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Combined application of saponin and chimeric toxins drastically enhances the targeted cytotoxicity on tumor cells

Iring Heisler[a], Mark Sutherland[a], Christopher Bachran[a], Philipp Hebestreit[b], Anna Schnitger[a], Matthias F. Melzig[b], Hendrik Fuchs[a,*]

[a]*Institut für Klinische Chemie und Pathobiochemie, Charité–Universitätsmedizin Berlin, Campus Benjamin Franklin, Hindenburgdamm 30, D-12200 Berlin, Germany*

[b]*Institut für Pharmazie–Pharmazeutische Biologie, Freie Universität Berlin, Königin-Luise-Str. 2+4. D-14195 Berlin, Germany*

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Abstract

Immunotoxins have to be administered in high doses due to low cytosolic uptake with the consequence of severe side effects. Recently we found that the cytotoxic activity from *Agrostemma githago* seeds can be attributed to a synergistic toxicity of a triterpenoid saponin and a ribosome-inactivating protein. Here we investigated whether saponins are able to enhance the efficacy of a receptor-specific chimeric toxin consisting of saporin-3, epidermal growth factor and a molecular adapter previously shown to reduce side effects on non-target cells. Pre-applied saponin enhances the target cell-specific cytotoxic effect, dependent on the cell line, between 3560- and 385,000-fold with an IC_{50} up to 0.67 pM. Non-target cells are not affected at the same concentration. At the optimal concentrations of the chimeric toxin and saponin application of either one of the components shows no cytotoxicity at all proving a synergistic effect. In the presence of saponin ligand-free saporin-3 does not exhibit any cytotoxic effect up to 0.1 nM providing further evidence for an increased specificity. This synergistic effect is in the same order of magnitude as in a mouse model. Our investigations clearly demonstrate that a combined administration of saponin and chimeric toxins opens up a promising perspective for tumor therapy.

Keywords: Recombinant chimeric toxin; Immunotoxin; Saporin; Epidermal growth factor; Saponin; Cancer therapy

1. Introduction

In context with targeted toxins for tumor therapy chimeric toxins (CT) are recombinant proteins or chemically coupled conjugates in which a cell-targeting moiety is combined with a cytotoxic agent. The latter is either a small radioactive molecule, a low molecular weight organic compound or the catalytic

Abbreviations: CT, chimeric toxin; PTD, protein transduction domain; RIP, ribosome-inactivating protein; Sap-3, His-tagged saporin-3; Spn, Saponinum album from *Gypsophila paniculata* L.

* Corresponding author. Tel.: +49 30 8445 2559; fax: +49 30 8445 4152.
E-mail address: hendrik.fuchs@charite.de (H. Fuchs).

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domain of a natural toxin. Usually the cell-targeting moiety is a natural ligand or an antibody fragment for cancer-associated endocytic surface antigens. In the latter case the CTs are also known as immunotoxins. Typical target antigens are the epidermal growth factor (EGF) receptor, the proto-oncogene receptor ErbB-2 (also known as HER-2 in humans), the interleukin-2 receptor or cancer-associated carbohydrates [1].

The first recombinant CT approved by the American Food and Drug Administration was Ontak. It consists of interleukin-2 and parts of diphtheria toxin. It was developed for the treatment of cutaneous T-cell lymphoma [2,3]. In addition to Ontak two further chemically coupled CTs, Zevalin [4,5] and Mylotarg [6], have been approved and several CTs are under investigation in clinical trials.

In spite of the clinical success of several newly developed CTs the effective cytosolic uptake of the drug remains a main problem requiring the application of large total doses to achieve the high local concentrations needed. This often leads to non-specific toxicity, which often results in diffuse endothelial damage and concomitant vascular leak syndrome (reviewed in Ref. [7]). An improvement for recombinant CTs was the development of a molecular adapter linking the toxic and targeting moiety. Due to cleavable endosomal and cytosolic peptides the adapter mediates cytosolic trapping of the toxin [8] which leads to reduced side effects on non-target cells in vitro [9].

In some CTs the natural membrane translocating sequence of the toxin is used to mediate endosomal membrane transfer. While this is possible e.g. for diphtheria toxin and *Pseudomonas* exotoxin other toxins like plant type I ribosome-inactivating proteins (RIP) lacking a translocating sequence must enter the cells using other to date unknown mechanisms. In the last decade, the use of protein transduction domains (PTD, also referred to as Trojan peptides or cell permeable proteins) capable of transporting effector molecules into cells has become an increasingly attractive mechanism to solve this problem (reviewed in Refs. [10,11]). Various peptide vectors derived from viral, bacterial, insect, and mammalian proteins possessing membrane transduction properties have been identified and tested for their ability to deliver compounds, proteins and DNA. In all these experiments, however, the PTD-mediated uptake was unspecific and thus independent of the cell type and the cellular surface receptors. To solve this problem a PTD was linked in the chimeric adaptertoxins mentioned above via an endosomal cleavable peptide to a target cell-specific moiety. The PTD is not exposed until binding of the chimeric adaptertoxin to a target cell-specific receptor, internalization and endosomal cleavage [9]. Nevertheless, the cytosolic uptake of these improved CTs is clearly lower than that of potent natural toxins.

An alternative for an enhanced but undirected uptake may be the use of saponins. Saponins are a highly diverse group of glycosides containing either a steroidal or triterpenoid aglycone to which one or more sugar chains are attached (reviewed in Ref. [12]). The adjuvant-active properties of saponins were recognized as early as the 1920s [13] and their use in commercial foot and mouth disease vaccines was described in 1951 [14]. Although the mechanism of action is unknown several studies have shown increased permeability of oral and gastric epithelia following oral administration of saponins resulting in increased nutrient uptake [12]. Soya saponins were shown to increase the permeability in vitro of rabbit jejunal mucosa to glycinin, a soya-derived protein [15]. An open cage-like immunostimulating complex (ISCOM) of cholesterol, lipid, immunogen and saponins from the bark of *Quillaja saponaria* Molina (soap bark tree) has found successful application as an active adjuvant for vaccination [16]. An interesting finding describes the cytotoxicity of the seeds from *Agrostemma githago* L. as a result of the combined action of saponin and agrostin, a type I RIP [17].

Based on these observations we tested the cytotoxicity of CTs in combination with Saponinum album from *Gypsophila paniculata* L. (Spn), which is structurally closely related to agrostemmasaponin. Both agrostemmasaponin and gypsosid, the main saponin of Saponinum album, are bisdesmosidic triterpene saponins with two branched sugar chains. One glycosidic sugar moiety is attached at pos. 3; the other sugar chain is attached via an ester bond at pos. 28 of the aglycone gypsogenin (see Fig. 1A for structure). The object of the investigations was to clarify whether the undirected enhancer effect of saponins could increase the specific cytotoxicity of receptor-targeted