Nanoparticles enhance therapeutic efficiency by selectively increased local drug dose in experimental colitis in rats

Alf Lamprecht, Hiromitsu Yamamoto, Hirofumi Takeuchi, Yoshiaki Kawashima*

Laboratory of Biopharmaceutics and Pharmaceutical Engineering, University Franche-Comté, Besançon, FRANCE (A.L.);
Laboratory of Pharmaceutical Engineering, Gifu Pharmaceutical University, Gifu, JAPAN (H.Y., H.T., Y.K.)
Running Title Page

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b) correspondence:
Alf Lamprecht
Inserm Erit-M 0323,
Faculty of Pharmacy
5 rue A. Lebrun
54001 Nancy Cedex, FRANCE
Phone: +33 3 83 68 22 97
Fax: +33 3 83 68 23 01
Email: Alf.Lamprecht@pharma.uhp-nancy.fr

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Abstract

Nanoparticles (NP) are proposed for targeted drug delivery to the inflammation site in severe cases of inflammatory bowel disease where state-of-the-art delivery devices fail. FK506 (tacrolimus) entrapped into NP was administered either orally or rectally to male Wistar rats suffering from a preexisting experimental colitis. Clinical activity score, colon/body weight index, and myeloperoxidase activity were determined to assess the inflammation. Tissue penetration experiments elucidated the processes involved in the proposed new therapeutic approach. The therapeutic effects of FK506 solutions as well as FK506-NP by oral route were minor. The myeloperoxidase activity and colon/body weight ratio decreased significantly ($P<0.05$) only after the rectal administration of FK506-NP while treatment by free drug was not different from colitis control in both, TNBS and oxazolone colitis model. NP allow an enhanced and selective drug penetration into the inflammation site as opposed to surrounding healthy tissue (healthy: FK506: 109±18nmol/cm²; FK506-NP: 51±13nmol/cm²; colitis: FK506: 79±28nmol/cm²; FK506-NP: 105±24nmol/cm²) presumably by protecting the encapsulated drug against influences from efflux systems and mucosal metabolism. The relative drug penetration into the inflamed tissue is about 3-fold higher compared to healthy tissue when using NP as drug carriers. The use of drug loaded NP offers several advantages compared to standard therapeutic strategies such as a higher selectivity in adhesion to and enhanced drug penetration into the inflamed tissue.
Introduction

The general principle of drug treatment in inflammatory bowel disease (IBD) is to induce remission of outbreaks and to prevent outbreaks during remission. The usually strong adverse effects of anti-inflammatory or immune suppressant drugs applied under these circumstances may require their strictly local application. Although many efforts have been made for a higher specificity of drug release by designing new drug delivery devices (Lamprecht et al., 2002), all marketed delivery systems still appear to be insufficiently selective (Lamprecht, 2003). This is due to the fact that the drug release mechanisms are based on physiological parameters which are not related to the inflammation and barely to its location. Drug delivery is usually either triggered by the luminal pH in the gastrointestinal tract, the enzymatic activity of colonic bacteria, or the transit time of the drug carrier normally initiating the drug release in the distal ileum. As the inflamed areas affect only limited sectors of the intestinal tissue and may vary from patient to patient, subsequently, still distinct drug loads are delivered unintentionally to non-inflamed regions. This misdirected drug release can be hardly excluded by the state-of-the-art drug delivery systems, thus remaining a general problem responsible for therapy failure and undesired adverse effects. The mainstays of the drug therapy of IBD are a variety of formulations of 5-aminosalicylic acid, the conventional and newer low bioavailability glucocorticoids, the nitroimidazole antibiotic metronidazole, and certain immunomodulating agents. Increased understanding of the mechanisms of inflammation in IBD has permitted the development of effective designer drugs (Egan and Sandborn, 1998). The proposed use of TNF-α monoclonal antibodies in the treatment of rheumatic arthritis and, more recently, in Crohn’s disease showed distinct success of the therapy due to the antibody specificity, which greatly reduces adverse effects. It was found, however, that patients develop antibodies against the treatment with an increased risk of infusion reactions and a reduced duration of response to treatment (Baert et al., 2003). Therefore, low molecular weight immune suppressant drugs may still be of interest, however, requiring the design of intelligent drug delivery systems providing a more local treatment. Tacrolimus (FK506), initially developed as an immune suppressive drug used to inhibit transplantation rejection, was
successfully applied in IBD to treat refractory ulcerative colitis where corticoid treatment failed (Fellermann et al., 1998; Hoshino et al., 1995; Aiko et al., 1997; Matsuhashi et al., 2000). These treatments were based on oral FK506 administration with macroscopic carriers (tablets, etc.) or parenteral injections resulting in a therapeutic success after systemic availability. However, these forms of treatment also provoke significant adverse effects (Finn, 1999) known to be a limiting factor of the therapy.

