International Journal of Pharmaceutics, 40 (1987) 201–206
Elsevier

IJP 01361

Magnetic drug targeting. I. In vivo kinetics of radiolabelled magnetic drug carriers

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(Received 11 May 1987)
(Accepted 20 June 1987)

Key words: Magnetic drug targeting; Radiolabelled magnetic drug carrier; In vivo kinetics; Mathematical model of drug carrier capture; Magnetic microparticle

Summary

The localization of intravenously injected magnetic drug carriers in the injured area of an organism is complicated by carrier capture in the liver and other normal organs and tissues. The in vivo kinetics of radiolabeled magnetically targeted drug carriers has been studied; one-exponent kinetics is shown. A mathematical model of carrier capture in animal tissues based on capture mechanism and mass-transfer processes in the circulating blood is proposed. Biodistribution and capture intensity of the carbohydrate- and albumin-coated magnetic microparticles of different sizes have been compared. For detecting small differences in the magnetic carriers' biokinetics, which are normally obscured by individual differences of the animals, a "one-animal" biokinetic test based on the proposed model proved to be effective.

Introduction

An important problem of practical medicine is the limitation of drug use because of different side-effects and complications. The localization of drug action in injured tissues is a promising way to solve this problem.

If the injured area is localized and its position is known, then the drug-containing magnetic carriers driven by externally applied magnetic field can help to prevent such complications. The possibility of drugs binding to magnetic carriers has already been investigated (Mosbach and Shroder, 1979; Widder et al., 1983; Akimoto and Morimoto, 1983; Torchilin et al., 1985); their biological effects in the experimental animals are reported (Widder et al., 1983). However, in vivo considerable accumulation of magnetic microparticles outside the area of magnetic field application usually takes place (Mosbach and Shroder, 1979; Akimoto and Morimoto, 1983; Widder et al., 1983). Being injected intravenously magnetic particles are rapidly captured by reticuloendothelial cells (Widder et al., 1983). Thus, a question appears: how the redistribution of a drug (compared with its routine administration in native form) can influence its physiological effect and subsequent patient safety?

We have studied spontaneous biodistribution of some potential magnetic drug carriers and their biokinetics. The main factors influencing the bio-

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distribution of microparticles are, evidently, their size and the character of their surface; that is why in our experiments we used microparticles of the same size but with different coatings, and microparticles with identical surface but differing in size.

Materials and Methods

Magnetic Fe_3O_4 particles (10–30 nm in diameter; electron microscopy data) were produced by alkaline precipitation of Fe^{2+} and Fe^{3+} salts solutions. Coating of magnetic particles with different materials was done by sonication of washed precipitate using Lab-Line Ultratip Labsonic System (Lab-Line Instruments, U.S.A.) in the presence of coating substance with subsequent incubation for 1 h at 90 °C or at room temperature.

Colloidal magnetite (about 5–7 nm in diameter) coated with dextran, was made as described in Molday and Mackenzie (1982).

Magnetic albumin microspheres were produced as described in (Widder et al., 1983) using 10 nm magnetite particles.

"Magnetic Sephadex" was prepared using Sephadex-G25 (Pharmacia, Sweden) as reported in (Torchilin et al., 1985); magnetic dextran particles, 1–3 μm in diameter, were prepared by the same technique.

A radioactive marker ^{99m}Tc was introduced into magnetite particles by coprecipitation of ^{99m}pertechnetate with magnetite. This technique offers high yield and firm marker binding to magnetic particles. The activity of ^{99m}Tc label can vary from 1 to 100 mCi/mg of preparation.

The radiolabelled preparations were injected into the marginal ear vein of male rabbits weighing 3.5 kg; the animals were first narcotized with Nembutal and were restrained under a γ-camera detector (Gamma 11, DEC, U.S.A.).

