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Rapid communication

One-week *in vivo* sustained release of a peptide formulated into *in situ* forming implants

Marianne Parent[a,*], Igor Clarot[a], Sébastien Gibot[b], Marc Derive[c], Philippe Maincent[a], Pierre Leroy[a], Ariane Boudier[a]

[a] Université de Lorraine, CITHEFOR, EA 3452, Nancy, France
[b] Université de Lorraine, INSERM U1116, Vandœuvre-lès-Nancy, France
[c] INOTREM, Vandœuvre-lès-Nancy, France

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ABSTRACT

The LR12 peptide has been reported to reduce the size of infarct and improve both cardiac function and survival in myocardial infarction in murine models, after daily repeated intraperitoneal injections. In order to protect peptide from degrading and to prolong its release, *in situ* implants based on biocompatible biodegradable polymers were prepared and both *in vitro* and *in vivo* releases were evaluated after subcutaneous administration to Wistar rats. A progressive and complete release was obtained *in vitro* in 3 weeks. *In vivo*, a 7-day sustained release was demonstrated after administrating the formulation once; bioavailability was improved by protecting the peptide against the degradation identified as a dimerization through disulfide bond formation. As a conclusion, *in situ* forming formulations are a suitable alternative for the therapeutic use of this peptide.

With 17.3 million deaths per year (a number expected to grow to more than 23.6 million by 2030), cardiovascular disease is the leading global cause of death. Related direct and indirect costs are estimated to be higher than $320.1 billion, including health expenditures and loss of productivity (Mozaffarian et al., 2015). As the immune system and the inflammation are now recognized as key players in the establishment and exacerbation of cardiovascular diseases, new therapeutic strategies have been emerging to control them. In this context, the triggering receptor expressed on myeloid-cells-1 (TREM-1) has been identified as an interesting target. This immune-receptor is expressed by neutrophils, macrophages and mature monocytes, and acts as an amplifier of the innate immune response during both infectious and sterile inflammation (Bouchon et al., 2001; Gibot et al., 2009; Zhou et al., 2013). Recently, the modulation of TREM-1 signaling by an inhibitory dodecapeptide (LR12, Fig. 1A) reduced the size of infarct and improved both cardiac function and survival in a murine model of myocardial infarction (Boufenzer et al., 2015). In that study, LR12 (5 mg/kg) was intraperitoneally (IP) administered once a day for 5 days. This therapeutic scheme should be advantageously replaced by a single injection of a sustained-release formulation. Moreover, LR12 oxidizes spontaneously in aqueous media with a short half-life ($t_{1/2}$) (approximately 14 h in phosphate buffer saline (PBS) *in vitro*, and 0.5 h in blood *ex vivo*): a disulfide bridge is formed between two peptides, generating a dimer (Fig. 1B). While still to be confirmed by further investigation, preliminary experiments on cells suggested that the dimer is devoid of pharmacological activity. Consequently, the formulation should both sustain LR12 release for at least a 5-day period and, as much as possible, protect the drug from degrading by dimerization.

In this contribution, the *in vitro* and *in vivo* release of LR12 (both the monomer and its main product of degradation, *i.e.* the dimeric form) from *in situ* forming implants have been evaluated. *In situ* forming implants are liquid formulations which, when injected into aqueous environments, precipitate as solid polymeric matrices entrapping the drug (Parent et al., 2013a). Sustained releases obtained with *in situ* forming implants have been described in the literature for a wide range of drugs with various physicochemical properties. Herein, poly-lactide-co-glycolide (PLGA, Resomer RG502H, 50:50 ratio LA:GA) and poly-lactide (PLA, Resomer R203S) were used as biocompatible biodegradable polymers due to their frequent use in *in situ* forming formulations and their lack of toxicity. Polymers were solubilized (18.7% w/w) in triacetin (TA, 74.8% w/w) before adding the drug (6.5% w/w). The concentration

* Corresponding author at: Université de Lorraine, CITHEFOR, EA3452, 5 rue A. Lebrun, BP 80403, F–54001, Nancy Cedex, France.
E-mail address: marianne.parent@univ-lorraine.fr (M. Parent).

