Probiotics during and after antibiotics

Designer probiotics are capable of more than reducing AAD

ABSTRACT

Each individual has a unique, relatively stable, intestinal microbiota which plays an important role in human health. One of the side effects of antibiotic intake is a disturbance of this microbiota which can result in antibiotic-associated diarrhoea (AAD). It was widely assumed that these disturbances were short-term but recently medium and long-term disturbances have been described. The exact clinical consequences of these microbiota disturbances are not yet clear but they are associated with a multitude of disorders such as IBS and allergy. Knowledge of the effect of antibiotics on the intestinal microbiota and ways of restoring their unique composition are therefore of clinical importance. A RTC performed by the Maastricht University Medical Centre showed that the probiotic Ecologic® AAD was not only able to reduce AAD but also to restore the intestinal microbiota towards the pre-antibiotic state.

INTRODUCTION

The gastrointestinal tract (GI-tract) comprises a complex bacterial ecosystem, i.e. the intestinal microbiota, colonizing the entire length of the gut and containing approximately hundred times as many genes as the human genome (1). The intestinal microbiota contains approximately $10^{14}$ bacteria and is composed of more than 1000 different species (2), of which anaerobes are a hundred times more abundant. In addition, the composition of the microbiota differs both along the gastrointestinal tract and from lumen to mucosa (3).

The microbiota plays an important role in human physiology. Firstly, the intestinal microbiota aids in the break-down of non-digested and indigestible polysaccharides, and supplies essential substrates like vitamins and short-chain fatty acids. It also provides colonization resistance by competing for substrates and adhesion sites and by producing antibacterial substances, thereby preventing the overgrowth of potential pathogens. Secondly, the intestinal microbiota affects mucosal barrier function by influencing the metabolism, proliferation and survival of the intestinal epithelial cells, the production and composition of mucus and by the strengthening of tight junctions (level 2) (4). Moreover there is an active cross-talk between the intestinal microbiota and the immune system via epithelial cells, specialised M cells and lamina propria DCs (5-6).

Though marked variations are present between individuals, within adults the intestinal microbiota is found to be relatively stable over time (7-8). However, several factors, among which the use of antibiotics, can disturb this microbiota.

ANTIBIOTICS AND ANTIBIOTIC-ASSOCIATED SIDE EFFECTS

Ever since the discovery of the first
antibiotic, penicillin, by Alexander Fleming in 1928, antibiotics have become one of the cornerstones in the prevention and treatment of infectious diseases. However, their use is not without side effects as it may lead to the development of antibiotic resistance or cause a disturbance of the intestinal microbiota. Moreover, antibiotics can have direct allergic and toxic effects on the intestinal mucosa, direct effects on immune cell function, and pharmacological effects on intestinal motility (9-11).

One of the most common and clinically manifest side effects of antibiotic use is antibiotic-associated diarrhoea (AAD), which can occur shortly after antibiotic intake to up to 8 weeks after cessation (12-13). The incidence of AAD ranges from 5-39%, depending on the definition of diarrhoea, the route of administration, the type of antibiotic used and host factors (13). In general, broad-spectrum antibiotics, amoxicillin, amoxicillin/clavulanic acid, clindamycin and cephalosporines are associated with a high risk of AAD (14). AAD can be divided into two types: non-specific AAD, which is usually mild and CDAD, which can lead to severe pseudomembranous colitis (15). Clostridium difficile is thought to be the causative agent in up to 20% of AAD (16). However, the exact pathogenesis of the majority of cases of AAD is not clear and is attributed to a disturbance of the intestinal microbiota. Overviews of the ecological disturbances due to different types of antibiotics have been published by Edlund et al. and Sullivan et al. (17-18). In these reviews it was generally found that the microbiota was only temporarily disturbed, returning to its original composition 1-2 months after cessation. However, mainly cultivation-based techniques were used, while it is estimated that less than 25% of the intestinal bacterial populations can be cultured (2,19-20). The application of culture-independent methods based on 16S rRNA provides a better and more comprehensive insight into the diversity of the intestinal microbiota and the ecological disturbances due to antibiotic treatment (21-25).

Antibiotic-induced disturbances of the intestinal microbiota can result in important functional differences in the microbial metabolome, i.e. the collective biochemical output of the microbiota, loss of colonisation resistance, a decreased intestinal barrier function and can influence the immune responses of the host (9).

