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Historical Perspectives

Nanoparticles—a historical perspective

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Abstract

The historical development of nanoparticles starting with Paul Ehrlich and then first attempts by Ursula Scheffel and colleagues and the extensive work by the group of Professor Peter Speiser at the ETH Zürich in the late 1960s and early 1970s are described from a personal point of view. Special attention is given to the years between 1970 and the early 1980s. Further developments resulting from this work are also followed, and focus is placed on especially interesting improvements such as nanoparticles for the delivery of drugs across the blood–brain barrier (BBB) and PEGylated nanoparticles with a prolonged blood circulation time.

Keywords: Nanoparticles; Historical development

Contents

1. Introduction

The concept of nanoparticles and drug targeting was inspired by a visit of one of the giants in science – Paul Ehrlich – to Karl Maria von Weber's opera "Der Freischütz" (Greiling, 1954). In this opera, so-called "Freikugeln", made by calling the spirit of the devil, play a central role. These Freikugeln always hit their goal, even if the rifleman did not aim properly or if the goal was out of reach. Paul Ehrlich (Fig. 1), who had been working for a long time on the staining of bacteria and tissues, after attending this opera thought that this concept of targeted delivery could greatly improve drug therapy. He called the delivery system that would be used in this type of therapy "Zauberkugeln" – English "Magic Bullets". Being a medical doctor with a great interest in bacteriology and immunology, he had something like antibodies in mind, but with this idea the concept of nanoparticles and of drug targeting was born.

2. The early years

The 1950s and the 1960s were characterised by tremendous progress in pharmaceutics: biopharmaceutics and pharmacokinetics were developed and as a result, retarded and controlled release became a major focus of attention. One of the pioneers in this field was Professor Peter Paul Speiser (Fig. 2) at the ETH (Swiss Federal Institute of Technology) in Zürich. Prof. Speiser's strategy for retarded and controlled release was a development of miniaturised delivery systems. His research group at first investigated polyacrylic beads for oral administration (Khanna and Speiser, 1969; Khanna et al., 1970; Speiser and Khanna, 1970), then focussed on microcapsules

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Fig. 1. Paul Ehrlich.

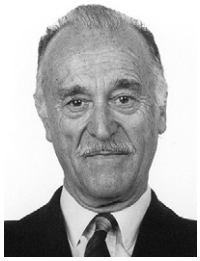

Fig. 2. Peter Paul Speiser. Reproduced with the permission of Prof. Dr. Peter P. Speiser, Forch, Switzerland.

(Merkle and Speiser, 1973) and in the late 1960s developed the first nanoparticles for drug delivery purposes and for vaccines.

Although his final objective was sustained drug release from nanocapsules that would be able to circulate in the blood after intravenous injection, in order to test the feasibility of a sustained release from such capsules, Speiser first focussed on the development of nanoparticles for vaccination purposes. Vaccinations against tetanus, diphtheria, and other infections require multiple injections to build up antibody levels in the body that are sufficient for protection. It was hoped that due to the sustained release properties of nanocapsules or nanoparticles a constant immune stimulation would be achieved, and only one injection would be required to achieve the necessary antibody response. Gerd Birrenbach was his first graduate student to work on this concept, starting in 1969, employing a process that they called "micelle polymerisation" (Birrenbach, 1973; Birrenbach and Speiser, 1976) (Fig. 3). In this process, an aqueous solution of tetanus toxoid or human IgG was solubilised in an outer hexane phase using large amounts of surfactants (about 35% of the mixture), i.e. bis-(2-ethyl-hexyl)-sodium succinate and polyoxyethylene-4-lauryl ether. Then acrylamide and *N*,*N*′-methylene-bis-acrylamide as a crosslinker were added, which partitioned into the aqueous phase. The polymerisation then was carried out by gamma irradiation, UV-irradiation, or by irradiation with visible light after addition of a photosensitizer, riboflavin 5′-sodium phosphate, and an initiator, potassium peroxodisulphate. After termination of the polymerisation, the particles were separated from the hexane phase after addition of methanol by ultracentrifugation or ultrafiltration. The antibody responses obtained with this preparation were very promising (Birrenbach, 1973; Birrenbach and Speiser, 1976). Nevertheless, these preparations were never further developed, most likely due to the high amounts of organic phase and surfactants required

