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Inhibition of bacterial adhesion to live human cells: Activity and cytotoxicity of synthetic mannosides

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ABSTRACT

Bacterial adhesion to glycosylated surfaces is a key issue in human health and disease. Inhibition of bacterial adhesion by suitable carbohydrates could lead to an anti-adhesion therapy as a novel approach against bacterial infections. A selection of five α-mannosides has been evaluated as inhibitors of bacterial adhesion to the polysaccharide mannan, as well as to the surface of live human HT-29 cells. Cell toxicity studies were performed to identify the therapeutic window for a potential in vivo-application of the tested carbohydrates. A previously published mannosidic squaric acid diamide was shown to be exceptionally effective as inhibitor of the bacterial lectin FimH.

1. Introduction

Carbohydrates are involved in numerous important biological events such as in cell recognition and cell adhesion [1]. They are found as part of cell surface glycoconjugates, making up a characteristic layer that is surrounding a eukaryotic cell and called its glycocalyx. There is an overwhelming molecular complexity of the glycocalyx which is interrogated by a class of specialized proteins, namely the lectins [2]. To learn more about carbohydrate–lectin interactions, synthetic glycosides and glycomimetics, respectively, have been utilized as modulators and inhibitors of the occurring molecular recognition processes [3–8]. As also adhesion of microbes to the surface of their target cells is frequently mediated by carbohydrate–protein interactions, its inhibition by suitable glycosides could provide means against, i.e., bacterial colonization and biofilm formation [9–12].

Bacteria use long hairy organelles, called fimbriae or pili, to facilitate adhesion to cell surfaces. One of the best characterized fimbriae are type 1 fimbriae, that comprise an α-D-mannoside-specific lectin at their tips, named FimH [13]. Type 1 fimbriae are critical virulence factors in uropathogenic *Escherichia coli* (UPEC) and widely distributed among Enterobacteriaceae [14]. A large collection of different mannosides and mannose conjugates, respectively, have been made and tested as inhibitors of type 1 fimbriae-mediated bacterial adhesion, primarily in vitro [15]. Only few examples have been published, where mannoside inhibitors of type 1 fimbriae-mediated bacterial adhesion have been tested with cells or in animal models, respectively [16–21]. Here, it has become our goal to examine a selection of most promising inhibitors of mannose-specific bacterial adhesion with live human cells (Fig. 1A) and test their cytotoxicity, in order to assess the therapeutic potential of these compounds.

Abbreviations: AzoMan, *E*-*para*-(*ortho*-methoxycarbonyl-phenylazo)phenyl α-D-mannoside; CRD, carbohydrate recognition domain; DMEM, Dubecco's modified eagle medium; EC_{50}, half-maximal effective concentration; *E. coli*, *Escherichia coli*; FBS, fetal bovine serum; HepMan, heptyl α-D-mannoside; IC_{50}, half-maximal inhibitory concentration; IP, inhibitory potency; MEM, minimal essential medium; MeMan, methyl α-D-mannoside; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; *p*NPMan, *para*-nitrophenyl α-D-mannoside; PBS, phosphate buffered saline; PBST, PBS + 0.5% Tween20; RIP, relative inhibitory potency; SAMan, *p*-[*N*-(4-ethylamino-2,3-dioxocyclobut-1-enyl)amino]phenyl α-D-mannoside; SE, standard error; SEM, standard error of the mean

