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Research paper

Increase of the duration of the anticonvulsive activity of a novel NMDA receptor antagonist using poly(butylcyanoacrylate) nanoparticles as a parenteral controlled release system

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Abstract

A novel non-competitive NMDA receptor antagonist MRZ 2/576 is a potent but rather short-acting (5–15 min) anticonvulsant following intravenous administration to mice as estimated by the prevention of maximal electroshock induced convulsions. This is most probably due to a rapid elimination of the drug from the central nervous system by transport processes that are sensitive to probenecid. Intravenous administration of the drug bound to poly(butylcyanoacrylate) nanoparticles coated with polysorbate 80 prolongs the duration of the anticonvulsive activity in mice up to 210 min and after probenecid pre-treatment up to 270 min compared to 150 min with probenecid and MRZ 2/576 alone. The results of this study demonstrate that polysorbate 80 coated poly(butylcyanoacrylate) nanoparticles used so far as a delivery system to the brain for drugs that do not freely penetrate the blood brain barrier can also be used as a parenteral controlled release system to prolong the CNS availability of drugs that have a short duration of action.

Keywords: NMDA receptor antagonist MRZ 2/576; Glycine$_B$ site; Choroid plexus; Probenecid; Nanoparticles; Polysorbate 80; Drug targeting; Controlled release

1. Introduction

The amino acid L-glutamate, the major fast excitatory neurotransmitter in the mammalian central nervous system (CNS), acts on different ligand operated ionotropic receptors, namely *N*-methyl-D-aspartate (NMDA), α-amino-2,3-dihydro-5-methyl-3-oxo-4-isoxazole-propanoic acid (AMPA), and kainate receptors and on several types of g-protein-coupled metabotropic receptors. Excessive activation of NMDA receptors following head or spinal cord trauma, ischaemia during stroke or hypoglycaemic conditions is implicated in acute over-excitation of the target neurons (the so called calcium overload) and excitotoxic cell death. Over-activation of NMDA receptors is also involved in chronic neurodegenerative processes and in a wide variety of neurological disorders such as Parkinson's and Alzheimer's disease, epilepsy, amyotropic lateral sclerosis, dementia, chronic pain, and psychiatric diseases such as schizophrenia, drug dependence, anxiety, and depression. Consequently, NMDA receptor antagonists have a powerful potential to protect the CNS from excitotoxic cell damage and, therefore, they became an increasingly attractive target for CNS drug development in the recent years [1].

MRZ 2/576 (8-chloro-4-hydroxy-1-oxo-1,2-dihydropyridazino[4,5-*b*]quinoline-5-oxide choline salt) is a novel non-competitive NMDA receptor antagonist acting at the strychnine-insensitive co-agonistic glycine$_B$ site of the NMDA receptor. Although MRZ 2/576 is only moderately potent in vitro (displacement of [^{3}H]MDL-105.519, a high affinity radioligand, binding to rat cortical membranes: IC_{50} 100 nM [2]) the drug is very potent in vivo (MES-test ED_{50} 7.7 mg/kg i.v. [2]) indicating a good systemic availability and penetration across the blood brain barrier [2], a property that most other glycine$_B$ antagonists are lacking [3]. However, the duration of activity in mice following intravenous administration of an aqueous solution (6 mg/kg) of the drug is rather short (5–15 min) as estimated by the prevention of maximal electroshock (MES) seizures suggesting a rapid clearance of the drug from the CNS. This has already previously been reported for other CNS-acting drugs, e.g. for glycine$_B$ antagonists of the 2-carboxyindole-series [3],

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the AMPA receptor antagonist NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[*f*]quinoxaline) [4] or the antiepileptic valproic acid [5]. In those cases specialized active transport processes at the choroid plexus sensitive to probenecid were responsible for removing the drugs from the CNS.

