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Original article

# Evaluation of anti-inflammatory effect of silver-coated glass beads in mice with experimentally induced colitis as a new type of treatment in inflammatory bowel disease

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ABSTRACT

*Background:* Recent studies point at the anti-inflammatory action of silver through induction of apoptosis of inflammatory cells *via* oxidative stress, promotion of wound healing as well as antimicrobial effect. Our aim was to design a new formulation based on silver and validate its anti-inflammatory activity in the mouse models of colitis.
*Methods:* Silver-coated glass beads were prepared using a magnetron sputtering method and a standard magnetron sputtering gun equipped with pure silver target. Colitis was induced by the *ic* administration of TNBS into colon (to mimic Crohn's disease) and addition of DSS to drinking water (to imitate ulcerative colitis). Evaluation of inflammation was performed based on macroscopic and microscopic scoring, quantification of the myeloperoxidase activity and colonic microflora analysis.
*Results:* Silver-coated glass beads administered *ic* alleviated intestinal inflammation in mouse models of colitis, induced by TNBS and DSS. This alleviation of colitis resulted principally from changes in the gut microflora. The anti-inflammatory action of the new formulation was associated predominantly with the presence of the silver nanolayer on the beads, and to a lesser extent the size of glass polymer units.
*Conclusions:* The application of the newly developed formulation employing silver-coated glass beads has the potential to be translated to clinical conditions for the efficient treatment of IBD.


## Introduction

Inflammatory bowel disease (IBD) is a group of chronic, recurring inflammatory conditions, represented by Crohn's disease (CD) and ulcerative colitis (UC). The former develops at 20–35 years of age and may concern any segment of the GI tract; however, the distal ileum and further parts are mostly affected. UC, which occurs predominantly at ages 15–30 [1] and 60–80 [2,3] is limited to the colon [4]. There are also differences in their microscopic image: CD develops within the entire bowel wall and UC affects the gut mucosa (for review see: [5]). Common for both diseases is that the alleviation of symptoms and maintenance of remission require a long-term treatment which in most cases is associated with the development of adverse side effects; with the current state of knowledge, there is no efficient cure available to the IBD patients. Moreover for some, like biologic drugs (*e.g.* infliximab), there is an unsatisfactory number of responders.

The main limitation in IBD treatment is our understanding – or rather the lack of thereof – of factors triggering the disease. Environmental, nutritional, and genetic background is often taken into consideration when studying the distorted response of the immune system leading to IBD (for review see: [5]). Since relatively recently, there is a major focus on the microbial content of the gut and its influence on the development of the inflammation and the course of the disease. For example, abnormal colonization of the ileal mucosa by adherent/invasive *E. coli* has been shown in IBD patients [6]. Importantly, both quantitative and qualitative

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changes in microbiota composition are regarded as a possible trigger of the inflammatory process in the gut [7].

Silver and its preparations have a long history of application for wound healing and anti-microbial agents (for review see: [8]). Importantly, the external use of silver is generally regarded as safe and without long-term adverse side effects; even when hepatotoxicity or bluish-gray discoloration of the skin (*argyria* – like symptoms) develop, they are reversible upon discontinuation of the treatment [9]. Recent studies point at anti-inflammatory action of silver through induction of apoptosis of inflammatory cells *via* oxidative stress and the above-mentioned promotion of wound healing ([8] and references therein). Noteworthy, the size of the silver particles used for the treatment of inflammation, as well as the ionization state (metallic silver *vs.* silver ions) may affect their interaction with biological membranes and passage to inflamed sites, resulting in differences in anti-inflammatory efficacy [10].

Several novel formulations based on silver have recently been reported and their anti-inflammatory activity validated in animal models. For example, nanocrystalline silver (NPI 32101) administered intracolonically (*ic*) or orally alleviated colitis in a rat model mimicking UC and was as effective as sulfasalazine [11]. Others include carbon nanoscrolls (CNSs) build of silver nanoparticles (AgNPs), graphene oxide (GO)-based nanocomposites, nanosized silica hybrid silver complex (NSS) [9,12,13] and silver nanoparticles complexed with a potent antioxidant, alpha-lipoic acid [14].

Our aim was to design a new formulation based on silver and validate its anti-inflammatory activity in the mouse model of colitis. In the course of the study, we developed silver-coated glass beads, which attenuated colonic inflammation in TNBS- and DSS-treated mice principally, but not exclusively through antimicrobial action. The application of this newly developed formulation has the potential to be translated to clinical conditions for the efficient treatment of IBD.

