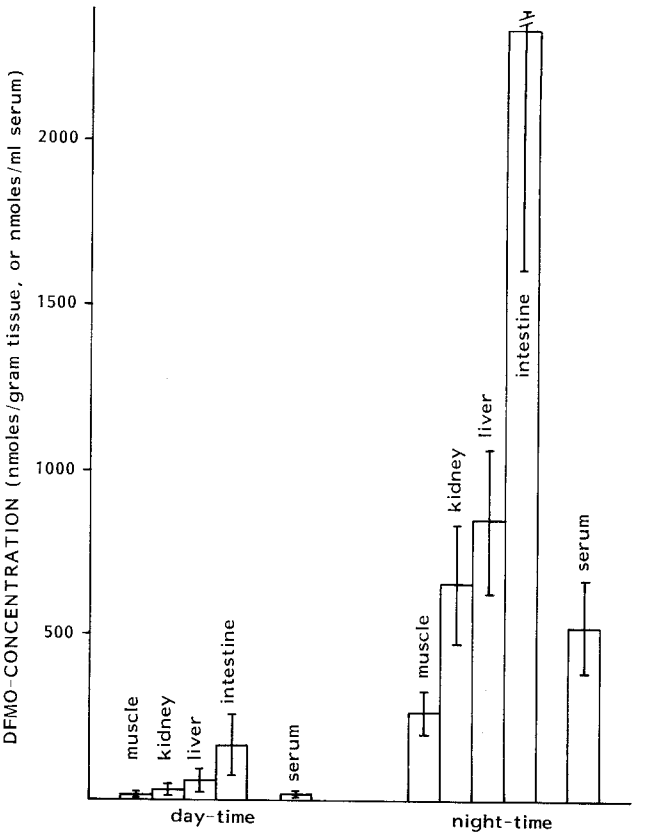

Fig. 5. DFMO concentrations in various organs of nude mice after oral administration during day-time (8 a.m. to 6 p.m.) and during night (8 p.m. to 8 a.m.). The animals were sacrificed at the end of the administration period

The experiments described above involved oral administration of DFMO overnight. Figure 5 shows a comparison between the DFMO concentrations achieved by administration during the daytime, in the light, and during the night-time, i.e. in the dark, for approximately the same period of time. In the dark, 10–15 times higher levels were attained than in the light in all tissues investigated. Such a difference is in agreement with the generally much lower consumption of drinking fluid by mice during the daytime.

The kinetics of the disappearance of DFMO after oral administration were studied by measuring drug levels in serum and tissues at various times after the substitution of DFMO solution by plain drinking water. In view of the possible variations in the drug dose actually received, the intake of DFMO was recorded for each individual mouse and only those animals which had taken a DFMO dose within the range of 0.9–1.8 mg/kg were used for this investigation. The drug was given overnight over a 14 h period. Figures 6 and 7 show that the level of DFMO remained

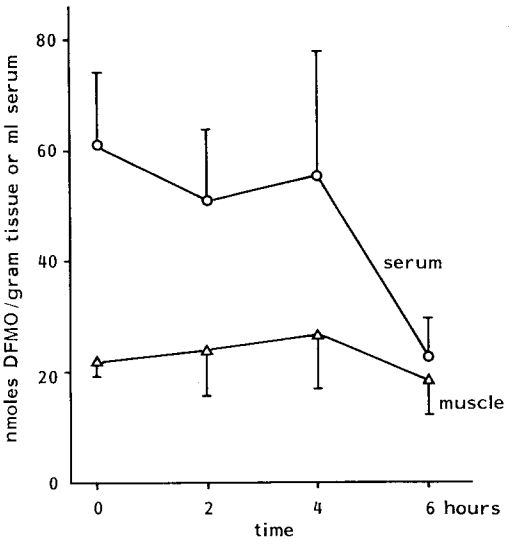

Fig. 6. Pharmacokinetics of DFMO in mouse serum after oral administration in nu/+ mice. DFMO was given overnight; during the subsequent 2–6 h the mice received normal drinking fluid (in the light period). For this experiment only mice that had received a dose in the range 0.9–1.8 g/kg were used. The mean dose was the same in all groups. *Points*, means; *bars* SD, for groups of 5–7 mice

constant during the first few hours after removal of the DFMO-containing drinking fluid. After 6 h, however, DFMO concentrations were reduced by 50%.

Discussion

As ornithine decarboxylase is a rapidly inducible enzyme [3], effective inhibition of its activity requires the continuous presence of inhibitor molecules. Our results demonstrate that the half-life of DFMO after i.p. administration in mice is very short. Although the levels in both serum and tissues can be increased by elevating the dose of DFMO, the short biological half-life prohibits the application of i.p. injections in many kinds of therapeutic studies in mice. In particular, in nude mice it is not feasible to give the animals injections repeatedly (several times per day) for an extended period of time.

To achieve more nearly continuous administration, DFMO is commonly given dissolved in drinking water. The present results indicate that the serum levels of DFMO that can be achieved by either oral or i.p. administration are well within the range of concentrations used for in vitro studies and are comparable to the levels attained in humans [1, 2]. However, a major problem with the oral administration of DFMO to mice via the drinking fluid is the lack of drug intake control, which may result in wide variations in drug concentrations between individual animals and at different times of the day (at least with the particular strain used in this investigation). The inter-mouse variations were found to be more pronounced than the daily variations observed within the same animal regarding water consumption. Therefore, it is recommended that the individual drug intake be carefully monitored, so that animals that do not conform the experimental requirements can be identified and excluded from the study.

The correlation found between DFMO concentrations and drug intake during overnight oral administration (Figs. 3 and 4) probably indicates that the animals had the same pattern of water intake consistently over the dark period. Surprisingly, drug levels appeared to remain constant for several hours after termination of the treatment (Figs. 6 and 7). This might be due to the absence of urinary excretion during the first hours of the light period and could be related to a certain degree of dehydration in these animals. These observations suggest that the diurnal variation in drug intake might be partly eliminated by combining oral treatment (which is effective during the night) with an additional i.p. injection given half-way through the light phase of the light-dark cycle. This may also apply to other drugs with similar pharmacodynamic properties.

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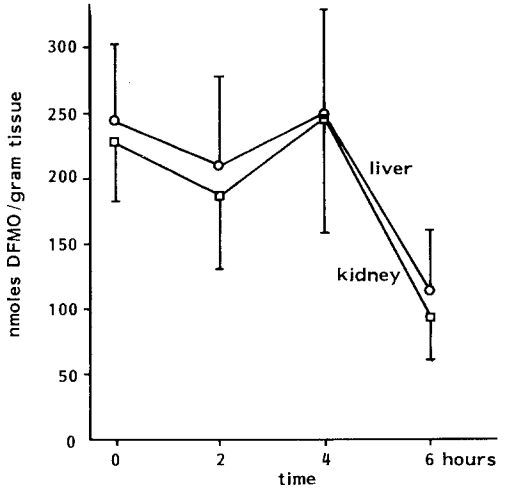

Fig. 7. Pharmacokinetics of DFMO in mouse tissues after oral administration in mice. Same animals as in Fig. 6

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