Moreover, a study in mice using qPCR showed that total bacterial numbers returned to normal within 1 week after cessation of antibiotic intake, but alterations in Bacteroides and segmented filamentous bacteria persisted more than 3 weeks (27).

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The clinical consequences of these antibiotic induced microbiota perturbation for the host are not yet clear. There is however, accumulating evidence indicating that a disturbance of the intestinal microbiota plays an important role in a multitude of disorders ranging from allergies to diarrhoea, irritable bowel syndrome (IBS), inflammatory bowel disease (IBD) and colorectal cancer (4,28-30). A disturbance of this intestinal microbiota due to antibiotics has been associated with local inflammation and a disturbed immunological functioning of the host (28,31-32). In addition, a number of epidemiological studies have demonstrated an association between the use of antibiotics during early childhood and an increased risk for acquiring allergy or asthma (33-36). Also, alteration of the intestinal microbiota due to antibiotic treatment was found to induce or exacerbate IBS (37). Thus, knowledge of the effect of antibiotic on the intestinal microbiota and ways of restoring their unique composition are of clinical importance.
PROBIOTICS FOR ANTIBIOTIC-ASSOCIATED SIDE EFFECTS

Probiotics are defined as "live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host". The treatment and/or prevention of AAD by probiotics have been subject of many trials and are reviewed in several meta-analyses and some systematic reviews (Table I). The meta-analyses by CREMONINI, D’SOUZA, SZAJEWSKA and JOHNSTON found combined relative risks of 0.37-0.44 in favour of probiotic over placebo treatment in the prevention of AAD (38-42). McFARLAND (43) and SAZAWAL (44) both conducted large meta-analyses (25 and 19 studies, respectively) including many different strains and types of probiotics. Both meta-analyses also showed that probiotics were efficacious in preventing AAD with a RR of 0.43 and 0.48, respectively. Moreover, McFARLAND stratified by probiotic type and showed that only S. boulardii, LGG and probiotic mixtures showed significant efficacy (43). SAZAWAL analysed the use of probiotics in the prevention of acute diarrhoea (n=34). Subgroup analyses were performed on type of diarrhoea (AAD:19 studies), age groups, products and quality of trial and showed that only type of diarrhoea and product influenced the effect (44).

Next to several meta-analysis also three systematic reviews were published (45-47). In the systematic review by HAWRELAK, evaluating only papers on LGG, 4 of 6 studies found a significant reduction of AAD, one study found a significant decrease in the number of days with diarrhoea and one study was negative (45). SCHEIKE reviewed 23 trials and found that probiotics were associated with a relative reduction of AAD of 52% compared to placebo with a number to treat (NTT) of 8 (47). Despite statistical heterogeneity between studies, detailed analysis revealed that the effects of probiotics were consistent. Moreover, a stratified analysis revealed that S. boulardii, LGG and multispecies products were more effective than monospecies products. Furthermore, no relation between trial quality and magnitude of effect on probiotic treatment was found and no adverse events were reported (47). The Cochrane database of

Table I – Meta-analyses and systematic reviews on the effect of probiotics in AAD

<table>
<thead>
<tr>
<th>Ref</th>
<th>Number of trials</th>
<th>Number of subjects (Adults/children)</th>
<th>Probiotics included</th>
<th>RR (95% CI)</th>
<th>Remarks</th>
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<tr>
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<tr>
<td>Meta-analyses</td>
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</tr>
<tr>
<td>55</td>
<td>9</td>
<td>1214 mixed</td>
<td>mix of single and multispecies</td>
<td>0.37 (0.26-0.53)</td>
<td>Only trials with S. boular</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 S. boulardi trials</td>
<td>0.39 (0.25-0.62)</td>
<td>dii were included</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 ‘non-yeast’ trials</td>
<td>0.34 (0.19-0.61)</td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>7</td>
<td>881 mixed</td>
<td>Lactobacillus spp. and S. boulardi</td>
<td>0.40 (0.27-0.57)</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>5</td>
<td>1076 mixed</td>
<td>S. boulardi</td>
<td>0.43 (0.23-0.78)</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>6</td>
<td>766 children mixed</td>
<td>mix of single and multispecies</td>
<td>0.44 (0.25-0.77)</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>25</td>
<td>2810 mixed</td>
<td>mix of single and multispecies</td>
<td>0.43 (0.31-0.58)</td>
<td>No significant results with ITT</td>
</tr>
<tr>
<td></td>
<td>6 CDD</td>
<td>354 adults</td>
<td>mix of single and multispecies</td>
<td>0.43 (0.31-0.62)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6 S. boulardi</td>
<td>0.37 (0.26-0.62)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>6 L. rhamnosus GG</td>
<td>0.31 (0.13-0.72)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7 Single strain probiotics</td>
<td>0.46 (0.21-1.03)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>7 Multiple strain probiotics</td>
<td>0.51 (0.38-0.68)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>mix of single and multispecies</td>
<td>0.59 (0.41-0.85)</td>
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</tr>
<tr>
<td>40</td>
<td>6</td>
<td>707 children</td>
<td>mix of single and multispecies</td>
<td>0.43 (0.25-0.75)</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>19</td>
<td>2050 mixed</td>
<td>mix of single and multispecies</td>
<td>0.48 (0.35-0.65)</td>
<td>Trial was on acute diarrhoea (n=34), but subgroup analyses was performed for AAD (n=19)</td>
</tr>
</tbody>
</table>