A new therapeutic approach is proposed on the basis of the strong cellular immune response occurring in the inflamed regions i.e., in general, an increased presence of neutrophils, natural killer cells, mast cells, and regulatory T cells, which play an important role in the pathophysiology of inflammatory bowel disease (Allison et al., 1988; Seldenrijk et al., 1989). Thus, it was hypothesized that particle uptake into those immune-related cells (Tabata et al., 1996) or the disrupted intestinal barrier at ulcerated regions (Stein et al., 1998) could allow the selective accumulation of the particulate carrier system in the desired area.

The increased adhesion of small particulate drug carriers to the inflamed tissue in ulcerative colitis led to a new therapeutic concept allowing a specific drug targeting in this disease (Nakase et al., 2000; Nakase et al., 2001; Lamprecht et al., 2001a). This approach is mainly based on two pathophysiological changes in the inflamed tissue, allowing the higher adhesion of the carriers in the inflamed tissue caused by elevated levels of mucus production and an intense particle uptake inside the colitis tissue as a result of an enhanced permeability and the presence of a highly increased number of immune related cells. This accumulation phenomenon was observed to be particle size dependent with an increasing effect for smaller particle diameters and highest efficiency for nanoparticles (NP) of around 100 nanometers (Lamprecht et al., 2001b).

FK506 containing polymeric NP were designed and tested for their therapeutic efficiency in two different rat colitis models after oral or rectal administration. In order to understand the mechanism of action of this new drug deliver strategy more clearly, drug and particle penetration studies allowed to elucidating the processes on tissue level ex-vivo.
Methods

Materials

Tacrolimus (FK506) was received as a kind gift from Fujisawa (Osaka, Japan). Poly(lactic-co-glycolic acid) 7520 (Mw 20,000Da), 2,4,6-trinitrobenzenesulfonic acid (TNBS), and o-dianisidine dihydrochloride were obtained from Wako Pure Chemical Industries (Osaka, Japan). Polyvinyl alcohol (Mw 20 kDa, 80% hydrolyzed), oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one) (OXA), Rhodamine 123, and troleandomycin were purchased from Sigma (Deisenhofen, Germany). All other chemicals were obtained from Nacalai Tesque Inc. (Kyoto, Japan) or Prolabo (Strasbourg, France) and were of analytical grade.

NP preparation and characterization

The preparation of NP was based on an oil/water emulsification-solvent evaporation method. Fix amounts of poly(lactic-co-glycolic acid) (210mg) and FK506 (30mg) were dissolved in 9ml methylene chloride. This solution was poured into 45ml of 5% w/w polyvinyl alcohol and an oil/water-emulsion was formed by ultrasonification for 3min. After evaporation of the solvent, NP were recovered by centrifugation at 55,000g for 30min and washed with distilled water. The washing step was repeated once before NP were resuspended in distilled water and lyophilized overnight.

NP were analyzed for their size distribution and their surface potential using a Photal laser particle analyzer LPA 3100 (Otsuka Electronics, Osaka, Japan) and a Zetasizer II (Malvern Instruments, Worcestershire, UK), respectively. FK506 entrapment within NP as well as the in-vitro drug release kinetics was determined by high performance liquid chromatography as described elsewhere in detail (Lamprecht et al., 2001c; Akashi et al., 1996).

