Microbubbles conjugated with knottin 2.5D

$MB_{Knottin}$

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Chemical name:	Microbubbles conjugated with knottin 2.5D	
Abbreviated name:	$MB_{Knottin}$	
Synonym:		
Agent Category:	Peptide	
Target:	$\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_5\beta_1$ integrin receptors	
Target Category:	Receptor	
Method of detection:	Ultrasound	
Source of signal / contrast:	Microbubbles	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure not available in PubChem.

Background

[PubMed]

Ultrasound is the most widely used imaging modality in clinical medicine (1) and its role in noninvasive molecular imaging with ligand-carrying microbubbles is expanding (2). Microbubbles are comprised of spherical cavities filled by a gas encapsulated in a shell. The shells are made of phospholipids, surfactant, denatured human serum albumin, or synthetic polymer. Ligands and antibodies can be incorporated into the shell surface of microbubbles. Microbubbles are usually 1–8 μm in diameter. They provide a strongly reflective interface and resonate to ultrasound waves. They are used as ultrasound contrast agents in imaging of inflammation, angiogenesis, intravascular thrombus, and tumors (3-5). They are also potentially used for drug and gene delivery (6).

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Integrins are a family of heterodimeric glycoproteins on cell surfaces that mediate diverse biological events involving cell–cell and cell–matrix interactions (7). Integrins consist of an α and a β subunit and are important for cell adhesion and signal transduction. The $\alpha_v\beta_3$ integrin is the most prominent receptor affecting tumor growth, tumor invasiveness, metastasis, tumor-induced angiogenesis, inflammation, osteoporosis, and rheumatoid arthritis (8-13). Expression of the $\alpha_v\beta_3$ integrin is strong on tumor cells and activated endothelial cells, whereas expression is weak on resting endothelial cells and most normal tissues. The $\alpha_v\beta_3$ antagonists are being studied as antitumor and antiangiogenic agents, and the agonists are being studied as angiogenic agents for coronary angiogenesis (12, 14, 15). The peptide sequence Arg-Gly-Asp (RGD) has been identified as a recognition motif used by extracellular matrix proteins (e.g., vitronectin, fibrinogen, laminin, and collagen) to bind to a variety of integrins, including $\alpha_v\beta_3$. Various radiolabeled antagonists have been introduced for imaging of tumors and tumor angiogenesis (16).

Microbubbles conjugated to either peptides or antibodies against integrins, cell adhesion molecules, and VEGF receptors have previously been studied for the noninvasive assessment and imaging of tumor angiogenesis (17-20). Cystine knot peptides (knottins) share a common disulfide-bonded framework and a triple-stranded β-sheet fold (21). The integrin-binding RGD motif was grafted into a knottin from trypsin inhibitor II of the squash plant (*Ecballium elaterium*). Knottin 2.5D (with three disulfide bonds; GCPQGRGDWAPTSCSQDSDCLAGCVCGPNGFCG-NH_2) was identified from a series of genetically engineered knottin peptides to have nanomolar binding to the $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_5\beta_1$ integrin receptors (22, 23). Willmann et al. (24) studied ultrasonic imaging of tumor vasculature using microbubbles conjugated with knottin 2.5D ($MB_{Knottin}$) in mice bearing human tumor xenografts.

Related Resource Links:

- Chapters in MICAD
- Gene information in NCBI (Knottin)
- Articles in OMIM

Synthesis

[PubMed]

Knottin 2.5D was prepared with solid-phase peptide synthesis (24). The linear peptide was folded in the presence of 2.5 mM reduced glutathione and 20% dimethylsulfoxide in 0.1 M ammonium bicarbonate (pH, 9). Both peptides were purified with high-performance liquid chromatography. Peptide purity and molecular mass were confirmed with MALDI-TOF mass spectroscopy and electrospray ionization mass spectrometery. Knottin was biotinylated at its *N*-terminus for conjugation with perfluorocarbon-filled microbubbles (1.5 ± 0.1 μm) through biotin-streptavidin interaction to yield $MB_{Knottin}$. Three other microbubbles were also prepared: microbubbles coupled to a biotinylated scrambled knottin peptide ($MB_{scrambled}$), microbubbles coupled to a biotinylated rat

anti-mouse $\alpha_v\beta_3$ integrin ($MB_{\alpha v\beta 3}$), and microbubbles coupled to a biotinylated c(RGDfK) (MB_{cRGD}). The number of ligands per μm^2 of microbubble surface was estimated to be ~7,600.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Willmann et al. (24) reported that $MB_{Knottin}$, $MB_{scrambled}$, $MB_{\alpha v\beta 3}$, or MB_{cRGD} (7×10^6/ml) perfused through the flow chamber coated with integrin-positive mouse angiosarcoma endothelial cells at a wall shear rate of 100 s^{-1} for ~3 min. There was a significantly ($P < 0.001$) greater number of $MB_{Knottin}$ attached to the endothelial cells (1.76 ± 0.49 MBs/cell) than the other three microbubble preparations ($MB_{scrambled}$, 0.15 ± 0.12; $MB_{\alpha v\beta 3}$, 0.60 ± 0.07; MB_{cRGD}, 0.74 ± 0.32). Pretreatment with excess c(RGDyK) reduced the binding of $MB_{Knottin}$ to 0.20 ± 0.16 MBs/cell ($P < 0.04$). All four microbubble preparations showed minimal binding (<0.13 MBs/cells) to integrin-negative mouse breast cancer cells (4T1).

Animal Studies

Rodents

[PubMed]

Willmann et al. (24) performed ultrasound assessment of microbubble binding in 9–19 mice bearing human ovarian adenocarcinoma SKOV-3 tumors at 4 min after injection of $MB_{Knottin}$, $MB_{scrambled}$, $MB_{\alpha v\beta 3}$, or MB_{cRGD} (7×10^6). The tumor video intensities were 11.9 ± 6.1, 5.2 ± 1.0, 5.6 ± 2.3, and 7.1 ± 7.9 for $MB_{Knottin}$, $MB_{scrambled}$, $MB_{\alpha v\beta 3}$, and MB_{cRGD}, respectively. The binding of $MB_{Knottin}$ to the tumors was significantly higher than that of the other microbubbles tested ($P < 0.05$). Pretreatment with excess c(RGDyK) (30 min before $MB_{Knottin}$) reduced the tumor video intensity to 4.7 ± 4.0 ($P < 0.05$). The muscle video intensity was 1.3 ± 0.3 for $MB_{Knottin}$. Immunofluorescence staining of frozen SKOV-3 tumor sections showed the localization of β_3 integrin to the tumor endothelial cells.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

NIH Support

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