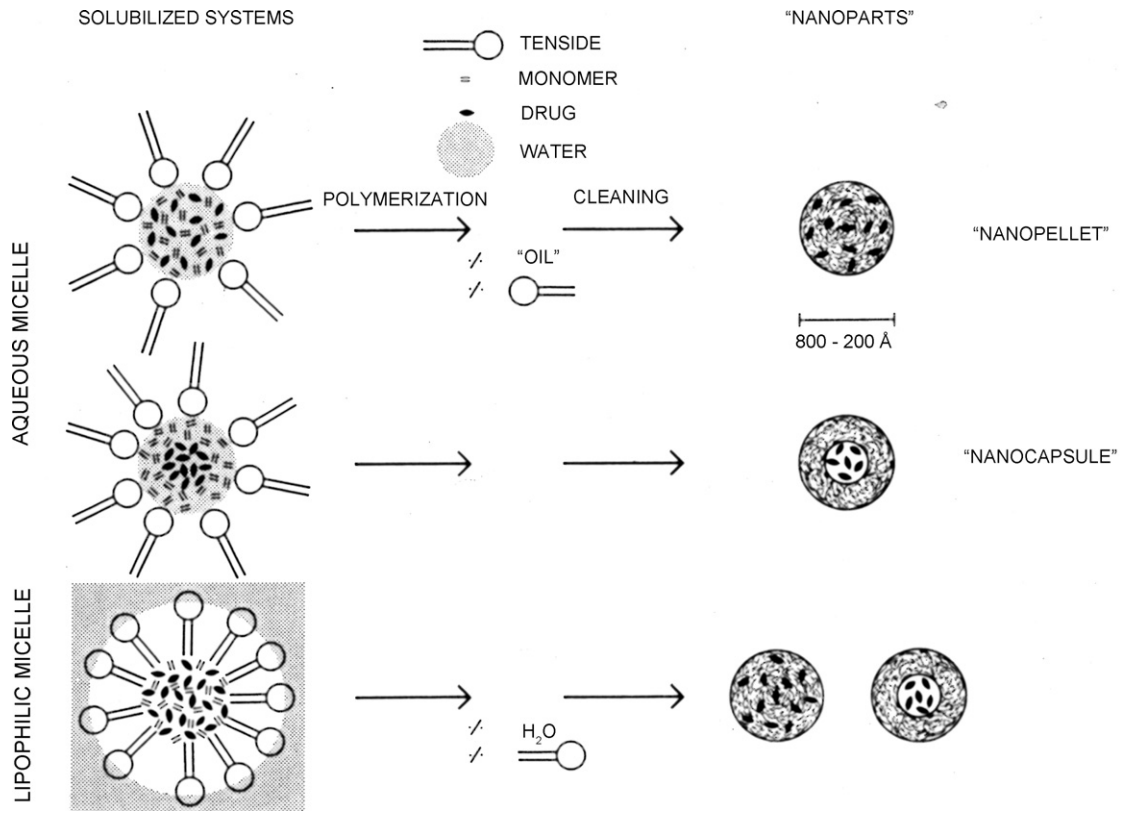

Fig. 3. Schematic drawing of the principle of nanoparticle preparation in the article Birrenbach, G., Speiser, P.P., 1976. Polymerized micelles and their use as adjuvants in immunology. J. Pharm. Sci. 65, 1763–1766. Reproduced with the permission of John Wiley & Sons, Hoboken, NJ, USA.

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Pharmazeutisches Institut der Eidgenössischen Technischen Hochschule Zürich

Studium der Mizellpolymerisation in Gegenwart niedermolekularer Arzneistoffe

1. Mitteilung: Herstellung und Isolierung der Nanopartikel, Restmonomerenbestimmung, physikalisch-chemische Daten

Von H. Kopf, R. K. Joshi, M. Soliva und P. Speiser

Zusammenfassung

Die vorliegende Arbeit beschreibt die Isolierung von bei der Mizellpolymerisation entstehenden Nanopartikeln sowie deren wichtigste physikalisch-chemische Daten. Zur Isolierung der Nanopartikel wurde neben einer Zentrifugiermethode zur schnellen Reinigung von den Lösungsmittelbestandteilen eine Methode zur genaueren Erfassung von bei der Mizellpolymerisation zurückbleibenden Acrylamid-Restmonomeren ausgearbeitet.

Summary

The present work deals with the isolation of micelle-polymerised nanoparticles as well as with their significant physicochemical properties. Furthermore a sensitive method for estimation of remaining Acrylamide monomers in washings gained during the purification of polymer product with help of centrifugation has been developed and suggested.

Fig. 4. First half page of the article Kopf, H., Joshi, R. K., Soliva, M., Speiser, P., 1976. Studium der Mizellpolymerisation in Gegenwart niedermolekularer Arzneistoffe. 1. Herstellung und Isolierung der Nanpartikel, Restmonomerenbestimmung, physikalisch-chemische Daten. Pharm. Ind. 38, 281–284. Reproduced with the permission of Edito Cantor Verlag, Aulendorf, Germany.

as well as the high toxicity of the acrylamide monomer. Later it was found out that this process did not yield nanocapsules with a shell-like wall as believed earlier but rather monolithic nanoparticles with a continuous matrix (Kreuter, 1981, 1983a).

