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Rhenium-coated glass beads for intracolonic administration attenuate TNBS-induced colitis in mice: Proof-of-Concept Study

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Abstract: In search for novel effective treatments in inflammatory bowel diseases, a new strategy employing glass beads coated with rhenium nanolayer has been developed and validated in the mouse model of 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis. Briefly, mice were randomly divided into 5 experimental groups: control (vehicle alone, Group 1); control treated with rhenium-coated glass beads (Group 2); TNBS (Group 3); TNBS treated with rhenium-coated glass beads (Group 4); and TNBS treated with uncoated glass beads (Group 5). Mice from Group 2, 4 and 5 were treated with respective beads (once daily, 5 beads / animal, i.c.) between D3–D6 post-TNBS/vehicle and evaluation of colonic damage was performed on D7, based on macroscopic scoring and clinical parameters. Severe colonic inflammation developed in post-TNBS mice (Group 3) [$P < 0.001$ vs. control (Group 1) for macroscopic score], which was significantly attenuated by treatment with rhenium-coated glass beads (Group 4) [$P < 0.01$ vs. TNBS (Group 3), for macroscopic score]. Neither rhenium-coated glass beads had any effect in control animals (Group 2), nor uncoated glass beads influenced TNBS-induced colitis (Group 5). In conclusion, a novel and attractive strategy for the treatment of colonic inflammation has been proposed; therapy with rhenium-coated glass beads already proved effective in the mouse model of TNBS-induced colitis, now requires further characterization in clinical conditions.

Key words: glass bead; mouse model of colitis; Crohn's disease; rhenium; rare elements; transition metal.

Introduction

Inflammatory bowel diseases (IBD), which include Crohn's disease (CD) and ulcerative colitis, are chronic inflammatory disorders of the gastrointestinal (GI) tract (for review, see: [1]). An increase in the incidence of IBD has recently been observed, in particular in well-developed countries and has become a very heavy burden for healthcare systems worldwide [2]. The etiopathology of IBD remains largely unknown, but the imbalance between the immune system-derived pro- and anti-inflammatory cytokines is believed to constitute the major trigger. Different approaches, which include non-steroidal anti-inflammatory agents (NSAIDS), corticosteroids, and biologicals ("anti-TNF's") have been employed to treat IBD depending largely on the advancement of the disease; ultimately, surgical intervention may be needed. Due to the fact that disease symptoms, rather than pathways involved in IBD development and underlying mechanisms are being targeted, the success rate in IBD treatment is still unsatisfactory.

Several novel therapeutic strategies have recently been proposed, of which a considerable number has been validated in basic studies and will now be verified in clinical studies. These include many biosimilars [3], as well as receptor ligands [4, 5] or endogenous enzyme inhibitors [6]. Some of these approaches are based on nanoparticle technology (for review, see: [7]). Following this trend, we have recently designed and validated in preclinical models a novel potential therapeutic approach in IBD employing glass beads, on which a silver nanolayer (50 nm thick) was deposited (unpublished results). Of note, the principal anti-inflammatory action of these silver-coated glass beads was associated with the restoration of dysregulated microflora in the colon (unpublished results).

Following the successful application of the silver nanolayer, we propose the use of rhenium-coated glass beads in the treatment of IBD. In this study, we describe the details of the design and preparation of rhenium-coated glass beads, and validation of this new approach using an animal model of colonic inflammation. Attenuation of TNBS-induced colitis in mice that was observed in this study strongly encourages further research on rhenium nanolayer to understand its anti-inflammatory action in the GI tract.

Materials and methods

Preparation of rhenium-coated glass beads

Borosilicate glass beads with the diameter from 0.9 to 1.4 mm were placed in a dedicated holder, which allows for uniform mixing of glass beads during rhenium layer deposition in magnetron sputtering unit. For deposition, simple magnetron sputtering method with the use of standard 2" magnetron sputtering gun equipped with pure rhenium (4N) target was chosen. The actual weight of glass beads per each deposition was set to about 0.5 g. After reaching the base pressure of 5×10^{-3} Pa in the vacuum chamber, pure argon (5N) was introduced to the working pressure of 0.7 Pa. Deposition of rhenium layer lasted 1800 s with sputtering power of 270 W.

