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Review Article

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Cycloastragenol: A Review of Preparation Methods, Structural modifications and Pharmacological Activities

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Abstract Cycloastragenol (CAG), a telomerase activator, is a tetracyclic triterpenoid isolated from *Astragalus membranaceus*. CAG can be prepared by hydrolysis of astragaloside IV (ASI) using three methods: acid hydrolysis, enzymatic hydrolysis and Smith degradation. In addition, the structure of CAG has been modified by chemical modification and microbial transformation, and many modification products have been obtained. According to the study, CAG was shown to possess several biological activities, including anti-aging, anti-cancer, anti-inflammatory, anti-osteoporosis, and anti-neurodegenerative diseases. This paper reviews the preparation methods, structural modifications and pharmacological activities of CAG that have been studied in recent years.

Keywords Cycloastragenol, Preparation Methods, Pharmacological Activities, Structural Modifications

Introduction

Astragalus membranaceus is an important and widely used herb that has been used for over 2000 years and is still used in various herbal formulations. And it has been used as a diuretic, anti-aging, anti-stress, anti-hypertensive, and antibacterial agent. ASI (see Figure 1 for the structure) was considered as the primary critical ingredient in *Astragalus membranaceus* [1]. CAG (molecular formula $C_{30}H_{50}O_5$, molecular weight 490.71, see Figure 1 for the structure), a tetracyclic triterpenoid compound and a secondary metabolite isolated from *Astragalus membranaceus*, which is a glycoside of ASI. Compared with ASI, CAG is more lipid soluble and more easily absorbed transdermally. It has been shown that encapsulation of CAG in phospholipid vesicles enhances the transport and delivery across the skin barrier [2], which can be a new practical approach to make it more bioavailable for transdermal absorption. During the screening of *Astragalus* extracts for active ingredients with anti-aging properties, CAG was found to have pro-cell proliferative activity and promote wound closure by activating telomerase [3]. Since then, CAG has come into the attention of researchers and has become a research hotspot. Nowadays, its preparation methods, structural modifications and pharmacological activities have been further understood. However, there are no comprehensive reviews of the preparation, structural modification and pharmacological effects of CAG, so this paper will review each of these three parts.

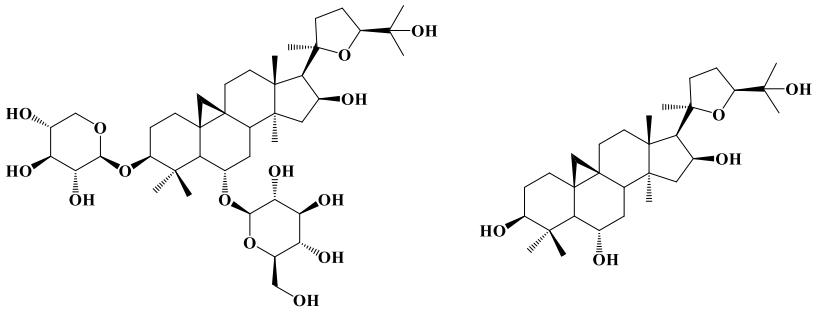

Figure 1. Structures of ASI and CAG

1. Preparation Methods of CAG

CAG is the aglycone of ASI, the structure of which is obtained by linking CAG to one molecule of xylose and one molecule of glucose through glycosidic bonds, respectively. Therefore, the preparation of CAG can be carried out by hydrolysis of ASI, and thus several methods for hydrolysis of glycosidic bonds can be investigated and used. The existing reports on the preparation of CAG are mainly acid hydrolysis, enzymatic hydrolysis and periodate degradation (Smith degradation).

Acid hydrolysis

Since the glycosidic bond has an acetal (ketone) structure, it is feasible for the hydrolysis reaction of ASI to occur under acidic conditions. The commonly used reagents are water or alcohol, and the commonly used catalysts are mainly dilute hydrochloric acid [4] and dilute sulfuric acid. Acid hydrolysis has the advantages of simple process and low preparation cost, but the structure of CAG is unstable in acidic conditions and easily converted to astragenol, which limits its application to industrial production.

