Pre-clinical Study Confirms Potential of Peptide Hydrogels As Localised Drug Delivery Vehicles

Susan J. Tzotzos

APEPTICO Research & Development GmbH, 1150 Vienna, Austria; e-mail: s.tzotzos@apeptico.com

Since the discovery of a self-assembling peptide sequence in the yeast protein zuotin, 25 years ago (Zhang et al, 1992; 1993), self assembling, hydrogel-forming, short synthetic peptides have become the object of intense investigation owing to their vast potential application in biomedicine and nanotechnology (Zhang S, 2003). Several molecular designs have emerged for the synthesis of self-assembling hydrogel-forming peptides, which fall roughly into four categories: amphiphilic peptides, short peptide derivatives, alpha-helix/coil-coil peptides and beta-sheet peptides (Markey et al, 2016). Beta-sheet peptides developed by Zhang and co-workers are based on the alternation of hydrophilic and hydrophobic amino acid residues with complementary side-chains, which self-assemble into anti-parallel beta-sheet rich fibres; above a critical gelation concentration (CGC) these fibres entangle/associate, forming 3D networks that entrap water molecules (Markey et al, 2017). The final hydrogel network topology can be controlled by manipulation of the fibre-fibre interactions, which are in turn defined by tailor-made design of the individual peptide monomers (Gao et al, 2017). A wide range of hydrogel-based biomaterials are currently under investigation, (Mehrban et al, 2014; Markey et al, 2016; Moore & Hartgerink, 2017), several of which are already finding application in regenerative medicine, as hydrogels provide ideal scaffolds for tissue culture (http://www.puramatrix.com/index.html).

An exciting application of hydrogels lies in their use as localised drug delivery vehicles. Small molecule, peptide and recombinant protein drugs incorporated into the hydrogel network as "guest molecules" can potentially be delivered to their local targets in the body (Koutsopoulos et al, 2009; Tang et al, 2014; Koutsopoulos, 2016)(Figure 1). Peptide hydrogels have the advantage of not involving harmful materials (e.g., toxic cross-linkers, etc.) to initiate the solution–gel transformation and the degradation products are natural amino acids, which can be metabolised. A study of the release of two commercial small molecule drugs, lidocaine (soluble under conditions used in the reported work) and flurbiprofen (insoluble) from hydrogel formed by the self-assembling, beta-sheet forming nonapeptide FEFKFEFKK, revealed that flurbiprofen showed favourable retention and release characteristics, although lidocaine retention was hampered by drug and peptide carrying the same electrostatic charge (Tang et al, 2014)(Figure 1). In the case of peptide drugs for example, release from directly injected, drug-suffused hydrogels would circumvent the problem of protease degradation and consequent reduced plasma half-life associated with gastrointestinal or intravenous routes of administration (Di L, 2015). Soluble proteins such as cytokines or growth factors may be tethered to the self-assembled peptide to provide internal stimuli to cells interacting with the peptide nanofibre hydrogel (Koutsopoulos, 2016).

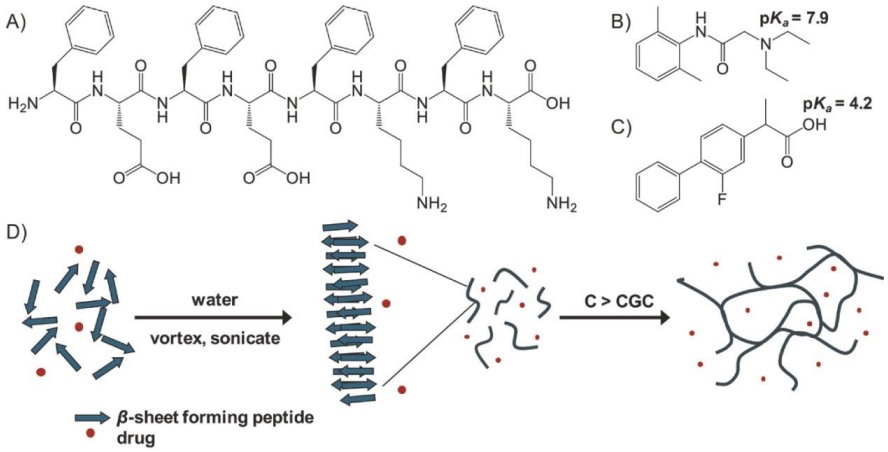

Figure 1. Chemical structure of (A) FEFEFKFK peptide, (B) lidocaine and (C) flurbiprofen. (D) Diagrammatic representation of the self-assembly and gelation processes of β-sheet forming peptides. (Tang et al; Int J Pharm. 2014 Apr 25;465(1-2):427-35. doi: 10.1016/j.ijpharm.2014.02.039).

Recently reported results of a study involving pre-clinical testing of radiolabelled hydrogels in mice, have shown conclusively that peptide hydrogels afford a safe and effective means to deliver drugs *in vivo* (Morris et al, 2017). Morris and co-workers used positron emission tomography (PET) studies and in vivo fluorescence imaging to assess the pharmacokinetics, biodistribution and metabolic fate of the peptide FEFKFEFKK (F9) and its hydrogel. For the PET studies, F9 was site-specifically labelled with 2-[^{18}F]fluoro-3-pyridinecarboxaldehyde ([^{18}F]FPCA) via oxime bond formation. For this it was necessary to functionalise the N-terminal amine of F9 with an amino(oxy)-group to give (Aoa)-F9. Functionalisation of the (Aoa)-F9peptide in this way permitted its radiolabelling with [^{18}F]FPCA (Figure 2).