The scanning electron microscope (SEM) observations of the microstructure of the coatings as well as energy dispersive spectroscopy (EDS) analysis of chemical composition were performed. In order to measure the thickness of the coating, the beads were embedded in the epoxy resin sample, than grinded and polished to obtain the desired cross-sections of beads. Such cross-section were observed using the scanning electron microscope (SEM) JEOL JSM-6610LV integrated with the Oxford Instruments EDS X-MAX 80 unit, which analyses specimen elemental chemistry.

Animals

Male balbC mice obtained from Animal Facility of the University of Lodz, Poland, weighing 22–26 g were used for all experiments. Mice were housed at a constant temperature (22–24°C) and maintained in sawdust-lined plastic cages under a 12-hour light/dark cycle with free access to laboratory chow and tap water ad libitum. The experimental protocol was in accordance to the European Communities Council Directive of September 22, 2010 (2010/63/EU).

Induction of colitis

Colitis was induced by intracolonic (i.c.) instillation of 2,4,6-trinitrobenzene sulfonic acid (TNBS), as described before [8]. Briefly, mice were lightly anesthetized with 1% isoflurane (Baxter Healthcare Corp., IL, USA) and TNBS (4 mg in 0.1 ml of 30% ethanol in saline) was instilled into the colon through a catheter inserted into the anus (3 cm proximally). Control animals received vehicle alone (0.1 ml of 30% ethanol in saline). Preliminary experiments demonstrated that the dose of TNBS used in this study induced reproducible colitis.

Experimental groups and treatments

Mice were randomly divided into 5 experimental groups: control (Group 1); control treated with rhenium-coated glass beads (Group 2); TNBS (Group 3); TNBS treated with rhenium-coated glass beads (Group 4); and TNBS treated with uncoated glass beads (Group 5). Mice from Group 2, 4 and 5 were treated with respective beads (once daily, 5 beads / animal, i.c.) between D3–D6 post-TNBS/vehicle and evaluation of colonic damage was performed on D7.

Evaluation of colonic damage

Animals were sacrificed by cervical dislocation. The colon was removed, opened longitudinally, rinsed with phosphate buffered saline (PBS), and immediately examined. Macroscopic colonic damage was assessed by an established semi-quantitative scoring system by adding individual scores for ulcer, colonic shortening, wall thickness, and presence of hemorrhage, fecal blood, and diarrhea, as described before [8]. For scoring ulcer and colonic shortening the following scale was used: ulcer — 0.5 points for each 0.5 cm; shortening of the colon: 1 point for >15%, 2 points for >25% (based on a mean length of the colon in untreated mice of 8.18 ± 0.19 cm, n = 6). The wall thickness was measured in mm: a thickness of n mm corresponds to n scoring points. The presence of hemorrhage, fecal blood, or diarrhea increased the score by 1 point for each additional feature.

Drugs and reagents

All drugs and reagents, unless otherwise stated, were purchased from Sigma-Aldrich (Poznań, Poland).

Statistics

Statistical analysis was performed using Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA). The data are expressed as means ± SEM Student t-test or one-way ANOVA followed by Newman-Keuls post-hoc test were used for all analyses. P values <0.05 were considered statistically significant.

Results

Glass bead coating contains uniform nanolayer of rhenium

In Figure 1, the SEM Image of the cross-section of the bead coated with rhenium layer is presented. The image contains an additional line representing the path, along which the chemical composition of the bead cross-section was obtained using the EDS method. The contents (% at.) for individual components along the path are also shown, under the SEM Image.

Of all individual components, the content of rhenium is of main interest, as it allows determination of the position of the rhenium layer covering the bead. The clear increase of rhenium content along the path points the beginning of the layer. The end of the layer is difficult to identify, as the content decreases too gently along the path, and is disturbed by local extremes.

In Figure 2, the SEM Image of the bead coated with the rhenium layer is presented. Under the SEM Image, the chemical composition of the bead surface revealed by the use of EDS method is shown. The obtained surface content for rhenium is of 0.91% at.,