Probenecid (*p*-[dipropylsulfamoyl]benzoic acid), an uricosuric agent (e.g. Benemid®, MSD) and a pharmacological research tool, prevents active transport of organic acids across epithelial barriers (kidney, brain) by competition with substrates at the carboxylic acid transporter. Upon intraperitoneal administration (200 mg/kg) 30 min before administration of MRZ 2/576 (20 mg/kg i.p.) probenecid prolongs the duration of the anticonvulsive activity of MRZ 2/576 up to 150 min demonstrating that the NMDA antagonist is also transported actively out of the brain [6].

Unfortunately, the use of probenecid as an auxiliary agent to improve the pharmacokinetic properties of MRZ 2/576 causes several problems because probenecid itself has pharmacodynamic activity, namely an uricosuric activity which represents an unwanted side effect. Furthermore, an intraperitoneal route of administration for the probenecid suspension is necessary since probenecid is poorly soluble in aqueous vehicles and, moreover, relatively high doses are required to inhibit the organic acid transporter.

The disadvantages of the probenecid approach may be overcome by the development of a galenical formulation that enhances the brain availability of the new glycine$_B$ antagonist. Recently, Alyautdin et al. [7] demonstrated in an animal model that the delivery of the opioid receptor agonist loperamide across the blood brain barrier by means of polysorbate 80-coated poly(butylcyanoacrylate) nanoparticles not only massively enhanced the central analgesic effect compared to a micellar solution of the drug in 1% polysorbate 80 but also prolonged the duration of loperamide action significantly.

The objective of the present study was to investigate if polysorbate 80-coated poly(butylcyanoacrylate) nanoparticles used so far as a delivery system to the brain for drugs that do not freely penetrate the blood brain barrier can also be used to prolong the CNS availability of drugs that have a short duration of action by employing a similar delivery mechanism. With both types of drugs the active principle would be released in brain compartments either after endocytosis by the brain blood vessel endothelial cells, transcytosis, and/or opening of tight junctions. For this reason nanoparticle formulations containing either MRZ 2/576 or probenecid were prepared and tested in vivo in an animal model for MES protection.

2. Materials and methods

2.1. Animals

Female NMRI mice (Charles River, Sulzfeld, Germany), body weight 18–24 g, were used for the in vivo studies. Water and standard laboratory chow were freely available to the animals.

2.2. Drugs and reagents

n-Butyl-2-cyanoacrylate was obtained from Sichel-Werke (Hannover, Germany), poloxamer 188 was a gift from C.H. Erbslöh (Düsseldorf, Germany), 1.0 N HCl and 1.0 N NaOH were purchased from Merck (Darmstadt, Germany), mannitol from Roquette (Lestrem, France), probenecid, dextran 70000 and polysorbate 80 from Sigma (Deisenhofen, Germany), and MRZ 2/576 (US Patent Application No. 08/686,346) was from Merz + Co., Preclinical R&D (Frankfurt, Germany).

2.3. Preparation of nanoparticles

Nanoparticles were prepared by emulsion polymerization [8] of 1.1% butylcyanoacrylate in an acidic medium (0.01 N hydrochloric acid or ethanol 10% (v/v) (pH 2.25)) using either dextran 70000 or poloxamer 188 at a concentration of 1.5% (w/v) as stabilizer. The following nanoparticle preparations were made (for a further description of the experimental groups see Table 1):

1. Unloaded nanoparticles used in the experimental group 9: poloxamer 188 was used as stabilizer and no drug was added to the polymerization medium (0.01 N HCl).
2. Nanoparticles containing MRZ 2/576 used in the experimental group 3: dextran 70000 was used as stabilizer and the drug (1 mg/ml) was added to the polymerization medium (0.01 N HCl) 30 min after beginning polymerization.
3. Nanoparticles containing MRZ 2/576 used in the experimental group 4: dextran 70000 was used as stabilizer and the drug (2 mg/ml) was added to the polymerization medium (ethanol 10% (v/v) (pH 2.25)) 90 min after beginning polymerization.
4. Nanoparticles containing MRZ 2/576 used in the experimental groups 6 and 7: poloxamer 188 was used as stabilizer and the drug (2 mg/ml) was added to the polymerization medium (ethanol 10% (v/v) (pH 2.25)) 5 min after beginning polymerization.
5. Nanoparticles containing probenecid used in the experimental groups 5 and 6: poloxamer 188 was used as stabilizer and the drug (1 mg/ml) was added to the polymerization medium (0.01 N HCl) 60 min after beginning polymerization.