Animal treatment

All animal experiments were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research
The TNBS and oxazolone rat models were chosen as well recognized experimental models (Morris et al., 1989; Ekström, 1998) that allow induction of colitis at an exact location. This permitted administration of rectal drug/NP in form of an exclusively local delivery to the inflamed tissue. Moreover, the differences in both models allowed characterizing the efficiency of the NP under varying pathophysiological conditions resembling Crohn’s disease and ulcerative colitis, respectively. Male Wistar rats (10 weeks; n=6/group) were treated by the following procedure in order to induce the TNBS model colitis: after light narcotizing with ether, the rats were catheterized 8cm intrarectal and 500µl of TNBS (140mg/kg) in an ethanol/water mixture was applied. Control groups received a rectal installation of an ethanol/saline mixture without TNBS instead. For 48h the rats were housed without treatment to maintain the development of a full inflammatory bowel disease model.

The second colitis model was based on the induction with oxazolone (Ekström, 1998). Rats were immunized with 300µl of a solution containing acetone/ethanol (1:4) and 12mg oxazolone was applied topically to the skin. The challenge was performed a week later by rectal administration of 10mg of the haptenating agent, oxazolone was administered per rectum in a total volume of 450µl of an ethanol/water mixture. Again, control groups received a rectal administration of ethanol/saline mixture without oxazolone instead. Similar to TNBS, for 48h the rats were housed without treatment to maintain the development of a full inflammatory bowel disease model.

During the treatment period, all animals received (orally or rectally) either 0.3ml of FK506 solution or FK-NP suspension once daily for 10 (TNBS-induced) or 6 (oxazolone-induced) consecutive days at a dose of 1mg/kg body weight. The oral administration was performed by gavage while rectal administration consisted of an 8cm intrarectal catheterization providing NP directly to the inflammation site. The control groups received only saline (colitis control), drug-free NP (NP control) or drug-containing particles (healthy control). The animals were sacrificed 24h after the last drug/particle administration and colon were resected for the determination of colon/body weight index and myeloperoxidase (MPO) activity.
Colitis activity was quantified with a clinical score assessing weight loss, stool consistency, and rectal bleeding as previously described elsewhere (Hartmann et al., 2000). Resected distal colon tissue samples were opened longitudinally and rinsed with iced phosphate buffer to remove luminal content and weighed. The colon/body weight index was calculated as a quotient of the colon wet weight compared to the total body weight of each rat. The MPO activity allowed quantifying the severity of the colitis as a reliable index for the infiltration of activated neutrophils into the inflamed tissue. Activities were analyzed according to a standard method (Krawisz et al., 1984).

*Creatinine clearance and blood urea nitrogen levels*

FK506 was administered to a new population of rats daily over five weeks by either oral or rectal route (FK506 solution or NP). This treatment period was required in order to ensure the full manifestation of the nephrotoxic adverse effect. Creatinine clearance and blood urea nitrogen levels were assessed at the end of the treatment. Creatinine in serum or urine and blood urea nitrogen (BUN) levels were determined with the respective kits from Biomérieux (Marcy-l’Etoile, France) according to the supplier’s instructions.

*Tissue penetration experiments*

The resected colonic rat tissue samples (healthy or inflamed from TNBS-colitis) were washed with ice-cold phosphate buffer pH7.4 and mounted in Ussing chambers with an area of 1.13cm². The general setup was slightly modified from those described in literature (Lampen et al., 1996). The tissue samples were preincubated for 30 min before the various drug formulations were added into the apical compartment (at final FK506 concentrations of 10-40μmol/l (Fig.5) or 4-16μmol/l (Fig.6A+B), optionally amiodarone (amio; 40μM) was added as P-glycoprotein (P-gp) inhibitor or/and troleandomycin (tro; 10μM) as cytochrome P450 3A (CYP) inhibitor and incubated for one hour. After the incubation was completed, the tissue samples were rinsed carefully and lyophilized. After extraction with acetonitrile from lyophilized tissue, drug content was determined by high performance liquid chromatography (Akashi et al., 1996).
Cryosections were analyzed by confocal laser scanning microscopy for their fluorescent signals after the incubation with either Rhodamine 123, a model compound affected by P-gp efflux systems, or Rhodamine 123 loaded NP. A Zeiss LSM 510 Laser Scanning Confocal Imaging System (Carl Zeiss, Jena, Germany), equipped with an argon ion and a helium/neon laser (Uniphase Laser Systems, San José, CA, USA) as well as a Zeiss Axioplan 2 microscope were used to investigate the various tissue samples.

