Abstract. The targeting of 5-aminosalicylic acid (5-ASA), a first-line therapeutic agent for mild to moderate active ulcerative colitis (UC), to the site of inflammation has remained a challenge and an unmet requirement in the treatment of UC. However, nanoscale carriers for targeted drug delivery are promising for pharmacotherapy, and nanoparticles improve the pharmacokinetics of the loaded therapeutics based on their physical properties. To design and prepare 5-ASA-loaded silicon dioxide nanoparticles (5-ASA-SiO$_2$ NPs), a micro-emulsion method was conducted, and their respective therapeutic effects were validated in a mouse model of UC. Cytotoxicity of 5-ASA-SiO$_2$ NPs was detected in vitro using the Cell Counting Kit-8 method. The therapeutic effect of 5-ASA-SiO$_2$ NPs was assessed based on their disease activity index (DAI), colon histopathology, myeloperoxidase (MPO) and levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). SiO$_2$ NPs were successfully prepared, and cytotoxicity of 5-ASA-SiO$_2$ NPs was identified as being similar to 5-ASA and SiO$_2$ NPs. DAI and colon histopathology scores in the normal dosage, high dosage and the 5-ASA-SiO$_2$ NP groups demonstrated a significant improvement when compared with the model group. DAI in the high dosage and 5-ASA-SiO$_2$ NP groups also demonstrated a significant improvement when compared with the normal dosage group. However, MPO, serum IL-6 and TNF-α levels in normal dosage, high dosage and 5-ASA-SiO$_2$ NPs groups were significantly lower than in the model group, and these indexes in the high dosage group and 5-ASA-SiO$_2$ NP group were significantly lower than that in the normal dosage group. Expression of IL-6 and TNF-α mRNA in colonic mucosa in the normal dosage, high dosage and 5-ASA-SiO$_2$ NP group was significantly lower than that in the model group. Colonic mucosal IL-6 and TNF-α mRNA expression in the high dosage and 5-ASA-SiO$_2$ NP groups was significantly lower than that in the normal dosage group (P<0.05). In conclusion, 5-ASA-SiO$_2$ NPs are a selective drug release system that target the inflamed colon, characteristics of UC, and can greatly increase therapeutic efficacy in UC.

Introduction

Ulcerative colitis (UC) is progressively emerging as a global threat (1), and an intractable disease by the World Health Organization (2). Colonic mucosal healing is currently considered the gold standard for treatment (3). 5-aminosalicylic acid (5-ASA), a conventional anti-inflammatory drug, has been a first-line therapeutic agent in the treatment of mild to moderate active UC for several decades, which allows for remission to be maintained (4). However, when 5-ASA is orally administered, a large quantity of the drug is absorbed by the upper gastrointestinal (GI) tract and enters into the systemic circulation, with only a fraction of the active compound reaching the inflamed target colon areas. As a result, targeting 5-ASA to the site of inflammation has remained a challenge and an unmet requirement in the treatment of UC. In the current study, 5-ASA-loaded silicon dioxide nanoparticles (5-ASA-SiO$_2$ NPs) were designed and prepared, and their therapeutic efficacy in a dextran sodium sulfate (DSS)-induced mice model of UC was investigated.

Materials and methods

Preparation and characterization of SiO$_2$ NPs and 5-ASA-SiO$_2$ NPs. SiO$_2$ NPs and 5-ASA-SiO$_2$ NPs were prepared and synthesized by the Dalian Institute of Chemical Physics, Chinese Academy of Sciences (Dalian, China). The brief procedures were illustrated in Fig. 1. The micro-emulsion method was used to prepare the SiO$_2$ NPs according to the literature (5). This method involved use of an appropriate amount of cyclohexane (31 ml), butanol (8 ml), polyoxyethylene nonylphenol ether (15 ml), ammonia water (0.5 ml), and distilled H$_2$O (6 ml) were mixed thoroughly for 5 min. Then, 20 mmol tetraethyl orthosilicate was added to the
mixture and reacted for 12 h. A total of 20 ml alcohol was then added into the mixture to stop emulsification. The NPs were collected by centrifuging the mixture at 8,000 x g for 5 min at room temperature, prior to being re-suspended in ethanol. The NPs suspension was added into a pressure bottle and mixed for 5 min in an 80˚C oil bath; and this step was repeated four times. The SiO₂ NPs were obtained by drying the suspension. After reaction with APTES and dehydrated toluene, the amination of SiO₂ NPs were obtained; then the amination of SiO₂ NPs (0.2 g) were anhydride modified by reacting with succinic anhydride (1 g), acetonitrile (10 ml) in a pressure bottle for oil bath at 80˚C with magnetic stirring. After reaction for 24 h, the anhydride modified SiO₂ NPs were collected with centrifuging (8,000 x g, 5 min), washing with acetonitrile and drying. SiO₂ NPs were dispersed in deionized water to prepare 1 mg/ml suspension. Following ultrasonic dispersion for 30 min, the suspension was taken out, and a small amount of dispersion suspension was drained by copper mesh. The morphology of the nanoparticles was observed by transmission electron microscopy (TEM). The anhydride-SiO₂ NPs (0.2 g), 5-ASA (0.2 g; TCI, Tokyo, Japan) and acetonitrile (20 ml) were mixed in a pressure bottle for another 24 h oil bath at 90˚C; and the drying precipitate was washed with dimethyl formamide (30 ml); then the target agent of 5-ASA-SiO₂ NPs were obtained after drying.