After 4 h polymerization was complete and the pH of the nanoparticle suspensions was adjusted to pH 6.5 with sodium hydroxide solution. Stirring of the suspensions was continued overnight in order to obtain an optimal amount of drug binding. Then the ethanol was removed (preparations 3 and 4) using a rotary evaporator (Büchi, Switzerland) at 40°C and 200 mbar. The suspensions were filtered through sintered glass filters (G3, pore size 16–40

Table 1
Preparations used in the different experimental groups

Experimental group	Description of the preparation	Total amount of MRZ 2/576 (mg/kg)	Probenecid (mg/kg)	Nanoparticles (mg/kg)	Percentage of drug bound to NP	Polysorbate 80 (mg/ml)
1	MRZ 2/576 solution, i.v.	6.0	–	–	–	–
2	MRZ 2/576 solution, i.p. + PBC suspension, i.p.	20.0	200.0	–	–	–
3	MRZ 2/576 bound to NP without PS 80 coating, i.v.	4.5	–	75.2	100	–
4	MRZ 2/576 bound to NP, i.v.	6.0	–	84.8	51.3[a]	21.7
5	Free MRZ 2/576 + PBC bound to NP, i.v.	6.1	14.4	86.2	0.0[a]/100[b]	23.2
6	MRZ 2/576 bound to NP + PBC bound to NP, i.v.	6.2	6.5	80.6	24.4[a]/100[b]	21.5
7	MRZ 2/576 bound to NP, i.v. + PBC suspension, i.p.	6.7	200.0	83.9	45.5	20.8
8	MRZ 2/576 + PS 80, i.v.	5.9	–	–	–	26.1
9	MRZ 2/576 + unloaded NP + PS 80, i.v.	5.8	–	100.8	–	21.4

[a] MRZ 2/576.
[b] Probenecid, NP, nanoparticles; PBC, probenecid; PS 80, polysorbate 80.

μm, and G4, pore size 10–16 μm, Schott, Germany) and lyophilized in a Christ Beta 1-lyophilisator (Christ Medizinischer Apparatebau, Osterode, Germany) for 48 h. Mannitol was added as a cryoprotectant at a concentration of 3% (w/v) prior to lyophilization.

2.4. Particle size measurement

Particle size measurement was performed by photon correlation spectroscopy using a Malvern Autosizer 2c (Malvern Instruments, UK) connected to a Malvern multi-8-bit correlator (type 7032 CN). Results are quoted as the *z*-average particle diameter (Z_{ave}) and the polydispersity index (Q).

2.5. Quantification of polymer content in the freeze-dried powder

The quantification of the poly(butylcyanoacrylate) content in the freeze-dried powder was performed by gas chromatography according to a modified method published by Langer et al. [9]. Freeze-dried nanoparticles were hydrolyzed under stirring with 1.0 N sodium hydroxide solution for 24 h. The resulting solution was neutralized and an aliquot was poured into a volumetric flask. An internal standard solution (2% *n*-propanol in methanol) was added and the mixture was diluted with methanol to a final volume of 10.0 ml. The butanol content of the solution was determined using a Hewlett-Packard 5890A gas chromatograph (Hewlett-Packard Company, USA) and calculated using a calibration curve. The polymer content of the freeze-dried powder was calculated based on the amount of *n*-butanol produced by hydrolysis of the nanoparticles.

2.6. Determination of drug loading of the nanoparticles

After dissolution of freeze-dried nanoparticles in 0.1 N sodium hydroxide solution the amount of drug (either MRZ 2/576 or probenecid) bound to nanoparticles was determined by HPLC and calculated using calibration curves. HPLC measurements were performed using a Kontron-HPLC connected to a K430A UV-detector (Kontron Instruments, UK). The analytical column used was a Purospher© 100 RP-18 endcapped (Merck, Darmstadt, Germany), and a mixture of acetonitrile, water and trifluoroacetic acid (40:60:0.03) was used as eluent. Detection of both drugs was performed at a wavelength of 256 nm.