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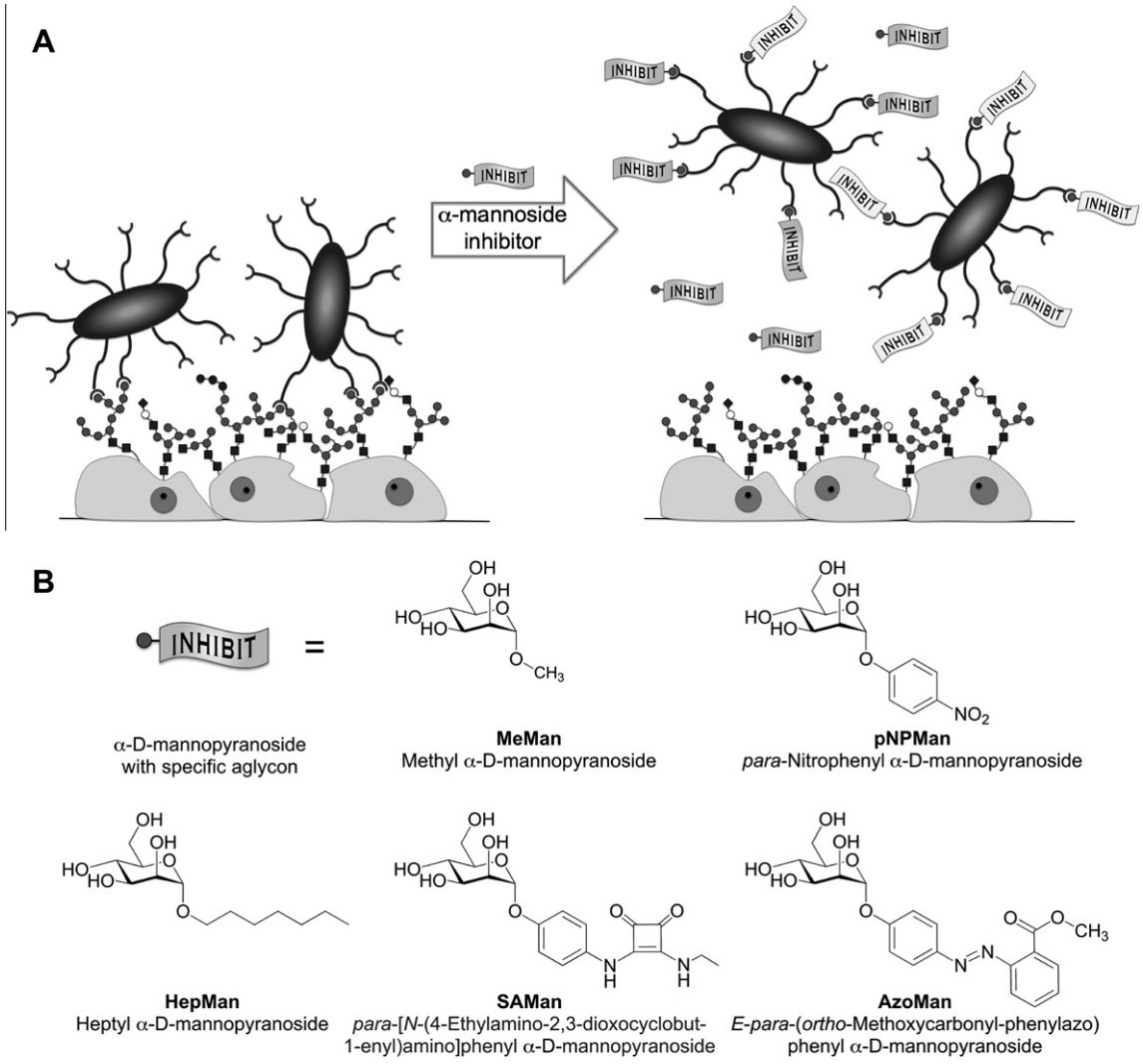

Fig. 1. Five synthetic α-mannoside inhibitors of bacterial adhesion to eukaryotic cells. (A) The cartoon illustrates fimbriae-mediated adhesion of bacteria to the glycocalyx of cells and its prevention by suitable α-mannoside inhibitors and (B) structures and names (abbreviations and IUPAC nomenclature) of tested α-mannoside inhibitors.

In the past, we have typically tested and ranked synthetic mannosides as inhibitors of bacterial adhesion to the polysaccharide mannan [15,22]. Owing to the known structure of the type 1 fimbrial lectin FimH [17,23,24], the affinity of α-D-mannoside ligands can be greatly improved by variation of the aglycone moiety, whereas the mannose glycone part must not be changed. Recently, we have added a very potent low-molecular weight mannoside to the collection (SAMan, Fig. 1B), which has the potential to serve as a lead structure for the development of FimH antagonists [25,26]. Hence, it is important to evaluate its inhibitory potency with human cells as well as to test its cytotoxicity. In addition, a novel azobenzene mannoside (AzoMan, Fig. 1B) was tested as anti-adhesive and both mannosides, SAMan and AzoMan, were compared to known inhibitors of type 1 fimbriae-mediated bacterial adhesion [27], namely methyl α-D-mannoside (MeMan), *p*-nitrophenyl α-D-mannoside (*p*NPMan), and heptyl mannoside (HepMan). The latter has recently been described as high-affinity ligand for FimH [17,28,29].

Highly glycosylated HT-29 mammalian colon cells were chosen to study bacterial adhesion, its inhibition, and cytotoxicity of the mannosidic inhibitors. The inhibitory potencies determined using HT-29 cells were compared to the results from a test, where the polysaccharide mannan was used as the adhesive layer.

2. Materials and methods

2.1. Synthetic mannosides (Fig. 1B)

Methyl α-D-mannoside (MeMan) and *para*-nitrophenyl α-D-mannoside (*p*NPMan) were purchased from Sigma–Aldrich and Senn Chemicals, respectively. For the synthesis of the squaric acid diamide conjugate SAMan, *p*NPMan was reduced to the corresponding amine and subsequently coupled to squaric acid diethylester to obtain the respective squaric acid monoamide [25]. This was in turn converted into the target squaric acid diamide SAMan by reaction with ethylamine [26]. Mannosides HepMan and AzoMan were synthesized by standard glycosylation of heptanol and *ortho*-(*para*-hydroxyphenylazo)benzoic acid methyl ester, respectively, according to the trichloroacetimidate method [30] followed by final deprotection. Purity of the synthesized mannosides was confirmed by analytical HPLC and/or elemental analysis.

2.2. Cultivation of bacteria

The GFP-tagged type 1 fimbriated *E. coli* strain PKL1162 was grown as published [22]. Details are described in the Supplementary material.