Assessment of drug loading efficiency and 5-ASA-SiO₂ NPs toxicity in Caco-2 cells. 5-ASA-SiO₂ NPs were dissolved in 1 M NaOH solution (pH=9.0) and mixed at 15 x g in a 50˚C oil bath for 12 h. The suspension was filtered with a 0.22 µm filter membrane (Thomas Scientific, Swedesboro, NJ, USA) and detected using high performance liquid chromatography (HPLC; Agilent Technologies, Inc., Santa Clara, USA). The loading efficiency of 5-ASA-SiO₂ NPs was calculated using the following equation: (Total 5-ASA NPs volume/total 5-ASA-SiO₂ NPs volume) x 100%. The detection was performed in triplicate.

Concentration of the logarithmic growth of Caco-2 cells (Cell Resource Center/PUMC, Beijing, China). in Minimum Essential Medium (MEM) was adjusted to 2x10⁵/ml. Cell suspension (100 µl) was added to one well of the 96-well plate and cultured in CO₂ incubator for 48 h. The medium in the plate was discarded and the cells washed with PBS twice. Then, 100 µl 5-ASA, 100 µl SiO₂ NPs and 100 µl 5-ASA-SiO₂ NPs solution were added into different wells and incubated for 12 h. For different treatments, the working concentrations of the solutions were adjusted by MEM to 15.6 µg/ml, 31.2 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml and 1 mg/ml. Following the incubation, the medium was replaced by 90 µl new medium with 10 µl Cell Counting Kit-8 (CCK-8) solution (Beijing Zoman Biotechnology Co., Ltd., Beijing, China) and incubated for another 2 h before recording the optical density (OD). The negative control group (wells with Caco-2 cells and CCK-8 solution) and the blank control group (wells with CCK-8 solution only) were also set up as described above. Each treatment was represented by three replicates.

The OD values in different wells were recorded using a microplate reader at 450 nm. The survival rates (%) of different treatments were calculated as (OD value in treatment group‑OD value in blank control group) / (OD value in negative control group‑OD value in blank control group) x 100%.

Colitis model. A total of 60 male healthy BALB/c mice (8-9 weeks of age, body weight 20-25 g) were provided by the Animal Center, Dalian Medical University (Dalian, China). Colitis in mice was induced by 5% (w/v) DSS supplemented in the drinking water for seven days (6,7). Mice were maintained in a room with constant temperature (22±1˚C) and a dark-light cycle (12/12 h), and housed in cages (<5 mice per cage). They were fed with standard laboratory food and water for one week before the experiments. All animal experiments were conducted in the accordance with the Institutional Animal Ethics Committee and Animal Care Guidelines of Dalian Medical University (Dalian, China) governing the use of experimental animals.

Mice were randomly divided into six groups (10 per group): Group A, control group; group B, model group (UC model mice); group C, normal dose 5-ASA group (200 mg/kg.day⁻¹ for 7 days); group D, high dose 5-ASA group (400 mg/kg.day⁻¹ for 7 days); group E, SiO₂ NPs group (100 mg/kg.day⁻¹ for 7 days); group F, 5-ASA-SiO₂ NPs group (100 mg/kg day⁻¹ for 7 days).
Results of IL-6 and TNF-α mRNA expressions in colonic samples of different groups have been presented in Table IV. IL-6 and TNF-α mRNA expressions in group B were significantly higher than those of group A. There was no significant difference between groups B and E. The IL-6, TNF-α and MPO levels were significantly reduced by administration of normal dose 5-ASA (group C) compared with the control group; and the levels were lower in groups D and F when compared with group C.

