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The effects of a growth hormone-releasing hormone antagonist and a gastrin-releasing peptide antagonist on intimal hyperplasia of the carotid artery after balloon injury in a diabetic rat model☆

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Abstract *Introduction:* Arterial restenosis after angioplasty/stenting has hindered coronary artery disease treatment, especially in diabetics. We theorized that gastrin-releasing peptide (GRP) antagonists and growth hormone-releasing hormone (GHRH) antagonists might decrease neointimal hyperplasia and restenosis in diabetic rats after common carotid arterial balloon injury.
Methods: Two separate experiments were conducted to test the effects of a GRP antagonist (RC-3095) and a GHRH antagonist (MZ-4-71) on vascular smooth muscle (VSM) growth. In a preliminary *in vitro* experiment non-injured human aortic vascular smooth muscle (VSM) proliferation was compared between growth media and control. In a second *in vivo* experiment,

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intimal and medial area, intima/media ratio (IM) and percent stenosis were compared between injured carotid arteries in twelve Zucker type II obese rats treated with subcutaneously injected RC-3095, MZ-4-71, or control media.
Results: In the *in vitro* experiment, decreased VSM cell growth was observed in GRP antagonist ($p < 0.05$) and GHRH antagonist groups ($p < 0.05$) compared to the control group. In the *in vivo* experiment, the GRP antagonist group had a decreased IM ratio (1.63 ± 0.41, $p < 0.05$) and an increased area of stenosis (98.78% $\pm$ 1.48 p = NS) compared to control (2.38 ± 1.09) while the GHRH antagonist group had decreased IM ratio (1.33 ± 0.58 SD, $p < 0.05$) and percent area of stenosis (78.84% $\pm$ 24.97, $p < 0.05$) compared to control (2.38 ± 1.09).
Conclusions: The significant decrease in both IM ratio and percent area of stenosis in the GHRH antagonist group supports the hypothesis that this peptide may reduce neointimal hyperplasia and restenosis.

Introduction

Percutaneous cardiac procedures, whether with balloon angioplasty or stent, are commonplace in cardiology practice today. However, arterial restenosis following angioplasty or stent placement continues to prevent the long-term successful treatment of coronary artery disease, particularly for patients with diabetes.[1,2] Diabetes has been demonstrated to be an independent risk factor for the development of such restenosis.[3]

The mechanisms of restenosis are complex and appear to result from interactions between vascular smooth muscle (VSM) cells and several growth factors resulting in VSM cell proliferation and neointimal hyperplasia.[4–13] While the use of drug-eluting stents containing antiproliferative agents, such as paclitaxel and sirolimus, has reduced the incidence of restenosis, this complication still persists and results in considerable morbidity and mortality, especially in diabetics.[14–16]

A variety of new antiproliferative agents are actively being researched for their potential as cancer therapy. However, the potential role of these agents in treating cardiovascular disease has not yet been investigated. Two peptide classes that appear to have great potential for this application are antagonists of bombesin/gastrin-releasing peptide (GRP) and antagonists of growth hormone-releasing hormone (GHRH).

GRP, in addition to its role of inducing release of gastrin, is an autocrine growth factor that is expressed in a variety of neoplasms and promotes angiogenesis. GRP antagonists have demonstrated retardation of tumor growth and inhibition of tumor microcirculation, possibly through reduced expression of epidermal growth factor (EGF) receptors or direct blockage of GRP receptors.

Similarly, GHRH antagonists have shown the ability in nude mouse models to inhibit growth of renal cell carcinoma, prostate adenocarcinoma, osteosarcoma, and lung carcinoma. The antitumor effect of GHRH antagonists rests partially on their ability to reduce serum levels and tumor levels of insulin-like growth factor 1 (IGF-1). The blockade of GHRH receptors and resultant inhibition of autocrine tumoral GHRH appear to be responsible for the antiproliferative effects of these agents.[17–46]

Potentially, the therapeutic effects of GRP antagonists and GHRH antagonists may be extended beyond oncology. Both GRP antagonists and GHRH antagonists have demonstrated the ability to reduce the growth of benign prostatic hypertrophy.[47,48] Thus, we hypothesized that both GRP antagonists and GHRH antagonists might decrease arterial restenosis by reducing VSM proliferation through mechanisms mediated by EGF, GRP, and IGF. Specifically, we were interested in determining whether GRP antagonist RC-3095 or GHRH antagonist MZ-4-71 might be effective in decreasing restenosis and neointimal hyperplasia in a diabetic rat model after experimental balloon injury to the arterial intimal layer.

Methods

Our preliminary investigation consisted of an *in vitro* experiment using human aortic VSM. For the cell proliferation assay, human VSM cell proliferation was determined using the BrdU Proliferation Assay Kit® (Oncogene Research Products, Cat# HTS01). Human aortic smooth muscle cells (passage 7) were grown to 80% confluence in a 75 cm^2 flask. Cells were detached using 2 mL 0.25% Trypsin/EDTA® (Gibco), which was washed out with 20 mL SMBM complete medium (Cambrex). This cell suspension was then used to seed a well plate. Each well received 100 μL of the cell suspension and 100 μL of SMBM complete medium. The plate was then incubated at 37 °C, in a 5% CO_2 atmosphere overnight to allow cells to attach. The medium was then drained and 200 μL of D-MEM/F-12 (Gibco) without serum was added to each well. Cells were serum starved in this medium for 20 h in a 37 °C, 5% CO_2 incubator. The medium was removed and replaced with 200 μL of treatment medium (either D-MEM/F-12 or SMBM complete) plus BrdU labeling material. Treatment medium contained either GRP antagonist RC-3095 or GHRH antagonist MZ-4-71 at a concentration of 1 pM. The doses reported in previous publications were used in order to determine the appropriate concentration of RC-3095[28,49–53] and MZ-41[18,19,54–56] for our experiment. Control wells had media with either 0.1% DMSO (the solvent for all the peptides), or lacked BrdU label, or had no label and no cells, or had label and media