Results and Discussion

The intravenous injection of any radiolabelled magnetic preparation into the rabbit ear marginal vein is followed by an almost immediate increase in the ^{99m}Tc count rate in the areas above heart and lungs (later in other organs), this is caused by the filling of blood vessels with blood containing radiolabelled particles (Fig. 1a). During the next 15–30 min the count intensity changes depending on the rate of carrier capture by different tissues. In those organs capable of accumulating microparticles, e.g. liver, the radioactivity increases; in other areas it decreases due to the elimination of radioactive particles from circulating blood (Fig. 2). Thus, magnetic particles are rapidly captured from blood by the liver (80%); they also accumulate in the spleen (2–10%), the kidneys (up to 5%), and the bones (up to 10%). Coarse particles (more than 5–7 μm diameter) also accumulate in the lungs (Fig. 1). When the radioactivity in different organs shows no more changes, the microscopy of the blood samples together with the absence of blood radioactivity shows complete disappearance of magnetic particles from the circulation. This indicates that all the particles injected are completely captured by different tissues.

The estimation of quantitative parameters of magnetic particle biodistribution is hindered by non-uniform shielding of γ-radiation in animal tissues. In certain cases, nevertheless, shielding coefficients may be excluded from the kinetic equations and the results of the external measurements of radioactivity may be used for the biokinetics description.

Let us consider the process of microparticle capture with tissues at the level of microcirculation (Fig. 3). A microparticle "m" moving with blood crosses the vessel segment "h". Assuming the single possibility of microparticle capture by a capture site "b" (the mechanism of the capture is not significant) particle "m" either crosses "h" section or is captured by "b" capture site. Supposing the capture probability being X_b and the corresponding probability of the passing through being $X'_b = 1 - X_b$, both X_b and X'_b should depend on the particle "m" properties and site "b" state.

The "v" vessel of any length may be represented as a set of the h_J segments, containing one capture site each. Thus, the probability of passing through the vessel "v" for particle "m" is defined as $X'_v = X'_1 \times \ldots \times X'_n$; corresponding capture

