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Effects of InlB321-CD in solution and hydrogel formulation on immortalized human keratinocyte cell line

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Internalin B (InlB) is an invasion protein of Listeria which facilitates its uptake into host cells by activating the receptor tyrosine kinase c-Met. It was proposed that activation via receptor dimerization is mediated through an InlB dimer. The dimerized fragment of Internalin B, InlB321-CD (1) (crystal dimer), was designed to stabilize the InlB dimer in solution. In binding studies and in in vitro scatter assays (1), InlB321-CD revealed to be a stronger agonist than monomeric InlB321 and Internalin B.

In human skin, mainly epithelial cells express the c-Met receptor which controls amongst others proliferation and migration. After being stimulated by its endogenous agonist hepatocyte growth factor (HGF), which is secreted by e.g. dermal fibroblasts, this receptor plays an important role in the regeneration of the epidermis.

In previous studies, the mitogenic properties of InlB321-CD have already been tested on immortalized dermal keratinocytes (2) and premature organotypic co-culture (3). Furthermore, InlB321-CD stimulation was shown in a wound healing assay (3).

In order to distinguish migration from proliferation in the present study, an in vitro 'wound healing' assay of a confluent HaCaT monolayer was performed subsequent to mitomycin C treatment which inhibits mitosis. Additionally, the present project aims at incorporating InlB321-CD in a formulation which is intended to be tested on modified organotypic co-culture in upcoming tests. For this purpose, a hydrogel formulation e.g. a hydroxyethylcellulose gel (HEC gel) was chosen since hydrogels were found to trigger wound healing in vivo and in vitro (4) even without any active compound. Moreover, hydrogels might be proper vehicles for protein drugs. The stability of InlB321-CD in this formulation should be investigated with regard to maintaining its mitogenic activity. Therefore, a proliferation assay was carried out on immortalized dermal keratinocytes subsequent to incubation with InlB321-CD that was incorporated in HEC gel versus InlB321-CD in solution.

Methods:
A confluent HaCaT monolayer was serum-starved (24 h), incubated with 10 µg/ml mitomycin C (2 h) and afterwards scratched with a pipette tip. The cells were washed with PBS and then incubated for 24 h with 0.5 nM InlB321-CD, 1 nM InlB321, 0.5 nM HGF or just medium. The

cultivation with medium served as negative control whereas that with HGF served as positive control. Partial 'healing' of the scratch area was documented with micrographs and quantified with an imaging software system (Olympus DP soft, Olympus).

In terms of proliferation assay, a hydroxyethylcellulose gel (0.6 %) was loaded with 25 nM InlB321-CD. The same concentration in PBS served as control. Storage conditions were 4 – 8°C for 24 hours and seven days, respectively, to check for any incompatibilities. Afterwards, the buffered InlB321-CD solution, the gel with active compound, and the plain formulation were diluted 50-fold with serum-free medium. Subsequently, serum-starved HaCaT monolayers were treated with 0.5 nM InlB321-CD incorporated in gel as well as in solution versus medium and plain HEC gel. After 24 hours of incubation, the proliferation was measured with an MTT assay.

Results: Subsequent to incubation with mitomycin C and 24 h after scratching, the monolayer of HaCaT cell line, treated with 0.5 nMdimeric InlB321 showed a significant smaller gap compared to incubation with medium. The cells treated with 0.5 nM HGF as positive control reduced the 'wound' gap as well, whereas 1 nM monomeric InlB321 did not stimulate the HaCaT cells. This result shows that InlB321-CD also acts as motogenic agent on immortalized dermal keratinocytes, whereas monomeric InlB321 does not show any migratory activity.

The proliferation assay showed the same mitogenic properties of InlB321-CD incorporated in HEC gel compared to InlB321-CD in solution both after 24 h and seven days of storage of the formulations. Furthermore, proliferation was higher by factor 1.8 than with plain HEC gel and medium. Hence, it can be concluded that InlB321-CD activity is not negatively affected by the gelling agent hydroxyethylcellulose.

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