**Metabolism experiments**

Rat intestine samples were again rinsed with cold phosphate buffer before cut into small slices of about 200mg tissue wet weight. Tissue samples were homogenized and 30min preincubated in gentamycin (10µg/ml) containing Dulbecco’s modified eagle medium. In inhibition experiments, troleandomycin was added prior to the preincubation step. FK506 solutions were added and incubated for 4 hours at 37°C. Thereafter, samples were lyophilized, FK506 was extracted with acetonitrile and quantified by high performance liquid chromatography (Akashi et al., 1996).

**Relative tissue penetration index**

A relative penetration index was calculated as the coefficient of drug amounts recovered from intestinal segments of colitis groups versus values from healthy samples at equivalent drug concentrations in the donor compartment. The recovered drug amount was normalized with respect to the tissue sample weight.

**Statistical Analysis**

The results were expressed as mean values ± S.D. For the pairwise comparison of healthy and colitis groups the Mann-Whitney U-test was used to investigate differences statistically. The statistical analysis of treatments with more than two groups was performed with Kruskal-Wallis test followed by Dunn’s test, except when normality and equal variance were passed it was followed by the Tukey test. In all cases, \( P < 0.05 \) was considered to be significant.
Results

FK506-NP were designed by using a biodegradable polyester (polylactide-co-glycolide75/25, Mw: 20kDa) providing a solidified polymer network allowing the entrapment of the drug. The particles exhibit a spherical shape with a mean particle size of 107±8nm. The encapsulation process led to a total drug load of 7.4±0.6% FK506 inside the NP. Due to the matrix structure of the particles, sustained drug release kinetics were achieved with a complete drug release after about one week (in-vitro drug release in phosphate buffer (pH 7.4): t_{10\%}: 1.3±0.2h; t_{50\%}: 42.1±3.8h).

For purposes of testing the therapy concept in a preexisting experimental colitis in rats, FK506 formulations were administered either orally or rectally in order to determine possible differences occurring by the gastrointestinal passage. In the TNBS model, clinical activity was found to be lower after the administration of FK506-NP compared to the groups receiving FK506 solution or saline (Fig. 1A). Although the observed effect after oral and rectal administration of FK506-NP was similar, a faster response to treatment was found when administered by rectal route (different from untreated control, rectal: day 8, oral: day 10; P < 0.05). MPO activity and the colon/body weight ratio were only slightly reduced when FK506 or FK506-NP were administered by oral route (Fig. 2A). Oppositely, after rectal administration distinct differences were observed between FK506 and FK506-NP treated groups (P < 0.05). The rectal administration of FK506-NP reduced both, MPO activity and colon/body weight ratio below the 50% level of untreated animals while the mitigating effect was minor for FK506 solution (Fig. 2B).

Slightly different tendencies for the clinical activity score were found in the oxazolone model (Fig. 1B). While rectal administration of NP provided also here the strongest reduction of clinical activity, the rectal FK506 solution was superior to the oral FK506-NP formulation although these differences were not statistically significant. The oral administration of FK506 solution and NP tended to be more efficient than in the TNBS model however only the colon/body weight index of oral FK506-NP was significantly lower compared to untreated colitis control (Fig. 2C). Similar to observations from the clinical activity, the rectal
administration of FK506 solution was mitigating the colitis to a higher extent although not reaching levels of rectal FK506-NP which were approaching values of a complete remission (Fig. 2D).

Nephrotoxic effects were assessed by BUN and creatinine clearance. BUN levels were increased after oral administration of FK506 solution while particle formulations reduced BUN values to a level comparable with untreated control (Fig. 3A). Endogenous creatinine clearance was significantly decreased in FK506 solution treated rats (Fig. 3B) while oral and rectal FK506-NP resulted in a creatinine clearance similar to the control group.

