Progress of α-Glucosidase Inhibitor in Mulberry Leavesand Its Hypoglycemic Effect

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Keywords: α-glucosidase inhibitor; mulberry leavesand; hypoglycemic effect.

Abstract: Diabetes has become one of the serious threats to human health. At present, Clinical drugs for diabetes treatment mainly include insulin, metformin, sulfonylurea and α-glucosidase inhibitor.The α-glucosidase inhibitor have attracted much attention for their unique advantages in the treatment of type Ⅱ diabetes.Natural Chinese herbal medicine has a history of more than 2000 years in the treatment of diabetes, and has rich clinical experience, so it is developed from natural plant materials α- Glucosidase inhibitors are of great significance.Mulberry leaves contain a variety of active substances such as flavonoids, polysaccharides, alkaloids and so on, which have inhibitory effect α- the activity of glucosidase can slow down the rise of blood sugar.Therefore, the treatment of diabetes has been recorded since ancient times.The paper reviewed the main active components of mulberry leaves and its research progress as an inhibitor of α- glucosidase and the mechanism of hypoglycemic action.

1. Introduction

Modern research shows that mulberry leaves contain a variety of active substances such as flavonoids, polysaccharides, alkaloids, etc., with various pharmacological effects of inhibiting blood sugar rise, lowering blood lipid and anti-aging[1].As one of the world's largest health problems in the 21st century, diabetes has been listed as the four major types of non-communicable diseases, and especially Type 2 diabetes is the most common.α- glucosidase inhibitors can reduce the postprandial hyperglycemia by delaying the absorption of carbohydrate in the intestine, and have its unique advantages in controlling blood glucose fluctuation and preventing and treating diabetic complications.[2]At present, α-glucosidase inhibitors used in clinical practice are only Acaporose, Voglipcan and Maglitazol. They have a complex preparation process, high production cost and obvious side effects, which is not conducive to long-term use.However, the substances extracted from plants have the characteristics of mild action, small side effects, multi-component, multi target and multi effect. Mulberry leaves have been used in the treatment of diabetes since ancient times, and its active ingredients such as polysaccharide, alkaloids and flavonoids have inhibitory activities against α-glucosidase. , especially its 1-deoxywiljicin is a strong inhibitor ofα-glucosidase.Therefore, a large number of studies have shown that mulberry leaves have a variety of pharmacological effects such as inhibiting blood sugar rise, lipid-lowering and anti-aging.[3]Study on mulberry leaves α- glucosidase inhibitors, as well as their regulation and treatment of diabetes, have important research value for mulberry leaf resource development and new diabetes drug development.

2. α-glucosinase inhibitors in mulberry leaves

There are many kinds of active ingredients in mulberry leaves, among which mulberry leaf polysaccharide, flavonoids and alkaloids have medicinal effects.They have antioxidant, antihypertensive, hypoglycemic and antibacterial activities. Among them, the hypoglycemic activity is mainly realized by lowering blood lipid, resisting oxidation, and inhibiting the apoptosis of β cells on the islet,and the inhibition of α-glucosidase is an important pathway.

2.1 Mulberry leaf polysaccharide (MLP)

Plant polysaccharides is a carboxyl or aldehyde polymer formed by a number of monoglyaccharide molecules through polymerization and dehydration of glycoside bonds.Mulberry leaves contain a large number of polysaccharides, which are mainly composed of galactose, glucose,rhamnose, pectinose, xylose and mannose.[4]Extensive research has shown that,MLP has Hypoglycemia, antioxidant, immunomodulation, anticoagulant, competitive inhibition of pancreatic lipase.[5][6][7]Ji Tao et al. [8] separated and purified the active ingredient extract of mulberry leaves and established the mode of α-glucosinase-Inhibitors using sucrose as a glucosidase , it is found that the components in mulberry leaves interact with each other. The results showed that the Inhibition of α-glucosinase with the compatibility of mulberry leaf polysaccharide and alkaloids was greater than that of acarbose combined with alkaloids, even if the inhibitory effect of mulberry leaf polysaccharide alone was less than that of acarbose.Han Aizhi et al. [9] found that the drug mulberry leaf polysaccharide had an obvious α-glucosidase inhibitor at the concentration of 0.25~2mg/ml, and had a dose-effect relationship.Cui hanzhao [10] isolated and purified crude polysaccharides from mulberry leaves, and obtained two components with glucosidase inhibitory effect. One group was composed of arabinose, glucose and galactose, and the other group was composed of rhamnose, arabinose, fructose, glucose and galactose.In the study of in vitro model, it was found that the activity of polysaccharides had a dose effect relationship.

