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The octapeptide NAP alleviates intestinal and extra-intestinal anti-inflammatory sequelae of acute experimental colitis

Markus M. Heimesaat[a,*], Eliezer Giladi[b], Anja A. Kühl[c], Stefan Bereswill[a,1], Illana Gozes[b,1]

[a] Department of Microbiology and Hygiene, Charité – Universitätsmedizin Berlin, Corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany

[b] Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

[c] Department of Medicine I for Gastroenterology, Infectious Disease and Rheumatology/Research Center ImmunoSciences (RCIS), Charité – University Medicine Berlin, Corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany

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ABSTRACT

The octapeptide NAP has been shown to exert neuroprotective properties and reduce neuro-inflammatory responses. The aim of the present study was to investigate if NAP provides anti-inflammatory effects in acute murine colitis. To address this, C57BL/6 j mice were challenged with 3.5% dextran sulfate sodium from day 0 until day 6 to induce colitis, either treated intraperitoneally with NAP or placebo (NaCl 0.9%) from day 1 until day 6 post-induction (p.i.) and subjected to in depth macroscopic, microscopic and immunological evaluations. Whereas NAP application did not alleviate macroscopic (i.e. clinical) sequelae of colitis, lower numbers of apoptotic, but higher counts of proliferating/regenerating colonic epithelial cells could be observed in NAP as compared to placebo treated mice at day 7 p.i. Furthermore, lower numbers of adaptive immune cells such as T lymphocytes and regulatory T cells were abundant in the colonic mucosa and lamina propria upon NAP versus placebo treatment that were accompanied by less colonic secretion of pro-inflammatory mediators including IFN-γ and nitric oxide at day 7 p.i. In mesenteric lymph nodes, pro-inflammatory IFN-γ, TNF and IL-6 concentrations were increased in placebo, but not NAP treated mice at day 7 p.i., whereas interestingly, elevated anti-inflammatory IL-10 levels could be observed in NAP treated mice only. The assessed anti-inflammatory properties of NAP were not restricted to the intestinal tract, given that in extra-intestinal compartments such as the kidneys, IFN-γ levels increased in placebo, but not NAP treated mice upon colitis induction. NAP induced effects were accompanied by distinct changes in intestinal microbiota composition, given that colonic luminal loads of bifidobacteria, regarded as anti-inflammatory, "health-promoting" commensal species, were two orders of magnitude higher in NAP as compared to placebo treated mice and even naive controls. In conclusion, NAP alleviates intestinal and extra-intestinal pro-inflammatory sequelae of acute experimental colitis and may provide novel treatment options of intestinal inflammatory diseases in humans.

1. Introduction

The octapeptide NAP (namely NAPVSIPQ) was initially identified as the smallest neuroprotective fragment of the activity-dependent neuroprotective protein (ADNP) [1]. Human ADNP is highly expressed within the central nervous system (CNS) including microglia and astrocytes [2] and also in the spleen, peripheral blood leukocytes and macrophages [3–5]. The neuroprotective effects of NAP have been assessed *in vitro* and in multiple *in vivo* models of neuronal morbidities including Alzheimer's disease, stroke, closed head injury, fetal alcohol syndrome and neonatal hypoxia [1,6–10]. The target of NAP activity was identified as microtubules [11], through direct interaction with microtubule end binding proteins [12], enlisting the microtubule associated protein tau to microtubules [13], which in turn protects against apoptosis induced by oxidative stress [14]. Immunomodulatory and anti-oxidative actions have been proposed as potential underlying mechanisms for the beneficial effects exerted by NAP as in the case of multiple sclerosis [15]. Anti-inflammatory, protective effetcs of NAP

Abbreviations: ADNP, activity-dependent neuroprotective protein; CNS, central nervous system; DSS, dextran sulfate sodium; GABA, γ-aminobutyric acid; H&E, hematoxylin and eosin; HPF, high power field; IFN, interferon; IL, interleukin; MLN, mesenteric lymph nodes; NO, nitric oxide; NOD, nucleotide-binding oligomerization domain; PBS, phosphate buffered saline; PLC, placebo; p.i., post-induction; qRT-PCR, quantitative real-time polymerase chain reaction; TLR, Toll-like receptor; TNF, tumor necrosis factor

* Corresponding author: Charité – University Medicine Berlin, CC5, Department of Microbiology and Hygiene, Campus Benjamin Franklin, FEM, Garystr. 5, D – 14195 Berlin, Germany.
E-mail address: markus.heimesaat@charite.de (M.M. Heimesaat).

