REVIEW

New insights into the links between anti-diabetes drugs and gut microbiota

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Abstract

In patients with type 2 diabetes mellitus (T2DM), the intestinal flora is out of balance and accompanied by leaky gut. The flora is characterized by an increase in mucus-degrading bacteria and a decrease in fiber-degrading bacteria. Short-chain fatty acids (SCFAs), as the major fiber-degrading bacteria fermentation, not only ameliorate the leaky gut, but also activate GPR43 to increase the mass of functional pancreatic β-cells and exert anti-inflammation effect. At present, the gut microbiota is considered as the potential target for anti-diabetes drugs, and how to reverse the imbalance of gut microbiota has become a therapeutic strategy for T2DM. This review briefly summarizes the drugs or compounds that have direct or potential therapeutic effects on T2DM by modulating the gut microbiota, including biguanides, isoquinoline alkaloids, stilbene and C7N-aminocyclic alcohols.

Key Words

- gut microbiota
- leaky gut
- functional pancreatic β-cells
- short-chain fatty acids (SCFAs)
- anti-diabetes drugs
- type 2 diabetes mellitus (T2DM)

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Introduction

A genome-wide association study in Chinese population has shown that there is marked reduction in microbiota diversity in patients with T2DM. The main flora of the T2DM patients is characterized by an increase in mucus-degrading bacteria and a decrease in the number of fiber-degrading bacteria. Compared with healthy people, the abundance of fiber-degrading bacteria *Bifidobacterium*, *Faecalibacterium prausnitzii*, *Roseburia* and *Bacteroides vulgatus* decreased significantly, while the mucus-degrading bacteria *Akkermansia muciniphila*, *Bacteroides caccae*, *Clostridium* and *Escherichia coli* increased in patients with T2DM (1, 2, 3).

In the intestinal epithelial cells, the mature mucus layer is characterized by the fact that the number of fiber-degrading bacteria is significantly higher than that of mucus-degrading bacteria, so that the mucus layer has a complete barrier function (4). For example, when mice are given a diet rich in dietary fiber, fiber-degrading bacteria emerge as the times require. The fiber-degrading bacteria can produce a large number of enzymes to depolymerize and ferment dietary polysaccharides, turning them into SCFAs that can be absorbed by the host. The energy supply of the intestinal flora comes from dietary fiber or glycoproteins in the mucus layer covering the intestinal epithelium, and energy is conserved. In a diet lacking dietary fiber, the flora needs to erode the glycoproteins of mucus layer to obtain energy, leading to the number of mucus-degrading bacteria much higher than that of fiber-degrading bacteria in the intestinal tract. At the same time, a low-fiber diet leads to a sharp increase in the number of gram-negative bacteria in the intestine, which will increase the content of lipopolysaccharide (LPS) and pathological organisms. LPS and pathological organisms will take the opportunity to adhere to the thinning mucosal layer, and pass through the intestinal epithelial layer through the following mechanisms: (i) LPS and pathological organisms activate TLR2/4 receptor and recruit MyD-88 aptamers. (ii) LPS and pathological organisms combine with Nod1. (iii) LPS and pathological organisms are phagocytosed and co-localized by dendritic cells.

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(iv) LPS and pathological organisms destroy the tight junctions of intestinal epithelial cells so that tight junction proteins (occludin and zonula occludens-1) and CB2 are reduced (5). Through the previous methods, they penetrate the intestinal mucosal barrier with enhanced permeability, smoothly transfer to the lamina propria and submucosa, and continue to transfer to the circulatory system and peripheral tissues. This translocation of LPS and pathological organisms in the intestine is called 'leaky gut' (6). Bacterial DNA was found in the blood, omental, subcutaneous and mesenteric adipose tissue in T2DM patients. A small amount of bacterial DNA can increase the expression of pro-inflammatory factors in subcutaneous adipocytes. As a 'barrier' for bacteria to enter the body from the intestine, mesenteric adipose tissue has the highest bacterial diversity and number compared to other adipose tissues. The most convincing evidence is that living bacteria are detected by catalyzed reporter deposition fluorescence *in situ* hybridization in the subcutaneous adipose tissue and the bacteria are encapsulated in the adipose tissue (7). In addition, the tight junctions are damaged, and the mRNA levels of occludin and zonula occludens-1 (zo-1) are significantly downregulated in diabetic mice. Clinical studies have confirmed that the fecal zonulin concentration of patients with T2DM is significantly higher than that of the normal population. High-fat-diet (HFD) mice and T2DM patients have elevated serum LPS levels. The mRNA levels of the inflammatory markers PAI-1, IL-1, TNF-α in mesenteric adipose tissue and the mature macrophage marker F4/80 in the subcutaneous adipose depot of mice increased (8). The previous evidence strongly indicates that the occurrence of T2DM is accompanied by the ongoing leaky gut.

