Biodegradable Nanoparticles for Targeted Drug Delivery in Treatment of Inflammatory Bowel Disease

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ABSTRACT

The use of nanoparticles for targeted oral drug delivery to the inflamed gut tissue in inflammatory bowel disease was examined. Such a strategy of local drug delivery would be a distinct improvement compared with existing colon delivery devices for this disease. An experimental colitis was induced by trinitrobenzenesulfonic acid to male Wistar rats. Rolipram, an anti-inflammatory model drug, was incorporated within poly(lactic-coglycolic acid) nanoparticles, which were administered once a day orally for five consecutive days. A clinical activity score and myeloperoxidase activity were determined to assess the inflammation, whereas an adverse effect index reflected the remaining neurotropic effect of rolipram resulting from its systemic absorption. All nanoparticle formulations proved to be as efficient as the drug in solution in mitigating the experimental colitis. The clinical activity score and myeloperoxidase activity decreased significantly after the oral administration of rolipram nanoparticles or solution. During the next 5 days when animals were kept without drug treatment the drug solution group displayed a strong relapse, whereas the nanoparticle groups continued to show reduced inflammation levels. The rolipram solution group had a high adverse effect index, whereas the rolipram nanoparticle groups proved their potential to retain the drug from systemic absorption as evidenced by a significantly reduced index. This new delivery system enabled the drug to accumulate in the inflamed tissue with higher efficiency than when given as solution. The nanoparticle deposition in the inflamed tissue should be given particular consideration in the design of new carrier systems for the treatment of inflammatory bowel disease.

The ordinary treatment of inflammatory bowel disease requires the frequent intake of anti-inflammatory drugs at high doses, which causes the absorption of those drugs from the small intestine, leading to significant adverse events. Therefore, several strategies have been followed such as the development of prodrugs that deliver drugs specifically in the large bowel after cleaving the active part from the hydrophilic carrier by specific bacterial enzymes in the colon (McLeod et al., 1994; Fedorak et al., 1995) and the development of solid dosage forms that release the drug in the colon in dependence of the physiological environment (Watts and Illum, 1997; Kinget et al., 1998; Tozaki et al., 1999). The administration of drugs by rectal route is also currently used. However, it is not effective when the inflamed tissues are located in the upper parts of the colon.

Although prodrugs lead to reduced adverse effects, a more comfortable dosage frequency cannot be achieved. Sustained drug release devices, e.g., pellets, capsules, or tablets, delivering the drug specifically in the colon for a longer time period have been developed. However, their efficiencies seem to be decreased in many cases due to the diarrhea, a symptom of inflammatory bowel disease that enhances the elimination and reduces the possible drug release time (Hardy et al., 1988; Watts et al., 1992). Thus, a carrier system that delivers the drug specifically and exclusively to the inflamed regions after oral administration for a prolonged period would be desirable. Such a system could reduce side effects significantly in the case of conventional chemical anti-inflammatory compounds.

As reported from previous work, drug carrier systems with a size larger than 200 μm are subjected to the diarrhea symptoms, resulting in a decreased gastrointestinal transit time and therefore to a distinct decrease in efficiency (Hardy et al., 1988; Watts et al., 1992).

Because nanoparticles can be designed to control drug release after oral administration, the development of such nanoparticles seems to be promising primarily to reduce the dosage frequency. In the case of colitis, a strong cellular immune response is known from the inflamed regions, i.e., in

ABBREVIATIONS: PLGA, poly[lactide-co-glycolide]; TNBS, trinitrobenzenesulfonic acid; NP, nanoparticle.
general, an increased presence of neutrophils, natural killer cells, mast cells, and regulatory T cells, which have an important role in the pathophysiology of inflammatory bowel disease (Allison et al., 1988; Seldenrijk et al., 1989; Probert et al., 1996). Moreover, it has been reported that microspheres and nanoparticles can be efficiently taken up by macrophages (Tabata et al., 1996). Thus, it may be expected that particle uptake into those immune-related cells or the disruption of the intestinal barrier function (Stein et al., 1998) could allow the accumulation of the particulate carrier system in the desired area. A subsequent increase of residence time that would be postulated for nanoparticles compared with existing drug delivery systems allows a dose reduction. Indeed, it has been demonstrated that microparticles containing dexamethasone showed previously promising results in colitis-induced mice (Nakase et al., 2000). In addition, the successful oral administration of drugs with strong adverse effects such as rolipram, the model drug in our study, may be a new medical approach.