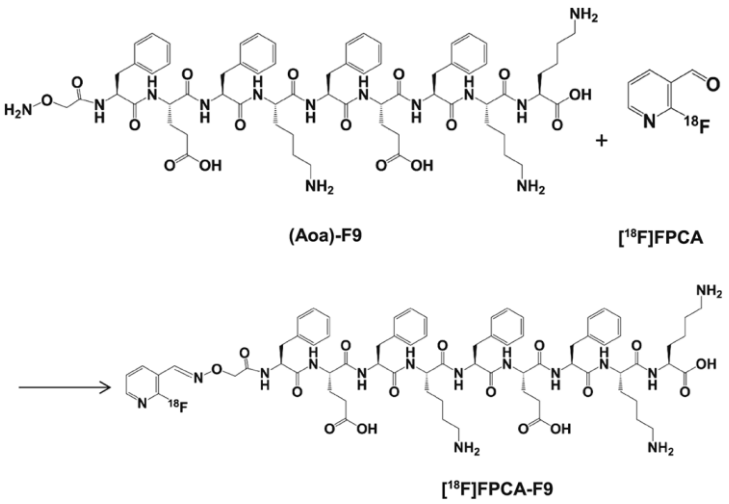

Figure 2. Reaction pathway showing (Aoa)F9 radiolabelling with [^{18}F]FPCA. (Morris et al, 2017; Journal of Labelled Compounds and Radiopharmaceuticals; JLCR-17-0004.R2, 4 AUG 2017 DOI: 10.1002/jlcr.3534).

Radiolabelled F9 ([^{18}F]FPCA-F9) was administered by intravenous injection into the tail vein of healthy C3H mice at a concentration below the CGC, in order to follow the fate of the peptide monomer in the bloodstream. Following intravenous injection, [^{18}F]FPCA-F9 monomer showed prompt bladder uptake, rapid renal excretion at 20 minutes and high stability in plasma. The *in vivo* behaviour of the hydrogel was followed by mixing together [^{18}F]FPCA-F9 monomer with unfunctionalised F9 peptide at a concentration above the CGC enabling co-assembly and formation of [^{18}F]FPCA-F9 hydrogel and then subcutaneously injecting the radio-labelled hydrogel into the flank of C3H mice. In contrast to the intravenously-introduced peptide, [^{18}F]FPCA-F9 peptide administered subcutaneously as a hydrogel showed gradual bladder accumulation and lower renal excretion, indicating gradual elution from the hydrogel as the latter physically disintegrated/disaggregated (Figure 3). The results of the PET experiments were complemented by the *in vivo* fluorescence imaging, using fluorescein isothiocyanate (FITC) N-terminally labelled F9 to follow the gradual disaggregation and long-term stability (over 5 days) of the hydrogel. An estimate of 1-4 days for the biological half-life of the F9 peptide was thereby obtained.

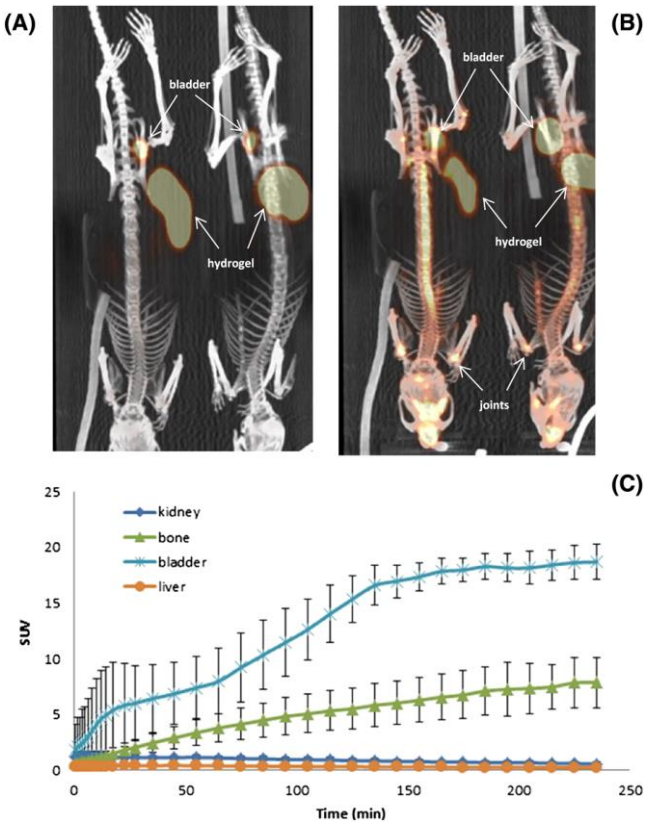

Figure 3. [^{18}F]FPCA-F9 hydrogel at (A) 60 seconds and (B) 4 hours postinjection. (C) TAC showing [^{18}F]FPCA-F9 biodistribution in bone and excretory organs (C3H mice, n = 3). (Morris et al, 2017; Journal of Labelled Compounds and Radiopharmaceuticals; JLCR-17-0004.R2, 4 AUG 2017 DOI: 10.1002/jlcr.3534). (SUV, standard uptake values).

The study by Morris et al characterises the *in vivo* behaviour and metabolic fate of a self-assembling, hydrogel-forming peptide. The work has confirmed that the behaviour exhibited by F9 is typical of natural, small peptides (Di L, 2015) and its *in vivo* biodistribution is not altered on account of its gel form. The striking feature of the results is the high plasma stability of labelled F9 monomers disaggregating from the hydrogel and the favourable biological half-life of the labelled F9 hydrogel itself. The results are encouraging for use of F9 hydrogels as targeted drug delivery vehicles and as biomaterials in general.

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