Helmut Kopf was Peter Speiser's second graduate student working on the same process. However, his task was to obtain the first nanoparticles for sustained intravenous drug delivery. He bound norephedrine HCl and 1-[4-(2-biphenyloxy)-butyl]-1-ethylpiperidinium bromide into the acrylamide nanoparticles and also performed a thorough chemical and physicochemical investigation of the nanoparticles and of the production process including a determination of residual monomers (Kopf, 1975; Kopf et al., 1976, 1977) (Fig. 4).

I was Prof. Speiser's third graduate student (Fig. 5) who did his dissertation on nanoparticles. My task was to encapsulate

Fig. 5. Jörg Kreuter. Reproduced with the permission of Prof. Dr. Jörg Kreuter, Bad Homburg, Germany.

or bind inactivated whole virus or subunit influenza antigens to nanoparticles. Because the high amounts of surfactants in the former process would have destroyed the influenza viruses, I employed another process, emulsion polymerisation or heterogeneous polymerisation in an aqueous surrounding medium (Kreuter, 1974). The name emulsion polymerisation is misleading, because this process also can be carried out in the absence of additional emulsifiers, depending on the solubility of the monomer in water. The presence of emulsifiers and hence the number of micelles does not affect the number of particles formed (Roe, 1968; Robb, 1969; Fitch, 1973; Kreuter, 1982). Methyl methacrylate was used as a monomer. Being used as a material for artificial bones as well as bone glue since the 1940s, methyl methacrylate was considered to be very biocompatible. In addition, the polymerisation process and nanoparticle formation could be carried out by gamma irradiation in water or phosphate buffered saline without addition of any other materials. The virus or the virus subunits could be partly encapsulated by polymerisation in the presence of the virus, and the antibody responses achieved with this preparation were much higher and the protection against infection was much better than with the classical adjuvants that were allowed for human vaccination, aluminium hydroxide or aluminium phosphate (Kreuter, 1974; Kreuter and Speiser, 1976a,b; Kreuter et al., 1976c; Kreuter and Liehl, 1978; Kreuter and Zehnder, 1978). Good but somewhat lower antibody titres as well as a good protection also were

achieved when the virus was added after polymerisation (Kreuter et al., 1976c). The industrial development of this nanoparticle preparation for human vaccination was stopped when the Sandoz company left the vaccine area in the early 1980s.

Richard Oppenheim from Melbourne, Australia, joined Prof. Speiser's group in 1974 as a visiting scientist for about a year and, as a result, also became one of the pioneers in nanoparticles. I remember him having many good ideas and fitting into our group of graduate students very well. We had a lot of fun. After his return to Melbourne, Richard Oppenheim together with his graduate student, Jennifer Marty, continued the work that he started in Zürich and developed gelatin and albumin nanoparticles using the desolvation that occurs upon addition of a desolvating agent such as an alcohol or an inorganic salt in high concentrations. This addition normally leads to coacervation, and microcapsules are formed by this process. Oppenheim and Marty adapted this process by terminating the addition of the desolvating agent shortly before phase separation occurs: In this state, the molecules are in a tightly packed, rolled-up conformation, and after crosslinking with aldehydes this structure can be maintained and nanoparticles are formed (Marty, 1977; Marty and Oppenheim, 1977; Marty et al., 1978; Oppenheim et al., 1978). Almost 20 years later this process was improved (Coester et al., 2000) and used by our group in Frankfurt for the transport across cell membranes of oligonucleotides (Wartlick et al., 2004a,b), peptide nucleic acids (PNAs) (Langer et al., 2000), and even genes (Rhaese et al., 2003). Cell targeting using these nanoparticles was improved by the covalent attachment of antibodies (Wartlick et al., 2004a,b; Balthasar et al., 2005), and the transport of drugs across the blood–brain barrier (BBB) was achieved by the covalent binding of apolipoprotein E (apo E) to the surface of human serum albumin nanoparticles produced using the method of Oppenheim and Marty (Michaelis et al., 2006). It may be noteworthy in this context that one of the closest friends of Jennifer Marty at that time in Melbourne, Jennifer Dressman, much later (1994) became my closest colleague here in Frankfurt.

Another pioneer in nanoparticles, Patrick Couvreur (Fig. 6) joined Professor Speiser's group for a couple of months in 1976. In Zürich he worked on the process developed by Birrenbach and Speiser, and after his return to Brussels found the lysosomotropic effects of the nanoparticles (Couvreur et al., 1977). Shortly after this he produced the first rapidly biodegradable acrylic nanoparticles made of poly(methyl cyanoacrylate) and poly(ethyl cyanoacrylate) (Couvreur et al., 1979). Later the nanoparticles were made out of poly(*n*-butyl, isobutyl, *n*-hexyl, and isohexyl cyanoacrylate) and became a success story (see below).