2.2 Alkaloid

Alkaloids are nitrogen-containing alkaline compounds present in nature, most with complex ring structure and biological activity.Mulberry leaves are rich in alkaloids such as 2-O-α-D-galactopyranosyl-1-deoxynojirimycin,Gal-1-DNJ 1-deoxyrijimycin and its derivatives,Fagomine,3-epi-fagomine,calystegine B2,1,4-dideoxy-1,4-imino-D-arabinitol and its derivatives etc. [11][12] Among them, 1-deoxynojirimycin(1-DNJ) is a potent α-glucosidase inhibitor endemic to mulberry leaves.Yagi et al. Found the natural DNJ in mulberry root bark for the first time, and then found that DNJ also existed in mulberry leaves and insects feeding on mulberry leaves.The structure of 1-DNJ is similar to that of D-glucose. The difference is that the protonated N atom replaces the O atom of glucose.It can bind with carboxylic acid ions in the active center of glucosidase through electrostatic interaction, thus competitively inhibiting the binding of glucosidase with glucose.However, because 1-DNJ is similar to glucose, it can also be absorbed into the blood after entering the small intestine and quickly excreted through the urine, so it cannot stay in the body for a long time.[13]In 2015, Su WeiLei et al. [14] found that there was no significant difference in the level of postprandial blood glucose between the oral 1-DNJ in mice group and the blank group at $P < 0.01$ and 2 h after $P < 0.05$, so DNJ derivatives were generally used for hypoglycemic treatment, and now 1-DNJ hydroxylated derivatives have been used as hypoglycemic drugs for the treatment of type II diabetes, such as miglitol.

2.3 Flavonoid

Flavonoids are another important active ingredient in mulberry leaves, containing about 0.01~0.03 of dry heavy mulberry leaves.The reported flavonoids in mulberry leaves include chlorogenic acid, rutin, Isoquercitrin, astragalin and quercetin[15].The main extraction methods of Flavonoids from mulberry leaves are ethanol extracting, supercritical carbon dioxide extraction, microwave extraction and so on.In production, the macroporous resin adsorption method is used to purify flavonoids from mulberry leaves.

The hypoglycemic mechanism of Flavonoids from mulberry leaves is to enhance the activity of hexokinase and SOD in liver, improve antioxidant capacity and promote insulin secretion. [16]It can also reduce the content of free fatty acids in serum, enhance the expression of protein kinase mRNA and protein in liver, and inhibit adenylate kinase 2 to achieve anti diabetic effect[17]and through inhibition α- glucosidase expression to reduce blood sugar.Hu Jingyi et al. [18] prepared and purified the components of mulberry leaves and in vitro inhibition test of α- glucosidase with each component

showed that the inhibition rate of flavonoids was higher than that of polysaccharides and lower than that of alkaloids.[18]

3. Screening of α- glucosidase inhibitors from mulberry leaves

In recent years, the screening methods of natural α- glucosidase inhibitors have been greatly developed. In general, it includes in vitro screening and in vivo screening.In vitro screening models mainly include 5 kinds:

(1) Screening model with4-nitrophenol- α- D-glucopyranoside as the substrate: pNPG was decomposed into pNP under the action of glucosidase, and the product content was detected by adding inhibitors.[19]

(2) Screening model with starch, sucrose and maltose as the substrate:It has the same principle with the screening model with pNPG as a substrate, but the false positive rate is lower.

(3) Microhole plate screening model:The 96 microhole plate replaced the test tube as the screening carrier to achieve high-throughput screening.

(4) screening model of immobilized enzymes:The N-terminal immobilization of α-glucosidase makes the binding of enzyme and substrate limited, which can better simulate the transmembrane situation of enzyme in vivoand and can be used to evaluate the effect of inhibitors in vivo.[20][21]

(5) Caco-2 cell screening model:Using Caco-2 cells which are similar to human intestinal epithelial cells in function and structure and using sucrose as substrate.Caco-2 cells were cultured by adding the test substance to the lumen of Caco-2 cells then the inhibitor effect was calculated from the concentration of free glucose on both sides of the cells.[22]

Some new vitro screening models have been developed in recent years.They achieve fast and efficient high-throughput screening of α-glucossidase inhibitors using new techniques.For example, the TLC method can quickly obtain the compound activity and the corresponding position. Momodels such as HPLC, CE and LC-MS perform activity analysis and screening.