In obesity or the early stage of T2DM, systemic insulin resistance can trigger a compensatory response, increase the workload of pancreas and enhance the insulin secretion by pancreas β-cells. Some evidence have proved that pancreas β-cells can be stimulated into a compensatory state by high-fat diet (HFD) with the upregulation of free fatty acid receptor 2 gene (Ffar2) in mice (9). Free fatty acid receptor 2 (FFAR2), also known as GPR43, is a subclass of the nutrition-sensitive receptor GPCR, and SCFAs are considered to be the endogenous ligands (10). In addition to the acetate existing in the blood, there is a small part of the binding form of SCFAs in the intestine. Most of them exist in an ionized state and require special transporters across the epithelial barrier. Leaky gut provides a new way for SCFAs to cross the barrier. Animal studies have shown that (i) In islets, FFAR2 can be activated by SCFAs, increasing the glucose-stimulated insulin secretion in mice through the Gαq/11 signaling pathway (11, 12), (ii) In endocrine L cells of colon, FFAR2 mediates the secretion of GLP-1 by coupling with the ligand SCFAs, which in turn promotes the proliferation of functional islet β-cells mass (13, 14). Besides, SCFAs can play a role in anti-inflammation and maintaining the integrity of the intestinal barrier.

The intestinal flora of patients with T2DM is seriously out of balance, and the mucus-degrading bacteria and LPS producing pathogenic bacteria are dominant. LPS and pathological organisms transfer through the intestinal epithelial cells, transfer to the submucosa through leaky gut to the submucosa, then into the mesenteric adipose tissue, and finally into the blood and peripheral tissues. The increase of fiber-degrading bacteria is accompanied by the increase of SCFAs, which strengthen the intestinal barrier function, reduces the degree of metabolic endotoxemia and improve the function of islet β-cells. This review summarizes the research progress of Biguanides, Isoquinoline alkaloids, Stilbene, C7N-aminocyclic alcohols on intensifying intestinal barrier and improving islet β-cells function by regulating microbiota dysbiosis.

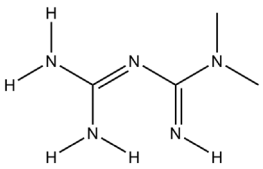
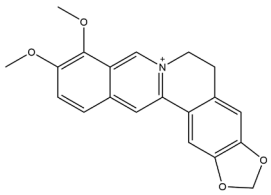
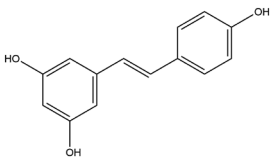
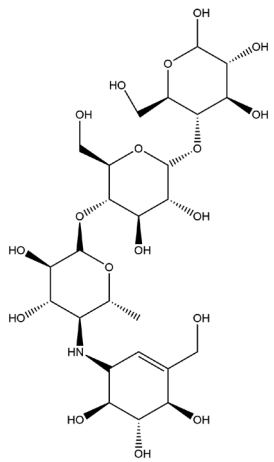
Drugs

Biguanides

Recent studies have shown that metformin can modulate the abundance of gut microbiota (Table 1). After metformin treatment on obese mice, the total amount of SCFAs in feces increased; the number of *Akkermansia muciniphila*, *Clostridium cocleatum* and the phylum Bacteroidetes elevated; the number of *Prevotella*, an opportunistic pathogen, reduced (15, 16). In T2DM patients treated with metformin, the abundance of *Escherichia, Roseburia, Faecalibacterium, Bifidobacterium* increased (17). *Roseburia, Faecalibacterium, Bifidobacterium* belonged to fiber-degrading bacteria. The number of *Clostridium, Intestinibacter* species and six OTUS abundances of *Bacteroides* reduced (18, 19).

As to the flora regulated by metformin, the number of fiber-degrading bacteria increased in patients, the mucus-degrading microbiota *Clostridium cocleatum* abundance increased in mice and decreased in patients. *Prevotella* had the ability to degrade mucin oligosaccharides, and its abundance reduced in HFD mice. The animal experiment treated with metformin showed that the mRNA expression for the canonical factors of the TLR/NF-κB signaling pathway (including TLR2, TLR4 and TNF-α)

Table 1 Drugs microbiota and the mechanism of ameliorating leaky gut (the chemical structure of the drug is drawn by ChemDraw).