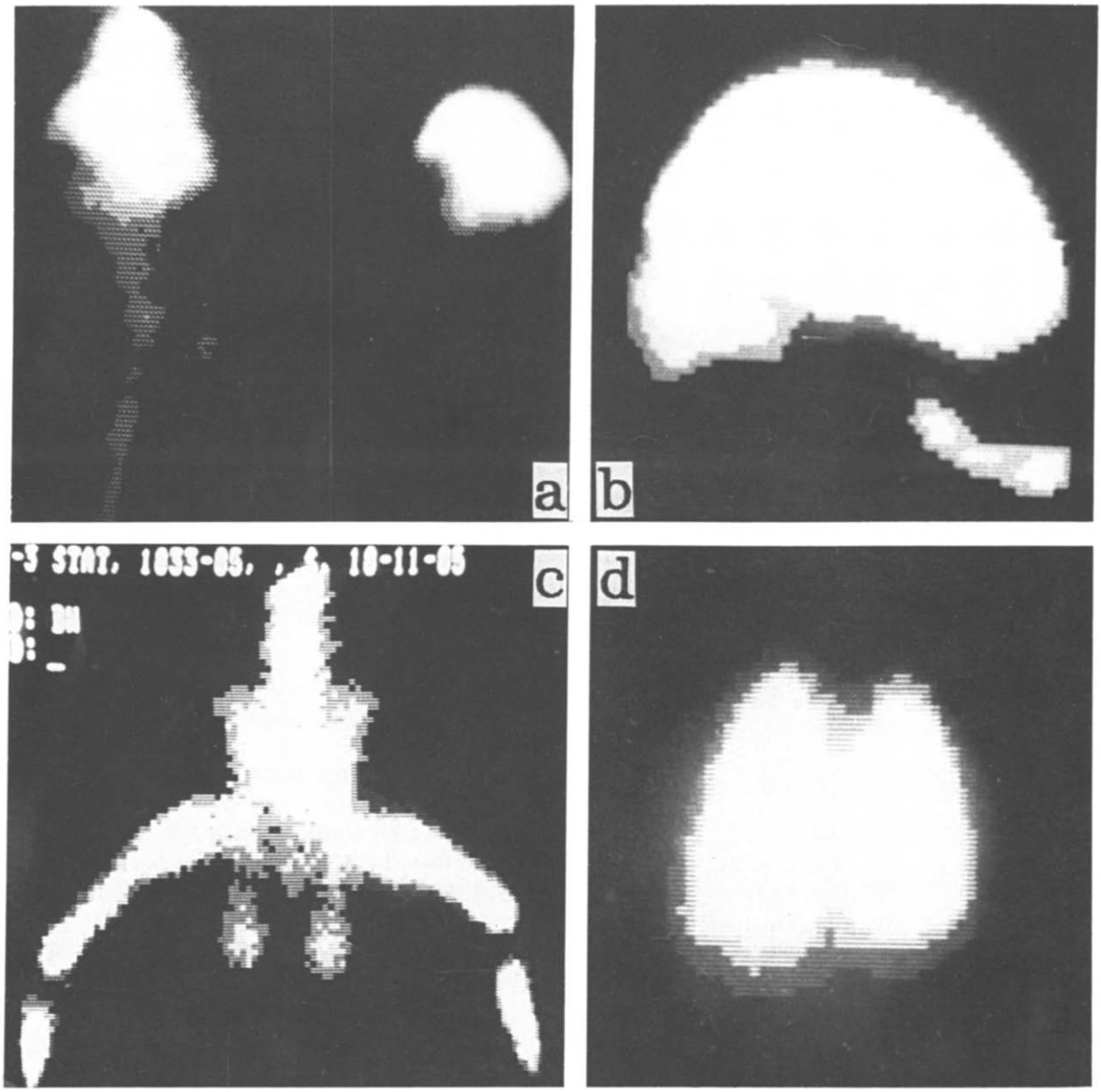

Fig. 1. Accumulation of ^{99m}Tc-labelled magnetic microparticles in the rabbit organs. a, b: capture by the rabbit liver of the magnetic microspheres (obtained from cross-linked albumin according to Widder et al. (1983). 100 mg of Tc-labelled microspheres, 1–2 μm in diameter, were administered intravenously. Pictures are taken at 1 min and 30 min after the injection (a: (top to bottom) heart and lungs, liver, abdominal aorta; b: liver); capture of the same preparation by the liver and spleen of another rabbit (b). c: accumulation of 20–30 nm sucrose-coated magnetic particles in rabbit bones. Picture is taken 20 min after administration of 10.1 mg of microparticles. d: accumulation of 50 μl of the particles of "Magnetic Sephadex", 20 μm in diameter, in the rabbit lungs.

probability is $X_v = 1 - X_1' \cdot X_2' \cdot \ldots \cdot X_n'$.

Any target can be considered as a network of vessels v_k, forming a set of routes for particle "m" entering target by any way (an example is presented on Fig. 3). Particle "m" moves along a route T_m with the probability X_{T_m}. This probabil-

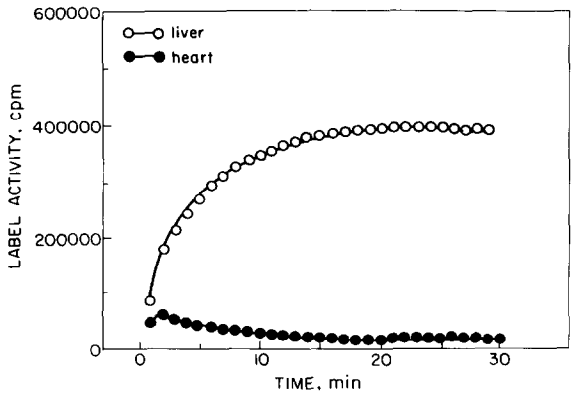

Fig. 2. Accumulation of ^{99m}Tc-labelled dextran-coated magnetic particles (20 nm in diameter, 12 mg injected) in rabbit liver.

ity is determined by the structure and the state of target blood flow system. Moving along the route particle "m" passes through a target with the probability $X'_m = \pi_v X'_v$ (the product of pass possibilities for all the vessels forming a route) or it is captured with corresponding probability $X_m = 1 - X'_m$. Thus, a probability of the passing through of the target of microparticle "m" (by any route) is

$$X' = \sum_{T_m} X'_m \cdot X_{T_m}$$

and the corresponding capture probability is $X = 1 - X'$.