Ussing chamber studies allowed the analysis of drug penetration into the different tissue samples and subsequent changes in dependency of the disease state. While tissue penetration of Rhodamine 123 in colitis tissue appeared to be lower than in healthy controls the penetration and especially the adhesion of NP is highly increased (Fig. 4, I+II). On the other hand, in healthy tissue Rhodamine 123 penetrated better than NP also demonstrating a reduced adhesion and subsequent low tissue concentration (III+IV). Drug concentrations that had penetrated the different tissue samples for varying drug concentrations elucidated this phenomenon quantitatively (Fig. 5A). Drug amounts inside inflamed tissue were slightly higher for FK506-NP than for free FK506 however drug levels after incubation with FK506-NP were significantly increased in inflamed compared to healthy tissue. Moreover, the penetration potential of FK506-NP was distinctly reduced in healthy colonic tissue samples against drug solution, thus, providing a distinct selectivity for the penetration into colitis tissue.

When inhibiting P-gp with amiodarone (FK506+amio), higher tissue levels were detected in both, healthy and inflamed tissue, while the increase was more dramatic for the inflamed samples (Fig. 5A). Compared to tissue concentrations after incubation with FK506+amiodarone, FK506-NP led to increased drug tissue levels in inflamed tissue but lowered the FK506 concentrations in healthy samples.

When colonic tissue samples were homogenized (in order to exclude influences by P-gp) and incubated together with FK506, higher metabolic drug degradation was observed in the inflamed tissue (Fig. 5B). Addition of CYP3A inhibitor troleandomycin reduced the metabolic activity generally and in colitis tissue and healthy control similar metabolism levels were obtained. FK506-NP achieved a higher preservation of
the incorporated drug compared to drug solution in colitis group at some drug concentrations while values with NP in healthy tissue were only slightly reduced.

The relative penetration of FK506 solution was higher into healthy compared to inflamed tissue, while differences were reduced in the presence of the inhibitors amiodarone and troleandomycin (Fig. 6). FK506-NP were however, able to completely inverse this penetration phenomenon leading to an about 3-fold higher drug concentrations inside the inflamed tissue than in healthy tissue.
Discussion

Still nowadays, standard drug delivery systems release the anti-inflammatory drug non-specifically to the colonic epithelium, independent from their healthy or inflamed state. Only few innovative therapeutic strategies have been proposed to eliminate this lack of specificity in drug delivery (Nakase et al., 2000; Nakase et al., 2001; Lamprecht et al., 2001a; Zhou et al., 1999; Jubeh et al., 2004). The selective adhesion of particulate drug carriers to the inflamed tissue in experimental colitis proved to be a promising therapeutic concept of specific drug targeting in IBD.

Standard drug formulations applied in the clinical studies with FK506 (Fellermann et al., 1998; Matsuhashi et al., 2000) released the drug during the intestinal passage followed by its systemic absorption. After oral administration, the effective dose of FK506 is lowered distinctly due to drug’s P-gp related efflux combined with inactivation from mucosal metabolism by CYP3A which cause a relatively low bioavailability (Kagayama et al., 1993; Shimomura et al., 2002) subsequently reducing the therapeutic efficiency. Thus, effects achieved in the reference group receiving drug solution orally closely reflect the properties of such a non-specific formulation.

The rectal administration of FK506 solution, reflecting drug availability of commercialized systems for rectal drug administration or standard colon delivery systems (pH-dependent release, prodrugs, etc.) demonstrated also relative limited efficiency. Rectally administered free FK506 aiming mainly for a local effect undergoes an increased drug efflux due to the higher expression levels of P-gp in the inflamed tissue (Farrell et al., 2000; Farrell and Kelleher, 2003). Moreover, an overall increasing mucosal metabolism in inflamed tissue related to the highly increased levels of lymphocytes and neutrophils, which express CYP3A (Stärkel et al., 1999) further reduce relevant drug concentrations at the site of action.

