Probiotics to prevent upper respiratory tract infections

KEYWORDS: Microbiota, probiotics, upper respiratory tract infections.

Abstract
Upper respiratory tract infections (URTIs) are illnesses caused by an infection of mucosal surfaces in the nose, sinuses, pharynx and/or larynx. These infections include the common cold, rhinosinusitis, tonsillitis and otitis media and are very common, especially among infants, children and elderly. URTIs are among the most common reasons for people to visit their doctor and they account for a large part of antibiotic use in high-income countries. Although antibiotics are very effective in clearing infections, they come with negative side-effects such as antibiotic resistance. This has prompted researchers to look into more natural alternatives, such as probiotics, for treatment and/or prevention of URTIs. Mechanisms by which probiotics may be effective are thought to be related to restoration of the microbial balance in the upper respiratory tract and stimulation of the immune response. Based on these scientific insights the probiotic formulation, Winclove 381 Respiratory, to prevent URTIs was developed.

INTRODUCTION
The importance of the microbial communities living in our intestinal tract for our health is well known. Due to extensive research we have learned that establishment of a symbiotic relation between the host and the gut microbiota in early life is crucial for optimal health later in life. However, only recently we have started to appreciate the role of the microbial communities that inhabit the upper respiratory tract. Nevertheless, the upper respiratory tract, just like our intestines, needs a variety of bacteria to maintain human health. The upper respiratory tract is an important site of pathogen colonization. In order to colonize and/or outgrow in the upper respiratory tract, bacterial pathogens must compete with each other and with commensal members. If the commensal microbiota is compromised, for example by use of antibiotics, antimicrobial nasal sprays, or a weakened immune system, pathogens may be able to cause upper respiratory tract infections (URTIs). Recently, studies have been published addressing the microbial communities that inhabit the upper respiratory tract (1,2). These studies show quite a difference between the key bacterial players in the respiratory tract compared to the ones in the gastro-intestinal tract. Data indicate that just like in the gastro-intestinal tract, imbalances in the microbiota seem to play an important role in the susceptibility to infections. A healthy upper respiratory tract microbiota provides us with local colonization resistance. A lack of commensals might lead to outgrowth of potential pathogenic bacteria, consequently resulting in respiratory illness (3).

PROBIOTICS
According to the latest definition by the World Health Organization, probiotics are live-micro-organisms that, when administered in adequate amounts, confer a health benefit to the host (4). Probiotic interventions are aimed to restore the microbial balance and/or prevent disturbances in this balance. Their action can take place at different levels, they: reduce the risk of outgrowth of potential pathogenic bacteria, restore lost bacterial or metabolic activities, or stimulate a specific immune response (5,6). Probiotics have shown extensive beneficial effects in the gastro-intestinal tract and have established positive effects in the treatment of e.g. diarrhea and functional gastro-intestinal disorders (7,8). Moreover, their effects seem to reach beyond the gut. Human clinical trials have shown that probiotics could also be effective in cases of allergies, mental disorders, periodontitis, vaginal infections and also in respiratory infections (9,10).

UPPER RESPIRATORY TRACT INFECTIONS
Upper respiratory tract infections (URTIs) are illnesses caused by an infection of mucosal surfaces in the nose, sinuses, pharynx and/or larynx. These infections include non-allergic rhinitis (the common cold), rhinosinusitis, pharyngitis, tonsillitis and otitis media. URTIs are a very common problem, especially among infants, children and elderly, and they account for 9% of all consultations in general practice (11).
URTIs can be caused by viruses or bacteria. The most common viruses causing URTIs are rhinoviruses, coronaviruses, parainfluenza viruses and influenza virus [11]. The most common bacterial pathogens causing URTIs are Group A streptococci, Mycoplasma pneumonia, Chlamydia pneumonia, Corynebacterium diphtheriae, Staphylococcus aureus and Streptococcus pneumonia [11]. For example pharyngitis in children is in 15-40% of the cases caused by a viral infection and 38-40% of the cases caused by a bacterial infection. In adults these numbers are respectively 30-60% and 5-10% [12].

