Study of Manganese Bacteriopheophorbide as a Potential Contrast Agent for Magnetic Resonance Tomography

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The use of manganese bacteriopheophorbide characterized by a high relaxation capacity and selectively accumulating in the tumor as a contrast agent for magnetic resonance tomography significantly improves tumor contrasting against the background of normal tissues. The pharmacokinetics and selectivity of accumulation were studied by diffuse reflection spectroscopy.

Key Words: *manganese bacteriopheophorbide; magnetic resonance tomography; contrast agent; diffuse reflection spectroscopy; selectivity*

Contrast agents (CA) are used for increasing tumor contrasting in magnetic resonance tomography (MRT). Coordination compounds of paramagnetic ions, primarily gadolinium and manganese complexes, represent the most frequently used group of CA, due to high paramagnetism of their ions. CA is to rapidly attain the maximum content in the tumor and selectively accumulate in it. Its concentration in the body should be maintained at a high level during the entire period of the diagnostic procedure and at the same time it should rapidly (less than within 24 h) decrease after the end of procedure.

The method for evaluating the relaxation capacity of CA in solution is simple [7]. At the same time, the study of the pharmacokinetics and selective accumulation of CA in tumors in comparison with the adjacent normal tissues involves some difficulties. The use of traditional methods, for example, extraction or isotope methods, and direct MRT examinations of animals with experimental tumors require unique expensive equipment and reagents. Gd and Mn compounds usually do not fluoresce, and therefore simple fluorescent methods [8] cannot be used.

Some compounds used as CA for MRT have absorption bands in the visible or near-infrared spectrum; the concentrations of these compounds in tissues and organs can be compared *in vivo* by the intensity of these absorption bands; this method can be also used for dynamic studies.

We evaluated the possibility of using manganese bacteriopheophorbide (Mn-Bpheid) as a CA for MRT *in vivo*.

MATERIALS AND METHODS

Mn-Bpheid was obtained at Weizman Institute of Science (Israel) by two-stage transmethylation of bacteriopheophorbide. This substance has two paramagnetic forms: with the central Mn(III) or Mn(II) ion with narrow optical absorption bands, their spectrum maximums about 835 and 775 nm, respectively.

The rates of magnetic relaxation $1/T_1$ in water solutions of both Mn-Bpheid forms of different concentrations were measured on a DBX 400 spec-

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trometer (Bruker) with magnetic field of 9.4 T and Larmor frequency of 400 MHz. The specific relaxation capacity R_1 was estimated by the relaxation rates from the equation:

$$R_1 = \frac{(T_1)^{-1} - (T_1)_0^{-1}}{C_{CA}},$$

where T_1 is relaxation rate in the presence of CA, $(T_1)_0$ relaxation rate without CA, and C_{CA} the CA concentration. The R_1 values for Mn-Bpheid were high: 41.3 and 29.1 $mM^{-1}sec^{-1}$ for Mn(II) and MN(III) forms, respectively.

Preliminary studies showed that the Mn(II) form was more liable to aggregation and precipitation in water solution in high concentrations, and hence the Mn(III) form of Mn-Bpheid was chosen for intravenous injections *in vivo*. Mn-Bpheid was injected to mice into the caudal vein in doses of up to 10 mg/kg (about 15 μmol/kg).

The pharmacokinetics and selective accumulation of Mn-Bpheid in the tumor were studied in 10 $(C57Bl/6\times DBA/2)F_1$ mice with Ehrlich tumor (about 1 cm in diameter) by diffuse reflection spectroscopy using a LESA-01 fiberoptic spectroanalyzer (Biospek).

Magnetic imaging was carried out on 10 nude mice (CD1) with C6 glioma (tumor diameter about 1 cm), to which Mn-Bpheid was injected in the tomograph chamber through a pre-installed catheter (BioSpec 47/30 (Bruker) tomograph with 4.7 T magnetic field. A radiofrequency coil (7.5 cm in diameter) [2-4] was used (interval between pulses TR 750 msec, re-focusing interval TE 15 msec, scanning area 5 cm, section thickness 2 mm, scan resolution 256×150), the T1-weighed spin-echo protocol was chosen.