[1] These authors contributed equally.

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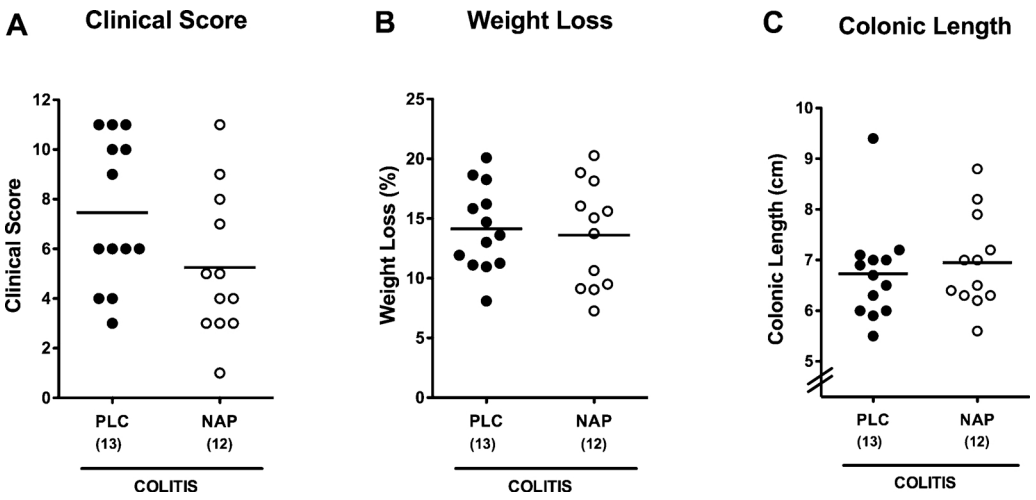

Fig. 1. Macroscopic sequelae of NAP treatment in mice with acute colitis.
In order to induce acute colitis, C57BL/6j wildtype mice were subjected to dextran sulfate sodium treatment from day 0 until day 6 post-induction (p.i.) as described in methods. Mice were treated either with synthetic NAP (open circles) or placebo (PLC; filled circles) from day 1 until day 6 p.i. Immediately before sacrifize at day 7 p.i., (A) clinical conditions were quantitated applying a standardized scoring system (see methods), (B) the relative loss of body weights between day 7 p.i. and day 0 determined (in%) and upon necropsy, (C) individual colonic lengths were measured (in cm). Means (black bars) and numbers of analyzed mice (in parentheses) are indicated. Data were pooled from three independent experiments.

