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Application of carbon nanotubes as the carriers of the cannabinoid, 2-arachidonoylglycerol: Towards a novel treatment strategy in colitis

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ABSTRACT

Aims: Treatment of colitis has remained a major clinical challenge. The cannabinoid, 2-arachidonoylglycerol (2-AG), has shown beneficial effects in colitis, however, poor solubility or rapid hydrolysis may limit its efficiency. According to the high biocompatibility of carbon nanotubes (CNTs) and their ability for controlled drug delivery, we aimed to prepare multi-walled CNTs-2-AG (MWCNTs-2-AG) complex in order to improve the pharmacological profile of 2-AG and evaluate the therapeutic potential of this nanocomplex in a rat model of colitis.
Materials and methods: Aminated MWCNTs-2-AG complex was prepared using acidified MWCNTs and then characterized by Fourier transform infrared spectroscopy and transmission electron microscopy. In vitro cytotoxicity of MWCNTs was evaluated. Colitis was induced by colonic instillation of trinitrobenzene sulfonic acid (TNBS) and the effects of 2-AG solution and various types of MWCNTs on the colonic tissue damage, inflammation, and oxidative stress were evaluated.
Key findings: Aminated MWCNTs and MWCNTs-2-AG complex exhibited significantly lower cytotoxicity than acidified MWCNTs. Once daily intrarectal application of MWCNTs-2-AG complex (containing 2 mg/kg of 2-AG) 2 days before and 8 days after the induction of colitis effectively reduced the macroscopic and microscopic injuries, malondialdehyde, tumour necrosis factor-α, and interlukin-1β concentrations, and myeloperoxidase activity. While, free 2-AG (2 mg/kg), and acidified or aminated MWCNTs showed no beneficial effects.
Significance: Amino-functionalized MWCNTs appear as the suitable carriers for 2-AG which provide a sustained concentration for this cannabinoid leading to the promising therapeutic effects in the experimental colitis.

1. Introduction

Colitis is an inflammatory intestinal disorder which may be associated with chronic course of relapse and remission. The infiltration of lymphocytes, neutrophils, mast cells, or macrophages and the release of inflammatory mediators may lead to the mucosal ulceration and disruption. Colitis is associated with variable signs and symptoms such as the recurring bloody diarrhea, abdominal pain, weight loss, and fecal incontinence. The etiology of the disease has remained elusive, meanwhile, the genetic susceptibility, environmental factors, or immunological disturbances may be involved in the pathogenesis of colitis [1]. Development of the abnormal immune or inflammatory responses are usually associated with the enhanced levels of pro-inflammatory cytokines which may result in the loss of the intestinal epithelial barrier integrity and function as well as the vascular congestion and oedema [2]. Colitis may lead to the serious extra-intestinal complications such as the colorectal cancer, hepatobiliary dysfunction, or neurological disorders [3–5]. Following the colonoscopy, patients may need hospitalization and receive nonsteroidal anti-inflammatory drugs or corticosteroids [6], however, the current treatment options are associated with limited efficiency, various side effects, or resistance [6,7]. This, has provoked increasing research efforts to develop novel treatment strategies. In recent years, the role of the endocannabinoid system in the physiology and pathophysiology of the gastrointestinal (GI) tract has been extensively reviewed. The endocannabinoids including anandamide and 2-arachidonoylglycerol (2-AG) exert various biological activities by activating two types of cannabinoid CB_1 and CB_2 receptors which are widely distributed throughout the gut [8]. Activation of these receptors results in the inhibition of nociception or hyperalgesia due to the inflammatory conditions as well as the reduction of GI motility [9,10]. The therapeutic potential of the endocannabinoid system in the experimental colitis has also been shown [11,12]. In this respect, pharmacological modulation of this ubiquitous signalling system appears as a promising therapeutic approach against the GI disorders. 2-AG (Fig. 1), a full agonist at both CB_1 and CB_2 receptors [13], has shown a variety of beneficial effects including the anti-colitis effect [14–16], however, the rapid hydrolysis [17] may limit the efficiency of this cannabinoid. Over the last few decades,

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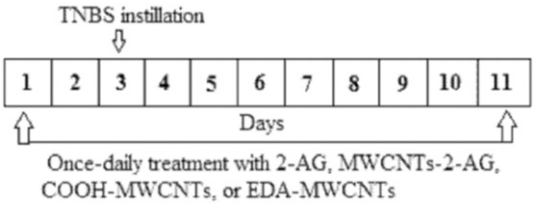

Fig. 1. A schematic diagram of the treatment schedule.

the promising advances in nanotechnology-based approaches have led to the development of more effective therapeutic options. In this context, carbon nanotubes (CNTs) have emerged as the advanced nanovectors for the delivery of a variety of compounds with short half-life or poor solubility. The superior mechanical properties, high thermoelectrical conductivities, and improved solubility or biocompatibility have made CNTs as suitable candidates for high-resolution imaging, biosensing, or controlled drug delivery [18–20]. Based on this background, we aimed to prepare multi-walled CNTs (MWCNTs)-2-AG complex and evaluate its ability to prolong the stability and therapeutic effects of 2-AG in an experimental model of colitis.