Enzymatic hydrolysis

Enzymes are proteins or RNAs produced by living cells that are highly specific and catalytically efficient for their substrates. β-xylosidase and β-glucosidase are combined to efficiently remove the two glycosyl groups of ASI, respectively. Several enzymes with the ability to hydrolyze the glycosidic bonds of ASI have now been identified and reported. It has been shown that ASI can be biotransformed to CAG using *Bacillus* sp. LG-502, attributed to the presence of glucosidase and xylosidase within this bacterium, and that the activity of glucosidase is much greater than that of xylosidase under experimental conditions [5]. A 1509bp gene for a heat-stable β-xylosidase (xln-DT) was cloned from *Dictyoglomus thermophilum* and expressed in E. coli BL21. The β-xylosidase Xln-DT showed optimal activity at pH 6.0 and 75°C and was heat stable and highly tolerant to xylose [6]. The researchers also demonstrated that two functional β-glucosidases Dth3 and β-xylosidase Xln-DT from *Dictyoglomus thermophilum* could be heterologously expressed in E. coli BL21. They remained relatively stable under stimulation at 75 °C and high sugar concentration. The optimal reaction conditions obtained were as follows, 75 °C, pH 5.5, 1 U of Dth3 and 0.2 U of Xln-DT. Under these conditions, the corresponding molar conversion of ASI to CAG was as high as 94.5% [7]. Two enzymes (β-xylosidase: Xyl-T; β-glucosidase: Bgcm) that have been applied to achieve efficient production of CAG have been developed. They have broad pH stability (Xyl-T: pH 3.0-8.0; Bgcm: pH 4.0-10.0), high catalytic efficiency (k_{cat}/K_m: 0.096 $mM^{-1}s^{-1}$ (Xyl-T) and 3.08 $mM^{-1}s^{-1}$ (Bgcm)), and optimal catalytic temperature (50 °C) [8], and are two promising candidates for application in the industrial production of CAG, which is attributed to the fact that it increases the CAG yield to 96.5%. Other types of enzymes need to be studied more to demonstrate their application in the hydrolysis of ASI and to improve the hydrolysis rates and product yields. Enzymes are efficient, specific, have mild reaction conditions, and have high product purity. However, at the same time, enzymes need suitable reaction temperature and pH, so the newly investigated enzymes should have a good thermal property and a wide and safer range of pH.

Periodate degradation (Smith degradation)

Periodate degradation, also called as Smith degradation, refers to the specific hydrolysis of the polysaccharide alcohol obtained by the reduction of periodate oxide by borohydride in a dilute acid environment. Its specificity is demonstrated by the fact that dilute acid hydrolysis selectively breaks the glycosidic bonds broken by periodate, whereas periodate oxidation is a selective oxidation reaction, i.e., it can only act at even di- or even tri-hydroxyl groups in the polysaccharide molecule. This degradation process is shown in Figure 2. A researcher obtained the following experimental conditions for Smith degradation with high yields. ASI was dissolved in 60% MeOH-H_2O solution, oxidized with 5 equiv. $NaIO_4$ for 12 h, followed by reduction with 3 equiv. $NaBH_4$ for 4 h, and finally acidified with 1 M H_2SO_4 at pH 2 for 24 h. Under the optimal conditions, CAG could be prepared from ASI at a yield of 84.4% [9]. This method is suitable for the hydrolysis of glycosides with unstable aglycone. The disadvantages of this method are the tedious operation steps, the need for multiple extractions leading to sample loss and solvent consumption, and the high cost of periodate oxidation products.

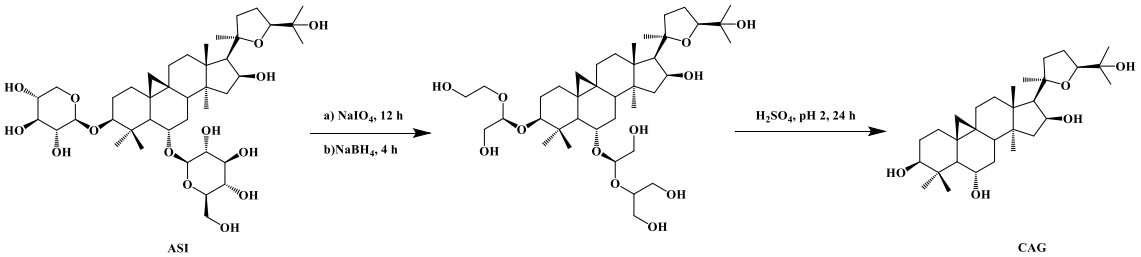

Figure 2: Smith degradation process from ASI to CAG

2. Structural Modifications of CAG

Structural modification is an important method to enhance the pharmacological activity, attenuate the toxic side effects and improve the physical properties of drugs, and is one of the important ways to obtain new drugs. In this paper, the researches of CAG in both chemical modification and microbial transformation are reviewed.