So, the probability of microparticle irreversible capture in any target is a function of the structure of target blood flow system, capture sites quantity and their state, and particle properties (for reversible capture additional parameters of capture sites, e.g. specific half-retaining time, should be introduced). In the case of a magnetic particle remaining on a blood vessel wall there are no specific capture sites. Nevertheless each small vessel section "h" is characterized by appropriate values of hydrodynamic parameters, magnetic field characteristics and specific topography, and can be considered in the frame of the model as an individual capture site.

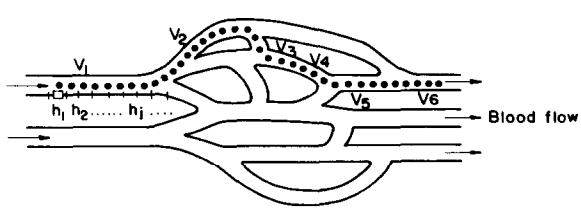

Fig. 3. Scheme of blood flow system of a target (see the text). ●●●, one of the possible particle "m" routes in a target; v, the vessels forming this particle route; h, vessel sections containing one capture site each, □, one of the capture sites "b".

In the case of description of N identical microparticles' behaviour, we can write microparticle concentration in blood entering the target (C) and leaving it (C') as: $C' = X' \cdot C$. Until the capture of particles does not cause pronounced changes in capture sites number and/or state, X' will be constant. This means that microparticles captured in any target can be characterized by a specific capture coefficient: $k = X = (C - C')/C$. In this case the accumulation rate of microparticles in any target "i" can be described by the equation:

$$\frac{dD_i}{dt} = k_i F_i C_i$$

where D_i is a fraction of a preparation dose accumulated in the target, and F_i is a volume blood flow through the target.

An organism (or its circulation system) can be considered as a set of individual "targets" (organs or their compartments) included into the total blood flow. Each of these targets is characterized by a definite value of blood flow volume F (expressed as ml/min or ml/min per 100 g of a tissue). Evidently, the rate of particles accumulation in the target can be expressed as:

$$\frac{dD_i}{dt} = k_i F_i C \qquad (1)$$

where C = particle concentration in the arterial blood:

$$-\frac{dC}{dt} = \sum_i \frac{dD_i}{dt} \cdot \frac{1}{V} \qquad (2)$$

V = the blood volume. At low doses the accumulation of the particles in the target should not affect its normal functions and, thus, k values can be considered as constant during the whole experiment. In this case it follows from Eqns. 1 and 2

that:

$$D_i = D_0 \frac{k_i F_i}{V\alpha}(1 - e^{-\alpha t}) \quad (3)$$

and

$$C = \frac{D_s}{V} = \frac{D_0}{V} e^{-\alpha t} \quad (4)$$

where

$$\alpha = \frac{1}{V} \sum_i k_i F_i$$

D_0 = the administered dose of the preparation, D_s = the dose of the preparation circulating with the blood at the given moment.

Thus, according to the model used, the capture of microparticles in organs and tissues can be described by the Eqns. 3 and 4. We have analysed the experimental data using linearizing (for the model described) coordinates $(\ln[I(t) - I(t + \tau)], t)$. All the results demonstrate very good agreement with the model (Fig. 4). Capture curves for all animals were linearized with $r = 0.95$–0.99 for body areas immoveable during breathing and $r \geqslant 0.8$ for heart and lungs.

The preparation capture in the target can change the intensity of further capture, which should result in linearity disturbances in coordinates proposed. It is also possible that the capture intensity can depend on the particle concentration (as a result of any cooperative effect, for example, particle aggregation). To check this possibility we have studied the capture of magnetite particles, 30 nm diameter, in rabbit tissues. The injection was done 3 times at 20 min intervals. The initial dose 2.2 mg and two subsequent doses of 10 mg each were administered. The radioactivity curves and their linear anamorphoses are presented on Fig. 5. The linearity of curves and their parallelism indicate the independence of the capture parameters (in the liver and other organs) on the particle concentration and preliminary capture (at least in the dose up to 22.2 mg for rabbit).