In vivo screening mainly through the establishment of diabetic animal model, compared with the changes of animal blood glucose after administration to screen inhibitors.The virtual screening method can also be used to screen inhibitors in vitro by using Autodock 3.0.5 in the environment of WISDOM.[23][24]

4. Hypoglycemic mechanism of α-glucossidase Inhibitors in mulberry leaves

4.1 Promote the repair of insulin β cells

Number of islets β cells is essential for maintaining islet function. The Bcl-2 gene family and the caspase family are the genes that control apoptosis.ZHANG Y et al.[25] found that Bcl-2 mediated cytochrome C release through the mitochondrial pathway, thus inhibiting caspase-3 release and apoptosis.Further studies showed that MLPII treatment for Rats with Diabetes Mellitus can up-regulate the expression of Bcl-2 protein,downregulation of Bcl2-related X (Bax) and caspase-3 protein expression in promoting apoptosis in islet cells. Moreover, MLPII significantly restored the Pancreas duodenum homeobox-1 (PDX-1) protein nuclear positioning, Increased m RNA and protein expression of islet cell PDX-1 and its downstream target glucose transporter 2 (GLUT2) and glucose kinase (GCK) in diabetic rats, improving the secretion capacity of insulin β cells.

4.2 Reducing oxidative stress and protecting islet cells

Oxidative stress refers to the imbalance between the production of reactive oxygen species (ROS) and reactive nitrogen (RNs) in the body, which leads to the excessive production of ROS and RNS and destroys protein and nucleic acid organisms.Oxidative stress not only interferes with insulin signal transduction and induces insulin resistance, but also damages islets β Cells, resulting in decreased insulin secretion and β Apoptosis.Ren Jiuchun [26] found that compared with the diabetic model group, the contents of 8-hydroxy-2-deoxyguanosine (8-hydroxy-2-deoxyguanosine) and Malondialdehyde concentration (MDA) in the pancreas of MLP treated rats decreased by 70.53% and 54.17%

respectively.MLP can significantly increase the activities of SOD, CATand GPx in the pancreas of diabetic rats. It is speculated that MLP can reduce the oxidative stress injury in the body tissue of diabetic rats, especially in the pancreas tissue.

4.3 a-glucosidase inhibition activities

Wu Hao [27] found that DNJ had the same inhibitory effect on maltase activity as acarbose.It has obvious inhibitory effect on maltase activity in a dose-dependent manner.In the system, DNJ showed a mixed inhibition of maltase, which was dominated by noncompetitive inhibition.The hydrogen atoms of 2-OH, 3-OH, 4-OH, 6-OH and - NH groups in DNJ structure can form hydrogen bonds with catalytic related amino acid residues, which play a synergistic role with hydrophobic interaction, thus interfering with the binding of substrate and maltase, and eventually leading to the inhibition of maltase activity.DNJ can also induce the conformation change of maltase, inhibit the polymerization and unfolding of maltase.

4.4 Regulation of insulin signaling pathway

AMPK is a protein kinase, its activation can enhance glucose uptake, fatty acid oxidation and insulin sensitivity.[28]PI-3K is a key enzyme in insulin signal transduction.Akt is a downstream molecule of PI-3K. Activated Akt can promote glycogen synthesis and glucose transport, which is the key molecule of insulin's ultimate effect.Fang Fei et al. [29] found that MLE could significantly increase AMPK α Thr^{172} phosphorylation level but for the total AMPK α. Akt protein expression and AKT Ser^{473} phosphorylation were not affected.So by adjusting AMPK α Phosphorylation is one of the important mechanisms for MLE to improve glucose uptake in insulin resistance. MLE is independent of insulin.The overexpression of JNK in islet tissue can increase the apoptosis of islet cells, and then block the signal transduction of insulin synthesis, hinder the signal transduction of insulin receptor, damage the synthesis of insulin, finally make the body produce insulin resistance, affect the absorption and utilization of glucose, and produce hyperglycemia symptoms.Liu Yinghui et al. [30] found that mulberry leaf polysaccharides and flavonoids can promote glucose absorption to a certain extent, effectively reduce JNK, IRS-1 gene and protein expression, enhance PDX-1 gene and protein expression, and significantly inhibit JNK signaling pathway.