2.7. Animal testing

For the characterization of the in vivo activity of glutamate antagonists the MES-test has proven to be a good index both for NMDA as well as for AMPA receptor antagonism [4,10]. Therefore, this convulsion model was used for estimating the ability of MRZ 2/576 to act as an NMDA receptor antagonist in vivo. The mice were divided into nine experimental groups comprising different numbers of animals according to the varying duration of drug activity in the different MRZ 2/576 preparations. Therefore, in groups 1 and 9, 15 animals, in groups 2, 3 and 7, 30 animals, in groups 4 and 6, 35 animals, in group 5, 40 animals and in group 8, 5 animals per group were used. The groups were treated with the preparations and by way of injection described in Table 1.

For the animal testing the freeze-dried nanoparticles were dispersed by ultrasonication in distilled water. Polysorbate 80 was added at a concentration of 2% (w/v) and nanoparticles were incubated for 30 min. Consequently, polysorbate 80 is adsorbed onto the surface of the nanoparticles whereas

the stabilizer used for the preparation process (either poloxamer 188 or dextran 70000) is interwoven with the surface nanoparticle polymer molecules as well as adsorbed to the particle surface. After this additional MRZ 2/576 was dissolved in the preparations for the experimental groups 4, 6 and 7 to obtain a final total concentration of 6 mg/kg MRZ 2/576 for the animal experiments. This step was performed to get similar MRZ 2/576 concentrations in all experimental groups (except group 2), thus to ensure comparability between the results yielded with the nanoparticle preparations and the aqueous MRZ 2/576 solutions. MRZ 2/576 solutions (experimental groups 1 and 2) were prepared using distilled water as solute since the drug is prone to agglomerate in saline or buffer solutions. The MRZ 2/576 + polysorbate 80 solution used in experimental group 8 was prepared by dissolving MRZ 2/576 in an aqueous polysorbate 80 solution.

For intravenous administration each mouse was injected with 0.25 ml of the described preparation via the tail vein. For the i.p. probenecid pre-treatment (experimental groups 2 and 7) probenecid was dispersed in an aqueous carboxymethylcellulose solution (0.5% (w/v)) at a concentration of 2 mg/ml; 0.25 ml of the suspension was injected intraperitoneally. Probenecid pre-treatment was performed 30 min before administration of the MRZ 2/576 solution/nanoparticle suspension.

At various times after dosing subgroups of five mice each were tested for MES protection. Maximal electroshock was performed with a 100 Hz current (20 mA shock intensity, 0.5 s shock duration, 0.9 ms impulse duration, Ugo Basile, Comerio, Italy) applied by corneal electrodes [11]. The presence of tonic convulsions was scored. Results are presented as percentage of mice protected in the MES-test, i.e. mice with no tonic extension of hind paws. All experiments were performed according to the animal rights commission allowance F 77-51 (Hessen).

3. Results

3.1. Nanoparticles

The unloaded nanoparticles (preparation 1) obtained by the preparation process had an average diameter of 228 nm and a narrow size distribution ($Q = 0.05$). The polymer content of the freeze-dried powder that also contained the cryoprotectant mannitol, the surfactant poloxamer 188 and sodium chloride, was 11.2% (w/w). The nanoparticles containing probenecid (preparation 5) had an average diameter of 251 nm and a polydispersity index of 0.053. The freeze-dried powder contained 10.8% poly(butylcyanoacrylate) and the drug loading was 16.7 mg probenecid/100 mg polymer. No free drug was present in the powder. Since large amounts of MRZ 2/576-loaded nanoparticles were needed for the animal experiments it was necessary to produce three batches of them. The nanoparticles used in the experimental group 3 (preparation 2) had an average diameter of 221 nm ($Q = 0.349$), the particles used in group 4 (preparation 3) had an average diameter of 335 nm ($Q = 0.509$) and the particles used in the experimental groups 6 and 7 (preparation 4) had an average diameter of 140 nm ($Q = 0.459$). The polymer content of the freeze-dried product was 14.3, 12.6 and 8.4%, respectively. 5.9, 4.4 and 3.6 mg of MRZ 2/576 were bound to 100 mg nanoparticles and no free drug was present in the freeze-dried powder.