Chemical modifications

Chemical modification method is to modify the structure of a drug by using chemical reaction. Generally, after structural modification, some new drugs can be obtained. These new drugs can become more active in certain activities compared to the original drug, and can even acquire new other physiological activities.

In 1987, two structural modifications of CAG were reported for the first time by a grope of researchers [4]. One was to make CAG ring-opening derivative, astragalosol **1**, by ring-opening reaction of cycloalkanes; the other was to obtain CAG acetylated derivatives **2** by using acetylation reaction of acetic anhydride. The chemical equations of the above two methods are shown in Figure 3.

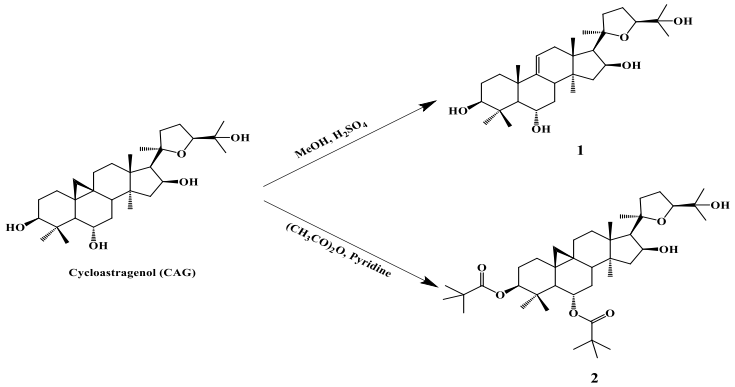

Figure 3. Synthesis of the ring-opening derivative and the acetylated derivative of CAG

In 2012, the structure of CAG was modified by Baeyer-Villiger reaction to obtain a framework of 3,5-seco-4,25,26,27,28,29-hexanor-triterpenoid [10]. The reaction principle was based on the acid digestion of CAG to compound **1**, followed by further oxidation to compounds **3** (20 ℃, 64% yield) and **4** (20 ℃, 72% yield) using Jones regent and different reaction temperatures, followed by oxidative rearrangement of compound **4** to obtain ester derivatives **5** and **6** using m-CPBA. The above reaction process is shown in Figure 4.