NP administered by oral route are expected to accumulate in the inflamed tissue to develop a more local effect by their accumulation at the site of action (Lamprecht et al., 2001a, Lamprecht et al., 2001b). Surprisingly, oral NP administration resulted in distinct lack of therapeutic efficiency which is seemingly based on a combination of several influencing parameters. Although freely available FK506 resulting...
from the initial drug release during the passage in the small intestine may be limited due to the slow onset of drug release, other factors such as degradation of the NP building matrix polyester by digestive enzymes may play a role. Besides, a distinct uptake into Peyer’s patches and other elements of the gut associated lymphoid tissue has been reported to lead to a systemic drug availability followed by hepatic metabolism (Florence et al., 1995). All these factors contribute partially to the reduced efficiency of orally administered FK506-NP. However, rectally administered FK506-NP showed a major enhancement of the therapeutic efficiency. Apparently, this is based on the adhesion of NP at the inflammation site. Oppositely to the orally administered NP, those particles were applied directly to the inflammation site, avoiding an early carrier degradation or uptake and therefore, did not risk a loss of efficiency.

The study determining the therapeutic efficiency of FK506-NP was performed in the TNBS and oxazolone colitis models in rats. Despite the fact that the relationship of the TNBS model to human disease is imperfect (Fiocchi, 1998; Neurath et al., 1995), it displays several Crohn’s disease-like features, especially full-thickness transmural mononuclear inflammation. Oxazolone colitis is a mucosal model of colitis that is an interleukin-4 driven, Th2 inflammation that has histologic features resembling the human disease, ulcerative colitis (Boirivant et al., 1998) being relatively superficial at the microscopic level.

The two colitis models led to generally comparable findings on the therapeutic efficiency. Although the treatment period was set shorter in the oxazolone model due to limited persistence of acute inflammation state, the therapy with FK506 was not less efficient. As submucosal swelling was reduced in oxazolone compared to TNBS model, less penetration depth was required for the drug or drug carriers in order to develop a full mitigating effect. Apparently, the lower tissue thickness in the oxazolone model led to general higher therapeutic efficiency of local FK506 treatment. This explains the higher efficiency of rectal FK506 solution and the observation that the rectal administration of FK506-NP approached values of a complete remission. Beside this effect of thickness, the dense infiltration of immune related cells in mucosal and submucosal tissue may essentially contribute to the metabolism of FK506 being responsible for reduced drug tissue concentrations in TNBS colitis samples.
Rectally administered FK506-NP clearly inhibited the elevation of BUN and prevented the reduction of creatinine clearance compared to solution groups. Moreover, measuring BUN and creatinine clearance revealed that FK506-NP levels did not differ from that of untreated control animals. Although this was also observed for orally given FK506-NP, it should be mentioned that in terms of therapeutic efficiency the rectal NP group was distinctly superior.

From the therapeutic point of view, beside the locally delivered drug concentration, drug penetration into the site of action is a major issue to address. Several factors are known to impede drug penetration into the mucosa beside the general tissue permeability, e.g. P-gp and CYP3A, which are altered in inflamed intestinal tissue. Studies in excised samples of healthy or inflamed tissue elucidated details on the mechanism of action of NP. While free FK506 exhibited a higher relative tissue concentration in healthy tissue, FK506-NP inversed this phenomenon in favor of increased drug amounts in the inflamed region. It might be concluded that the lower drug penetration into healthy tissue in the case of NP is mainly based on the reduced NP adhesion. When, however mucus production and NP capture in ulcerations are enhanced, a higher NP accumulation tendency is observed leading to higher drug concentrations inside inflamed tissues. Moreover, the entrapment of FK506 inside NP obviously prevented exposure of the drug to P-gp efflux and simultaneously to its mucosal metabolism. This protective effect of NP against P-gp and CYP3A in colitis tissue is apparently complementary which explains the supplementary increase of drug concentration inside the inflamed tissue compared to FK506 when the P-gp is inhibited.

For an increased therapeutic effect it seems not to be essentially necessary that the majority of NP enter the inflamed tissue to release the entrapped drug. As it became evident by the confocal fluorescence images most of the NP were located inside the mucus layer and ulcerated regions. The subsequent local drug concentration with NP at this site was much higher compared to concentrations that could be reached with drug solution alone. Subsequently, P-gp and CYP3A capacities could be saturated by a mechanism of high local drug dose which was proposed similarly for nanoparticulate drug delivery across the blood brain barrier (Pardridge, 2002).
Both factors the higher selective adhesion to and increased drug penetration in the inflamed region, in combination lead to a distinctly increased therapeutic efficiency of NP. Such a high selectivity is not reached by any other drug delivery approach in colitis.

