ANTI-INFLAMMATORY EFFECT OF METFORMIN ON MICROBIOTA IN NON-DIABETIC PEOPLE LIVING WITH HIV

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Introduction

Persons living with HIV (PLWH) on antiretroviral therapy (ART) present with increased risk of inflammatory comorbidities such as fatty liver, neurocognitive and cardiovascular diseases and certain cancers. Persistent inflammation on ART has been linked with elevated microbial translocation of bacterial and fungal products. We and others demonstrated that translocation of bacterial lipopolysaccharide (LPS) and fungal (1,3-glucan) (BDI) reduce inflammation (Mehray, CID 2018) in association with non-AIDS comorbidities in PLWH (Mors JADIS 2012, Hoening CDO 2018, Hoening J neonutri 2019).

Metformin, an anti-diabetic drug, was shown to be anti-aging and reduce inflammation by inhibiting mTOR signaling in animal models and diabetic people. Metformin activity was dependent on the presence of a gut microbiota as antibiotic therapy inhibits metformin effect in mice, and intravenous injection of the drug did not reduce inflammation in rat models. Moreover, metformin use was associated with changes in the gut microbiota and increased colonization of Akkermansia muciniphila, a beneficial bacterium associated with reduced inflammation in diabetic people, and healthy men. Metformin, through direct immunomodulatory effect, or indirectly via modification of the microbiota, appears as a promising therapy to counteract inflammation and dysregulated immune responses in cancer and inflammatory diseases, including HIV infection. Hence, we evaluated the effect of 12 weeks of metformin therapy on microbial translocation, inflammation and bacterial gut community composition in non-diabetic PLWH under ART.

Methods

In the LILAC clinical trial (CHRC/CTN PT 072, NCT02689306), we recruited 22 PLWH under ART for more than 3 years, with HIV viral load <<400 copies/ml, and a CD4/CD8 ratio below 0.7 to select participants with a higher risk of inflammation. Participants were non-diabetic (HbA1c <6%).

We asked participants to take metformin (850 mg bid) daily for 12 weeks. Dose reduction was applied to those taking diuretics with only 500 mg bid. Blood and stool samples were collected at baseline (V1), after 12 weeks of metformin (V2), and 12 weeks after metformin discontinuation (V3) to assess a carryover effect. Plasma markers of microbial translocation and inflammation were assessed by ELISA for bacterial lipopolysaccharide (LPS), soluble CD14 and LPS binding protein (LBP). Fungal translocation was assessed in plasma by measuring the 1,3-β-D-glucan (BDG) using the Fungal assay. Serum short chain fatty acids (SCFA) were measured by liquid chromatography – mass spectrometry (LC-MS). To assess composition of the microbiota DNA was extracted from at least 10g of frozen stools in the presence of Proteinase and Lysysyme. We analysed bacterial microbiota composition by sequencing of the V3-V4 region of the 16S rRNA gene. Data were filtered and analyzed using QIIME. Taxonomic assignment of amplicon sequence variants (ASV) was performed against the Silva database 122 and the UNITE database 122. Variations in microbiota composition were analyzed using R and the Lefse algorithm.

Population Characteristics

Table 1: Clinical characteristics of study participants (n=22)

<table>
<thead>
<tr>
<th>Age</th>
<th>Female male (n)</th>
<th>Ethnicity</th>
<th>CD4 count (copies/µL)</th>
<th>CD4/CD8</th>
<th>Viral load (copies/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>56</td>
<td>Female (2) Male (20)</td>
<td>Caucasian (14) Black (8)</td>
<td>428.9</td>
<td>817.5</td>
</tr>
<tr>
<td>Min.</td>
<td>41</td>
<td>141</td>
<td>351</td>
<td>0.2</td>
<td>undetectable</td>
</tr>
<tr>
<td>Max.</td>
<td>69</td>
<td>1082</td>
<td>1867</td>
<td>0.7</td>
<td>&gt;400</td>
</tr>
</tbody>
</table>

Results

Figure 4. Participants lost weight after metformin treatment, in association with increased plasma levels of GDF-15 but not Ghrelin. Median weight loss 1.4 kg. Plasma GDF-15 at baseline and V2 correlated with weight changes (ρ) and weight at V2 (ρ). Friedman test

Figure 5. Metformin increased Akkermansia muciniphila abundance in stools. 16S rRNA gene quantity was stable between visits as assessed by qPCR. Abundance of Akkermansia muciniphila, a beneficial microbe associated with reduced inflammation in obesity and diabetes, was increased after metformin treatment as assessed by qPCR. V1 = baseline, V2 = after 12 weeks of metformin, V3 = 12 weeks after metformin discontinuation. Friedman test.

Figure 6. Serum levels of butyric acid were increased after metformin treatment. Short chain fatty acid (SCFA) propionate, succinate and butyric acids were measured in serum by LC-MS. A significant increase of butyric acid was observed after metformin discontinuation. Note: excluding data from the 2 participants with high level of baseline butyric acid, a statistically significant difference appears between V1 and V3 (p<0.05). V1 = baseline, V2 = after 12 weeks of metformin, V3 = 12 weeks after metformin discontinuation. Friedman test.

Conclusions

A 12-week metformin therapy in non-diabetic PLWH under ART was safe. Metformin treatment reduced participant’s weight by a median of 0.6 kg, in association with an increase in plasma levels of the growth factor GDF-15. This effect was noticed in absence of a glucose lowering effect.

Metformin slightly decreased inflammation concomitantly with an increased abundance of butyric acid producing bacteria, such as Lachnolochlinochlostridium and Lachnospiraceae, a family of bacteria depleted in PLWH. Indeed, increased levels of plasma butyric acid but not other short chain fatty acids were detected after metformin treatment. Increased abundance of A. muciniphila, a beneficial microbe also depleted in PLWH, was also observed. Hence, metformin appears to partly reverse dysbiosis observed in ART-treated PLWH. As metformin use was safe, a longer metformin treatment may further reduce inflammation and prevent non-AIDS comorbidities in PLWH or people with other inflammatory diseases.

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