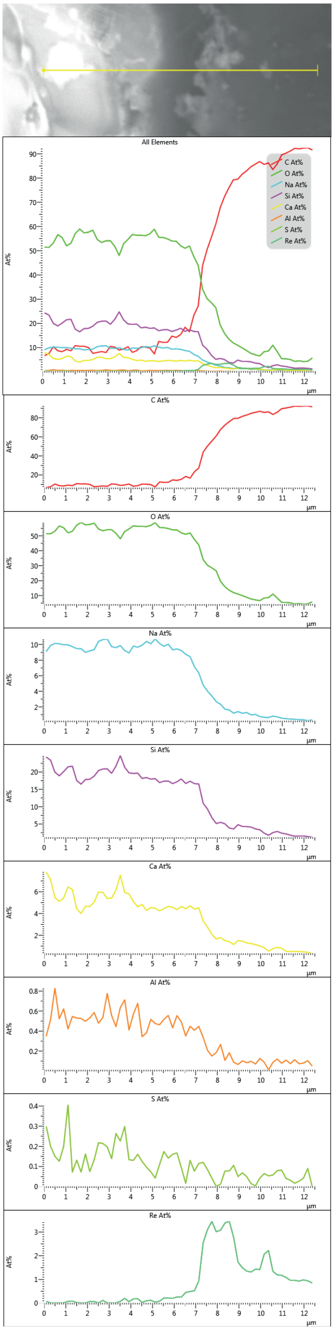
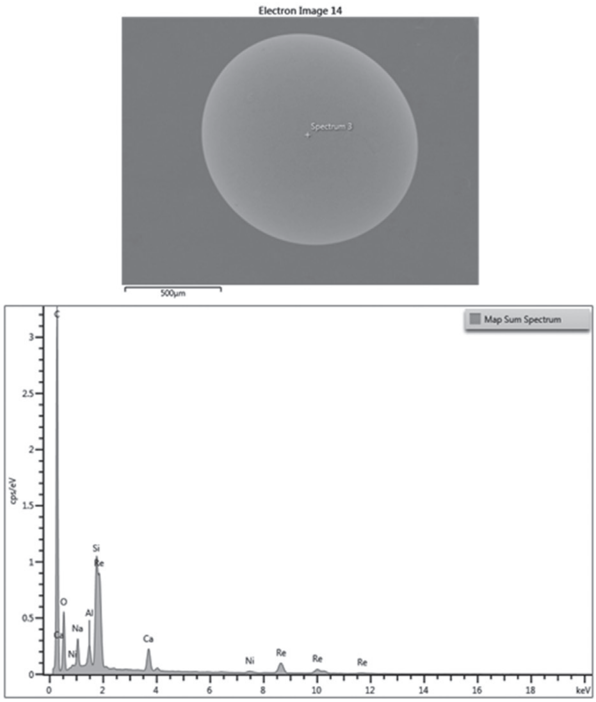


Fig. 1. The scanning electron microscope (SEM) Image for the cross-section of the bead coated with rhenium layer (top panel). The chemical composition of the bead cross-section along the path indicated in the SEM Image (bottom panels).



Element	Atomic %
C	80.02
O	14.70
Na	1.02
Si	2.15
Ca	0.70
Al	0.39
Ni	0.11
Re	0.91
Total:	100.00

Fig. 2. Bead coated with rhenium nanolayer (top panel) and chemical composition of the bead surface revealed by the energy dispersive spectroscopy (EDS) method (bottom panels).

which can be three times smaller than that obtained for the layer cross-section presented in Figure 1. For each registered component, the obtained surface distribution was uniform, what indicates a good coverage of the bead with deposited layer using the magnetron sputtering.

Treatment with rhenium-coated glass beads attenuates TNBS-induced colonic damage in mice

We used a well-established mouse model of colitis induced by TNBS. The i.c. administration of TNBS (Group 3) resulted in reproducible increase in macroscopic damage score and change in body weight over time compared with control animals (Group 1) (Fig. 3). Treatment with rhenium-coated glass beads (once daily between D3–D6 post-TNBS/