Results of IL-6 and TNF-α mRNA expressions taken from eyeball blood samples and MPO from colon samples were presented in Table III. The IL-6, TNF-α and MPO levels in group B significantly increased compared with group A. However, IL-6 and TNF-α in groups C-E were significantly reduced when compared with group B. The levels of IL-6 and TNF-α were lower in groups D and F when compared with group C.

Discussion
The colon is the terminal section of the digestive tract, and thus orally administered drugs targeting the colon must pass through a detrimental environment including digestive
enzymes, pH and bacteria. Use of a novel oral colon-specific drug delivery system has confirmed that it is difficult to release a drug in the upper GI tract, and thus, the drug is released rapidly into the colon following oral administration, allowing for the accumulation of the drug in the inflamed target regions (10). Investigating this system would hopefully result in a higher local drug concentration at the target site and, consequently, reduced required doses, toxicity and systemic side effects. Nanopharmacology has gained interest in the clinic, and nanomedicines are promising therapeutics employed in the treatment of diseases at the molecular level (11). NPs have received considerable attention as a novel medical technology due to their non-toxic, biocompatible, biodegradable and mucoadhesive properties. Among the various pharmaceutical excipients, the SiO$_2$ NPs are the most common. Their particle size is adjustable, they can be easily prepared and they are toxicologically safe, thus are favored by numerous researchers (12). NPs are an encouraging strategy to enhance the therapeutic benefit of UC (13-16), however, NPs in UC therapy remain in the initial stages.

The silica NPs demonstrated a mean size distribution and NP diameter of 90 nm in the present study, which is appropriate for uptake of NP in inflamed colonic mucosa (17). The particle size of the targeted delivery system is a major parameter to control and affect the particle transition into enzymes, pH and bacteria. Use of a novel oral colon-specific drug delivery system has confirmed that it is difficult to release a drug in the upper GI tract, and thus, the drug is released rapidly into the colon following oral administration, allowing for the accumulation of the drug in the inflamed target regions (10). Investigating this system would hopefully result in a higher local drug concentration at the target site and, consequently, reduced required doses, toxicity and systemic side effects. Nanopharmacology has gained interest in the clinic, and nanomedicines are promising therapeutics employed in the treatment of diseases at the molecular level (11). NPs have received considerable attention as a novel medical technology due to their non-toxic, biocompatible, biodegradable and mucoadhesive properties. Among the various pharmaceutical excipients, the SiO$_2$ NPs are the most common. Their particle size is adjustable, they can be easily prepared and they are toxicologically safe, thus are favored by numerous researchers (12). NPs are an encouraging strategy to enhance the therapeutic benefit of UC (13-16), however, NPs in UC therapy remain in the initial stages.

The silica NPs demonstrated a mean size distribution and NP diameter of 90 nm in the present study, which is appropriate for uptake of NP in inflamed colonic mucosa (17). The particle size of the targeted delivery system is a major parameter to control and affect the particle transition into enzymes, pH and bacteria. Use of a novel oral colon-specific drug delivery system has confirmed that it is difficult to release a drug in the upper GI tract, and thus, the drug is released rapidly into the colon following oral administration, allowing for the accumulation of the drug in the inflamed target regions (10). Investigating this system would hopefully result in a higher local drug concentration at the target site and, consequently, reduced required doses, toxicity and systemic side effects. Nanopharmacology has gained interest in the clinic, and nanomedicines are promising therapeutics employed in the treatment of diseases at the molecular level (11). NPs have received considerable attention as a novel medical technology due to their non-toxic, biocompatible, biodegradable and mucoadhesive properties. Among the various pharmaceutical excipients, the SiO$_2$ NPs are the most common. Their particle size is adjustable, they can be easily prepared and they are toxicologically safe, thus are favored by numerous researchers (12). NPs are an encouraging strategy to enhance the therapeutic benefit of UC (13-16), however, NPs in UC therapy remain in the initial stages.

