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Cycloastragenol: An exciting novel candidate for age-associated diseases (Review)

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Abstract. Cycloastragenol (CAG) is a triterpenoid saponin compound and a hydrolysis product of the main active ingredient in *Astragalus membranaceus* (Fisch.) Bunge. An increasing body of evidence has indicated that CAG has a wide spectrum of pharmacological functions, which are attracting attention in the research community. The aim of the present review paper was to review and elucidate the advanced study of CAG. The focus was on advanced studies of CAG in English and Chinese databases; the literature was collected and reviewed to summarize the latest efficacy, pharmacokinetics and adverse reactions of CAG. Extensive pharmacological effects have been attributed to CAG, including telomerase activation, telomere elongation, anti-inflammatory and anti-oxidative properties; CAG has also been reported to improve lipid metabolism. Clinical research has demonstrated that CAG activates telomerase in humans and ameliorates various biomarkers. CAG is absorbed through the intestinal epithelium via passive diffusion and undergoes first-pass hepatic metabolism. Within a certain dose range, oral CAG is relatively safe; however, underlying mechanisms associated with CAG are not clear, and thus, we should be aware of potential adverse reactions associated with CAG. According to existing studies and clinical trials, CAG is safe and has broad application prospects. However, further studies are required to fully understand its efficacy and potential adverse reactions, and to ensure the proper use of CAG is applied to treat diseases clinically.

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Abbreviations: CAG, cycloastragenol; TCM, traditional Chinese medicine; TERT, catalytic reverse transcriptase enzymes; TERC, telomerase RNA component; IPE, inositol trisphosphate; cAMP, cyclic adenosine monophosphate; Akt, protein kinase B; c-Src, proto-oncogene tyrosine-protein kinase; ERK, extracellular signal-regulated kinase; MEK, ERK kinase; JAK2, Janus kinase 2; STAT5b, signal transducer and activator of transcription 5b; HIV, human immunodeficiency virus; IPF, idiopathic pulmonary fibrosis; AIDS, acquired immunodeficiency syndrome; AG, astragaloside IV; CCI, cyclocephaloside I; CCE, cyclocanthoside E; FXR, Farnesoid X receptor; NAFLD, non-alcoholic fatty liver disease; MCD, methionine- and choline-deficient L-amino acid diet; NASH, non-alcoholic steatohepatitis; ROS, reactive oxygen species; T-AOC, total antioxidant capacity; T-SOD, total superoxide dismutase; HYP, hydroxyproline; MDA, malondialdehyde; ER, endoplasmic reticulum; IRE1, inositol-requiring enzyme 1; TXNIP, thioredoxin-interacting protein; NLRP3, NLR family pyrin domain containing 3; AMPK, 5'AMP-activated protein kinase; BMD, bone mineral density; NOAEL, no-observed-adverse-effect level; UGT, UDP-glucuronosyltransferase; GRAS, generally recognized as safe

Key words: *Astragalus membranaceus* (Fisch.) Bunge, cycloastragenol, telomerase activator

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1. Introduction

Astragalus membranaceus (Fisch.). Bunge is a plant used in traditional Chinese medicine (TCM), with known 'Qi tonifying' adaptogenic effects, as documented in the Chinese Materia Medica. *Astragalus membranaceus* (Fisch.) Bunge has been used in TCM for over 2,000 years and is still being use in various herbal preparations. It is known to enhance immune functions; protect the liver; act as a diuretics; and have anti-aging, anti-stress, antihypertensive, and extensive antibacterial properties (1). Astragaloside IV (AG) is the main compound found in *Astragalus membranaceus* (Fisch.) Bunge while cycloastragenol (CAG), an algycone of AG, is a triterpenoid saponin compound obtained from AG hydrolysis products (Fig. 1) (2).

The Geron Corporation in cooperation with The Hong Kong University of Science and Technology screened natural compounds from *Astragalus membranaceus* extracts. CAG

was identified as an anti-aging compound that promotes telomerase activity and wound closure (3). CAG is currently the only compound known to activate telomerase in humans (4), making it a great prospect for development. This study reviews the efficacy, pharmacokinetics studies, and adverse reactions of CAG, and provides a basis for further study into CAG.