2. Materials and methods

2.1. Preparation of MWCNTs-2-AG complex

According to the reduced toxicity and improved dispersibility of amine-modified CNTs [21], we aimed to prepare aminated MWCNTs-2-AG complex. Meanwhile, we initially used acidified MWCNTs instead of the direct aminization of MWCNTs as the carboxylation of CNTs prior to the aminization facilitates further aminization and elevates the reactivity of CNTs [22]. Amine-modification of MWCNTs was performed as previously reported [22,23] with some modifications. Briefly, 500 mg of acidified MWCNTs (Plasmachem GmbH, Berlin, Germany) and 50 ml of 98% thionyl chloride ($SOCl_2$, Sigma Aldrich, Germany) were sonicated for 40 min using ultrasonic system (Tecna 6, Tecno-Gaz, Italy) at 70% amplitude and stirred using a magnetic stirrer (IKA, Germany) at 25 °C for 48 h. Then, the suspension was filtered with 0.45 μm pore-sized microporous membrane (Sartorius, Germany), washed with tetrahydrofuran for 5 times in order to remove the excess $SOCl_2$, and vacuumed at 25 °C for 25 min. The residue was reacted with 50 ml of ethylenediamine (EDA) (Sigma Aldrich, Germany) and stirred for 10 h. The suspension was filtered, washed 5 times in tetrahydrofuran, vacuumed for 25 min, dialyzed in the deionized distilled water using a dialysis bag (MW cut-off 14 KD) for 72 h, and vacuumed to obtain amine-functionalized MWCNTs. In order to prepare amine-modified MWCNTs-2-AG complex, 2-AG (Tocris Bioscience, UK) (50 μM) was added to the mixture of aminated MWCNTs and PBS (0.25% w/v), stirred at 25 °C for 24 h, and centrifuged (Sigma-3k30, Germany) at 10,000 rpm for 20 min. Following the removal of supernatant, the sample was washed with PBS, re-centrifuged at 10,000 rpm for 20 min, and dispersed in 10 ml of PBS.

2.2. Characterization of MWCNTs

In order to characterize the chemical structures of MWCNTs, Fourier transform infrared (FTIR) spectrophotometer (Shimadzu, Japan) was used. Transmission electron microscopy (Philips CM12 TEM) was applied to assess the morphologies of MWCNTs.

2.3. Cytotoxicity assay

The potential cytotoxic effects of MWCNTs were evaluated by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (Sigma Aldrich, Germany) colorimetric assay [24]. Briefly, rat pheochromocytoma PC12 cells in the phase of exponential growth were seeded in 96-well plates (Nunc, Denmark) at a density of 10^4 cells/well and incubated in 5% CO_2 incubator at 37 °C for 24 h. The viability of cells exposed to the serial dilutions of COOH-MWCNTs, EDA-MWCNTs, and EDA-MWCNTs-2-AG (10, 20, 30, 50, 100, and 200 μg/ml in PBS) was evaluated at 1st, 3rd, and 7th day of incubation by adding 20 μl of MTT (stock solution: 5 mg/ml in PBS) to each well. Following the incubation at 37 °C for 4 h, the culture medium was removed and 100 μl of dimethyl sulfoxide was added to each well to dissolve the formazan crystals. Then, the plate was subjected to low-speed oscillation for 10 min to completely dissolve the crystals and the absorbance (Abs) was determined at 570 nm by a microplate reader (Anthos 2020, Anthos Labtec Instruments, Austria). For the calculation of cell viability, the groups including the control group (non-exposed cells), blank control group (culture medium), and blank experimental group (MWCNTs without PC12 cells) were considered as follows;

Cell viability (%) = [Abs of treated group − Abs of blank experimental group/Abs of control group − Abs of blank control group] × 100

The results were presented as the mean ± SEM of six independent experiments.

2.4. In vivo experiments

2.4.1. Animals

Male Wistar rats weighing 300–320 g were housed individually under the standard laboratory conditions (temperature: 22 ± 1 °C, humidity: 55–65%) on a 12-h light/dark cycle. Animals had free access to water but were deprived of food 12 h prior to the induction of colitis. The procedures were carried out in accordance with the National Institutes of Health guidelines for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and approved by the local Ethics Committee.

2.4.2. Animal grouping and induction of the colitis

Animals were randomly assigned into the following groups; intact (n = 6), vehicle-treated (n = 6), vehicle-treated colitic rats (n = 10), and once-daily intrarectally-treated with 2-AG (0.5, 1, and 2 mg/kg) dissolved in dimethyl sulfoxide-Tween 80-saline (1:1:18) [25,26] or 1.5, 3, and 6 mg/kg of MWCNTs-2-AG complex (containing 0.5, 1, and 2 mg/kg of 2-AG, respectively), COOH-MWCNTs, or EDA-MWCNTs (n = 10/group) 2 days before the induction of colitis and 8 days thereafter (Fig. 1). In order to induce the colitis, each rat was anesthetized with ketamine (80 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.) and then a 8 cm long polyethylene catheter with an external diameter of 2 mm was slowly inserted into the lumen of the colon through the anal canal followed by 1 ml flush of 0.9% saline solution and manual palpation of the abdomen to remove any fecal matter. Afterwards, 120 mg/kg of 2,4,6-trinitrobenzene sulfonic acid (TNBS, Sigma Aldrich, Germany) was dissolved in 0.9% NaCl-ethanol (50:50, v/v) and instilled into the colon in a volume of 1 ml [27]. Animals were held in a head-down position for 30 s to prevent the flowing out of the enema. Following the recovery, animals were housed individually and daily monitored for food intake, body weight, and diarrhea.

2.4.3. Collection of the blood and tissue samples

24 h after the last treatment, rats were anesthetized with ketamine (80 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.) and the blood samples were collected from the portal vein of the animals in order to determine the serum contents of the pro-inflammatory cytokines including the tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). For macroscopic and microscopic assessments of the colonic damage and determination of myeloperoxidase (MPO) activity and malondialdehyde (MDA) content, animals were sacrificed by cervical dislocation and the abdomen of each rat was opened by a midline incision, the colon was excised, opened, and rinsed with cold 0.9% saline.