Table II. Primer information.

| Gene   | Sequence (5’-3’)                        | T$_m$ (°C) | Production length (bp) |
|--------|----------------------------------------|------------|------------------------|
| IL-6   | Forward: CCACTTCACAAGTCGGAGGCTTA       | 78         | 169                    |
|        | Reverse: CCAGTTTGGTAGCATCCATATTTC      |            |                        |
| TNF-α  | Forward: TATGGGCCGAC ACCCTACA          | 80.6       | 199                    |
|        | Reverse: GGAGTAGAC AAGGTACAACCCATC    |            |                        |
| GAPDH  | Forward: AAATGGTTGAAGGT CGGTGTAAC     | 75.6       | 90                     |
|        | Reverse: CAACAATCTCCACTTTGCCACTG      |            |                        |

IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.
 cytotoxicity investigations demonstrated that compared to SiO₂ NPs, 5-ASA SiO₂ NPs demonstrated the strongest cytotoxicity. The lower toxicity of 5-ASA SiO₂ NPs compared to SiO₂ NPs may be explained by the toxicity limiting the availability of bound compared to free drug.

In vitro cytotoxicity investigations demonstrated that there was only marginal difference between the three agents when the concentration was lower than 62.5 µg/ml. A significant difference could be detected when the concentration was higher than 250 µg/ml, with the SiO₂ NPs having the strongest cytotoxicity. The lower toxicity of 5-ASA SiO₂ NPs compared to SiO₂ NPs may be explained by the toxicity limiting the availability of bound compared to free drug.

For all exposures, >70% survival percentage proved to be safe. In vitro cytotoxicity investigations demonstrated that there was only marginal difference between the three agents when the concentration was lower than 62.5 µg/ml. A significant difference could be detected when the concentration was higher than 250 µg/ml, with the SiO₂ NPs having the strongest cytotoxicity. The lower toxicity of 5-ASA SiO₂ NPs compared to SiO₂ NPs may be explained by the toxicity limiting the availability of bound compared to free drug.

Table III. Protein levels of IL-6, TNF-α and MPO after treatment of 5-ASA-SiO₂ NPs.

| Group | n  | IL-6 (pg/ml) | TNF-α (pg/ml) | MPO (U/mg) |
|-------|----|--------------|---------------|------------|
| Group A | 10 | 53.2±9.0     | 42.2±7.0      | 2.0±0.8    |
| Group B | 9  | 315.8±31.1a  | 284.5±27.1b   | 34.5±2.2a  |
| Group C | 10 | 226.2±17.5b   | 153.3±17.9b   | 25.7±2.0b  |
| Group D | 10 | 126.5±8.9c   | 84.0±9.3c     | 10.3±1.3c  |
| Group E | 9  | 317.8±18.9a  | 282.0±22.7a   | 34.1±1.7a  |
| Group F | 10 | 135.1±10.9d,c | 87.5±7.0d,c   | 10.9±1.4d,c |

P<0.01 vs. group A; P<0.01 vs. group E; P<0.05 vs. group B; P<0.05 vs. group C. IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; MPO, myeloperoxidase; 5-ASA-SiO₂ NPs, 5-aminosalicylic acid-loaded silicon dioxide nanoparticles.

Table IV. mRNA expression of IL-6, TNF-α in colonic mucosa.

| Group | n  | IL-6 | TNF-α |
|-------|----|------|-------|
| Group A | 10 | 1.1±0.3 | 1.2±0.3 |
| Group B | 9  | 26.1±1.6a | 15.0±1.6c |
| Group C | 10 | 10.3±1.0b,c | 4.7±0.9b,c |
| Group D | 10 | 4.6±0.7c | 2.0±0.5c |
| Group E | 9  | 25.5±1.7b | 14.7±1.3b |
| Group F | 10 | 4.7±0.5a,b,c | 1.8±0.4a,b,c |

P<0.01 vs. group A; P<0.01 vs. group E; P<0.05 vs. group B; P<0.05 vs. group C. IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

The general toxicity of the new system could be considered as minimal. However, we did not come to the conclusion that the toxicity of 5-ASA SiO₂ NPs or SiO₂ NPs was lower compared with 5-ASA, as previously indicated in the study of Pertuit et al (13). Together, the results of the present study demonstrate that 5-ASA-SiO₂ NPs were successfully prepared, which may be considered for use in animal experimentation.

The severity of disease can be assessed using different parameters. The therapeutic effects of IBD can be assessed with IBD disease activity, including clinical, endoscopic, histological and radiological assessment tools (19). The clinical activity score allows the assessment of the severity of the diseases in the living animal. The treatment efficacy of 5-ASA-SiO₂ NPs was assessed in UC model mice. The DAI scores was significantly decreased in the 5-ASA-SiO₂ NPs group; the efficacy was even comparable to 5-ASA dose of 400 mg/kg day⁻¹ for 7 days; D, high dose 5-ASA group (400 mg/kg day⁻¹ for 7 days); E, SiO₂ NPs group (100 mg/kg day⁻¹ for 7 days); F, 5-ASA-SiO₂ NPs group (100 mg/kg day⁻¹ for 7 days).