Since the drug-loaded nanoparticles were prepared in the presence of the drug (either MRZ 2/576 or probenecid) the respective drug is incorporated into the particles as well as adsorbed onto the particle surface. However, it is not possible to precisely determine analytically how much of the total amount of drug bound to the nanoparticles is incorporated into the particle matrix or ab- or adsorbed onto the particle surface.

3.2. Animal testing

The results of the experimental groups 1 and 2 are shown in Fig. 1. Five minutes after administration of a MRZ 2/576 solution (group 1) 60% of the animals were protected from MES-induced convulsions. Ten minutes later 20% of the mice were still protected but drug effects disappeared after 30 min. Probenecid pre-treatment 30 min before administration of a MRZ 2/576 solution (group 2) prolonged the duration of drug action up to 150 min when 20% of the maximal possible drug effect can still be extrapolated from the slope of the curve.

The results of the experimental groups 3 and 4 are shown in Fig. 2. Incorporative binding of MRZ 2/576 to nanoparticles without polysorbate 80 coating prolonged the anticonvulsive activity of the drug up to 60 min (group 3). Further extension of the duration of MRZ 2/576 activity was yielded after incorporating the drug into nanoparticles

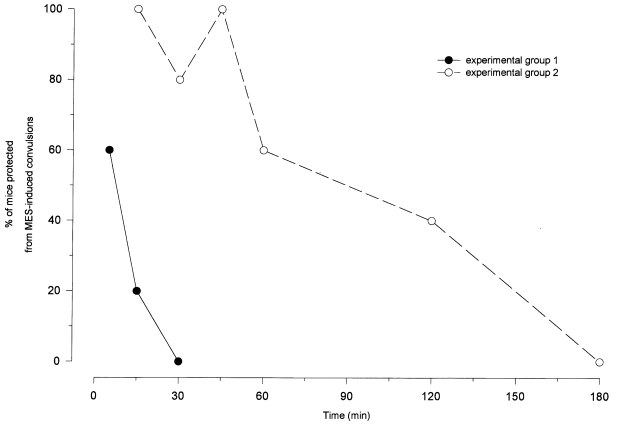

Fig. 1. The duration of the anticonvulsive activity of an aqueous MRZ 2/576 solution (6 mg/kg, i.v.) in the MES-test in mice is rather short (group 1) but after probenecid pre-treatment (200 mg/kg, i.p.) 30 min before administration of a MRZ 2/576 solution (20 mg/kg, i.p.) it is prolonged up to 180 min (group 2).

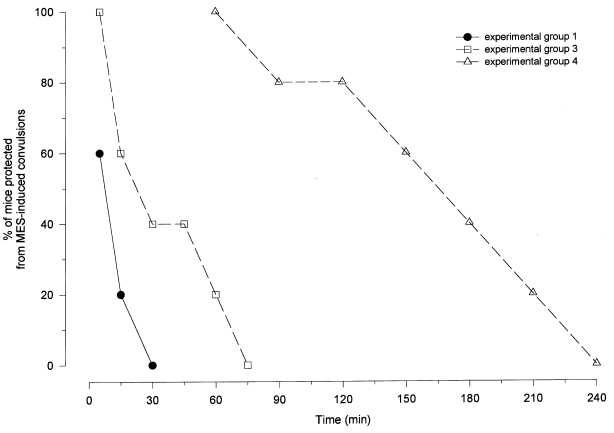

Fig. 2. In comparison with the duration of the anticonvulsive activity of a MRZ 2/576 solution (6 mg/kg, i.v., group 1) the activity of the glycine$_B$ antagonist is prolonged after binding the drug (4.5 mg/kg) to uncoated nanoparticles (group 3) and it is further prolonged after coating the MRZ 2/576 (6 mg/kg)-loaded nanoparticles with polysorbate 80 (group 4).

and coating the drug-loaded particles with polysorbate 80 (group 4). This galenical formulation prolonged the MRZ 2/576 activity up to 210 min.