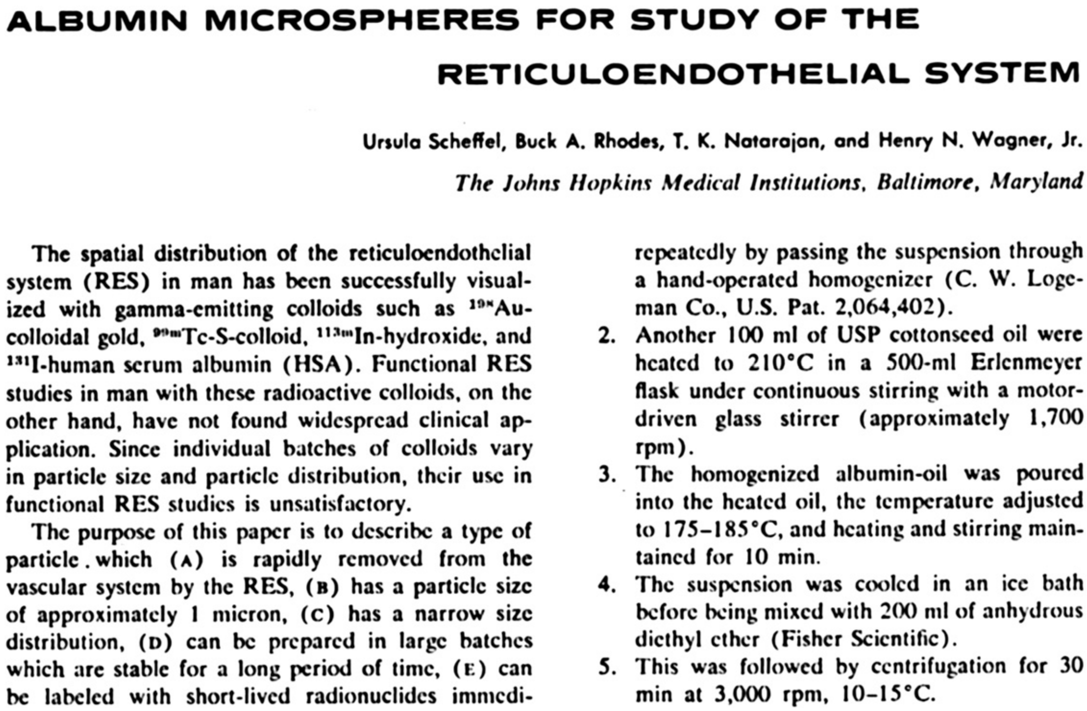
Fig. 6. Patrick Couvreur. Reproduced with the permission of Prof. Dr. Patrick Couvreur, Paris, France.

Unknown to our group in Zürich at that time, another type of nanoparticles was developed in the Department of Radiological Science at the Johns Hopkins Medical Institutions in Baltimore. This group implemented a process in which albumin was dissolved in water, and then this solution was emulsified in cottonseed oil (Zolle et al., 1973; Scheffel et al., 1973) (Fig. 7). By heating to 175–185 °C, the protein was denatured, and nanoparticles of a size between 300 and 1000 nm resulted. These particles were labelled with ^{99m}Tc, and body distribution studies and studies of the reticuloendothelial system (RES) were performed. Kramer (1974) introduced these nanoparticles for drug delivery purposes and bound mercaptopurine to them. Kramer must have left the nanoparticle field since I could not find other reports from him in this area. Some years later Widder et al. (1978, 1979a,b) used the same process and incorporated doxorubicin as well as magnetite particles into nanoparticles produced by this process. These particles could then be targeted by an external magnetic field. A localization in predesigned areas of rat tails was possible with these magnetic albumin nanoparticles, and very promising results were obtained in rats with a Yoshida tumour, a rat tail tumour (Widder et al., 1983a,b). However, it has to be considered that this tumour is located in a thin long extremity. It is much more difficult to focus the particles by a magnetic field deeper inside the body. For this reason, magnetic targeting has not so-far found broad application.

Interestingly, many years later, in 1987 or 1988, I met with Dr. Scheffel and Prof. Wagner. Because of my cooperation with the Nuclear Medicine Department in Frankfurt, I was invited to the Radiology Science Department at the Johns Hopkins Medical Institutions in Baltimore and gave a seminar about nanoparticles. To my big surprise, the people there were totally unaware of the development of nanoparticles for drug delivery purposes. They told me that they never had thought in this direction, and they were delighted to see the developments in nanoparticles and the resulting possibilities for drug therapy.

In Japan, another group took advantage of this process. Sugibayashi et al. (1977, 1979a,b) bound 5-fluorouracil to the albumin nanoparticles, and found denaturation temperature-dependent differences in drug release as well as in the body distribution in mice after intravenous tail vein injection. A 20% increase in life span was observed after intraperitoneal injection of the nanoparticles into Ehrlich Ascites Carcinoma-bearing mice.