![Figure 5: Histology image of colon issues obtained from different groups.](image)

The colons of animals in group E presented a similar degree of mucosal injury as group B. The degree of colon injury was reduced in group C. Conversely, the injured colon mucosa of animals in groups D and F was relieved significantly compared with the model group, with fewer edema and shallow ulcers. A, control group; B, model group (UC model mice); C, normal dose 5-ASA group (200 mg/kg day⁻¹ for 7 days); D, high dose 5-ASA group (400 mg/kg day⁻¹ for 7 days); E, SiO₂ NPs group (100 mg/kg day⁻¹ for 7 days); F, 5-ASA-SiO₂ NPs group (100 mg/kg day⁻¹ for 7 days).
After the resection of the colon, different inflammatory markers can be determined to evaluate the therapeutic efficacy. A frequently used parameter is the activity of MPO. MPO is an enzyme that is observed in mammalian granulocytes and the MPO activity is used to determine the infiltration of tissue with these cells. Inflammation can be assessed by measuring cytokine levels, for example, TNF-α and IL-6 are suitable, as their expression levels (as well as MPO expression) are closely associated with the occurrence and development of UC. These mediators have been demonstrated as being highly expressed in UC samples (20) with a several-fold increase of MPO levels also reported throughout the colonic mucosa in patients with UC (21). Thus, these three factors are excellent indicators for the severity of inflammation. In the current study, the significant decrease of IL-6, TNF-α and MPO in UC model mice after exposure to 5-ASA SiO₂ NPs was observed. The therapeutic potential of this delivery system appears similar to the application of high dose 5-ASA. It was concluded that a much lower dose of 5-ASA SiO₂ NPs achieved similar results of high dose of 5-ASA in treating UC. NPs delivered through oral administration were determined to accumulate in the inflamed tissues and therefore developed a more local effect through their accumulation at the site of action (14,17,22). Due to the tighter bond between the drug and carrier system, 5-ASA SiO₂ NPs present the advantage of targeting the drug to the inflamed area. The efficient binding of the 5-ASA to the SiO₂ NPs is the key issue in the process. Additional associated studies confirmed the therapeutic benefits of targeting drug-loaded nanoparticles to the site of the disease and to mitigate the clinical symptoms compared with conventional dosage forms (23,24). In recent years, the incidence rate of UC in China has increased markedly (25), and therefore, the application 5-ASA-SiO₂ NPs would decrease the financial cost and increase the safety of the treatment.

In conclusion, 5-ASA-SiO₂ NPs is a selective drug release strategy that targets the inflamed tissue and highly increases therapeutic efficacy. The 5-ASA-SiO₂ NPs at low dosage can achieve similar effects to high dosages of 5-ASA. As this drug delivery system is transposable to the majority of drugs in this therapeutic context, it represents a promising alternative for future innovative treatments of colon diseases.

Acknowledgements

The current study was supported by Science and Technology Agency of Liaoning Province (grant no. 2012225018) and the Science and Technology Bureau of Dalian (grant no. 2013E15SF154).