The results of the experimental groups 5, 6 and 7 are shown in Fig. 3. Probenecid pre-treatment 30 min before administration of MRZ 2/576 bound to nanoparticles (group 7) prolonged the drug effects up to 270 min. Upon administration of a mixture of MRZ 2/576-loaded nanoparticles and probenecid-loaded nanoparticles (group 6) animals were protected from MES-induced convulsions up to 120 min. Better results were achieved with the preparation used in group 5; coadministration of free MRZ 2/576 together with probenecid-loaded nanoparticles prolonged drug action in comparison with group 6 for a further 60 min.

The experimental groups 8 and 9 served as controls. In group 8 polysorbate 80 was added to an aqueous MRZ 2/576 solution. No protection of the mice in the MES-test could be observed after 15 min. Group 9 was treated with a simple mixture of unloaded nanoparticles, MRZ 2/576 and polysorbate 80. This suspension was administered immediately upon preparation giving the components no equilibration time for sorptive binding of MRZ 2/576 or polysorbate 80 to the particles. Forty percent protection could be observed after 5 min, 20% after 15 min and no protection could be observed 30 min or later after dosing.

Further controls (data not shown) confirmed that neither the aqueous vehicle nor polysorbate 80 nor unloaded nanoparticles nor probenecid had any protecting effects in the MES-test itself.

4. Discussion

The present study confirms that MRZ 2/576 is a potent systemically-active NMDA receptor antagonist as shown by injection of a simple aqueous solution of the drug (group 1, Fig. 1). The maximal possible drug effect (i.e. 60% of the animals are protected from MES-induced convulsions) was yielded 5 min after administration indicating a good penetration of the drug to the CNS. In addition, the compound is highly potent with a low ED_{50}-value in the MES-test [2].

The results after probenecid pre-treatment (group 2, Fig. 1) suggest that MRZ 2/576 is actively transported out of the brain by probenecid-sensitive carrier systems. Additionally, probenecid may increase plasma levels of MRZ 2/576 by inhibition of its renal excretion or displacement of the drug from plasma binding sites. At the present state it is not yet possible to differentiate between probenecid effects on the choroid plexus and on the kidney.

The results obtained with the MRZ 2/576-loaded nanoparticle preparations (groups 3 and 4, Fig. 2) strongly confirm that nanoparticles can serve as drug delivery systems for the targeting of MRZ 2/576 to the CNS after parenteral administration. Studies concerning drugs that do not freely penetrate the blood brain barrier (e.g. the above mentioned loperamide [7], the leu-enkephalin analogue dalargin [12], the quaternary ammonium compound tubocurarine [13] or the chemotherapeutic drug doxorubicin [unpublished data]) demonstrated that these drugs were delivered successfully to the brain by binding them to poly (butylcyanoacrylate) nanoparticles and coating these particles with polysorbate 80. After intravenous injection of drug-loaded nanoparticles without polysorbate 80 coating no pharmacological effects were observed demonstrating that the coating is necessary to achieve an uptake of the particles into the brain. The outcome of our studies confirm these findings. This is evident when comparing the results of the experimental groups 3 and 4. After administration of uncoated MRZ 2/576-loaded nanoparticles (group 3, Fig.

