International Journal of
Radiation Oncology
biology • physics

www.redjournal.org

Biology Contribution

A Novel Peptide to Treat Oral Mucositis Blocks Endothelial and Epithelial Cell Apoptosis

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Received Aug 2, 2011, and in revised form Dec 6, 2011. Accepted for publication Jan 4, 2012

Summary

A therapeutic role for a cytoprotective peptide fragment of antrum mucosal protein (AMP)-18 was sought in three hamster models of radiation-induced oral mucositis. AMP peptide reduced mucosal erythema and ulceration and enhanced recovery, possibly by its antiapoptotic effects shown in cultures of endothelial and epithelial cells. AMP peptide did not ameliorate the antitumor effects of concomitant radiation in a lung xenograft model, thereby providing additional support for a new agent to treat oral mucositis.

Purpose: No effective agents currently exist to treat oral mucositis (OM) in patients receiving chemoradiation for the treatment of head-and-neck cancer. We identified a novel 21—amino acid peptide derived from antrum mucosal protein-18 that is cytoprotective, mitogenic, and motogenic in tissue culture and animal models of gastrointestinal epithelial cell injury. We examined whether administration of antrum mucosal protein peptide (AMP-p) could protect against and/or speed recovery from OM.

Methods and Materials: OM was induced in established hamster models by a single dose of radiation, fractionated radiation, or fractionated radiation together with cisplatin to simulate conventional treatments of head-and-neck cancer.

Results: Daily subcutaneous administration of AMP-p reduced the occurrence of ulceration and accelerated mucosal recovery in all three models. A delay in the onset of erythema after irradiation was observed, suggesting that a protective effect exists even before injury to mucosal epithelial cells occurs. To test this hypothesis, the effects of AMP-p on tumor necrosis factor-α—induced apoptosis were studied in an endothelial cell line (human dermal microvascular endothelial cells) as well as an epithelial cell line (human adult low-calcium, high-temperature keratinocytes; HaCaT) used to model the oral mucosa. AMP-p treatment, either before or after cell monolayers were exposed to tumor necrosis factor-α, protected against development of apoptosis in both cell types when assessed by annexin V and propidium iodide staining followed by flow cytometry or ligase-mediated polymerase chain reaction.

Conclusions: These observations suggest that the ability of AMP-p to attenuate radiation-induced OM could be attributable, at least in part, to its antiapoptotic activity.

Keywords: Oral mucositis, Apoptosis, Endothelial cells, Epithelial cells, Gastrokine 1

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X. Wu and P. Chen made equal contributions to this study.

Supported by National Institutes of Health grant R21 DE018811 to F. G. Toback.

Conflict of interest: The animal experiments of this study were funded by NephRx Corporation, of which F. G. Toback is a founder. S. T. Sonis is a partner in Biomodels, at which the animal studies were performed.

Acknowledgment—The authors thank Margaret M. Walsh-Reitz and Patrick Cunningham for valuable discussions.

Int J Radiation Oncol Biol Phys, Vol. 83, No. 3, pp. e409—e415, 2012
0360-3016/$ - see front matter
doi:10.1016/j.ijrobp.2012.01.006

Introduction

Oral mucositis (OM) is a common, dose-limiting toxicity of many radiation and chemotherapeutic antineoplastic regimens (1). It is characterized by breakdown of the oral mucosa and development of ulcerative lesions that result in pain, decreased quality of life, and increased health care resource use (2). In severe cases, OM leads to adverse modifications of antineoplastic treatment. There is currently no effective intervention for the prevention or treatment of OM.

We identified a 21−amino acid peptide derived from the 18-kDa antrum mucosal protein (AMP-18) that exhibits protective, mitogenic, and motogenic properties on epithelial cells (3). AMP-18, also known as gastrokine 1, is constitutively expressed in epithelial cells of the gastric antrum (4), where it may protect barrier function and structure.

We have suggested that the protective effects of antrum mucosal protein peptide (AMP-p) may be attributable to its capacity to limit the loss of tight junction (TJ) proteins after injury (5). From the standpoint of OM, it seemed that an agent that targeted TJs, multiple proteins that bind together epithelial cells, might enhance the integrity of the oral mucosa and provide a novel interventional approach to preventing or treating OM (6). Consequently, we tested the effects of AMP-p on radiation-induced OM in three established models of the syndrome in hamsters (7, 8).

Methods and Materials

Materials

Human AMP-p (LDALVKEKKLQGKGPGGPPPK) and scrambled peptide (GKPLGQPGKVPKLDGKEPLAK) (3, 5) were synthesized by GenScript (Piscataway, NJ). Recombinant human tumor necrosis factor (TNF)-α and interferon gamma were purchased from PeproTech (Rocky Hill, NJ).