References

1. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benhaimiol EI, Panaccione R, Ghosh S, Barkema HW and Kaplan GG: Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology 142: 46-54.e2; quiz e30, 2012.
2. Lakatos L and Lakatos LP: Is the incidence of inflammatory bowel diseases increasing in Eastern Europe? Postgrad Med J 82: 332-337, 2006.
3. Pinetón de Chambrun G, Peyrin-Biroulet L, Lémann M and Colombel JF: Clinical implications of mucosal healing for the management of IBD. Nat Rev Gastroenterol Hepatol 7: 15-29, 2010.
4. Podolsky DK: Inflammatory bowel disease. N Engl J Med 347: 417-429, 2002.
5. Min Wang, Chen Chen, Jiping Ma and Jie Xue: Preparation of superhydrophobic cellulose-like silica nanoparticles with tunable water adhesion. J Mater Chem 21: 6962-6967, 2011.
6. Hoffmann JC, Pawlowski NN, Kühä AA, Höhne W and Zeitz M: Animal models of inflammatory bowel disease: An overview. Pathobiology 70: 121-130, 2002-2003.
7. Hartmann G, Bidlingmaier C, Siegwardt B, Albrich S, Schulze J, Tschoppe K, Eigler A, Lehr HA and Endres S: Specific type IV phosphodiesterase inhibitor rolipram mitigates experimental colitis in mice. J Pharmacol Exp Ther 292: 22-30, 2000.
8. Cooper HS, Murthy SN, Shah RS and Sedergranj DJ: Clinopathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest 69: 238-249, 1993.
9. Murano M, Maemura K, Hirata I, Toshikawa K, Nishikawa T, Hamamoto N, Sasaki S, Saitoh O and Katsu K: Therapeutic effect of intracolonomically administered nuclear factor kappa B (p65) antisense oligonucleotide on mouse dextran sulphate sodium (DSS)-induced colitis. Clin Exp Immunol 120: 51-58, 2000.
10. Lamprecht A: IBD: Selective nanoparticle adhesion can enhance colitis therapy. Nat Rev Gastroenterol and Hepatol 7: 311-312, 2010.
11. Hock SC, Ying YM and Wah CL: A review of the current scientific and regulatory status of nanomedicines and the challenges ahead. PDA J Pharm Sci Technol 65: 177-195, 2011.
12. Borbat PP, Costa-Filho AJ, Earle KA, Moscicki JK and Freed JH: Electron spin resonance in studies of membranes and proteins. Science 291: 266-269, 2001.
13. Pertuit D, Moulari B, Betz T, Nadaradjiame A, Neumann D, Jamali L, Retouvelet B, Pellequer Y and Lamprecht A: 5-amio salicylic acid bound nanoparticles for the therapy of inflammatory bowel disease. J Control Release 123: 211-218, 2007.
14. Lamprecht A, Yamamoto H, Takeuchi H and Kawashima Y: Nanoparticles enhance therapeutic efficiency by selectively increased local drug dose in experimental colitis in rats. J Pharmacol Exp Ther 315: 196-202, 2005.
15. Moulari B, Pertuit D, Pellequer Y and Lamprecht A: The targeting of surface modified silica nanoparticles to inflamed tissue in experimental colitis. Biomaterials 29: 4554-4560, 2008.
16. Kshirsagar SJ, Bhalekar MR, Patel JN, Mohapatra SK and Shewale NS: Preparation and characterization of nanocapsules for colon-targeted drug delivery system. Pharm Dev Technol 17: 607-613, 2012.
17. Lamprecht A, Schäfer U and Lehr CM: Size-dependent biodistribution of micro- and nanoparticulate carriers to the inflamed colonic mucosa. Pharm Res 18: 788-793, 2001.
18. Schmidt C, Lautenschlaeger C, Collnot EM, Schumann M, Bojarski C, Schulzke JD, Lehr CM and Stallmach A: Nano- and microscaled particles for drug targeting to inflamed intestinal mucosa: A first in vivo study in human patients. J Control Release 165: 139-145, 2013.
19. Walsh AJ, Bryant RV and Travis SP: Current best practice for disease activity assessment in IBD. Nat Rev Gastroenterol Hepatol 13: 567-579, 2016.
20. Zhou YH, Yu JP, Liu YF, Teng XJ, Ming M, Lv P, An P, Liu SQ and Yu HG: Effects of Ginkgo biloba extract on inflammatory mediators (SOD, MDA, TNF-α, NF-kappa B, IL-6) in TNBS-induced colitis in rats. Mediators Inflamm 2006: 92642, 2006.
21. Deyo RS, Witkon K, Chen AI, Hadiyane C, Weinstein MI and Pellecchia C: Interleukin-8 and neutrophil markers in colonic mucosa: A first in vivo study in human patients. J Control Release 87: 1447-1452, 1992.
22. Lamprecht A, Ulbrich N, Yamamoto H, Schäfer U, Takeuchi H, Maincent P, Kawashima Y and Lehr CM: Biodegradable nanoparticles for the targeted drug delivery in treatment of inflammatory bowel disease. J Pharmacol Exp Ther 299: 775-781, 2001.
23. Niebel W, Walkenbach K, Béduneau A, Pellequer Y and Lamprecht A: The targeting of specific type IV antiinflammatory drugs by microspheres to colitis therapy. J Pharmacol Exp Ther 299: 775-781, 2001.
24. Pan J, Wu Y, Li Z, Chen J, Wang Z and Yu B: Niosomes enhance healing In TNBS-induced colitis. J Control Release 175: 51-58, 2012.
25. Ng SC: Epidemiology of inflammatory bowel disease: Focus on Asia. Best Pract Res Clin Gastroenterol 28: 363-372, 2014.