The described NP demonstrate the possibilities in innovative drug delivery for the treatment of IBD by targeted delivery based on factors directly related to the location and intensity of the inflammation. FK506-NP allow a selective adhesion to the inflamed tissue as some kind of drug reservoir with a reduced drug availability at the healthy surrounding tissue. Additionally, these NP provide a selectively enhanced drug penetration into the inflamed tissue compared to free drug while in healthy tissue this phenomenon is inversed. The NP deposition and penetration in the inflamed tissue as therapeutic strategy appears to be a promising approach in the design of new carrier systems for the treatment of IBD.
References


**Footnotes**

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Legends for Figures

**Fig. 1:** Clinical activity score in TNBS (A) or oxazolone (B) model during the whole experimental period after either oral or rectal drug administration ( ■ = colitis control; △ = FK506 solution oral; ○ = FK506 NP oral; ▲ = FK506 solution rectal; ● = FK506 NP rectal; NP controls being similar to the colitis control as well as error bars are not shown for clarity reasons; n = 6) * P < 0.05 compared with colitis control rats given saline.

**Fig. 2:** MPO activity (left) and colon/body weight ratio (right) on day 14 in TNBS (A,B) or oxazolone (C,D) colitis model after either oral (A,C) or rectal (B,D) administration of FK506 and FK506-NP, respectively. Data are shown as mean ± S.D for n = 6 animals. * P < 0.05 compared with colitis control rats given saline, ** P < 0.05 compared with rats given FK506 solution.

**Fig. 3:** Blood urea nitrogen (A), and creatinine clearance (B) levels after 5 weeks with daily (oral or rectal) administration of either FK506 solution or FK506-NP. Data are shown as mean ± SD for n = 6 animals. * P < 0.05 compared with colitis control group.

**Fig. 4:** Representative confocal laser scanning microscopic images of cryosections after tissue penetration experiments showing the accumulation of either RH123 or RH123 trapping NP in inflamed colonic tissue (I, II) and in healthy colonic tissue (III, IV). Scale bars represent 100µm (a = apical; b = basolateral side of the tissue sample).

**Fig. 5:** (A) Penetrative behavior of FK506, FK506 in combination with a P-gp inhibitor or FK506-NP into either healthy or inflamed tissue samples was compared for different drug concentrations (n=4). Data are shown as mean ± S.D. * P < 0.05 compared with colitis samples incubated with FK506 solution at the equivalent initial drug concentration, ** P < 0.05 compared with tissue from healthy samples incubated...
with FK506-NP at the equivalent initial drug concentration. (B) FK506 metabolism in colonic tissue homogenate is shown in presence or absence of the cytochrome P450 3A inhibitor troleandomycin (n=4). Data are shown as mean ± S.D. * P < 0.05 compared with healthy tissue samples incubated with FK506 solution at the equivalent initial drug concentration, ** P < 0.05 compared with colitis tissue samples incubated with FK506 solution at the equivalent initial drug concentration.

Fig. 6: a relative penetration index demonstrated the inversion of drug tissue concentration inside colitis versus healthy group by the use of NP compared to FK506 solution (n = 4). * P < 0.05 compared with FK506 solution; ** P < 0.05 compared with FK506+amiodarone and FK506+troleandomycin.
Figure 1

A

TNBS

clinical activity score

day

B

OXA

clinical activity score

day
Figure 3

A

BUN [mg/dL]

control
FK506oral
FK506rectal
NPoral
NPrectal

* * *

B

creatinine clearance [ml/min/100g]

control
FK506oral
FK506rectal
NPoral
NPrectal

* * *
Figure 4
Figure 5

A

FK506 tissue conc. [nmol/cm²]

FK506 [µM]

B

FK506 metabol. [pmol/mg tissue/h]

FK506 [µM]
Figure 6