AMP-p treatment of radiation-induced OM in hamsters

LVG Syrian Golden hamsters (weight, approximately 92 g; all male) were anesthetized (ketamine and xylazine), and the left buccal pouch was everted, fixed, and isolated by use of a lead shield (7). OM was induced by radiation directed at the exposed cheek pouch. AMP-p or saline solution (vehicle) was administered by subcutaneous injection once daily. Similar daily subcutaneous dosing of peptide drugs (*e.g.*, insulin) has been well tolerated in patients including the head-and-neck cancer population.

Mucositis was scored from 0 for normal to 4 for marked ulceration (7). Digital images of the mucosa were randomly numbered and then graded in blinded fashion by at least two trained evaluators. A score of 3 or more marks the development of mucosal ulceration, a clinically relevant and important outcome (9). This protocol was approved by the Animal Care and Use Committee and performed at Biomodels (Watertown, MA).

In the acute model ($n = 16$) a single dose of radiation (40 Gy) was given at a rate of 3.2 Gy/min. Subcutaneous dosing of AMP-p (40 mg/kg) began 5 days before radiation (Day −5) and continued until Day 15, excluding the day of radiation (Day 0). Vehicle (saline solution) was administered to control animals by use of the same schedule. Mucositis was evaluated starting on Day 6 and subsequently on alternate days.

For the fractionated radiation model, animals ($n = 20$) were each given eight doses of 7.5 Gy on Days 0 to 3 and Days 7 to 10 at a rate of 3.3 Gy/min. AMP-p (40 mg/kg) or vehicle was administered subcutaneously on Day −5 to Day −1, Days 4 to 6, and Days 11 to 15.

For animals ($n = 20$) treated with fractionated radiation and cisplatin, eight radiation doses of 6.5 Gy were given on Days 0 to 3 and Days 6 to 9 and two cisplatin doses of 5 mg/kg were given on Days 0 and 6 as an intraperitoneal injection 2 h before radiation. AMP-p (40 mg/kg) was administered subcutaneously on Days −5 to −1, Day 4, and Day 5, and vehicle was administered to control animals by use of the same schedule.

Effect of AMP-p on radiation treatment of lung tumor cells *in vivo*

National Institutes of Health H460 human lung cancer cells (10^5) were implanted in the lower left flank of nude rats ($n = 24$). Focal radiation treatment (4 Gy on Days 0, 4, and 9) began when tumors reached a mean volume of approximately 100 mm^3 (Day −5), or tumors were left untreated. One radiation-treated group received AMP-p (40 mg/kg) daily from Day −5 to Day 15, whereas the other group and the untreated group received vehicle (phosphate-buffered saline solution) on the same schedule. Tumor volume was measured on alternating days throughout the duration of the study. We evaluated the differences in tumor volume observed among the different treatment groups by calculating the individual area under the curve for tumor growth and comparing these values using a Mann-Whitney rank sum test.

Cell culture

Adult human dermal microvascular endothelial cells (HDMECs) (Applied Cell Biology Research Institute, Kirkland, WA) were grown in endothelial basal medium (EBM)-2 supplemented with 5% fetal bovine serum (FBS), microvascular endothelial cell growth medium-2 (EGM-2 MV) BulletKit (Lonza, Basel, Switzerland), and 100 U/mL of penicillin/streptomycin (Invitrogen, Paisley, Scotland). Human adult low-calcium, high-temperature keratinocyte (HaCaT) cells (10) (Cell Lines Service, Eppelheim, Germany) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen) supplemented with 5% calf serum (Sigma-Aldrich, St Louis, MO).

Flow cytometry analysis of cell apoptosis

HDMECs (in DMEM and 5% FBS) and HaCaT cells (in DMEM and 0.5% FBS) were untreated (control), treated with TNF-α (50 ng/mL) only, pretreated with AMP-p (8 μg/mL) for 2 h before TNF-α was added, or treated with AMP-p (8 μg/mL) added 2 h after TNF-α. This dose of AMP-p was chosen as the lowest concentration that showed the most significant effect based on a dose−response curve (0−10 μg/mL) in the presence of 50 ng/mL of TNF-α. At the end of the study period (8 h), cell apoptosis was determined by flow cytometry analysis (FACScanto; BD Biosciences, San Jose, CA) of annexin V− and propidium iodide (PI)−stained cells (Vybrant apoptosis assay kit; Invitrogen), which characterized early and late apoptosis, respectively.