Drugs	Changes in gut microbiota	Model	Mechanism	References
Metformin 	↑*Escherichia*, ↑*Roseburia*, ↑*Faecalibacterium*, ↑*Bifidobacterium*, ↓*Clostridium*, ↓*Intestinibacter* ↓*Bacteroides*	Human	The mRNA expression for the canonical factors of the TLR/NF-κB signaling pathway (including TLR2, TLR4 and TNF-α) downregulated.	(15, 16, 17, 18, 19)
	↑*Clostridium cocleatum*, ↑*Akkermansia muciniphila* ↑*Bacteroidetes* ↓*Prevotella*	Mice	The mRNA and protein expression of occludin and zo-1 were upregulated.	
Berberine 	↑*Bacteroidetes*, ↑*Escherichia-Shigella*, ↑*Enterobacter*, ↑*Akkermansia muciniphila*, ↑*Escherichia coli*, ↓Clostridiaceae, ↓*Prevotella*, ↓*Streptococcus*	Mice	The level of serum LPS reduced. The protein expression of TLP2/4 and TNF-α was downregulated.	(22, 23, 24, 25, 26)
Resveratrol 	↑*Bacteroides*, ↑*Blautia*, ↑*Parabacteroides*, ↑*Ruminococcus*, ↑*Bifidobacterium*, ↓*Enterococcus faecalis*, ↓Proteobacteria, ↓*Akkermansia muciniphila*	Mice	The mRNA expression of zo-1, zo-2, occludin, claudin-1, JAM-A, the major mucin gene (MUC1, MUC2, MUC3) and transcription factor (Relmβ, Reg3γ) elevated. The expression of inflammatory factors IL-6, IL-16, IL-1β and TNF-α, MCP-1 decreased.	(27, 28, 29)
Acarbose 	↑*Faecalibacterium*, ↑*Prevotella*, ↑*Bifidobacterium longum*, ↑*Lactobacillus gasseri*, ↓*Enterococcus faecalis*, ↓*Clostridium* ↑*Bacteroides*, ↑*Blautia*, ↑*Bifidobacterium*, ↓*Desulfovibrio*, ↓*Ruminiclostridium*	Human Mice		(31, 32, 33)

downregulated. The mRNA expression and protein level of tight junction-related factors occludin and zo-1 were upregulated, Transmission electron microscopy indicated the reduced gap between intestinal epithelial cells (15, 16). Metformin can adjust the flora in the right direction, along with restoring the normal permeability of intestinal epithelium and protecting the intestinal barrier structure. In addition, Metformin microbiota is also related to the improvement of islet β-cells function. The reduction in abundance of multiple OTUs in *Bacteroides* could increase the homeostasis model assessment-β value (HOMA-β), indicating that the islet β-cells function was simultaneously enhanced (19). *Bifidobacterium* could reduce the serum levels of pro-inflammatory factors such

as IL-1β and TNF-α, and effectively prevent the occurrence of insulin resistance. After metformin treatment, the decrease in *Clostridium* abundance and the increase of *Bifidobacterium* abundance in adolescence were negatively correlated with HbA_{1c} levels (20, 21).

Isoquinoline alkaloids

In obese mice, the total content of SCFAs in feces increased after berberine treatment. The concentration of butyrate increased from 6 μg/mL to 12 μg/mL. The composition of the phylum Bacteroidetes increased and the number of fiber-degrading bacteria *Escherichia, Shigella, Enterobacter*, *Akkermansia muciniphila* elevated (22, 23). Berberine could reduce the abundance of Clostridiaceae, *Prevotella*, and *Streptococcus*, and increase gram-negative bacteria like *Escherichia coli*. *Prevotella* and *Escherichia coli* were both mucus-degrading microbiota (24, 25).

In intestinal epithelial cells, berberine could play a similar role to metformin. Berberine targeted intestinal microbiota could downregulate TLP2/4 and TNF-α protein levels in T2DM mice. The tight junction zo-1 protein staining strongly marked the cytoplasm of intestinal epithelial cells and made the brush border markers uniform distributed in diabetic mice. Berberine also reduced the serum LPS level and increase the height of the intestinal mucosa in diabetic mice (26). On the whole, the characteristic of berberine on gut flora is that the abundance of fiber-degrading bacteria is higher than that of mucus-degrading bacteria. In addition, berberine microbiota has the potential to enhance the function of islet β-cells. Treatment with berberine alone on mice could decrease TG level, while HDL showed the opposite tread (23). In clinic, the decrease in TG/HDL ratio not only predicted dyslipidemia, but also indicated the occurrence of insulin resistance to some extent. Increasing the TG/HDL ratio implied that the drug had the effect of inhibiting insulin resistance. Studies revealed that Clostridiaceae, *Prevotella* and *Streptococcus* were positively correlated with the homeostasis model assessment of insulin resistance (HOMA-IR) index (25). Lowing HOMA-IR index could alleviate insulin resistance in patients with T2DM.