2. Pharmacodynamics

Efficacy

Efficacy base on pre-clinical studies

Telomerase activation. Telomeres are located at the ends of linear chromosomes capped by nucleoprotein structures that consist of tandem repeats of hexameric sequences (TTAGGG in vertebrates) bound by a dedicated set of proteins. Telomeres shorten with every mitotic event. Telomerase is a ribonucleoprotein complex that lengthen telomeres and fundamentally consists of catalytic reverse transcriptase enzymes (TERT) and a telomerase RNA component (TERC). A key function of telomeres is to protect chromosomal ends from fusion and degradation by capping chromosomal ends. Cells recognize critically short telomeres as DNA damage; therefore, the shortest telomere, rather than average telomere length, is critical for cell viability and chromosomal stability (5). CAG activates telomerase, lengthens telomeres, and exerts a variety of pharmacological effects as introduced below (Fig. 2).

Telomerase activation and lengthening of telomeres. Several studies have shown that CAG activates telomerase both *in vitro* and *in vivo*. CAG activates telomerase and lengthens telomeres in a telomerase-dependent way *in vitro*; therefore, CAG decreases the percentage of critically short telomeres and DNA damage in the cell. CAG could activate telomerase in various cell types, including hematopoietic progenitors and mouse embryonic fibroblast haplo-insufficient for the TERC (MEF *Terc*$^{+/-}$) cells. *In vivo*, dietary supplementation of CAG increases TERT expression in tissues such as bone marrow, lungs, heart, brain, and liver. Further, CAG rescued short telomeres in 1- and 2-year old female mice. However, data have shown that dietary supplementation with CAG does not impact the mean or maximum longevity of female mice (6,7). Therefore, a necessary relation between telomerase activity and longevity does not likely exist.

The above study led to another question: How does CAG activate telomerase? Telomerase activity in human embryonic kidney HEK293 fibroblasts increases upon treatment with CAG and is not meditated via common secondary messenger pathways, including Ca^{2+}, inositol trisphosphate (IP3), cyclic adenosine monophosphate (cAMP), and protein kinase B (Akt). However, CAG induced the phosphorylation of extracellular signal-regulated kinase (ERK) in many cell lines, including HEK293 and HEK-neo keratinocytes, as well as cells from lung, brain, mammary, endothelial, and hematopoietic origins. Further, proto-oncogene tyrosine-protein kinase (c-Src), ERK kinase (MEK), and epidermal growth factor receptors are involved in CAG-induced ERK phosphorylation. CAG may activate telomerase and other cellular effects by activating the Src/MEK/ERK pathway (8). Our previous research showed that CAG induces expression of Janus kinase 2 (JAK2) and signal transducer and activator of transcription 5b (STAT5b) and enhances phosphorylation of STAT5b following an increase in TERT expression. Further, expression of molecules downstream from the JAK/STAT signaling pathway was also increased, suggesting that CAG activates telomerase through the JAK/STAT signaling pathway (9). In addition, CAG-mediated activation of telomerase is associated with the cAMP response element-binding protein (CREB) (3). The above preliminary studies of the mechanisms associated with CAG demonstrate that it activates telomerase and is related to the CREB, MAPK, and JAK/STAT pathways; however, further investigation is needed to comprehensively understand the mechanisms.

Telomerase activation and anti-immunosenescence. A prevailing view is that the immune system gradually ages during the process of senescence. Weak immune systems lead to an increased susceptibility to infections, which accelerates the process of senescence. The immune system consists of immune organs, immune cells, and active immune substances and is associated with senescence (10). Immune cells include T lymphocytes, B lymphocytes, and phagocytes, in which the number of lymphocytes is related to immune function. CAG enhanced both CD8(+) T lymphocytes and CD4(+) T lymphocytes, telomerase activity, and proliferative capacity *in vitro* (11,12). CD8(+) T lymphocytes from human immunodeficiency virus (HIV)-infected human donors treated with CAG showed an increase in telomerase activity, modest retardation of telomere attrition, increased proliferative potential of CD8(+) T lymphocytes, and enhanced cytokine/chemokine production, thus enhancing the antiviral potential. CAG-induced increases in telomerase activity are blocked by MAKP and ERK inhibitors; therefore, it is likely that CAG-associated enhanced antiviral functions are mediated through the ERK/MAPK pathway (13). In addition to T lymphocyte proliferation in HIV-infected human donors, CAG enhances normal human T lymphocyte proliferation.