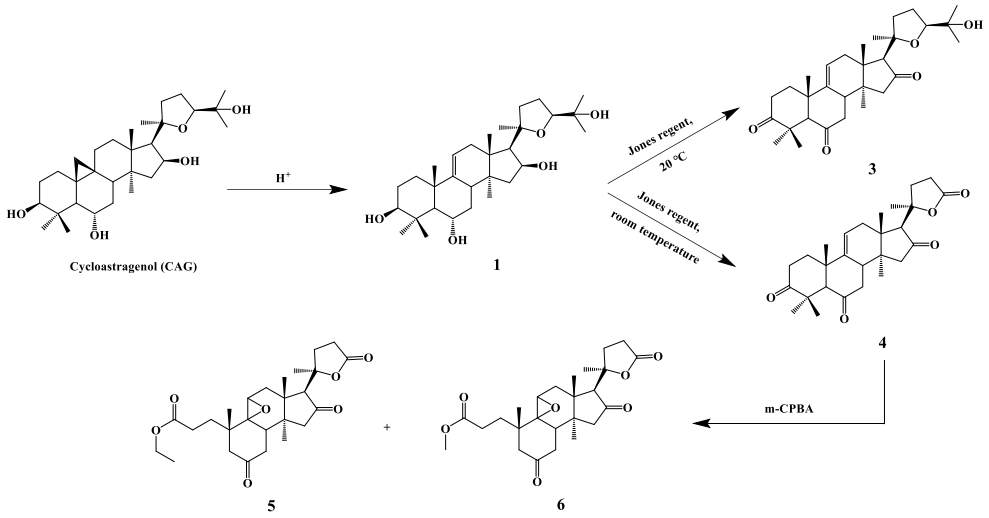

Figure 4. Synthesis of CAG Ester Derivatives through Baeyer-Villiger Oxidation

In 2018, some researchers have structurally modified CAG and its acid-hydrolyzed derivative **1** by oxidation, condensation, alkylation, acylation and elimination reactions. The process is shown in Figure 5. This study also reported that derivatives **7-11** all exhibited strong inhibitory activity on NF-κB (nuclear factor kappa-B) signaling, leading to inhibition of NF-κB transcriptional activation and suppression of cell proliferation [11].

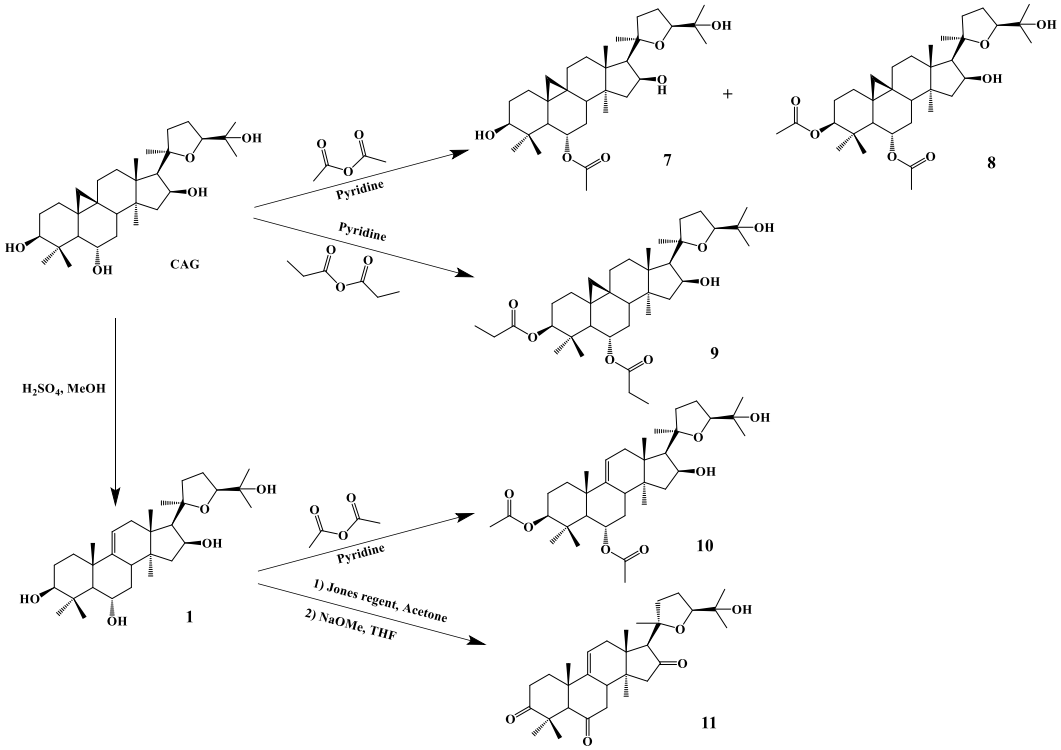

Figure 5. Synthesis of acetylated, propionylated and oxidized products of CAG

In fact, some glycosides of CAG also have specific biological activities. In 2017, a method to synthesize glycosides of CAG was developed [12] and the results have been published in *The Journal of Organic Chemistry*, so they will not be provided in this paper.

Microbial transformation

Microbial transformation uses some enzymes produced by microorganisms in vivo or in vitro during their life activities, thus modifying and transforming the structure of compounds. This method has become an important method to modify the structure of drugs due to its advantages of mild conditions, harmlessness, high efficiency and good regioselectivity, especially it can carry out complex transformations that cannot be accomplished by chemical modification methods. Since the products generated after microbial transformations of CAG are diverse and confusing, the structural formulae of the derivatives will not be provided in this paper.