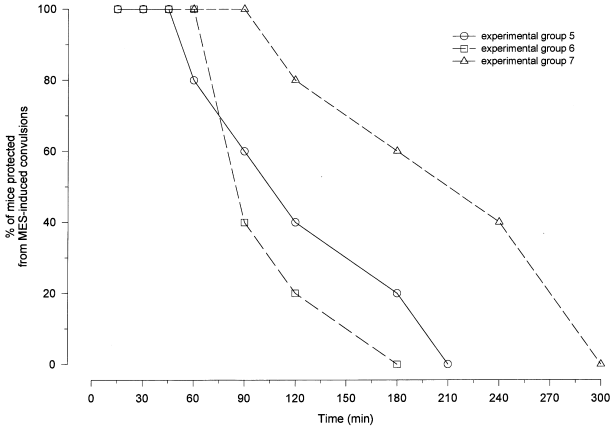

Fig. 3. Intravenous injection of free MRZ 2/576 (6.1 mg/kg) together with probenecid (14.4 mg/kg) bound to nanoparticles (group 5) is more effective in increasing MRZ 2/576 bioavailability than intravenous injection of MRZ 2/576 (6.2 mg/kg)-loaded nanoparticles together with probenecid (6.5 mg/kg)-loaded nanoparticles (group 6). The longest duration of MRZ 2/576 activity was yielded in experimental group 7 after probenecid pre-treatment (200 mg/kg, i.p.) 30 min before administration of MRZ 2/576 (6.7 mg/kg, i.p.) bound to nanoparticles.

2) drug effects were observed for 60 min although it is well known that after intravascular injection uncoated nanoparticles are rapidly captured by the reticulo-endothelial system (RES) resulting in an accumulation of the particles in the liver, the spleen, the lungs and the bone marrow [14,15]. It is possible that uncoated MRZ 2/576-loaded nanoparticles indeed were captured by the RES, and while the polymer was biodegraded the particles continuously released the drug into the blood-stream resulting in elevated plasma and brain levels of MRZ 2/576. The formulation used in group 3 thus may have acted as a parenteral sustained release system.

A much longer anticonvulsive effect of nanoparticle-bound MRZ 2/576, however, was obtained after polysorbate 80 coating of the MRZ 2/576-loaded nanoparticles (group 4, Fig. 2). As previously shown [16], the coating of nanoparticles with polysorbate 80 effectively increased the brain levels of nanoparticles. Accordingly, this coating also yielded elevated brain levels of MRZ 2/576 bound to nanoparticles. The formulation used in group 4, therefore, increased CNS availability of MRZ 2/576 after parenteral administration considerably and to a much higher extent than without polysorbate 80 coating (group 3). As a result, this formulation yielded the longest duration of drug effects in the MES-test (up to 210 min). However, it has to be kept in mind that polysorbate 80 in contrast to other surfactants such as poloxamine 908 does not lead to a significant prolongation of the blood circulation time or higher blood levels compared to uncoated particles [16].

Taking the results of the control groups (groups 8 and 9) into account, it can be concluded that the enhancement of the duration of MRZ 2/576 activity by polysorbate 80 is not a property of this surfactant by itself. Both preparations, an aqueous polysorbate 80 solution of MRZ 2/576 (group 8) as well as a mixture of MRZ 2/576, polysorbate 80, and unloaded nanoparticles (group 9) fail to produce anticonvulsive effects for more than 15 min. This duration of MRZ 2/576 action is similar to the results obtained with a simple MRZ 2/576 solution (group 1) demonstrating that polysorbate 80 is only effective in increasing MRZ 2/576 activity when used as a coating material on MRZ 2/576-loaded nanoparticles.

The results of the experimental groups 3 and 4 also show that in contrast to the above mentioned studies concerning drugs that do not freely penetrate the blood brain barrier, in our study the targeting aspect is not the main concern since MRZ 2/576 freely enters the brain. Just as important is the sustained release of the glycine$_B$ antagonist from the nanoparticles to compensate for the decreasing brain levels of MRZ 2/576 caused by the active transport of the drug out of the brain. The sustained release of MRZ 2/576 from the particles can be achieved by entrapment of the drug into the particle matrix (incorporative binding). Since cyanoacrylate nanoparticles are biodegraded by a surface erosion process [17] and not by bulk degradation, the drug is released continuously over a time frame of several hours (matrix-controlled release) until the particles are completely degraded. Studies concerning the degradation of poly(butylcyanoacrylate) particles report an almost complete hydrolysis in serum within 3.5 h [18]; other authors found a massive degradation of particles in buffer solutions (pH 7) containing esterases [19]. Our findings are in good correlation with these data since group 4 yielded drug effects for 3.5 h. Furthermore, our studies performed with nanoparticle formulations containing MRZ 2/576 only bound by sorption onto the particle surface (sorptive binding) showed no protection of the animals in the MES-test 30–45 min after dosing (data not presented here). This is most probably due to a burst release of the drug by desorption from the particle surface in the systemic circulation or in the CNS demonstrating that a significant prolongation of MRZ 2/576 activity is only obtained after incorporative binding of MRZ 2/576 to the particles.