Systematic reviews

<table>
<thead>
<tr>
<th>Ref</th>
<th>Number of trials</th>
<th>Number of subjects (Adults/children)</th>
<th>Probiotics included</th>
<th>RR (95% CI)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>6</td>
<td>692 mixed</td>
<td>L. rhamnosus GG</td>
<td>0.13 (0.02-0.94)</td>
<td>Only trials with LGG were included and RR range based on 4 of 6 positive studies</td>
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<td></td>
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<td></td>
<td>0.32 (0.09-1.11)</td>
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</tr>
<tr>
<td>47</td>
<td>23</td>
<td>3365 mixed</td>
<td>mix of single and multispecies</td>
<td>0.48 (0.37-0.63)</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>10</td>
<td>1986 children</td>
<td>mix of single and multispecies</td>
<td>0.49 (0.32-0.74)</td>
<td>No significant results with ITT</td>
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</table>
systematic reviews published a review on probiotics for the prevention of paediatric AAD. The per protocol analysis showed a pooled relative risk of 0.49 in favour of probiotic use. However, the intention to treat analysis showed non-significant results (46).

Only a limited number of studies investigated the effect of probiotics in the prevention of CDAD. As approximately 20% of AAD is CDAD (16), this is probably due to the large sample size required to detect a significant difference.

Thus there is compelling evidence that probiotics can be efficacious in the treatment and prevention of AAD. However, few studies have investigated the mechanism of action on the (potential) efficacy of probiotics which included the effect on antibiotic induced perturbations of the intestinal microbiota. Most have focussed on survival of ingested probiotic strains, emergence of antibiotic resistance or prevention of disturbances in specific bacterial groups (48-52). In addition, mainly culture-dependent approaches were used and the effect on the total microbiota was rarely investigated.

Recently, a study was conducted by the Maastricht University Medical Centre (MUMC) investigating the effect of a multispecies probiotics (Ecologic® AAD) on bacteriological, immunological and clinical parameters in healthy volunteers during and after amoxicillin use.

The study showed that there is a clear association between the disruption of the intestinal microbiota and the development of diarrhoea. Moreover, the study showed that intake of Ecologic® AAD significantly reduced the occurrence of diarrhoea-like bowel movements. This was probably due to a better restoration of the microbiota as a intestinal microbiota more similar to the pre-antibiotic state was observed in the volunteers treated with Ecologic® AAD compared to placebo (53-54). The latter was also observed in a study looking at the effect of a probiotic mixture during amoxicillin/clavulanic acid intake in healthy volunteers using a combination of culture and terminal restriction fragment length polymorphism. The results of both studies suggest therefore that a restoration of the intestinal microbiota is one of the mechanisms of probiotics efficacy.

CONCLUSION

It is becoming accepted that antibiotic treatment can cause medium to long term ecological disturbances of the intestinal microbiota even if AAD is not present. Even though the clinical consequences of these antibiotic induced microbiota perturbations are not yet clear, accumulating evidence indicates an important role in a multitude of disorders. Probiotics have not only shown to be efficacious in preventing AAD but also in restoring these antibiotic induced microbiota perturbations.

Large prospective human intervention trials are needed to determine to what extent antibiotic induced disturbances of the intestinal microbiota are permanent, what their clinical implications are and if probiotic supplementation is able to either prevent or reverse a prolonged imbalance. In addition, it would be interesting to identify host-related, microbiota-related or environmental risk factors to characterize subjects at risk and who would benefit from probiotic intake.

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