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Employment of magnet-susceptible microparticles for the targeting of drugs

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Abstract—It has been demonstrated in cats that magnet-susceptible microspheres and liposomes containing neuromuscular blocking agents (dipyronium, pyrocurinum and diadonium) caused a deeper inhibition of the neuromuscular transmission in the limb placed in the magnetic field than in the control limb located beyond the field. The microparticles containing a short-acting neuromuscular blocking agent diadonium appeared to have the highest selectivity of action. The present method allows a pronounced neuromuscular block in a target area to be achieved without noticeable effect on P_{CO_2} of the exhaled air.

The search for ways of increasing the selectivity of action of drugs, of reducing their side effects and toxicity is one of the urgent problems of modern pharmacology. To this end, various techniques are used which enable targeted transport of agents to the target organs. This provides an increase in local concentration of agents in a particular area and at the same time a decrease of the total dose of the drug. Targeted transport of drugs within the vascular compartment (Gregoriadis 1984) can be achieved by pH-sensitive liposomes (Yatvin et al 1980a; Connor et al 1984) which can selectively release the agents during pH changes, e.g. in the inflammation focus; thermosensitive liposomes releasing their content in the presence of local hyperthermia (Yatvin et al 1980b; Sullivan & Huang 1985); and also by various techniques of drug microcapsulation with ferromagnetics (Widder et al 1978; Ibrahime et al 1983; Ovadia et al 1983; Markevicha et al 1980). Magnet-controlled transport has made it possible to achieve a selective increase in the concentration of antineoplastic agents (Tankovich et al 1985), X-ray contrast substances (Bykov et al 1987) and anti-bacterial agents (Prilutskaya 1985) in the target organ.

The present study was devoted to the possibilities of selective action of neuromuscular blocking agents enclosed in magnet-susceptible microparticles on the neuromuscular transmission in certain groups of muscles. For this purpose microcarriers were needed that would provide a high concentration of the agent in the target organ. Magnet-susceptible microspheres (MMS) on the basis of insoluble polyelectrolyte complexes and liposomes (ML) were employed.

Materials and Methods

The possibility of achieving a selective muscle relaxation with the help of neuromuscular blocking agents enclosed in HMS and ML was tested in cats, 2·7–3·5 kg. Tracheostomy was performed under ether anaesthesia and a catheter was introduced into the jugular vein. The animals were then given pentobarbitone sodium (30 mg kg^{-1}, i.v.). To prevent possible hypoxia associated with the action of neuromuscular blocking agents artificial ventilation was started in the animals before the experiment. The neuromuscular transmission in the gastrocnemius muscles of the cat was estimated according to the value of evoked potentials (EP) of muscles during electric stimulation of sciatic nerves by supramaximal rectangular pulses (0·5 Hz). EP were registered by bipolar needles, and 10 consecutive responses were averaged. One hind limb was placed in a magnetic field with strength of about 0·158 MA m^{-1}. The other hind limb, which remained beyond the field, served as control. EP in both limbs were registered continuously throughout the experiment.

Neuromuscular blocking agents with different duration of action were used: dipyronium (long-acting), pyrocurinum (medium-acting) and diadonium (short-acting) (Kharkevich 1986).

MMS from an insoluble polyelectrolyte complex were prepared by mixing in saline of the solutions of nucleic acid, polyethylenimine, neuromuscular blocking agent and ferroparticles. MMS were 0·7–2 μm in diameter and were injected i.v. One mL of suspension contained 350 μg of diadonium, 130 μg of pyrocurinum or 20 μg of dipyronium (single neuromuscular blocking dose per 1 kg b.w.). The animals were injected with 0·4 mL kg^{-1}.

The liposomes consisted of phosphatidylcholine and cholesterol in a molar ratio 5:2 and 7:2. The lipid film for ML was prepared in a rotor evaporator under vacuum. A buffer solution containing gelatine-stabilized ferroparticles, and neuromuscular blocking agents diadonium and dipyronium were then added to the lipid film. The resulting suspension was then sonicated under nitrogen at ice-melting temperature for 60-100 s. The size of ML ranged from 0·1 to 2 μm. The drugs not enclosed in liposomes were removed by dialysis for 24 h. The content of diadonium and dipyronium in 1 mL of the suspension was 100 μg mL^{-1} and 10 μg mL^{-1}, respectively.

The data were expressed as mean ± s.e.m. Statistical differences in the data were analysed by Student's *t*-test.