In several different diseases, the proinflammatory cytokine tumor necrosis factor-α forms a necessary element in the chain of pathophysiological events leading to inflammation. Among the agents known to inhibit tumor necrosis factor-α production rather than block its function, attention has focused on cAMP-elevating phosphodiesterase inhibitors. Rolipram has initially been developed and studied as an antide-pressant drug (Wachtel, 1983). Recently, the potential therapeutic use of rolipram in tumor necrosis factor-α-dependent disease has been demonstrated in several animal models (Nyman et al., 1997; Ross et al., 1997; Hartmann et al., 2000).

The aim of this project was to test in vivo the targeting potential of nanoparticles to the inflamed tissue. A previous study (Lamprecht et al., 2001a) proved an increased nanoparticle deposition in the inflamed tissue of the colon compared with the healthy control. This accumulation allows a potential nanoparticulate drug delivery system to stay in the inflamed area for a longer time. Here, we evaluated the therapeutic efficiency of this drug carrier system using the experimental colitis rat model. To compare the efficiency of the nanoparticle treatment, control rats received the free drug or drug-containing particles.

Preparation Method of Biodegradable Nanoparticles.

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Materials and Methods

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clinical score ranging from 0 (healthy) to 4 (maximal activity of colitis).

**Myeloperoxidase Activity.** The measurement of the myeloperoxidase activity was performed to quantify the severity of the colitis. It is a reliable index of inflammation caused by infiltration of activated neutrophils into the inflamed tissue. Activities were analyzed according to Krawisz et al. (1984). Briefly, distal colon specimen was minced in 1 ml of hexadecyltrimethylammonium bromide buffer (0.5% in 50 mM phosphate buffer) on ice and homogenized. The homogenate was sonicated for 10 s, freeze-thawed three times, and centrifuged at 10,000 rpm for 3 min. Myeloperoxidase activity in the supernatant was measured spectrophotometrically. Supernatant (0.1 ml) was added to 0.167 mg/ml of o-dianisidine hydrochloride and 0.0005% hydrogen peroxide, and the change in absorbance at 460 nm was measured. One unit of myeloperoxidase activity was defined as the amount that degraded 1 μmol of peroxidase per minute at 25°C.

**Characterization of Systemic Adverse Effect.** The neurotropic effects of rolipram after oral administration in rats were described in detail by Wachtel (1983). Following these procedures, the adverse effect of rolipram was determined after administration of the drug. One hour after the drug or particles were administered, the behavior of rats was observed through transparent cages. The incidence of forepaw shaking and grooming was counted for 60 min by an observer unaware of the animal’s treatment. The following criteria were used: rapid repetitive forepaw shaking and grooming.

**Statistical Analysis.** The results were expressed as mean values ± S.D. The Mann-Whitney-Wilcoxon U test was used to investigate differences statistically because the number of animals in each group was relatively low. However, in all statistical analysis normality and equal variance was passed. Therefore, the Student’s t test was also applied to examine significance of differences. In all cases, $P < 0.05$ was considered to be significant and the marked significant differences are valid for both statistical test systems.

### Results

All nanoparticles were prepared with poly[DL-lactide-coglycolide], a biocompatible and biodegradable polymer that is now well established for use in humans. Nanoparticles were characterized in terms of size, polydispersity, surface potential, encapsulation efficiency, and drug release (Table 1). As it can be seen in Fig. 1, nanoparticles prepared by the sonication method had a spherical shape, submicrometer size, and were relatively monodispersed. Figure 2 illustrates the in vitro drug release profiles obtained for the two formulations later used in the in vivo experiments, by representing the percentage of rolipram release with respect to the amount of rolipram encapsulated. Drug release occurred in two phases: a first initial burst release and a sustained release of the drug over 1 week resulting from the diffusion of the drug through the polymer. The repeated washing steps after the preparation reduced the adsorbed drug amount on the nanoparticle surface to a minimum, subsequently, the initial burst release was lower than 30% of drug released within the first 2 h for both formulations (Fig. 2, inset).

To evaluate the therapeutic value of rolipram-containing nanoparticles, the effect of the carrier system was studied on preexisting colitis. On day 3, all animals received an intrarectal application of TNBS except the healthy control group. Before this time point, animals showed no clinical problems. After inducing the experimental colitis the clinical score increased rapidly and consistently for the next 3 days for all groups (Fig. 3). The inflamed tissue showed an extremely increased mucus production in the area of distal colon compared with the histology of healthy gut sections from the control group. Significant damages of the intestinal tissue, e.g., ulceration, have been observed (Fig. 4).