5. Problems and Prospects

At present, diabetes has become a major human disease due to excessive nutrition. Many drugs for the treatment of diabetes in the market need long-term medication with high cost and large side effects. Now natural drugs from plants are needed. Mulberry leaves are easy to obtain and their extracts can be obtained through a large number of microbial products, which has a great prospect.nowadays, most of the studies on the active components of mulberry leaves only stay in the stage of in vitro activity screening and animal experiments, and only 1-DNJ derivatives have entered the market. In addition, there is a lack of toxicological research and further separation and purification of the active components of mulberry leaf extract. In the future, it is necessary to carry out clinical research on the specific components of mulberry leaf and develop its market application.

References

[1] Wang Yawen, Duan Xiaofeng, Cheng Xiaoxiao, Zhang Miaoxin, Li Li, Zhao Ding. The regulating effect of mulberry leaf and its extract on intestinal function [J]. Journal of Chinese Pharmaceutical Sciences,2020,29(11):780-792.

[2] Day Hossain, Abhishek Kumar Das, Sumit Ghosh, Parames C. Sil,An overview on the role of bioactive α-glucosidase inhibitors in ameliorating diabetic complications,Food and Chemical Toxicology,Volume 145,2020,111738,ISSN 0278-6915

[3] Bisma Jan, Rabea Parveen, Sultan Zahiruddin, Mohammad Umar Khan, Sradhanjali Mohapatra, Sayeed Ahmad,Nutritional constituents of mulberry and their potential applications in food and pharmaceuticals: A review,Saudi Journal of Biological Sciences,2021,ISSN 1319-562X,

[4] Cheng Ying, Zhao Jun. determination and component analysis of polysaccharide in mulberry leaves [J]. Chinese Journal of clinical pharmacology, 2017,33 (18): 1803-1805 + 1809

[5] Guo Mudan, Hu Guang. Extraction technology and pharmacological action of mulberry leaf polysaccharide [J]. Journal of Chongqing University of Technology (NATURAL SCIENCE), 2020,34 (03): 205-209 + 239

[6] Chen Yongli, Huang juntong, Wang Ling, Yao Kai, Jia Dongying. Chemical composition of Polysaccharides from mulberry leaves and their inhibitory effect on pancreatic lipase [J]. Food science and technology, 2021,46 (03): 162-166

[7] Xiaolan Chen, Haifeng Yang, Jiping Jia, Yu Chen, Jing Wang, Haifeng Chen, Chunmao Jiang,Mulberry leaf polysaccharide supplementation contributes to enhancing the respiratory mucosal barrier immune response in Newcastle disease virus–vaccinated chicksPoultry Science,Volume 100, Issue 2,2021,Pages 592-602,ISSN 0032-5791

[8] Jitao, Sushulan, Guo Sheng, et al. Study of multicomponent efficacy interaction of mulberry leaves based on α-glucosinase inhibitory activity [J]. Chinese Journal of Traditional Chinese Medicine, 2016,41 (11): 1999-2006

[9] Han Aizhi, Wang Lijun, Jia Qinghua, et al. Optimization of extraction process and hypoglycemic activity of Polysaccharide from Morus alba leaves [J]. Brewed in China, 2017, 36 (8): 139-143

[10] Hanzhao. Extraction, separation, purification and activity of Polysaccharide from mulberry leaves of Xinjiang [d]. Tarim University, 2019

[11] Shen X Y, Song M, Hang T J. Identification and characterization of alkaloids in mulberry leaf by hyphenated LC-MS techniques [J]. Chinese Journal of Pharm Anal, 2016, 36: 1737-1744.

[12] Shang L, Ouyang Z, Zhao M, et al. HPLC-MS-MS Analysis and Determination of Alkaloids of Mori Folium[J]. Chinese Journal of Experimental Traditional Medical Formulae, 2014, 20: 47-51.