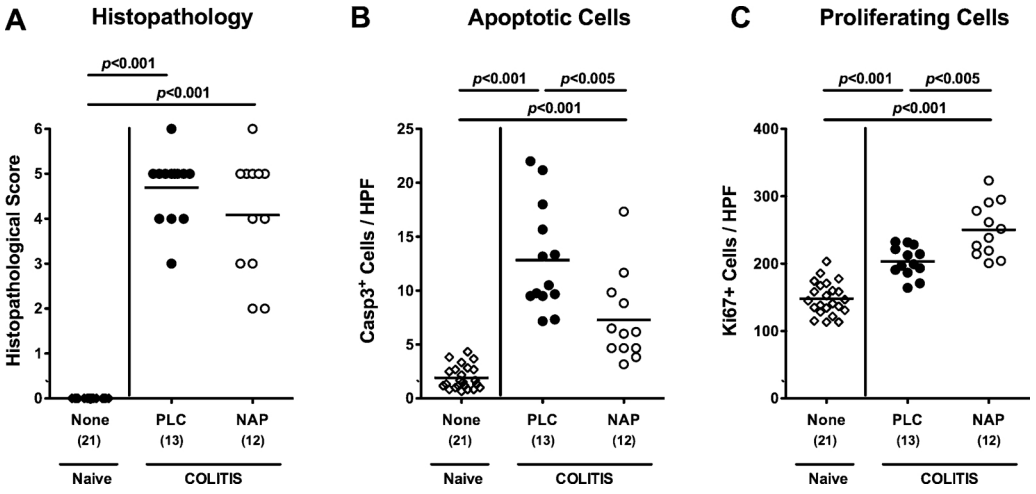

Fig. 2. Microscopic sequelae of NAP treatment in mice with acute colitis.
Upon induction of acute colitis by dextran sulfate sodium (from day 0 until day 6), mice were treated either with synthetic NAP (open circles) or placebo (PLC; filled circles) from day 1 until day 6 post-induction (p.i.). At necropsy (i.e. day 7 p.i.), (A) histopathological mucosal changes were quantitatively assessed in hematoxylin and eosin stained colonic paraffin sections applying a standardized histopathological scoring system (see methods). Furthermore, the average numbers of (B) apoptotic (positive for caspase-3, Casp3) and (C) proliferating colonic epithelial cells (positive for Ki67) from at least six high power fields (HPF, 400× magnification) per animal were determined microscopically in immunohistochemically stained colonic paraffin sections at day 7 p.i. Naive and untreated (None; open diamonds) mice served as negative controls. Means (black bars), level of significance (*p*-value) determined by Mann-Whitney *U* test and numbers of analyzed animals (in parentheses) are indicated. Data were pooled from three independent experiments.

have been shown in a mouse paradigm of closed head injury [16], and in a model of brain injury associated with diabetes [17]. Excitotoxicity during brain development (modeling cerebral palsy) also benefited from NAP treatment, providing neuroprotection as well as protecting developing oligodendroglial cells [18].

Inflammatory bowel diseases (IBD) are of multifactorial etiology and constitute chronic inflammatory conditions of the gastrointestinal tract (GIT) with acute episodes [19–21]. Whereas Crohn's disease may affect the entire GIT with the terminal ileum as predilection site ("ileitis terminalis"), ulcerative colitis is restricted to the large intestines [20,21]. In a previous study, we were able to demonstrate that NAP application resulted in potent anti-inflammatory effects in acute murine small intestinal inflammation mimicking key features of Crohn's disease [22]. Remarkably, beneficial properties of NAP treatment were not restricted to the intestinal tract, given that extra-intestinal and even systemic collateral damages of inflammation could be ameliorated [23]. This prompted us in the present study for the first time to elucidate potential immune-modulatory effects of NAP in another acute intestinal inflammation model, but of different location, namely in acute murine colitis that was induced by the barrier-damaging agent dextran sulfate sodium (DSS) and mirrors key features of acute episodes of IBD such as ulcerative colitis [24,25].

2. Materials and methods

2.1. Ethics statement

All animal experiments were conducted in accordance with the local ethical committee (Tel Aviv University, Israel, registration number 01-16-016). Animal welfare was monitored twice daily by assessment of clinical conditions including body weight.

2.2. Mice, colitis induction and treatment

C57BL/6 j WT mice were obtained from Harlan (Envigo, Jerusalem, Israel). In order to avoid potential sex-related confounding effect [26], only female mice were included in the study. For induction of acute colitis, three month old mice were treated with 3.5% (wt/vol) dextrane sulfate sodium (DSS; 40 kDa, MP Biomedicals, Illkirch, France) in drinking water (*ad libitum*) for 6 days. 24 h before necropsy, DSS was withdrawn and mice received regular tap water only as described previously [24,26,27]. The intake of DSS solution was controlled daily.

From day 3 until day 6 post-induction (p.i.), mice were either treated with synthetic NAP (1.0 mg per kg body weight; in NaCl 0.9%) or NaCl 0.9% (placebo, PLC) once daily via the intraperitoneal route. A potential antimicrobial effect of the NAP solution was excluded as described previously [28]. Naive mice (i.e. without colitis and without treatment; None) served as negative controls. Mice were kept in cages