A method of microbial transformation in *Cunninghamella blakesleeana* (*C. blakesleeana*) NRRL 1369 by ring cleavage (9,19-cyclopropane ring) followed by methyl migration, was first reported in 2010 [13]. This researcher went on to report in 2013 the microbial transformation of CAG using *Glomerella fusarioides* ATCC 9552 and *Mycobacterium* sp. NRRL 3805 for the microbial transformation of CAG. Both of these fungi are capable of catalyzing the hydroxylation reaction of CAG with concomitant dehydrogenation, cyclization and Baeye-Villiger oxidation reactions [14]. Among them, the former mainly catalyzed the dehydrogenation and oxidative ring-opening reactions of CAG to produce two hydroxylated products of CAG. The latter mainly catalyzed the oxidation reaction of CAG to produce one oxidation product of CAG. In 2012, three filamentous fungi, namely *Cunninghamella elegans* (*C. elegans*) AS 3.1207, *Syncephalastrum racemosum* (*S. racemosum*) AS 3.264, and *Doratomyces stemonitis* (*D. stemonitis*) AS 3.1411, were used as tools for the microbial transformation of CAG, yielding 15 metabolites. Among them, *C. elegans* was able to perform hydroxylation reactions on 28- and 29-CH_3 groups. *S. racemosum* efficiently catalyzed rearrangement reactions to form an unusual ranunculane backbone which was further substituted by different side chains at C-19; and *D. stemonitis* performed carbonylation reactions mainly on 3-OH. Specifically, *S. racemosum* also catalyzed an unexpected ring expansion reaction to generate the rare 9(10)α-homo19-nor-cycloartane backbone [15]. In 2015, an investigator of *C. blakesleeana* NRRL 1369 and *Glomerella fusarioides* ATCC 9552 were studied. The enzyme systems of these two fungi can catalyze hydroxylation, cyclization, dehydrogenation and oxidation reactions of CAG leading to structural modification [16]. In the same year, the biocatalysis of CAG by two filamentous fungi, namely *S. racemosum* AS 3.264 and *Alternaria alternata* AS 3.4578, was studied and 16 and 14 metabolites were obtained, respectively, which included ring expansion products, C-12 hydroxylation products, C-3 or C-6 acetylation products, 9,19-ring cleavage and methyl migration products, and astragenol derivatives [17]. In 2018, microbial transformation of CAG by *Mucor racemosus* (*M. racemosus*) AS 3.20 was studied. *M. racemosus* could catalyze ring expansion and epoxidation reactions to form 3β,10β-epoxy- or 6α,19α-epoxy-9,10-seco-cycloartane structures. They can extend the lifespan of Caenorhabditis elegans at 50 μM [18]. In 2019, microbial transformation of CAG by the fungi *Mucor subtilissimus* (*M. subtilissimus*) AS 3.2456 and *Aspergillus oryzae* (*A. oryzae*) AS 3.407 and produced 19 metabolites. *M. subtilissimus* can catalyze hydroxylation and carbonylation reactions while the fragile 9,19-cyclopropane ring remains intact. *A. oryzae* tends to catalyze hydroxylation, acetylation and ring expansion reactions [19]. In the same year, endophytic fungi isolated from *Astragalus* species were isolated and used to study the microbial transformation of CAG. Oxygenation, oxidation, epoxidation, dehydrogenation, and ring cleavage reactions were observed on the cycloartane (9,19-cyclolanostane) nucleus. Among the derivatives, someone has significant activity of telomerase activation [20]. In 2021, some researchers used *Astragalus* species originated endophytic fungi (*Penicillium roseopurpureum*, *Alternaria eureka*, *Neosartorya hiratsukae* and *Camarosporium laburnicola*) on 20(27)-octanor-cycloastragenol , a thermal degradation product of CAG, underwent microbial transformation, including hydroxylation, oxidation, ring cleavage and methyl migration, to obtain a series of derivatives with strong telomerase activity [21].

3. Pharmacological Activities of CAG

Telomerase activation and anti-aging

Telomeres are known to be repetitive "TTAGGG" nucleotide sequences found at the ends of chromosomes, which become shorter as the number of cell divisions increases and are involved in the conservation of cellular DNA. However, its intracellular shortening can cause cellular senescence. Telomerase, an enzyme that acts on telomeres, can slow down or even reverse intracellular telomere shortening, thus protecting cellular DNA from damage and contributing to cell division and proliferation and prolonging life span, making it a popular target for anti-aging.