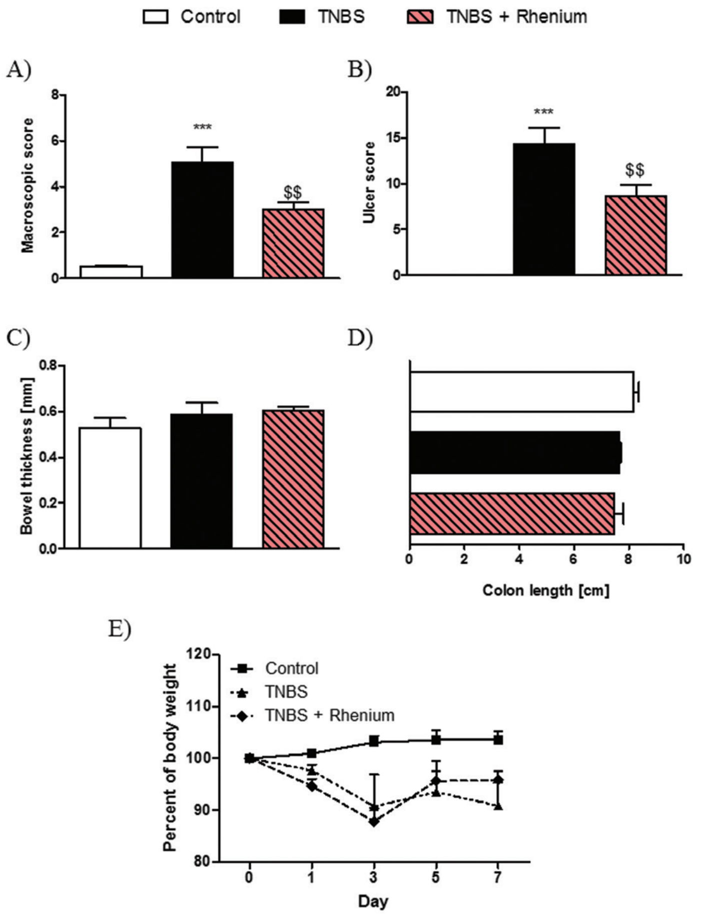


Fig. 3. The treatment with rhenium-coated glass beads (once daily, 5 beads / animal, i.c.) between D3–D6 post-TNBS/vehicle attenuated TNBS-induced colitis in mice. Figure shows data for macroscopic score (A), ulcer score (B), bowel thickness (C), colon length (D) and changes in body weight (E) for control (vehicle alone, Group 1), TNBS (Group 3) and TNBS treated with rhenium-coated glass beads (Group 4). Neither rhenium-coated glass beads had any effect in control animals (Group 2), nor uncoated glass beads influenced TNBS-induced colitis (Group 5) (data not shown).

***P <0.001, as compared to control mice. $$ P <0.01 vs. TNBS-treated mice. Data represent mean ± SEM of 6–8 mice per group.

vehicle, 5 beads / animal, i.c.) significantly improved colitis in TNBS-treated mice (Group 4) as shown by lowered macroscopic score (Fig. 3A) and ulcer score (Fig. 3B), and had no effect on bowel thickness (Fig. 3C), colon length (Fig. 3D), or animal body weight (Fig. 3E).

Neither rhenium-coated glass beads had any effect in control animals (Group 2), nor uncoated glass beads influenced TNBS-induced colitis (Group 5), suggesting a pivotal role of rhenium nanolayer in the inflamed colon.

Discussion

In this study, we observed that the i.c. administration of rhenium-coated glass beads in TNBS-treated mice significantly attenuated colitis, as shown by decreased macroscopic colon damage and ulcer scores compared to animals treated with TNBS alone. This is the first report on the application of rhenium-coated glass beads as a novel potential strategy in the treatment of colonic inflammation.

Silver, gold, copper and some other metals (zinc, cerium, cobalt) have been used in several forms and formulations (colloids, nanolayers, and nanoparticles), mainly as anti-bacterial and anti-inflammatory agents (for review, see: [7]). In contrast, the literature on rhenium is very scarce. Due to its high melting point (3185°C), rhenium has principally been employed in high temperature-resistant materials that serve in manufacturing jet engine components, for improving engine life, performance and operating efficiency [9]. In medicine, rhenium has been used for preparation of silica-coated magnetite nanoparticles [10] and colloids [11] labelled with Re-188, which are intended for magnetic targeted radiotherapy or treatment of rheumatoid arthritis.

The major issue concerning the application of rhenium coatings and nanoparticles is their instability, mainly due to oxidation by air and the occurrence of mixed oxidation states [9]. The uncertainty about the possible therapeutic effect of such formulations is a serious drawback in any clinical treatment.

In this preliminary study we have not only obtained relatively stable form of rhenium coating, but also successfully applied rhenium-coated glass beads in TNBS-treated mice, in which we observed a significant attenuation of colitis. Our results encourage further studies on the potential use of rhenium as an anti-inflammatory agent in IBD. The studies that will now follow should examine the mechanism of action of rhenium in the inflamed colon; imperatively, it needs to be determined whether rhenium coating interacts with dysregulated intestinal microflora, injured colonic epithelium or components of the immune system in the inflamed gut.

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Conflict of interest

None declared.

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