Usually an infection starts by direct invasion of the mucosa of the upper airways by the pathogen. The pathogens have to fight the commensal healthy microbiota present in the airways and overcome the local and systemic immune system. Usually respiratory infections are self-limiting and will resolve spontaneously. Common symptoms of URTI includes nasal congestion, sore or scratchy throat, fever and coughing. However, sometimes an infection left untreated can impair breathing and swallowing in such a way that hospitalization is necessary, especially when fever and diarrhea are symptoms as well. Treatment depends on the cause of the infection, however most treatments are aimed at alleviating the symptoms and not at treating the cause. Nevertheless, in some case antibiotics or antivirals are administered. Because antibiotics are associated with many side-effects and can promote bacterial resistance and secondary infections, they will have to be used cautiously [13,14]. Alternative therapies that support the commensal microbiota in the upper respiratory tract are highly desired.

PROBIOTICS AND UPPER RESPIRATORY TRACT INFECTIONS

Several studies and meta-analyses have shown that probiotics can be effective in preventing URTIs. In addition, recent studies suggest that probiotics may reduce the risk of various symptoms of URTIs. A recent Cochrane meta-analysis of 13 clinical studies including 3720 infants, children and adults, showed that probiotics were more beneficial in preventing URTIs compared to placebo. This review concluded that: despite the heterogeneity of the RCTs, probiotics were found to reduce the number of people experiencing episodes of acute URTI by 47%. Furthermore, overall the duration of an episode of URTI was reduced almost two days. Both these positive results may further reduce the use of antibiotics and cold-related school absence [15]. A second review included 14 RCTs and concluded that probiotics in children have a modest effect both in diminishing the incidence of URTIs as well as the severity of the symptoms [16].

Several possible mechanisms have been proposed to explain the beneficial effects observed in clinical studies [17]. In general these mechanisms can be divided into local and systemic effects. The first is based upon competitive exclusion of the pathogens by the probiotic bacteria. It has been hypothesized that especially recurrent infections are caused by the constant shedding of pathogens from biofilms within the nasal and nasopharyngeal cavity that are able to reach the more inaccessible niches such as the sinuses and middle ear and cause infection [18-20]. When administering a probiotic food supplement the bacterial strains can temporarily colonize the upper respiratory tract, thereby inhibiting and excluding pathogens in the upper respiratory tract and preventing their shedding into other niches. This microbiota management can not only prevent the healthy microbiota from being disturbed, it also is able restore the microbiota.

The systemic pathway is through immune-modulation. Probiotic strains are able to elicit a strong immune response against invaders of the respiratory tract. This is done via the innate immune system (enhancing phagocytic activity), via acquired immunity (inducing specific immunoglobulins) and by enhancing local immunity (increasing the production of T_{H}1 cells and cytokines). It is now thought that these combined strengths are responsible for the beneficial effect of probiotics in URTIs. For example in common cold infections, the symptoms like a stuffy nose and sore throat are the body’s inflammatory response toward a virus, not a direct action of the virus itself. Probiotic microorganisms may soften the immune system’s reaction by reducing the body’s inflammatory response.

WINCLOVE 381 RESPIRATORY

To offer a natural alternative for the prevention of URTIs Winclove has developed Winclove 381 Respiratory. Winclove 381 Respiratory consists of the following probiotic strains: Streplococcus oralis 89a, Lactobacillus rhamnosus LB21, Lactobacillus rhamnosus WGG, Bifidobacterium animalis subsp. lactis W12, Bifidobacterium animalis subsp. lactis W51, and Lactococcus lactis W19, blended on a carrier matrix consisting of rice starch, maltodextrin, inulin and vitamin C. The total cell count of the formulation is 1.0 x 10^9 colony forming units (cfu)/gram. The bacterial strains in Winclove 381 Respiratory have been selected based on their in vitro capacity to inhibit URTI related pathogens, and to positively influence the immune system. Winclove 381 Respiratory is developed as preventative therapy for URTIs. Two strains in the formulation, S. oralis 89a and L. rhamnosus LB21, have been added to the formulation for their in vitro capacity to inhibit URTI related pathogens such as S. pneumoniae, H. influenzae, M. catarrhalis, and S. pyogenes. In addition, they have shown positive effects in preventing ear- and throat infections in several clinical trials [21-26]. S. oralis is a natural member of the microbiota of the upper respiratory tract.