In 2011, it was shown that telomerase activity in human embryonic kidney HEK293 fibroblasts was increased in a CAG concentration-dependent manner. And phosphorylation of ERK (extracellular signal-regulated protein kinase) was induced by CAG in a time- and dose-dependent manner. cells in a time- and dose-dependent manner in HEK293 cells and HEK-neo keratinocytes. Investigators found that c-Src, MEK (ERK kinase) and the epidermal growth factor receptor, the Src/MEK/ERK pathway, are involved in CAG-induced ERK phosphorylation [22]. In 2014, it was shown that CAG stimulated telomerase activity in human neonatal keratinocytes and rat neuronal cells, and induced CREB (cAMP response element binding) activation, which then induced the expression of tert and Bcl-2. In addition, this study showed that oral administration of CAG for 7 days attenuated depression-like behavior in experimental mice [3]. In the same year, the efficacy and tolerability of CAG in anti-aging was evaluated in 20 volunteers in a drug experiment, and the results showed that these volunteers showed a significant improvement in their visible signs of aging after administration of a preparation containing CAG [23]. Klotho is a functional protein with anti-aging activity represented by lifespan extension. In 2019, it was shown that klotho deficiency reduces telomerase activity by altering the expression of TERF1 (telomeric repeat binding factor 1) and TERT (telomerase reverse transcriptase), leading to impairment of differentiation potential, pluripotency, cellular senescence and apoptosis in stem cells. This suggests that klotho and telomeres regulate the aging process of stem cells through the telomerase subunits TERF1, POT1 (protection of telomeres 1) and TERT using TGFβ (transforming growth factor-β), insulin and Wnt signaling. Also, CAG treatment partially reversed telomerase decline [24]. In 2020, CAG was shown to upregulate TERT but could be blocked by the telomerase inhibitor TMPyP4 [25]. In the same year, CAG was reported to reduce protein expression levels of p16, c-C3, and Bax and upregulate Bcl-2 expression, which protected cells from high-glucose stress-induced senescence and apoptosis. In addition, CAG reversed the high-glucose-induced downregulation of the expression profile of TERT and the shortening of telomere length in a glucose concentration-dependent manner [26]. In summary, CAG has excellent anti-aging activity by targeting increased telomerase activity, however, the signaling pathways and targets of its action need to be further complemented.

Anti-inflammation

In 2015, it was shown that cycloastragenol could inhibit ROS-associated endoplasmic reticulum stress and then suppress TXNIP (thioredoxin-interacting protein)/NLRP3 inflammasome activation and modulate AMPK (adenosine 5'-monophosphate (AMP)-activated protein kinase) activity, thereby improving endothelial dysfunction by suppressing inflammation and reducing apoptosis [27]. In 2017, a study concluded that CAG significantly reduced the expression of CD69 and CD25 on the surface of Con A (concanavalin A)-activated CD3+ T cells and inhibited the proliferation of activated lymphocytes. In addition, CAG significantly inhibited the Th1 cytokines IFN (Interferon)-γ, TNF (tumor necrosis factor), and IL-2, the Th2 cytokines IL (interleukin)-4, IL-6, IL-10, and the Th17 cytokine IL-17 A in Con A-activated lymphocytes, suggesting that CAG has important anti-inflammatory activity [28]. In 2018, it was shown that mRNA overexpression of NLGP3, caspase1, IL-18, and IL-6 was found to be significantly reduced in ISO (isoproterenol)-induced primary cardiac fibroblasts after CAG treatment, suggesting that CAG can effectively inhibit ISO-induced cardiac fibrosis by inhibiting the NLRP3 inflammasome pathway [29]. In 2019, it was shown that CAG dose-dependently reduced the levels of pro-inflammatory cytokines, including IL-1β, TNF-α, and IL-6, in mouse psoriasis skin, serum, and IMQ (imiquimod)-stimulated bone marrow macrophages. In addition, CAG selectively regulated macrophage function by inhibiting the NLRP3 inflammasome complex and suppressed NLRP3 inflammasome-mediated coking effect to ameliorate IMQ-induced psoriasis-like skin