Stilbene

Fecal microbiota of mice receiving resveratrol (RSV) flora transplantation was characterized by increased abundance of fiber-degrading bacteria *Bacteroides*, *Blautia*, *Parabacteroides*, *Ruminococcus* and *Bifidobacterium*. The abundance of *Enterococcus faecalis*, Proteobacteria and mucus-degrading bacteria *Akkermansia muciniphila* decreased (27, 28). The existence of a large number of Proteobacteria indicated that the gut flora is in an imbalanced state (29).

Compared with HFD mice, mice treated with RSV flora not only had more complete intestinal morphology and lower intestinal permeability under a fluoroscopy electron microscope, but also showed upregulated mRNA levels of the key markers of the intestine integrity zo-1, zo-2, occludin, claudin-1 and JAM-A. Relmβ secreted by goblet cells was beneficial for enhancing intestinal barrier function and integrity. The RSV flora could significantly elevate the mRNA expressions of major mucin genes (MUC1, MUC2, MUC3) and transcription factors (Relmβ, Reg3γ) in mice, which were close to normal levels. RSV flora could also reduce the serum levels of inflammatory factors IL-6, IL-16, IL-1β, TNF-α and low-grade inflammation marker MCP-1 to close to normal levels (27). The previous results suggest that RSV flora can reinforce intestinal structural integrity and mucosal barrier function, reduce the occurrence of inflammation and affect islet β-cells function. Experiments illustrated that transplanting resveratrol-treated mice fecal flora into HFD mice could improve the blood glucose homeostasis and insulin resistance (30). Indeed, the fasting blood glucose decreased, hyperinsulinemia improved, and the area under the curve in oral glucose tolerance test and insulin tolerance test decreased significantly. The previously described illustrated that resveratrol-induced fecal microbiota could reduce blood glucose level and improve insulin sensitivity. In addition, the HOMA-IR index value of HFD mice was twice that of mice received fecal transplantation from resveratrol-treated mice (27).

C7N-aminocyclic alcohols

In obese mice, acarbose treatment could increase the content of butyrate and the abundance of fiber-degrading bacteria *Bacteroides*, *Blautia* and *Bifidobacterium*, while the number of *Desulfovibrio*, *Ruminiclostridium* decreased (31). In T2DM patients, the abundance of *Faecalibacterium*, *Prevotella*, *Bifidobacterium longum*, *Lactobacillus gasseri* increased. The number of *Enterococcus faecalis* and mucus-degrading bacteria *Clostridium* abundance reduced (32, 33).

Acarbose also has the potential to regulate the imbalance of the flora. Due to lack of relevant research, the relationship between acarbose-regulated flora and leaky gut is unknown. However, evidence revealed that the acarbose had the effect of enhancing pancreatic islet β-cell function. The level of glycosylated hemoglobin decreased in patients treated with acarbose, which was

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associated with increased abundance of *Bifidobacterium longum* and *Lactobacillus gasseri*. Decreasing the abundance of *Enterococcus faecalis* resulted in a decrease in LPS level, thus preventing the progression of chronic low-grade inflammation and systemic insulin resistance (34, 35).

Discussion

It is well known that a long-term high-fat/low-fiber diet can easily induce the occurrence of T2DM. Due to the lack of fiber intake, patient's flora is out of balance and characterized by the predominance of pathogenic and mucus-degrading bacteria. Over time, a large number of mucus-degrading bacteria obtain energy to survive by degrading the mucus layer of the intestinal epithelium. LPS, produced by gram-negative pathogenic bacteria in intestine, can transfer through the intestinal epithelium into intestinal cells and even in peripheral tissues (adipose, skeletal muscle, liver), causing severe metabolic inflammation. Living bacteria was also detected in blood and omental, subcutaneous and mesenteric adipose tissue in diabetic patients. Both LPS and living bacteria can directly promote the M1-like polarizaiton of macrophages in visceral adipose tissue and even islets, causing systemic insulin resistance and functional decline of β-cells.