Furthermore, the bacterial strains L. rhamnosus WGG and B. animalis subsp. lactis W12 (genetically identical to respectively L. rhamnosus LGG and B. animalis subsp. lactis BB12), have been added to the formulation because they have shown in previous clinical trials to prevent common cold [27]. The last two strains in the formulation, B. animalis subsp. lactis W51 and Lc. lactis W19, have been added to further improve the immune boosting function of the formulation. The capacity of these two strains on modulating the immune system has been measured in vitro by T_{H}1 cell induction and the production of cytokines.
An in vitro screening of a subset of Winclove's probiotic strain collection was performed in collaboration with the Wilhelmina Children's Hospital Utrecht, the Netherlands, to investigate the capacity of the bacterial strains to trigger differentiation of a naïve T cell into either T\(_{H1}\), T\(_{H2}\), T\(_{H17}\) or T\(_{reg}\) cells (28). After co-culture of peripheral blood mononuclear cells (PBMCs) with probiotic bacteria, the induction of CD4\(^+\) T cell subsets was analyzed based on gene expression levels of signature transcription factors using reverse transcriptase PCR. For Winclove 381 Respiratory strains were selected for their capacity to induce T\(_{H1}\) cells, which was measured by the induction of T-bet gene expression. The results of the screening are found in figure 2. As can be seen from figure 2, Lc. Lactis W19 is a very good inducer of the differentiation of naïve T cells into Th1 cells.

Another property for which a subset of Winclove's probiotic strain collection was screened was the in vitro ability to modulate the production of cytokines by mononuclear cells. Peripheral blood mononuclear cells (PBMCs) were isolated from three healthy adult donors.
The effects of the probiotic strains on cytokine production by antigen-stimulated peripheral blood mononuclear cell (PBMC) were evaluated. Cell-free supernatants were collected of 24 h cultures of PBMCs with individual probiotic strains. Cytokine profiles were measured by the multiplex immunooassay of Lumineux as described by De Jager et al. (29). Production of IFN-γ and TNF-α, which are cytokines mainly produced by T_{h}1, cells, was measured and compared to control, see figure 3A and 3B. Data are mean ±SEM values of three or four healthy donors. As can be seen from figure 3A and 3B, B. animalis subsp. lactis W51 and Lc. lactis W19 are able to induce the production of IFN-γ and TNF-α. These cytokines will strengthen the T_{h}1 immune response against pathogens in the respiratory tract.

CONCLUSIONS AND FUTURE DIRECTIONS

With this article we aimed to stress the importance of the microbiota for the prevention of upper respiratory tract infections. Living in a time where the drawbacks and the risks of antibiotic treatment are being more recognized, alternatives treatment options such as probiotics are highly desirable for doctors. A large majority of clinical trials conducted on prevention and treatment of URTIs with probiotics have shown positive outcomes (15,16). Probiotics such as Winclove 381 Respiratory could offer an alternative treatment solutions. An important aspect that should be addressed before developing a probiotic product is safety. All the probiotic strains in Winclove 381 have the Qualified Presumption of Safety status (QPS) or an extensive safety file and have been tested independently for their efficacy on pathogen inhibition and/or immune parameters. Since probiotic strains can show synergistic effects but also competition, testing of the final formulation for effectivity, preferably in vivo, is desired. Besides the food supplement Winclove 381 Respiratory, Winclove is further investigating to develop more products that could alleviate or prevent upper respiratory tract infections. At the moment a medical device, aimed at reducing the risk of recurrent ear and throat infections is being developed. Further research is planned to show effectivity of these formulations in vivo.

REFERENCES