inflammation in mice [30]. Expression of TLR2, IκBα, MyD88 and NF-κB (p65) and the concentrations of TNF-α, IL-1β, and IL-6 were significantly reduced after CAG treatment. The above effects make CAG useful in the treatment of sepsis-induced brain injury [31]. In 2020, it was shown that CAG could significantly reduce MMP (matrix metalloproteinase)-9 activity, prevent degradation of tight junctions, and subsequently improve blood-brain barrier disruption. In addition, CAG was able to significantly upregulate SIRT1 expression in ischemic brains, while reducing p53 acetylation and the ratio of Bax to Bcl-2 in ischemic brains. In addition, CAG inhibited the nuclear translocation of NF-kB p65 and therefore suppressed the mRNA expression of pro-inflammatory cytokines, including TNF-α and IL-1P, and inhibited the activation of microglia and astrocytes in the ischemic brain. The above results suggest that CAG may exert neuroprotective effects in ischemic brain injury by upregulating SIRT1 and inhibiting apoptosis and inflammation in the ischemic brain [32]. In 2021, CAG was demonstrated to bind to the active site of autophagy- related gene 4-microtubule-associated proteins light chain 3 complex, which explains the reduced autophagic flux in asthma caused by CAG. Also, CAG was shown to inhibit the infiltration of inflammatory cells and the secretion of inflammatory cytokines, including IL-5, IL-13, and immunoglobulin E [33].

Anti-cancer

AAA (abdominal aortic aneurysm) is a common and rapidly developing degenerative aortic disease characterized by extracellular matrix degradation, chronic inflammatory infiltration, oxidative stress, and apoptosis of vascular smooth muscle cells. CAG was reported in 2018 to exert a protective effect on AAA by downregulating MAPK (mitogen-activated protein kinase) signaling pathway, thereby reducing inflammation, oxidation, VSMC (vascular smooth muscle cell) phenotypic transition and apoptosis, and expression of MMPs, and increase elastin biosynthesis [34]. In 2022, CAG was shown to inhibit the progression of experimental AAA by a mechanism that includes reduction of MMP-2 activity, protection of elastin, and reduction of calcification [35]. In addition, CAG was shown in 2020 to be potentially beneficial in the treatment of lung cancer through autophagy and the p53/AMPK/mTOR signaling pathway [36]. It has been shown that by inactivation of Src and Janus-activated kinases (JAK1/2) activation, CAG can induce negative regulation of STAT3 phosphorylation at tyrosine 705. This impaired STAT3 protein translocation and its DNA-binding activity, which can further inhibit cell proliferation by causing massive apoptosis. In addition, CAG has been shown to enhance the anticancer effect of paclitaxel-induced gastric tumor cells [37]. But in combination with paclitaxel, more animal studies are needed to assess the safety of its application in humans.

Anti-neurodegenerative diseases

Aβ (Amyloid-beta) is known to be one of the etiologies triggering neurodegenerative diseases. It has been shown that CAG attenuates $A\beta_{1-42}$-induced apoptosis in bEnd.3 cells and increases the expression of tight junction scaffold proteins. CAG also increases the efflux of soluble Aβ across the BBB (blood-brain barrier) by upregulating P-gp and downregulating the RAGE (receptor for advanced glycation end products) expression [38]. In Aβ-injected mouse brains (cortex and hippocampus), the expression of NeuN (neuronal nuclear protein) was significantly reduced. In addition, expressions of Nrf2 (nuclear factor erythroid 2-related factor 2), HO-1 (heme oxygenase 1), p-TrKB, and BDNF were also reduced in parallel. Expression of MAPK, such as p-JNK (phospho c-Jun-N-terminal kinase), p38, and ERK1/2 (phosphoextracellular signal-related kinase), were also downregulated. CAG was shown to reduce or reverse the abnormal alterations in the above indices caused by Aβ. CAG was also shown to reduce activated microglia and inflammatory cytokines, and also had significant modulatory effects on apoptosis and memory impairment [39].