Results

It has been shown in control experiments that the magnetic field of 0·199 MA m^{-1} strength was without noticeable effect on the neuromuscular transmission at the chosen parameters of stimulation. Under these conditions the administration of neuromuscular blocking agents dipyronium, pyrocurinum and diadonium was associated with a decrease of the amplitude of potentials identical in both limbs of the animal. The injection of

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MMS and ML not containing neuromuscular blocking agents was without effect on the neuromuscular transmission.

a) *Magnet-susceptible microspheres.* On injection of MMS containing dipyronium its action tended to be more marked in the limb placed in the magnetic field. The results obtained, however, were not consistent enough and therefore, no significant differences were revealed.

The experiments with pyrocurinum-containing MMS have demonstrated that 1 min after the injection the amplitude of EP in the limb placed in the magnetic field decreased to 47·5±4·7% of the baseline level. Over the same period the amplitude of EP in the control limb was 75·5±2·0%. Maximum effect was reached 3 min after the administration. The amplitude of EP in the limb placed in the field decreased to 31±2·5%. Meanwhile, no significant increase in the neuromuscular blocking action was observed in the control limb. After 3 min the mean amplitude of potentials was 73·2±2·8%. After 5 min the restoration of the neuromuscular transmission began as was evident from a certain increase in the EP. By 10 min after the administration of pyrocurinum the neuromuscular transmission was almost completely restored (Fig. 1).

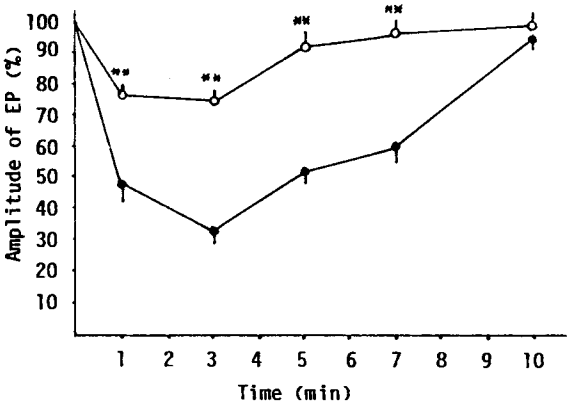

FIG. 1. Action of pyrocurinum-containing magnet-susceptible microspheres on the amplitude of evoked potentials (EP) in gastrocnemius muscles of the cat. (○) EP amplitude in control limb, (●) EP amplitude in the limb placed in the magnetic field. Asterisks indicate statistical significance of results. ** $P<0.01$.

The experiments with diadonium showed an even greater efficiency of the targeted transport. Thus, 3 to 5 min after administration of diadonium the amplitude of EP made up 23±1·5% of the original level in the limb placed in the magnetic field, whereas in the control limb it was 51·2±2·9%. After 20 min the amplitude of potentials in both limbs was completely restored.

A special experimental series was devoted to the assessment of the action of diadonium-containing MMS on P_{CO_2} of the exhaled air in spontaneously breathing animals. The administration of diadonium-containing (140 $\mu g\ kg^{-1}$) MMS caused the inhibition of the neuromuscular transmission in the limb placed in the magnetic field by 65±1·7% and was without effect on P_{CO_2} of the exhaled air. Diadonium injected in the same dose in control experiments increased P_{CO_2} by 35±1·1%. The administration of diadonium without MMS in doses causing a 65% inhibition of the neuromuscular transmission was followed by an increase in P_{CO_2} by 55%, and a higher inhibition of the neuromuscular transmission was always associated with a dramatic depression of respiration.

b) *Magnet-susceptible liposomes.* After i.v. administration of ML containing dipyronium (20 $\mu g\ kg^{-1}$) a decrease of EP amplitude was observed in gastrocnemius muscles of both limbs. The responses in the limb placed in the magnetic field diminished by 45±3·7% and in the control by 7·5±1·5%. With increasing doses of dipyronium-containing ML the selectivity of action decreased.

The administration of diadonium-containing ML in submyoparalytic dose (115 $\mu g\ kg^{-1}$) caused a 70±4% inhibition of EP in the limb placed in the magnetic field and in the control one by only 15±9%. Maximum effect was observed 3 min after the administration and complete restoration after 9 min (Fig. 2).