We have compared the clearance rate and bio-

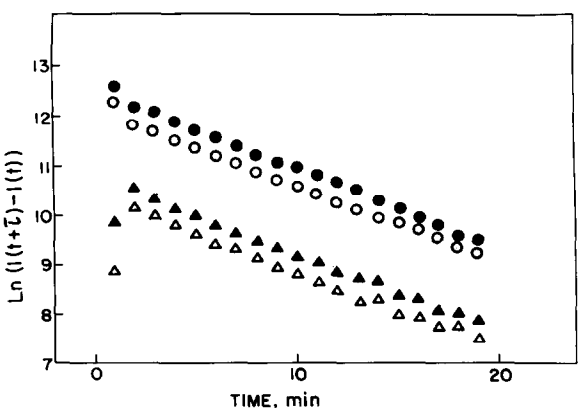

Fig. 4. Linear representations of the curves shown on Fig. 2. ●, ○, liver; ▲, △, heart. (Each curve is processed with two different values of τ.)

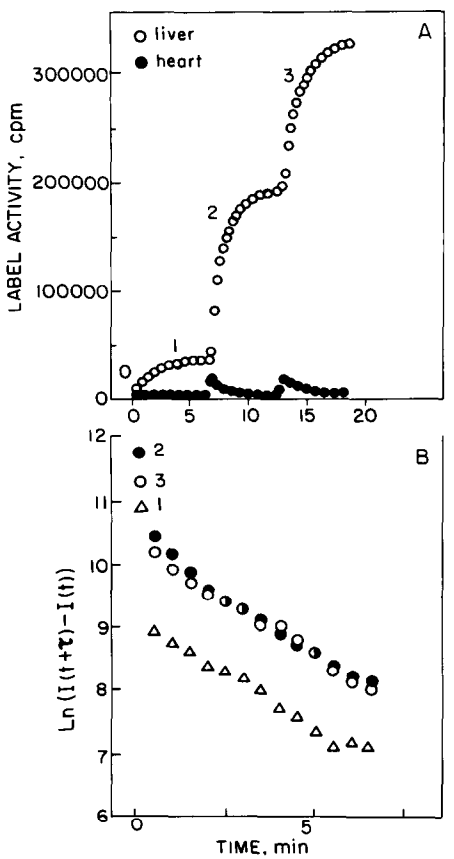

Fig. 5. Label activity in the rabbit liver and heart (A) after the injection of 2.2 (1), 10 (2) and 10 (3) mg of radiolabelled magnetite particles; and linear representations of liver capture curve sections corresponding to consequence of injections (B).

kinetics of different magnetic particles. It was found that only one type among the particles studied – namely, dextran-coated magnetite of 5–7 nm diameter – is able to circulate in the blood for a long time. Other particles are eliminated from the blood with a half-capture time of ca. 5–15 min and accumulate mainly in the liver. The differences between microparticles of different types influence the capture parameters to a lesser degree than individual variations between animals. The particles mainly accumulate in the liver, spleen and kidney. Carbohydrate- (e.g. sucrose) coated particles are captured partially by bones (see Fig. 1).

As we have shown a nil effect of small doses of preliminary captured microparticles on further capture process, we have used this observation to compare biokinetics of different particles in a "one-animal" test. The low doses (less than 1 mg each) of different magnetic particles were subsequently injected at 10–20 min intervals (each dose was injected after radioactivity distribution in the body became constant); the first of the preparations injected was administered a second dose at the end of the experiment for the control of capture parameter stability. A "one-animal" test permits detection of fine differences in magnetic microparticles biokinetics hidden normally by individual differences between the animals.

In general, coarse particles (1–3 μm diameter) are trapped by liver cells 1.5 times more rapidly than fine particles (10–30 nm). Among 10–30 nm particles albumin-coated ones are captured from the blood 2 times faster than dextran-coated particles and 1.5 times faster than sucrose-coated ones. There is no difference between dextran and heparin coating. In the case of large particles (1–3 μm), particles coated with dextran are captured 1.5–2 times more slowly than albumin-coated ones.

Thus, we have demonstrated the applicability of a "one-animal" test for the estimation of the capture parameters for different magnetic microparticles. This creates an opportunity for the development of a prognosis method for the prediction of the biological effect of a targeted magnetic drug on the basis of a mathematical model using experimentally determined capture parameters and physicochemical properties of any magnetic carrier.

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