Independently from Prof Speiser's group, polyacrylamide particles crosslinked with *N*,*N*′-methylene-bis-acrylamide were produced by the group of Ingvar Sjöholm in Upsala, Sweden (Ekman et al., 1976). Instead of the hexane/bis-(2-ethylhexyl)-sodium succinate/polyoxyethylene-4-lauryl ether system, Ekman et al. used a mixture of toluene and chloroform (4:1) and as a surfactant poloxamer 188 (Pluronic® F68). They incorporated human serum albumin as a model macromolecule into these particles. The amounts of surfactant required for the production of these nanoparticles was much lower than in the Birrenbach–Speiser process; the presence of the albumin proba-



ALBUMIN MICROSPHERES FOR STUDY OF THE RETICULOENDOTHELIAL SYSTEM

Ursula Scheffel, Buck A. Rhodes, T. K. Natarajan, and Henry N. Wagner, Jr.

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The spatial distribution of the reticuloendothelial system (RES) in man has been successfully visualized with gamma-emitting colloids such as ^{198}Au-colloidal gold, ^{99m}Tc-S-colloid, ^{113m}In-hydroxide, and ^{131}I-human serum albumin (HSA). Functional RES studies in man with these radioactive colloids, on the other hand, have not found widespread clinical application. Since individual batches of colloids vary in particle size and particle distribution, their use in functional RES studies is unsatisfactory.

The purpose of this paper is to describe a type of particle which (A) is rapidly removed from the vascular system by the RES, (B) has a particle size of approximately 1 micron, (C) has a narrow size distribution, (D) can be prepared in large batches which are stable for a long period of time, (E) can be labeled with short-lived radionuclides immedi-

repeatedly by passing the suspension through a hand-operated homogenizer (C. W. Logeman Co., U.S. Pat. 2,064,402).

2. Another 100 ml of USP cottonseed oil were heated to 210°C in a 500-ml Erlenmeyer flask under continuous stirring with a motor-driven glass stirrer (approximately 1,700 rpm).
3. The homogenized albumin-oil was poured into the heated oil, the temperature adjusted to 175–185°C, and heating and stirring maintained for 10 min.
4. The suspension was cooled in an ice bath before being mixed with 200 ml of anhydrous diethyl ether (Fisher Scientific).
5. This was followed by centrifugation for 30 min at 3,000 rpm, 10–15°C.

Fig. 7. First half page of the article Scheffel, U., Rhodes, B.A., Natarajan, T.K., Wagner Jr., H.N., 1973. Albumin microspheres for study of the reticuloendothelial system. J. Nucl. Med. 13, 498–503. Reproduced with the permission of the Society of Nuclear Medicine, Reston, VA, USA.

bly aided the homogenisation and particle formation. Polymerisation in the Sjöholm group was carried out at 30 °C using a catalyst system of Temed® and ammonium peroxodisulphate. Later this group changed from the crosslinked polyacrylamide particles to polyacryldextran (Edman et al., 1980) and to polyacryl starch (Arthursson et al., 1984) nanoparticles to improve the biodegradability. They entrapped enzymes into them to improve enzyme stability or for their targeting to the reticuloendothelial system. Furthermore, antigens were entrapped for vaccination purposes to stimulate an immune response (Arthursson et al., 1985).

Poly(lactic acid) and its copolymer poly(lactic-co-glycolic) acid belong to the most important polymers for parenteral administration. First developed for transplantation purposes and later as microcapsules, approved by the regulatory authorities and being on the market in many forms, they also represent very promising materials for nanoparticles. Because both polymers are only soluble in organic solvents and cannot be polymerised directly to form nanometer-sized particles like the acrylates, the preparation of particles of this size represented a challenge. Robert Gurny (Fig. 8), who started to study Pharmacy in Zürich and later went to Geneva to continue his career, was the first to develop poly(lactic acid) nanoparticles for drug delivery purposes during his stay as a visiting scientist at the Purdue University in Gil Banker's laboratory (Gurny et al., 1981). Together, this group produced nanoparticles using testosterone as a model drug, poly-(D,L-lactic acid) as the polymer, and poloxamer 188, polysorbate 80 or sodium lauryl sulphate as the surfactants. Then they investigated the particles' physicochemical characteristics, storage stability, drug release, and histopathology after i.m. injection in rats. At that time, these researchers described the

Fig. 8. Robert Gurny. Reproduced with the permission of Prof. Dr. Robert Gurny, Geneva, Switzerland.

particles as "pseudolatices" because they were manufactured by compartimentilisation from previously polymerised bulky material, in contrast to latices that are produced during polymerisation in situ (like the acrylates). Nevertheless, due to their particle size and application purposes Robert Gurny's particles definitely fall under the definition of nanoparticles.

