

# Conjugation of curcumin-loaded lipid nanoemulsions with cell-penetrating peptides increases their cellular uptake and enhances the anti-inflammatory effects in endothelial cells

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## Keywords

biodistribution; cell-penetrating peptides; curcumin; endothelial cells; lipid nanoemulsions

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## Abstract

**Objectives** To prepare and characterize *in vitro* and *in vivo* lipid nanoemulsions (LN) loaded with curcumin (Cm) and functionalized with a cell-penetrating peptide.

**Methods** Curcumin-loaded lipid nanoemulsions (CmLN) functionalized with a nona-arginine peptide (R9-CmLN) have been obtained, characterized and optimized for size, entrapment efficiency and *in vitro* Cm release. The interaction of R9-CmLN with human endothelial cells (HEC) was investigated using cultured EA.hy926 cells, and *in vivo* biodistribution studies were performed using C57BL6 mice.

**Key findings** When used in therapeutically relevant concentration, R9-CmLN have low haemolytic activity, low cytotoxicity on HEC, and show anti-inflammatory effects by reducing the monocytes adhesion to TNF- $\alpha$  activated HEC. Moreover, HEC uptake and internalization of R9-CmLN was significantly higher compared to the non-functionalized CmLN. *In vivo* biodistribution studies in mice revealed a higher accumulation of R9-CmLN in the liver and the lungs compared to CmLN and the body clearance of the both nanoformulations after 72 h.

**Conclusions** Cell-penetrating peptides-functionalized CmLN have superior characteristics compared to their non-functionalized counterparts: are more efficiently internalized by the cells, produces anti-inflammatory effects in HEC and when administrated *intravenously* in mice exhibit increased accumulation in the liver and the lungs, suggesting their potential therapeutic applications in different inflammatory pathologies localized in the liver or the lungs.

## Introduction

Curcumin (1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, Cm) is a hydrophobic polyphenol isolated from the root of the *Curcuma longa*, a curry spice that has been widely studied and its high therapeutic potential well established. It was reported that Cm displays anti-inflammatory and antioxidant properties and anti-proliferative, anti-invasive and anti-angiogenic activity.<sup>[1]</sup> The anti-inflammatory properties are the consequence of

modulation of the activity of several transcription factors such as nuclear factor (NF)- $\kappa$ B,<sup>[2]</sup> activator protein (AP)-1,<sup>[3]</sup> hypoxia inducible factor (HIF)-1,<sup>[4]</sup> signal transducer and activator of transcription 3 (STAT 3),<sup>[5]</sup> peroxisome proliferator-activated receptors (PPAR) $\gamma$ ,<sup>[6]</sup> NF erythroid 2-related factor 2 (Nrf2)<sup>[7]</sup> and signalling pathways such as extracellular signal-regulated kinase (ERK),<sup>[8]</sup> p38 mitogen-activated protein kinase (p38 MAPK)<sup>[9]</sup> and Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT).<sup>[10]</sup> These effects result in down-regulation of