RESULTS

Absorption bands with maximums at about 835, 775, and 680 nm were detected in the tissue absorption spectra after injection of Mn-Bpheid Mn(III) form. The first of these bands is due to Mn(III) form absorption. The band with the spectral maximum at 680 nm seems to be due to generation and accumulation of chlorine (biochemical degradation product of bacteriochlorophyll derivatives); its intensity monotonously increased throughout the experiment. The appearance of absorption band with the spectral maximum at 780 nm is due to the formation of the Mn-Bpheid Mn(II) form as a result of reduction of injected Mn(III) form by endogenous reducers, *e g.* ascorbic acid, which is present in animals in high concentrations [5].

Accumulation of both Mn-Bpheid forms was evaluated by the intensity of their absorption bands. The concentration of Mn-Bpheid Mn(III) form in the tumor peaked 10-20 min after injection and then slowly decreased (Fig. 1, *a*). The concentration of Mn-Bpheid in normal tissue was significantly lower and rapidly decreased. This provided high selectivity of CA accumulation in the tumor in comparison with normal tissue (selectivity index >4) during several hours. The dynamics of Mn(II) concentration was similar (Fig. 1, *b*), but the selectivity of its accumulation in the tumor was lower.

High selectivity of Mn-Bpheid accumulation together with pronounced paramagnetic characteristics of Mn ions led to a significant (1.4 times)

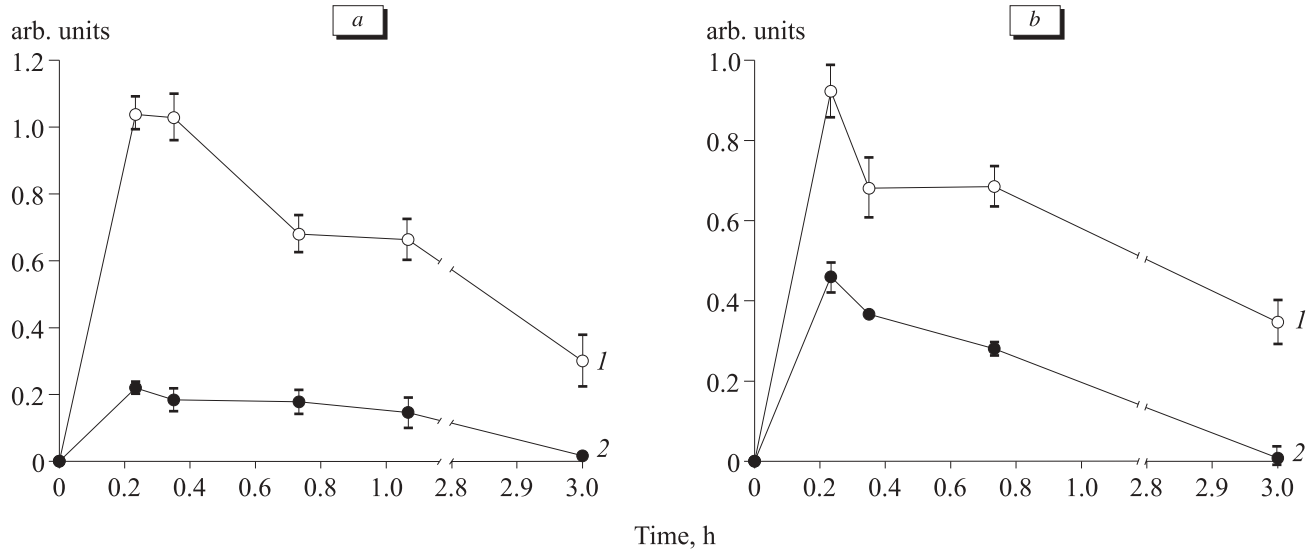

Fig. 1. Accumulation of Mn-Bpheid *in vivo*. *a*) Mn(III) form, 8 mg/kg; *b*) Mn(II) form. *1*) tumor; *2*) normal tissue.