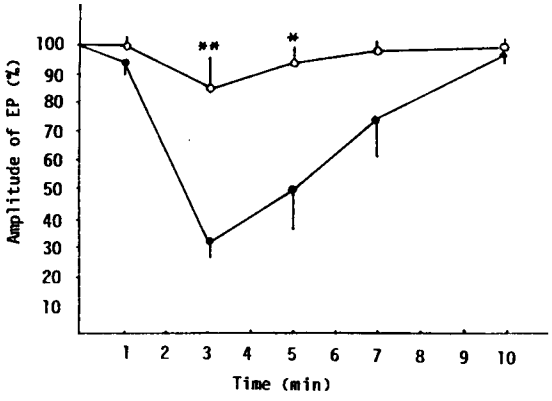

FIG. 2. Action of diadonium-containing magnetic liposomes on the amplitude of evoked potentials (EP) in gastrocnemius muscles of the cat. (○) EP amplitude in control limb, (●) EP amplitude in the limb placed in the magnetic field. Asterisks indicate statistical significance of results. * $P<0.05$, ** $P<0.01$.

Liposomes containing diadonium or dipyronium but without ferromagnetic particles inhibited the neuromuscular transmission equally in both limbs. In this case the action of neuromuscular blocking agents developed gradually reaching a maximum 20-25 min after the injection.

Discussion

The possibility of increasing the concentration of antineoplastic agents in the target organs and their effect with the help of magnetic fields has been reported (Widder et al 1978; Tankovich et al 1985; Sugibayashi et al 1982). A local effect of thrombolytic agents enclosed in the ferrofluid was observed by Rusetsky et al (1986). Magnet-susceptible erythrocytes containing acetylsalicylic acid can provide a local concentration of the agent needed for the prevention of platelet aggregation (Samokhin & Domogatsky 1987).

It has been shown in our experiments with neuromuscular blocking agents enclosed in magnet-susceptible MMS and ML that they provide a more marked inhibition of the neuromuscular transmission in the animal limb placed in the magnetic field. This effect seems to be related to the rise in concentration of neuromuscular blocking agents in the tissues of the target limb. The magnetic field alone is without effect on the neuromuscular transmission. Moreover, the magnetic field failed to enhance the effect of the neuromuscular blocking agents not enclosed in the microparticles.

The rise in local concentration of the drugs in the target limb is achieved by the retention of ferromagnetic-containing microparticles in the heterogeneous magnetic field. The range of distribution of magnetic carriers in the magnetic field is determined by their size, as well as the strength and gradient of the field. In a high-strength field this range is small and this may lead to aggregate formation in the vascular lumen and provide an additional factor promoting the retention of MMS and localization of the effect of the agent. In this period MMS are destroyed and the release of the agent is at its highest.

The employment of ML is also associated with aggregation in the magnetic field. As a result the lipid membrane is broken and its content is released rapidly. This leads to a significant rise in concentration of the neuromuscular blocking agent in the area placed in the magnetic field.

It should be noted that magnetic transport failed to achieve an absolute selectivity of the drug action. The decrease of EP amplitude was observed not only in the limb placed in the magnetic field but also in the control one, though to a lower extent. The highest selectivity of action was observed with short-acting agent diadonium. The duration of action seems to be one determinant of the selectivity of magnet-controlled transport. It is essential that the drug released from magnetic microparticles after having exerted the desired effect on the target organ undergoes a fast biodegradation to be without systemic effect.

The neuromuscular blocking agents entrapped in MMS and ML appeared to produce a shorter effect in our experiments than during conventional administration of the same agents not enclosed in magnet-susceptible microparticles (Kharkevich 1986). This may be due to the uptake of microparticles by reticuloendothelial cells of the liver and spleen. This effect has been described for albumin microspheres (Senyei & Widder 1981) and polymeric carriers (Ibrahime et al 1983). Kharkevich et al (1986) have shown that 30 min after i.v. injection 33 and 6%, respectively, of the injected ML are detected in the liver and spleen (Kharkevich et al 1986).

The uptake of microcarriers is decreased by 20–25% during targeted transport compared with free circulation in blood. A few hours after the injection, however, most of the particles are deposited in the reticuloendothelial system in spite of the presence of magnetic vector. Thus, without efficient decrease of the uptake of magnet-susceptible microcarriers by reticuloendothelial cells they cannot be used as a universal tool for targeting of drugs. At the same time, if it is necessary to achieve a high local concentration of a drug within a short time of injection, e.g. as with neuromuscular blocking agents, magnet-controlled delivery appears to be effective.

Conclusions. Magnet-susceptible microspheres and liposomes allow the provision of a predominant action of drugs in a magnetic field within a short time of their injection. Maximum efficiency of targeted transport was observed with the short-acting neuromuscular blocking agent diadonium.

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