Anti-osteoporosis

GIOP (glucocorticoid-induced osteoporosis) is mainly characterized by reduced bone mineral density and susceptibility to fracture in the presence of glucocorticoid overdose. In 2020, it was shown that CAG significantly increased ALP (alkaline phosphatase) activity, mineralization, RUNX2 (runt-related transcription factor 2), osteocalcin, osteopontin and mRNA of type I collagen in MC3T3-E1 cells and DEX (dexamethasone)-treated

MC3T3-E1 cells [25]. In addition, CAG was also shown to improve the chondrogenic differentiation of hAMSCs (human adipose-derived mesenchymal stem cells) and maintain a stable active chondrocyte phenotype. These findings suggest that CAG is beneficial for cartilage regeneration [40]. In the models of aging-related bone loss induced by D-galactose, CAG improves bone formation, reduces the number of osteoclasts, attenuates bone microarchitectural degeneration, and enhances bone biomechanical properties [41]. A study in 2022 showed that CAG can inhibit osteoclast formation and function by modulating RANKL (receptor activator of nuclear factor-κB ligand)-induced intracellular signaling pathways [42], thus exerting an anti-osteoporosis function.

Other pharmacological activities of CAG

CAG has been shown to improve cardiac dysfunction and remodeling by inhibiting AKT1-RPS6KB1 signaling to promote myocardial autophagy and suppressing MMP-2 and MMP-9 expression, suggesting its potential to treat congestive heart failure [43]. CAG has also been reported to alleviate AMI (acute myocardial infarction) symptoms in rats through regulation by multiple proteins associated with inflammatory regulation (ERK1/2, PLCG1, IKBKG, and ZEB1), cardiomyocyte apoptosis (Bax, COX2, and GSK3β), and myocardial function (RhoA and DSC2) [44]. A study has shown that CAG dose-dependently reduces cytoplasmic lipid droplets in 3T3-L1 adipocytes and stimulates 3T3-L1 preadipocyte calcium influx. Therefore, it could be a potential therapeutic candidate for the alleviation of obesity and hyperlipidemia [45]. FXR (farnesoid X receptor) is a potential drug target for the treatment of NAFLD (non-alcoholic fatty liver disease). CAG was shown to stimulate the transcriptional activity of FXR, which significantly reduces lipid accumulation in the liver induced by a high-fat diet, accompanied by a decrease in blood glucose and protection of the liver [46]. CAG was studied to restore D-galactose-induced circadian rhythm behavioral disturbances in aging mice [47]. CAG was shown to be a TERT activator in human epidermal stem cells, which may act by activating Wnt/β-catenin signaling to interfere with the proliferation and migration of these cells [48]. In addition, the inhibitory effect of CAG on UGTs (UDP-glucuronosyltransferases) was investigated and it was shown that CAG can competitively inhibit UGT1A8 and non-competitively inhibit UGT2B7 [49]. In addition, CAG was reported that can inhibit UGT1A8 by inhibiting opening of mPTP (mitochondrial permeability transition pore) to prevent mitochondrial dysfunction [50]. The pharmacological activities of CAG found so far is not comprehensive and needs to be further investigated in various aspects.

Conclusion

CAG is an activated form of ASI and is widely known since it was shown to be a telomerase activator. It has been found that the conversion from ASI to CAG can be achieved by three feasible methods, namely acid hydrolysis, enzymatic hydrolysis and Smith degradation, compared to enzymatic digestion, which can achieve large-scale industrial production of CAG. In addition, some researchers have carried out structural transformation and modification of CAG by chemical modification and microbial transformation. Microbial transformation can obtain complex structural transformation that cannot be accomplished by chemical modification, and more metabolic derivatives of CAG were obtained, which provides more basis for the development of structural transformation of CAG. In addition, CAG has been shown to have various pharmacological activities, such as anti-aging, anti-inflammatory, anti-cancer, and anti-neurodegenerative diseases. However, for the nature of CAG itself, although CAG has some advantageous activities, it exhibits some toxic effects outside of certain safety limits [51]. It was found that CAG needs to undergo hepatic first pass effect in vivo and its oral bioavailability is low [52, 53], so the better application is dermal administration. In addition, phospholipid vesicle-encapsulated CAG nanoparticles have been successfully prepared, and this drug-loaded nanoparticle can better facilitate the transdermal delivery of CAG [2]. In conclusion, this paper reviews the preparation methods, structural modifications and pharmacological activities of CAG, which provide a certain basis and help for the subsequent research on the related aspects of CAG.

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