The first review article about nanoparticles appeared as early as 1978 (Kreuter, 1978). Towards the end of these early years the definition of nanoparticles for pharmaceutical and medical purposes also was developed. Initially, as mentioned above, it was believed that these particles were capsules, nanocapsules. Oppenheim (1981) and, more comprehensively, Kreuter (1983b, 1994a) presented a definition[1] that later was adopted by the Encyclopaedia of Pharmaceutical Technology (Kreuter, 1994b) and the Encyclopaedia of Nanotechnology (Kreuter, 2004). The

[1] ***Definition of Nanoparticles***: Nanoparticles for pharmaceutical purposes are defined by the Encyclopedia of Pharmaceutical Technology [7] as solid colloidal particles ranging in size from 1 to 1000 nm (1 μm). They consist of

advantage of this definition was that nanoparticles could be defined independently of their respective structure and production methods.

3. Later developments

What were in my opinion the most noteworthy later developments? The absolutely most important application of nanoparticles of course is their employment for cancer therapy. Already Sugibayashi et al. (1979b) and Widder et al. (1983a,b) but more importantly Couvreur et al. (Brasseur et al., 1980; Couvreur et al., 1986) demonstrated the benefit of employing nanoparticles for this purpose. In many cases a significantly enhanced efficacy (Brasseur et al., 1980; Chiannilkulchai et al., 1989), and in some cases a reduction in toxic side effects (Couvreur et al., 1982, 1986) were both observed. The possibility to take advantage of the enhanced permeability and retention effect (EPR effect) (Maeda and Matsumura, 1989) was already described by Grislain et al. (1983). Grislain et al. showed a significant concentration of the nanoparticles in the primary tumour location of a Lewis Lung Carcinoma as well as higher concentrations in the lungs, probably due to an accumulation in lung metastases. The poly(isohexyl cyanoacrylate) nanoparticle preparation with bound doxorubicin later was subjected to a clinical trial (Kattan et al., 1992). The doxorubicin-loaded nanoparticles exhibited a higher therapeutic index than free doxorubicin and also showed promise in overcoming multidrug resistance. Although unexpected side effects such as fever, bone pain or allergic reactions were noted, they were well tolerated and were all rapidly reversible.

Later it was demonstrated that, in addition to anticancer drugs, nanoparticles also enabled an improvement in the delivery of antiinfective drugs (Henry-Michelland et al., 1987; Youssef et al., 1988; Fattal et al., 1989) as well as nucleic acids, DNA fragments and genes (Bertling et al., 1991; Chavany et al., 1992; Rhaese et al., 2003), and for the treatment of AIDS (Löbenberg and Kreuter, 1996; Löbenberg et al., 1998).

One of the most promising applications of nanoparticles is their use for the transport of drugs across the blood–brain barrier. This barrier represents an insurmountable obstacle for a large number of drugs, including anticancer drugs, antibiotics, and a variety of central nervous system (CNS)-active drugs, especially neuropeptides. Already around 1980 Peter Speiser had the idea to use i.v. injected nanoparticles for brain delivery. I remember telling him that this was not a good idea – actually I said "*stupid idea*" – because of the tight junctions between the brain capillary endothelial cells and the powerful biochemical barrier properties of these cells that would make the BBB impermeable for the particles. About twelve years later, a friendly academic guest from Moscow, Renad Alyautdin visited me and suggested the same idea as Speiser. At that time, my total disbelief in this possibility was already waning because my graduate student Gerrit Borchard had spent some time in the laboratory of Ken Audus in Lawrence, Kansas, and had observed that nanoparticles coated with certain surfactants including polysorbate 80 (Tween® 80) were taken up by brain capillary endothelial cells in tissue cultures (Borchard et al., 1994). So Renad Alyautdin manufactured poly(butyl cyanoacrylate) nanoparticles in my lab, and I advised him to coat these particles with polysorbate 80. After his return to Moscow, he could indeed observe very significant CNS effects in animals treated with the hexapeptide dalargin (Alyautdin et al., 1995; Kreuter et al., 1995) (Fig. 9), loperamide (Alyautdin et al., 1997), or tubocurarine (Alyautdin et al., 1998) bound to the nanoparticles coated with polysorbate 80. Being administered in a free form, these agents do not produce any CNS effects because they cannot pass across the BBB. A big forward leap then was achieved when Svetlana Gelperina – another Russian scientist that had worked for some time in my lab – together with her group showed that very significant brain concentrations of doxorubicin, 65-fold above detection limit, could be achieved when the drug was bound to the polysorbate 80-coated poly(butyl cyanoacrylate) nanoparticles, whereas the concentrations of all control formulations remained below the detection limit (Gulyaev et al., 1999). Later we could demonstrate that this formulation was able to significantly enhance the survival time of rats bearing the very aggressive glioblastoma 101/8 implanted intracranially. A total tumour remission was shown repeatedly in 20–50% of the animals (Steiniger et al., 2004). No increase in toxicity was observed with these nanoparticles (Gelperina et al., 2002).