Starting from day 6, rats received orally either rolipram solution or rolipram nanoparticles daily for five consecutive days, only the colitis control group received saline instead. The clinical activity score was used to evaluate the severity of the colonic inflammation and the colitis control group proved to be an excellent model of inflammation as evidenced by the highly increased clinical activity. All drug-receiving groups showed a decrease of inflammation severity after a lag time

### Table 1

| Polymer mass, particle diameter, zeta potential, and encapsulation efficiency of PLGA nanoparticles used in this in vivo study ($n = 3$) |
|----------------------|------------------|------------------|
| Nanoparticle Batch   | 5020             | 5005             |
| Molecular weight     | 20,000           | 5,000            |
| Diameter (nm)        | 473.9 ± 13.9     | 332.2 ± 6.4      |
| Zeta potential (mV)  | $-2.9 ± 0.1$     | $-19.9 ± 2.2$    |
| EE (%) (±SD)         | 87.8 ± 1.9       | 80.2 ± 2.1       |

EE, encapsulation efficiency.
of 24 to 48 h. The difference between drug-treated groups and colitis controls became significant on day 9. During the whole rolipram treatment period the clinical activity was lowered by free drug and by the two drug carrier formulations as well. After the 5 days without drug treatment, the free drug group showed a strong relapse, whereas for the NP groups continuously reduced clinical activity scores were observed.

Because rolipram undergoes a very strong first-pass effect (Krause and Kühne, 1988) the plasma concentration of the drug could not readily be determined. Therefore, the strong visible neurotropic effect has been used as an indicator as reported from previous work (Wachtel, 1983). Grooming and forepaw shaking indicated the intensity of the neurotropic adverse effects as a proof of systemic drug absorption. This showed an enormous difference between rats receiving rolipram as free drug in solution and those receiving rolipram entrapped in nanoparticles (Fig. 5).

On day 11 (24 h after the last drug administration), the first series of animals was sacrificed and colon/body weight ratio and myeloperoxidase activity were determined to quantify the inflammation. The drug-treated groups showed a distinct decrease in the colon/body weight ratio compared with the colitis control group (Fig. 6a). The differences between free drug and the nanoparticle formulations were not significant. Furthermore, the myeloperoxidase activity in samples obtained from the inflamed colonic tissue was examined (Fig. 6b). Here also, an enormous difference between rolipram-treated and control groups was found. As observed previously, no significant differences for the mitigating effect of all rolipram-receiving groups were observed.

The remaining animals were sacrificed on day 15, i.e., 5 days after the last drug administration. Whereas the colitis control group showed a continuously strong colonic inflam-
mation reflected by a high clinical activity score, the rolipram groups responded differently to the lack of drug treatment. The group initially treated with free drug showed a distinct relapse of the inflammation, manifested by clinical activity score as well as an increased colon/body weight ratio and myeloperoxidase activity. This relapse was almost as severe as the inflammation of the nontreated colitis control group regarding clinical activity and colon/body weight ratio. However, the myeloperoxidase activity was still significantly lower than in the colitis control group. In contrast, the nanoparticle groups showed rather no deterioration in colon/body weight ratio and myeloperoxidase activity after 5 days without drug treatment. Only the clinical score showed a slight increase during this period. The neurotropic adverse effect was observed to be slightly higher than in the colitis control group, but no significant difference between free drug and nanoparticle groups was found.

**Discussion**

The behavior of the proposed nanoparticulate drug delivery system was examined with a specific view to therapeutic activity after oral administration. Owing to its simplicity and reproducibility the TNBS colitis model was selected. Furthermore, it is a relevant model because it involves the use of an immunological hapten and develops a chronic inflammation rather than an acute mucosal injury (Yamada et al., 1992). The experimental colitis model in rat after the intrarectal administration of TNBS (Morris et al., 1989) should allow an in vivo characterization of the particulate carrier systems under the influence of chronic inflammation symptoms.

As expected, significant damage of the intestinal tissue, e.g., ulceration, have been observed. In these areas a high amount and infiltration activity of immune-related cells was found (Elson et al., 1995). Thus, an enhanced uptake of administered particles by these cell types could be expected, which resulted in an advantageous accumulation of the carrier system in the inflamed area. Moreover, the inflamed distal colon tissue showed an extremely increased mucus production in the areas surrounding the ulceration compared with the histology of healthy gut sections from the control group. This observation supports the hypothesis of particle accumulation in the inflamed area because small polymeric particles were found to be attached to the healthy intestinal mucus (McClellan et al., 1998).