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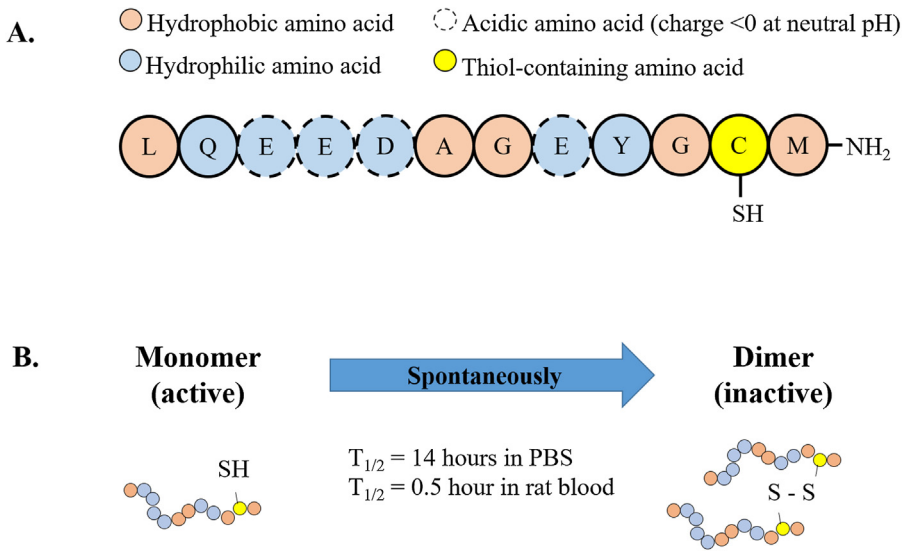

Fig. 1. A) Murine sequence of LR12 monomer peptide (Mr 1341 g/mol, calculated pI ~ 3.6) and B) main pathway of degradation by dimerization.

of peptide used in the formulation allowed to subcutaneously inject a dose of 80 mg LR12/kg to the animals. This can be compared to the total dose of 25 mg/kg of free LR12 administered *via* several IP injections in the study, which demonstrated the benefit of LR12 in myocardial infarction (Boufenzer et al., 2015). This dose is compatible with a slow delivery of LR12 from the reservoir formulation during the time of experiment, and it is likely without safety issues. Additionally, with this concentration, the viscosity of the formulation remained suitable for an easy injection.

Formulations were prepared and *in vitro* release experiments were performed in physiological buffer saline according to previously reported protocols (Parent et al., 2013b). An HPLC-UV method was developed, then validated for selectivity, precision, accuracy and linearity according to the FDA guidelines, and used to quantify LR12 (monomer and dimer) released in the aqueous medium (Fig. 2). The chromatographic system was the same as previously described (Parent et al., 2016). The elution was isocratic (86/14% v/v of water/acetonitrile + 0.1% trifluoroacetic acid) with 20 µL of injected sample. The detection was set at 220 nm. Linearity was verified for monomer and dimer between 1.0 and 50.0 µg/mL ($y = 14.30x - 17.55$ and $R^2 = 0.994$ for monomer, $y = 14.15x - 11.87$ and $R^2 = 0.996$ for dimer). Peptides remaining in the implants were also quantified with the same method after

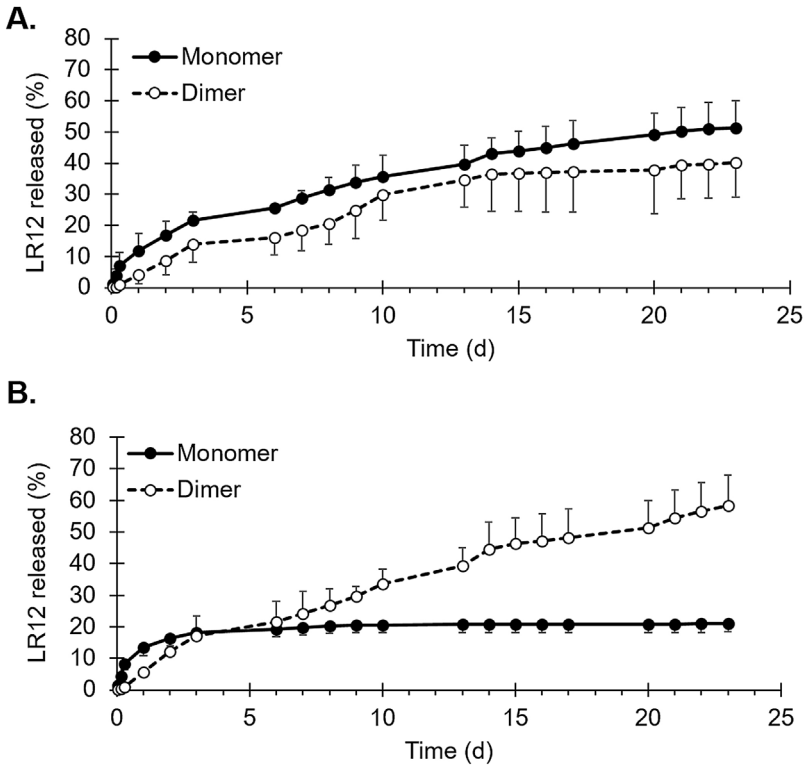

Fig. 2. *In vitro* release profiles of LR12 (monomer and dimer) obtained from *in situ* implants made of TA and of either PLGA (A) or PLA (B). Results are presented as mean ± sd of three experiments.