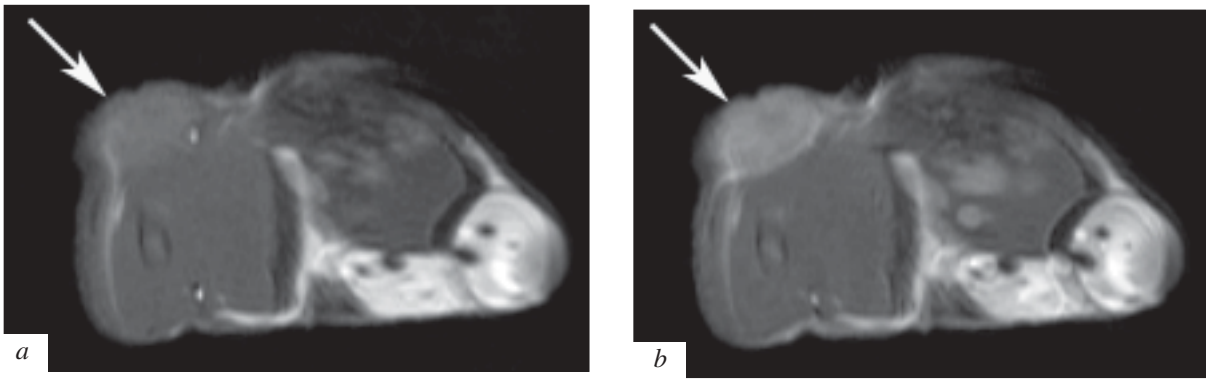

Fig. 2. Lateral MRT scans of CD1 mouse with C6 glioma. *a*) before injection; *b*) 5 min after intravenous injection of Mn-Bpheid. Arrow shows the tumor.

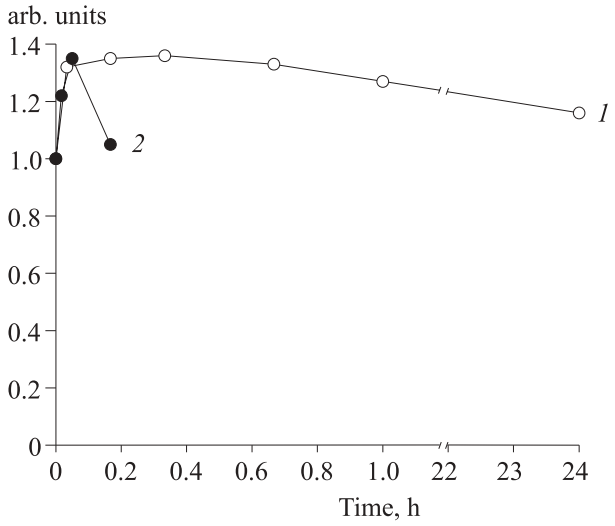

Fig. 3. Increase in MRT contrasting of C6 glioma in CD1 mice. *1*) 15 μmol/kg Mn-Bpheid; *2*) 500 μmol/kg Gd-DOTA.

increase in the tumor MRT contrasting against the background of adjacent normal tissue (Fig. 2) even after its injection in a low dose (about 15 μmol/kg).

The reference CA Gd-DOTA (Dotarem) widely used in clinical MRT exhibited an effect comparable to that of Mn-Bpheid under the same conditions being used in a much higher dose (about 500 μmol/kg; Fig. 3).

Hence, Mn-Bpheid is characterized by high specific relaxation capacity and selectivity of accumulation in the tumor and can be recommended for further studies as a CA for MRT.

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