Whether it is diet therapy or drug intervention, intestinal flora is an important site to alleviate the progression of diabetes, which is closely related to metabolic inflammation, insulin resistance and islet function. Experiments showed that changes in dietary conditions could cause rapid fluctuations in the abundance of the flora. For example, the abundance of *Akkermansia muciniphila*, *Bacteroides ovatus*, *Bacteroides caccae* and *Eubacterium rectale* changed significantly when the mice equipped with human sequencing flora were subjected to daily fiber-free/fiber-rich alternating diet (4). However, the adjustments to the diet require personalized customization, which will not be discussed in this review. The experiment observed that the mucus layer of the intestinal epithelium of gram-free mice was thinner than that of normal mice, indicating that microbial colonization is a necessary factor for maintaining the thickness of the mucus layer. The serum levels of LPS and TNF-α decreased in obese mice receiving antibiotic treatment, suggesting that flora regulation can improve the metabolic inflammatory state. In summary, as the mucus layer of intestine is damaged, some targeted drugs would be the first choice to modulate the imbalanced flora.

This review mainly focuses on the drugs against T2DM by regulating intestinal microecology and flora. For metformin, its therapeutic effects and adverse reactions are involved in microorganisms. The analysis of intestinal genome samples of patients with T2DM after taking metformin showed a significant increase in the abundance of *Escherichia*. With the increase of the abundance of *Escherichia coli*, the increase in virulence factor LPS will lead to metabolic inflammation and an increase of white blood cell count. In addition, the increased hydrogen production by *Escherichia coli* promoted the production of propionate and butyrate to maintain blood sugar steady state, but it might also facilitate the synthesis of hydrogen sulfide by sulfate-reducing bacteria, causing adverse reactions such as abdominal distension and intestinal discomfort (18). After metformin treatment, increased *Akkermansia* used glycosylated proteins in the epithelial mucus layer as the main carbon and nitrogen sources to produce SCFAs, especially acetate and propionate, during the fermentation process. Some researchers believed that *Akkermansia* was beneficial to maintain the functional integrity of the intestinal mucosa, that could be used to explain the effect of metformin on improving glucose tolerance. The amount of *Akkermansia* in T2DM is controversial. Previous experiments suggested that the abundance of *Akkermansia* decreased in patients with T2DM. *Akkermansia* could strengthen the integrity of the intestinal epithelium by adhering to the intestinal epithelium without causing inflammation in the body. In order to study the direct effect of *Akkermansia*, HFD mice were given living *Akkermansia* orally and showed marked increase in the thickness of the mucus layer and a decrease in the incidence of endotoxemia. In contrast, a metagenomic study illustrated that *Akkermansia* abundance in patients with T2DM elevated. As far as I am concerned, I agree with the latter conclusion. First of all, most of the intestinal flora in the human are identified through genomics rather than cultured *in vitro*. Therefore, whether *Akkermansia*, existing in the complex human environment, can play the previously mentioned role remains to be further studied. Secondly, it is assumed that *Akkermansia in vivo* is beneficial to maintain the integrity of intestinal epithelium and improve intestinal permeability. The mechanism of muciniphila-derived EVs induced AMPK phosphorylation in a dose-dependent manner, resulting in upregulation of occludin expression in the intestinal epithelium, and ameliorating the loss of barrier integrity induced by LPS (36). However, there are more than one mechanism for cunning pathological microorganisms to increase intestinal permeability, and the increase in *Akkermansia* abundance

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cannot completely prevent leaky gut. Finally, the flora of T2DM patients is characterized by an increased abundance of most gram-negative pathogenic bacteria. The number of *Akkermansia* belonging to the genus gram-negative bacteria is far less than that of pathogenic bacteria. The latest research indicated that the presence of living bacteria detected in subcutaneous adipose tissue of T2DM patients further confirmed the previous conclusion. In addition to metformin, berberine can also increase the abundance of *Akkermansia*. *Sutterella*, *Allisonella*, *Akkermansia*, *Bacteroides* and *Paraprevotella* that existed in healthy male volunteers before the drug intervention were participants in the gastrointestinal side effects after the intervention, leading to an increase in the incidence of gastrointestinal adverse reactions (37). *Akkermansia* and *Bacteroides* in patients are known in T2DM patients, and the drugs mentioned in the review all have different degrees of gastrointestinal adverse reactions.

In summary, drug-targeted flora could correct the imbalance of the gut flora in T2DM patients and alleviate the leaky gut, so as to improve insulin resistance and indirectly enhance the function of islet β-cells. At present, with the exception of metformin, the relationship between the flora regulated by other drugs and adverse reactions is not clear. Moreover, the powerful evidence used to prove leaky gut was the presence of living bacteria in adipose tissue. Whether the drug-regulated flora can reduce the number of live bacteria in adipose tissue needs to be proved by further research. The code of the relationship between the drug and the flora requires a lot of experiments to unravel, which can then minimize the incidence of adverse reactions as the drug exerting a therapeutic effect.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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