The mechanism of drug delivery across the BBB by means of nanoparticles was for a long time obscure and still is not totally elucidated. However, experiments with apolipoproteins adsorbed to the surface of the nanoparticles (Kreuter et al., 2002) and recently with apolipoproteins E covalently bound to human serum albumin nanoparticles manufactured by an improved method of Oppenheim and Marty (Michaelis et al., 2006) demonstrated the likely involvement of these lipoproteins. Therefore, it presently appears that after injection the polysorbate 80-coated nanoparticles adsorb these lipoproteins from the blood. The adsorbed lipoproteins then seem to interact with the respective receptors of the brain capillary endothelial cells followed by endocytotic uptake of the particles by these cells (Ramge et al., 2000). Further transport of the nanoparticle-bound drug into the brain interior then may be possible after its release within these cells and diffusion into the brain or by transcytosis of the nanoparticles. This, however, still remains unknown.

Maincent et al. (1984, 1986) were the first to demonstrate that nanoparticles also can improve the oral bioavailability of drugs. Later, insulin (Damgé et al., 1988) and a practically insoluble drug, avarol (Beck et al., 1994) attached to nanoparticles were delivered by this route. The oral uptake of ^{14}C-labelled poly(methyl methacrylate) nanoparticles had been demonstrated earlier (Nefzger et al., 1984).

At the same time, nanoparticles for ocular delivery of drugs were developed. Eye drops lack a sufficient bioavailability, i.e normally around 1% and maximally 5%. A large portion of the

macromolecular materials and can be used therapeutically as drug carriers, in which the active principle (drug or biologically active material) is dissolved, entrapped, or encapsulated, or to which the active principle is adsorbed or attached.

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Brain Research 674 (1995) 171–174

BRAIN RESEARCH

Short communication

Passage of peptides through the blood–brain barrier with colloidal polymer particles (nanoparticles)

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Abstract

Transport of the hexapeptide dalargin across the blood–brain barrier was accomplished using a nanoparticle formulation. The formulation consisted of dalargin bound to poly(butyl cyanoacrylate) nanoparticles by sorption, coated with polysorbate 80. Intravenous injection of this formulation to mice resulted in an analgesic effect. All controls, including a simple mixture of the three components (drugs, nanoparticles, and surfactant) mixed directly before i.v. injection, exhibited no effect. Analgesia was also prevented by pretreatment with naloxone. Fluorescent and electron microscopic studies indicated that the passage of the particle-bound drug occurred by phagocytic uptake of the polysorbate 80-coated nanoparticles by the brain blood vessel endothelial cells.

Keywords: Nanoparticle; Dalargin; Leu-enkephalin analog; Polysorbate 80; Blood–brain barrier; Peptide delivery to the brain

Fig. 9. First half page of the article Kreuter, J., Alyautdin, R.N., Kharkevich, D.A., Ivanov, A.A., 1995. Passage of peptides through the blood–brain barrier with colloidal polymer particles (nanoparticles). Brain Res. 674, 171–174. Reproduced with the permission of Elsevier, Amsterdam, The Netherlands.

instilled eye drop solution, therefore, drains via the lacrimal duct into the nose where it is rapidly absorbed and can lead to side effects. In order to improve the bioavailability of eye drops, one of the most important ophthalmic drugs, pilocarpine, was bound to nanoparticles, and indeed an improved bioavailability was attained (Gurny, 1981; Harmia et al., 1986; Diepold et al., 1989).

The second very important nanoparticle development was the preparation of nanoparticles with covalently attached poly(ethylene glycol) (PEG) chains. Uncoated nanoparticles are rapidly opsonised and removed by the macrophages in the liver (Kupffer cells), spleen, lungs, and bone marrow, that all belong to the RES. Illum and Davis (1983), Illum et al. (1987) found that by coating of the nanoparticles with poly(oxy-ethylene)-poly(oxy-propylene) block copolymers (poloxamer 338 = Pluronic® F 108 and poloxamine 908 = Tetronic® 908) their blood circulation time could be prolonged and the liver uptake reduced very significantly. Tröster et al. (1990), Tröster and Kreuter (1992) later systematically investigated a large number of surfactants for their properties to prolong the blood circulation time and to achieve an increased redistribution into different organs that do not belong to the RES.