[13] Hu Xueqin. Hypoglycemic and lipid-lowering mechanism of 1-deoxynojirimycin from mulberry leaves and screening of its producing bacteria [D]. Hefei University of technology, 2017

[14] Su WeiLei, Yang Jing, Sheng LinBO, et al. Drug release rate and hypoglycemic effect of mulberry milk and 1-deoxynojirimycin sustained release granules [J]. Sericulture science. 2015,41 (1): 120-126

[15] Zhang Yue, Tang Qinyue, Dai Juan, Wei Xiangyi, Yan Jiali, Jiang Yan. Preparation of Flavonoids from mulberry leaves and its antioxidant activity in vitro [J]. China food additive, 2020,31 (09): 47-52

[16] Chen Lingling, Liu Wei, Chen Jianguo, bu Wenlei. Study on the mechanism of mulberry leaf flavonoids regulating blood glucose in diabetic mice [J]. Chinese Journal of clinical pharmacology, 2010,26 (11): 835-838

[17] Huang Liansha, Liu Tonghua, Sun Wen, Xu Guangyuan, Guo Xuan, Li Yingzhen, Chen Shuhui, Zhou Peng. Effect and mechanism of Flavonoids from mulberry leaves on blood glucose level in diabetic rats [J]. Chinese Journal of experimental prescriptions, 2018,24 (16): 152-156

[18] Hu Jingyi, Lei Ling, Liu ASE, Deng Wenlong. Study on the inhibition of α-glucossidase in mulberry leaves [J]. Pharmacology and Clinical of Chinese Medicine, 2006 (06): 44-45

[19] K. Anam, R.M. Widharna and D. Kusrini, 2009. α-Glucosidase Inhibitor Activity of Terminalia Species. International Journal of Pharmacology, 5: 277-280.

[20] Yang, J.; Liu, X.; Sun, S.; Liu, X.; Yang, L. Screening platform based on robolid microplate for immobilized enzyme-based assays.ACS Omega 2017, 2, 5079–5086.

[21] Deng Shiren,Xia Linbo,Zhu Xiamin,Zhu Jiang,Cai Mingchen,Wang Xin. Natural Alpha-Glucosidase Inhibitors Rapid Fishing from Cyperus Rotundus Using Immobilized Enzyme Affinity Screening Combined with UHPLC-QTOF MS.[J]. Iranian journal of pharmaceutical research: IJPR,2019,18(3).

[22] O'Brien Patrick,Corpe Christopher Peter. Acute Effects of Sugars and Artificial Sweeteners on Small Intestinal Sugar Transport: A Study Using CaCo-2 Cells as an In Vitro Model of the Human Enterocyte. [J]. PloS one,2016,11(12).

[23] Nguyen, T.T.H., Ryu, HJ., Lee, SH. et al. Discovery of novel inhibitors for human intestinal maltase: virtual screening in a WISDOM environment and in vitro evaluation. Biotechnol Lett 33, 2185 (2011). https://doi.org/10.1007/s10529-011-0675-8

[24] Thi Thanh Hanh Nguyen,Hwa-Ja Ryu,Se-Hoon Lee,Soonwook Hwang,Jaeho Cha,Vincent Breton,Doman Kim. Discovery of novel inhibitors for human intestinal maltase: virtual screening in a WISDOM environment and in vitro evaluation[J]. Biotechnology Letters,2011,33(11).

[25] ZHANG Y, REN C, LU G, et al. Anti - diabetic effect of mulberry leaf polysaccharide by inhibiting pancreatic islet cell apoptosis and ameliorating insulin secretory capacity in diabetic rats ［J］. Int Immunopharmacol,2014,22(1): 248 - 257

[26] Ren Chunjiu, Sun Yongliang, Li Weiqing, Cui Weizheng, Wang Yanwen, Gao Zhao Ju, Mou Zhimei. Protective effect of mulberry leaf polysaccharide MLPⅡ on oxidative stress of pancreatic tissue in diabetic rats [J]. Silkworm Science, 2017,43 (04): 671-676

[27] Wu Hao. Regulation and inhibition of microbial derived 1-deoxynojirimycin biosynthesisStudy on the mechanism of α- glucosidase[D]. Guangxi University, 2019

[28] Zhang Jingyi, Bao Cui, Li Jing. Research progress of AMPK signaling pathway in diabetic cardiomyopathy [J]. Chinese Pharmacology Bulletin, 2017,33 (11): 1496-1499

[29] Fang Fei, Luo Mingli, Su Nan, Wu Xinrong. Effect of Mulberry Leaf Extraction on Insulin Resistance to HepG2 Cell Gose Intake and Its Molecular Mechanism [J]. Pharmacy, 2012,47 (11): 1452-1456

[30] Yinghui Liu, Xin Mou, Diyi Zhou, Chengmin Shou. Study on the mechanism of effective constituents of Morus alba to improve insulin resistance based on JNK signaling pathway[J]. Chinese Journal of Traditional Chinese Medicine,2019,44(05):1019-1025.