A size-dependent particle deposition in the gastrointestinal tract of healthy rats was reported in previous studies (Jani et al., 1989, 1990; Desai et al., 1996). These studies reported an increase of recovery for smaller particles in the whole gut: we observed the same phenomenon, but the size dependence was less pronounced. On the contrary, there was a strong influence of the particle size in the experimental colitis model (Lamprecht et al., 2001a). In our study the highest deposition amount was found for nanoparticles (particle diameter 100 nm) inside the inflamed tissue of the colon (14.5 ± 6.3% of the total administered particle mass). Two major reasons can be stated for the more distinct size-dependent deposition in the case of colitis. First, smaller particles are taken up more easily by macrophages in the area of active inflammation. Second, the strong increased mucus production leading to a thicker mucus layer in the inflamed areas allows a higher amount of particle adherence. Smaller par-
articles can be better bound to the mucus layer due to an easier penetration into the layer with respect to their relatively small size. In addition, both phenomena may occur at the same time.

The choice of an optimal particle size for the design of a particulate carrier system has to be discussed, based on two major influencing factors. It has to be kept in mind that by increasing the particle size, a higher drug-loading capacity can be reached, which allows the transport of higher drug amounts with less polymer. On the contrary, we observed a tendency of a higher deposition rate and a better targeting index for smaller particles. Combining these two effects, we prepared nanoparticles with a diameter between 200 and 500 nm allowing the production of optimal nanoparticles including efficient drug loading and still having a size range that promises a high targeting efficiency.

The use of biodegradable polymers for nanoparticle preparation was preferable for this application to prevent complications with long-term deposition of nanoparticles or any residual component inside the ulcerated tissue. Moreover, polyvinyl alcohol-coated nanoparticles were found to be very efficient in delaying nanoparticles degradation in the gastrointestinal tract (Landry et al., 1998).

Rolidpram has proven to have an anti-inflammatory potential in our experimental colitis model as reported previously by Hartmann et al. (2000).

The clinical activity score, colon/body weight ratio, and myeloperoxidase activity decreased significantly after the oral administration of rolipram nanoparticles or free drug. Nanoparticles formulations proved to be as efficient as the free drug in mitigating the experimental colitis. The local anti-inflammatory effect was achieved by the controlled drug release during the nanoparticle deposition period in the inflamed colon areas.

After the final rolipram administration, the group receiving free drug showed a strong relapse, whereas this was not observed in animals fed with rolipram nanoparticles. This suppressed relapse might be due to an accumulated deposition of nanoparticles observed in the first part of this study, which would retain the drug carrier in the inflamed regions of the colon for up to several days.

On the other hand, the reduction of neutrophilic effects that was observed after the administration of the rolipram nanoparticles is based on the reduced availability of rolipram during the gastrointestinal passage by the surrounding polymeric carrier. An additional advantage seems to be that only during the gastrointestinal passage by the surrounding polyvinyl alcohol-coated nanoparticles for up to several days.

This also could explain an enhanced attachment of nanoparticles to the inflamed mucus areas. However, a decreased surface potential as the here-tested formulation NP-5005 did not lead to significant pharmacological differences based on this nanoparticle property.

Conclusions

Polymeric particulate carrier systems are expected to target the inflamed tissue in inflammatory bowel diseases. This new delivery system allows the desired drug to accumulate in the inflamed tissue with high efficiency. Compared with approaches from previous works, this includes two major advantages. The drug is concentrated at its site of action, which reduces possible adverse effects and enhances the effect of the administered dose. Moreover, the sustained drug release allows pharmacological effects to be extended due to the prolonged presence time of the carrier system at the targeted inflamed area. This deposition of NP in the inflamed tissue should be given particular consideration in the design of new carrier systems for the treatment of inflammatory bowel disease.

References


Jani PU, Halbert GW, Langridge J, and Florence AT (1989) Nanoparticle formulations proved to be as efficient as the free drug in mitigating the experimental colitis. The local anti-inflammatory effect was achieved by the controlled drug release during the nanoparticle deposition period in the inflamed colon areas.

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On the other hand, the reduction of neutrophilic effects that was observed after the administration of the rolipram nanoparticles is based on the reduced availability of rolipram during the gastrointestinal passage by the surrounding polymeric carrier. An additional advantage seems to be that only a low uptake of nanoparticles into Peyer’s patches and their translocation has been reported (Florence et al., 1995), which can prevent an uncontrollable spreading of the nanoparticles during their transportation through the gut.

The remaining adverse effect that was observed for both nanoparticle preparations might be due to the initial burst effect as observed in vitro (30% released in 2 h; Fig. 2). This burst release leads to some drug release in the stomach and small intestine, which is in favor of drug absorption from the upper regions of the gut and subsequent adverse effects of rolipram.

Charge interactions are reported to further enhance binding of macromolecules to the inflamed tissue because it has been shown that ulcerated tissues contain high concentrations of positively charged proteins that increased the affinity to negatively charged substances (Nagashima, 1981).


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