The covalent attachment of PEG chains, therefore, was a logical and promising step forward. At first, the PEG chains were bound to poly(lactic acid) nanoparticles by Gref et al. (1994) and independently by Bazile et al. (1995). Later PEG also was bound to poly(hexadecyl cyanoacrylate) nanoparticles (Peracchia et al., 1997, 1999). Both types of nanoparticles, the PEGylated poly(lactic acid) and the poly(hexadecyl cyanoacrylate) (PHDCA) nanoparticles, significantly prolonged the blood circulation time and reduced the liver uptake. The PEGylated PHDCA nanoparticles also accumulated in the brain at a 4–8-fold higher concentration than non-PEGylated PHDCA nanoparticles after intravenous injection into rats (Brigger et al., 2002). Moreover, the PEGylated particles accumulated to a 11-fold higher degree in an intracerebrally implanted 9L glioblastoma compared to the adjacent brain area. Non-PEGylated PHDCA nanoparticles also accumulated in this tumor but to a three-fold lower degree than the PEGylated particles. Unfortunately, after binding of doxorubicin to these nanoparticles no significant therapeutic improvement was obtained in 9L glioblastoma-bearing rats (Brigger et al., 2004).

4. Nanocapsules

As mentioned above, Peter Speiser's initial goal was to develop nanocapsules, which, however, was not feasible with the manufacturing systems he was using. Arakawa and Kondo (1980) prepared poly(N^{α}, N^{ε}-L-lysinediylterephthaloyl) particles, containing hemolysate, of a size of about 200–500 nm by a process called electrocapillary emulsification. The electron microscopic department at the ETH Zurich tried to determine the morphology of these particles. However, no clear-cut evidence could be provided if the particles indeed were nanocapsules with a shell-like structure or just monolithic particles.

Nanocapsules definitely were produced by Al Khouri Fallouh et al. (1986) using an oil-in-water system. Isobutylcyanoacrylate was dissolved in an oil–ethanol mixture with an excess of the ethanol. This mixture also contained the drug in dissolved or dispersed form. It was then slowly injected into water containing a non-ionic surfactant such as poloxamer 188. Due to the large excess of ethanol, the oil phase was finely dispersed in the aqueous phase, and the isobutylcyanoacrylate monomer polymerised exclusively at the oil/water interphase, and nanocapsules with an oily interior were formed. As mentioned above, insulin could be encapsulated into these particles, and oral application of these particles led to a prolonged hypoglycaemic effect (Damgé et al., 1988).

5. Conclusions

Nanoparticles for pharmaceutical and medical application are around now for over 35 years. At first, they appeared to many people to be pharmaceutical curiosities with no or only extremely limited application: I still remember a congress in the early 1980s when I heard a comment of an industrial scientist at one of my posters who said nanoparticles would die out after my retirement. Now there are numerous reports and studies conducted every year and their number is increasing exponentially. The first commercial nanoparticle product containing a drug (Abraxane™, human serum albumin nanoparticles containing paclitaxel) appeared on the market at the beginning of 2005. Nanoparticles for diagnostic purposes have been marketed now for over 10 years. A second product based on poly(isohexyl cyanoacrylate) nanoparticles (Doxorubicin-Transdrug®) loaded with doxorubicin is presently being developed by the company BioAlliance in Paris for the treatment of resistant hepatocellular carcinomas and a Phase I/II clinical trial has been conducted.

Why was and is the way of nanoparticles from bench to practice so long and winding? A prime reason is that these systems are so novel and alter the body distribution and the performance of bound drugs so significantly that much basic research was required before nanoparticles could be developed for marketing. In addition, the pharmaceutical industry was oblivious to the potential of these systems. It is interesting to note in this context that although most research with nanoparticles was carried out in Europe, the first product, Abraxane™, was put on the market by a US company, Abraxis Oncology.

Nanoparticles definitely do have a great potential for anticancer drug delivery and tumour targeting, because they can exploit the above mentioned EPR effect. Another very promising area for nanoparticles is the possibility to deliver drugs that normally cannot cross the blood–brain barrier to the brain after intravenous injection, and I expect further major breakthroughs in this field. A further area where breakthroughs can be expected is their application for nucleic acid and gene delivery. Last not least, the poor bioavailability of ophthalmic drugs needs serious attention, and nanoparticles as well as liposomes have the potential to significantly improve the situation.

There still is some confusion over the nomenclature of nanoparticles: the terms “nanoparticles”, “nanocapsules”, “nanospheres”, “microcapsules”, “microspheres”, “colloidal carriers”, and “latices” all are used interchangeably for the same thing: particles in the nanometer size range for the delivery of drugs or other biologically active materials. This makes a literature search difficult, because expressions like microcapsules and microspheres also include larger particles. Terms like “latices”, on the other hand, are mainly used for non-pharmaceutical applications. For this reason, some 25 years ago, attempts were made to introduce a comprehensive definition. Such a definition for pharmaceutical purposes: “Nanoparticles are solid colloidal particles ranging in size from 10 to 1000 nm (1 μm). They consist of macromolecular materials in which the active principle (drug or biologically active material) is dissolved, entrapped, encapsulated and/or to which the active principle is adsorbed or attached”, has now found entry into the relevant specialised Encyclopaedias (Kreuter, 1994b, 2004).

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