Very interesting results were obtained with the nanoparticle preparations containing probenecid (groups 5 and 6, Fig. 3). Following intravascular administration probenecid solutions exhibit only a relatively short duration of probenecid action since the drug has a dose-dependent short plasma half-life [20]. Therefore, and due to its poor water/saline solubility, for animal testing usually doses of probenecid up to 200 mg/kg are administered intraperitoneally as a suspension to delay absorption of the drug into the bloodstream. Zamboni et al. [21] report the administration of probenecid (600 and 1200 mg/kg) by oral gavage. Mostly the drug to be tested is subsequently given as a second bolus injection via the tail vein. This procedure causes a lot of distress to the animals and may therefore impair sensible trials such as body temperature measurements as well as behavioural studies. By comparing the results obtained with one intravenous injection of particle-bound probenecid and MRZ 2/576 together (group 5, Fig. 3) with the results of the intraperitoneal probenecid pre-treatment 30 min before intraperitoneal administration of MRZ 2/576 (group 2, Fig. 1) the advantage of using nanoparticle-bound probenecid is clearly proven since even better results were achieved with a 14-fold lower probenecid concentration (14.4 versus 200 mg/kg) and just a single injection of probenecid and MRZ 2/576 together.

The best preparation in our study, however, was the preparation used in experimental group 7 (Fig. 3), MRZ 2/576 bound to nanoparticles and i.p. probenecid pre-treatment. This group yielded 100% efficacy for 90 min, and a 20% efficacy can still be extrapolated to 270 min. Group 7 was even better than group 6, MRZ 2/576 and probenecid bound to nanoparticles. When comparing both groups (and of course also group 6 to the other groups) it must be noted, however, that in order to avoid nanoparticle overload of the animals in group 6 the dose of particle-bound MRZ 2/576 was halved to compensate for the addition of the same nanoparticle amount in the form of probenecid-loaded nanoparticles (see Table 1). This preparation was then brought to a total of 6 mg/kg with free MRZ 2/576. As a result only 25% of MRZ 2/576 was particle-bound. Since the free

amount does not contribute significantly to the anticonvulsive effect, group 6 showed an efficacy that was close to group 2 (free MRZ 2/576 i.p. and free probenecid i.p.). Nevertheless, group 6 required a 30-fold lower probenecid dose and a three-fold lower MRZ 2/576 dose.

In conclusion, the present study confirms that binding MRZ 2/576 to nanoparticles is a suitable method to prolong the action of the glycine$_B$ antagonist in the CNS. The results obtained with uncoated nanoparticles (group 3) demonstrate that sustained release alone enhances the bioavailability of this drug. Therefore, the development of an oral MRZ 2/576 sustained release formulation producing constant plasma and brain levels of MRZ 2/576 over several hours may be advantageous for the long-term treatment of chronic neurological disorders and psychiatric diseases. In contrast, acute cases of cerebral ischaemia (e.g. stroke) require a rapid intravenous administration of the drug and a duration of drug activity of at least 120–180 min. Therefore, a simple solution of this drug can not be used since it has a very short half-life. Repeated dosing or a continuous i.v. infusion of the drug would be necessary. However, injection of polysorbate 80-coated MRZ 2/576 nanoparticles (group 4) represents a much easier and convenient way to improve the pharmacokinetic properties of MRZ 2/576.

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