## Materials and methods

### *Drugs and reagents*

Borosilicate glass beads were purchased from Merck (Warsaw, Poland). All drugs and reagents, unless otherwise stated, were purchased from Sigma-Aldrich (Poznań, Poland).

### *Preparation of silver-coated glass beads*

Silver-coated glass beads (850–1400 μm and 5 μm) were prepared as described previously [15], using a magnetron sputtering method and a standard 2" magnetron sputtering gun equipped with pure silver (4N) target. Deposition of silver layer lasted 360 s with the sputtering power of 270 W. To confirm the correctness of the method, scanning electron microscope (SEM) observations of the microstructure of the coatings as well as energy-dispersive X-ray spectroscopy (EDS) analysis of chemical composition were performed (data not shown).

### *Animals*

Male BALB/c mice (22–28 g) were obtained from the Nofer Institute of Occupational Medicine (Lodz, Poland). Animals were housed at a constant temperature (22 ± 2 °C), on a 12 h light/dark cycle in sawdust-lined plastic cages with free access to chow and tap water. Animal protocols were approved by the Medical University of Lodz Animal Care Committee (Protocol #9/ŁB16/2016). All efforts were made to minimize animal suffering and to reduce the number of animals used. Groups of 8–10 animals were used in all experiments.

### *Induction of colitis*

#### *TNBS model*

Colitis was induced by the *ic* administration of TNBS, as described previously [16]. 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 administered into colon through a catheter inserted 3 cm proximally to the anus. Previous experiments showed that the dose of TNBS used in the study induced reproducible colitis.

#### *DSS model*

Colitis was induced by addition of DSS to drinking water starting from day 0 to day 5 (4% wt/vol; molecular weight 40,000; MP Biomedicals, Aurora, OH, Lot No. 5237K), as previously described [17]. On days 6 and 7 the animals received water without DSS. Control animals received tap water only. Animal body weight was monitored daily and mean water and food consumption was recorded.

### *Pharmacological treatments*

Non-coated glass beads and silver-coated glass beads (5 beads/animal, *ic*) were administered once daily on days 3–6 from induction of colitis. The number of beads per animal was selected based on Siczek et al. [15]. Control animals received saline as vehicle (100 μL, *ic*). Vehicle had no effect on the observed parameters.

### *Evaluation of inflammation*

#### *TNBS model*

Animals were killed by cervical dislocation 7 days after TNBS application. The colons were instantly removed, opened longitudinally, washed with saline, and immediately examined. Macroscopic colonic damage was assessed using an established semi-quantitative scoring system by adding individual scores for ulcers, colonic shortening, and wall thickness, presence of hemorrhage, fecal blood and diarrhea. For ulcer score and colonic shortening, the following scale was used: 0.5 points for each 0.5 cm of ulcerated tissue; shortening of the colon, 1 point for >15% and 2 points for >25% (based on the mean length of the colon in untreated mice of 8.38 ± 0.11, n = 8). The wall thickness was measured in mm. The presence of hemorrhage, fecal blood, or diarrhea increased the score by 1 point for each additional feature. The macroscopic scoring was performed in a blind manner.

After macroscopic scoring, segments of the distal colon were stapled flat, mucosal side up, onto cardboard and fixed in 10% neutral-buffered formalin for 24 h at 4 °C. Samples were then dehydrated, embedded in paraffin, sectioned at 5 μm on a microtome, and mounted onto slides. Subsequently, sections were stained with hematoxylin and eosin and examined using a Motic AE31 microscope (Ted Pella, Redding, CA, USA). Photographs were taken using a digital imaging system consisting of a digital camera (Moticam 2300, Ted Pella, Redding, CA, USA) and image analysis software (MoticImages Plus 2.0, Motic Deutschland GmbH, Wetzlar, Germany). Microscopic total damage score was determined in a blind manner based on the presence (score = 1) or absence (score = 0) of goblet cell depletion, the presence (score = 1) or absence (score = 0) of crypt abscesses, the destruction of mucosal architecture (normal = 1, moderate = 2, extensive = 3), the extent of muscle thickening (normal = 1, moderate = 2, extensive = 3), and the presence and degree of cellular infiltration (normal = 1, moderate = 2, transmural = 3).

To assess granulocyte infiltration and to quantify the myeloperoxidase activity (MPO), the method adapted by Fichna et al. was