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Peptides do not prevent cleavage of endoglin to produce soluble endoglin

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ABSTRACT

MMP14 cleaves membrane bound endoglin to produce soluble endoglin (sEng), an anti-angiogenic factor that causes endothelial dysfunction in preeclampsia. A recent publication proposed peptides with an amino acid sequence straddling the MMP14 cleavage site on endoglin decreases sEng release. This may be an exciting therapeutic approach and requires validation. We administered peptides to JAR cells, and primary placental explants and endothelial cells. The peptides had no effect on sEng production, and did not block sEng production in HEK293 with MMP14 and endoglin overexpressed. Peptides with an amino acid sequence encompassing the cleavage site do not prevent sEng production *in vitro*.

Introduction

Soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) are anti-angiogenic factors that cause endothelial dysfunction seen in preeclampsia [1]. Adenoviral over-expression of sEng and sFlt1 in rats phenocopies the hallmarks of severe preeclampsia [1,2]. Furthermore, serum sEng is markedly increased in women with preeclampsia [3]. Such studies highlight the critical role of sEng in severe preeclampsia.

Matrix metalloproteinase 14 (MMP-14; MT1-MMP) cleaves membrane bound endoglin at a specific peptide sequence close to the transmembrane domain, producing sEng [4]. We demonstrated that this mechanism plays a role in placental production of sEng [5]. Conceivably, blocking the cleavage of endoglin by MMP14 could represent a drug strategy to decrease sEng.

Recently, Valbuena-Diez et al. showed peptides with a sequence straddling the same amino acid sequence on transmembrane endoglin at the MMP14 cleavage site can reduce sEng release both *in vitro* and *in vivo* [6]. Conceivably, the peptides may work by blocking sEng release through competitive inhibition.

Given the therapeutic potential for such peptides to treat preeclampsia, we sought to independently validate this important finding in four separate *in vitro* systems including primary human tissues and a plasmid over-expression system.

Materials and methods

Generation of endoglin peptides

Two sets of small peptides were ordered from Auspep (Pty Ltd, Victoria, Australia). One, therapeutic peptide, has an identical sequence to the MMP14 cleavage site on

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membrane bound endoglin (TSKGLVLP – cleavage site peptide). The second, a control peptide, is identical to a sequence in the extracellular portion of endoglin distant to the cleavage site. (GPRTVTVK – control peptide). The peptides were purified to >90% purity by Auspep, as previously published [6] (Fig. 1).

JAR cells

JAR cells (choriocarcinoma cell line) were grown in RPMI (Gibco) supplemented with 10% FCS and 1% penstrep (Life Technologies). Cells were plated at 140,000 cells/cm^2 and the following day treated with endoglin-like peptides for 24 h as previously published [6].

Tissue collection

Informed written consent was obtained for collection. Placentas and umbilical cords were obtained from term caesarian pregnancies. Tissue was obtained immediately following delivery, and washed with ice-cold sterile PBS for further processing.

HUVEC isolation

Human umbilical vein endothelial cells were obtained as described previously [7] and grown in M199 (Gibco, Australia) containing 10% FCS (Sigma, Castle Hill, NSW, Australia), 100 μg/ml heparin (Sigma) and 20 μg/ml Endothelial Cell Growth Supplement (Sigma).

Isolation and culture of placental explants

Villous tissues were cut from the mid-portion of the placenta (avoiding maternal and fetal surfaces), dissected into 1–2 mm fragments, and three pieces placed into each well of a 24 well plate. Treatments were administered as indicated below. For ELISA analysis, secreted protein levels were normalized against placental explant weight.

HEK293-T over-expression system

Plasmids encoding MMP14 (Origene, Rockville, MD, USA), Endoglin (Gift from Dr. Kelly Walton) or vector alone were transfected into HEK293T cells using Lipofectamine 2000 (Life Technologies, Mulgrave, Australia).

Peptide treatment of primary HUVECs, placental explants and HEK293-T cells

Cells and tissues were treated with peptides at either 250 or 500 μM for 48 h and media collected for analysis of sEng.

ELISA analysis of sEng

sEng levels were measured in conditioned culture media using the human endoglin ELISA (R&D systems, Minneapolis, MN, USA) carried out according to manufacturer's instructions. Optical density was determined using a BioRad X-Mark microplate spectrophotometer (BioRad, Hercules, CA, USA) and endoglin levels were determined using the BioRad Microplate manager 6 software.

Statistical analysis

Continuous variables were compared using an unpaired *t*-test to assess parametric data or a Mann Whitney *U* for non-parametric data. $P \leqslant 0.05$ was considered significant. Analysis was undertaken using GraphPad Prism (GraphPad Software, CA, USA).

Results and discussion

We obtained two sets of peptides with the same amino acid sequence used in a report suggesting endoglin-like peptides could reduce sEng release [6]. The candidate therapeutic (named 'cleavage site peptide') is the same amino acid sequence that includes the specific glycine–leucine region on membrane endoglin where MMP-14 cleaves membrane bound endoglin to produce sEng (Fig. 1). The second peptide is identical in sequence as an extracellular

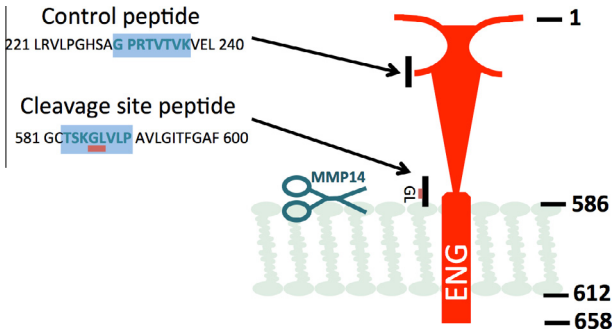

Fig. 1. Schematic of endoglin-like peptides to inhibit soluble endoglin release. One peptide (named 'cleavage site peptide') is the candidate therapeutic. Of 8 amino acids in length, it is the same amino acid sequence that encompasses the specific glycine–leucine region of membrane bound endoglin where MMP-14 cleaves to produce sEng. The second peptide is also of 8 amino acids in length, and is identical to the native endoglin sequence at an extracellular region distant from the MMP-14 cleavage site ('control peptide').