An *Astragalus membranaceus* root extract (HTA; not a single purified compound) has also been shown to be a telomerase activator. However, CAG is a more effective telomerase activator, increasing telomerase activity and the proliferative potential of human CD4 and CD8 T cells more than that of HTA. Further, CAG has been shown to enhance the anti-viral ability of cells and has the potential to ameliorate acquired immunodeficiency syndrome (AIDS); however, the anti-viral properties of CAG have not yet been proven in animal models.

Telomerase activation and anti-pulmonary fibrosis. Within the past decade, numerous diseases or maladies have been associated with mutations in telomerase and/or are known to accelerate telomere loss, including AIDS, congenital dyskeratosis, aplastic anemia, and idiopathic pulmonary fibrosis (IPF). CAG, a telomerase activator, has the possibility to treat these diseases or maladies; to this end, several studies have been conducted. CAG dose-dependently hindered bleomycin-induced fibrosis in mTERT heterozygous mice (TRET+/-mice). However, CAG did not alter the inflammatory response to bleomycin-induced fibrosis, suggesting that the underlying mechanisms are not related to the anti-inflammatory properties of CAG. Prevention against

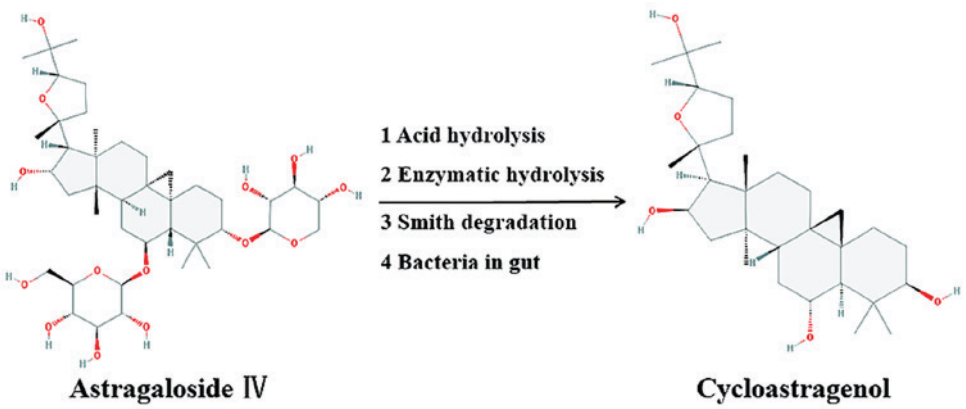

Figure 1. A schematic for cycloastragenol synthesis.

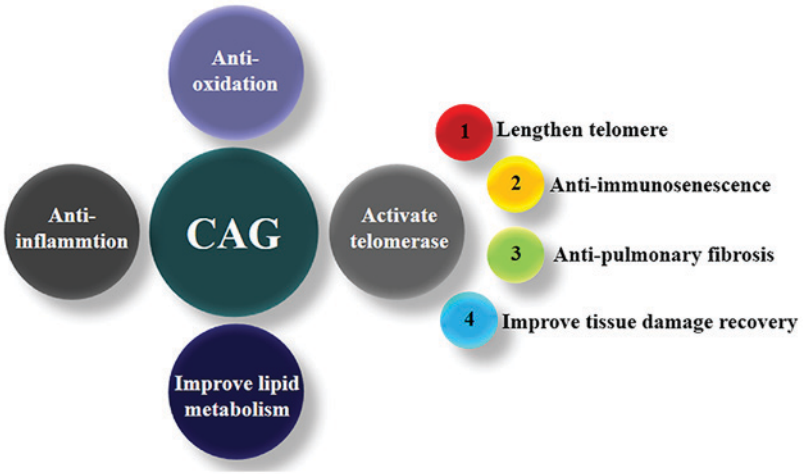

Figure 2. Pharmacological effects of CAG. CAG, cycloastragenol.

fibrosis and suppression of senescence in cells are dependent on telomerase activation. Further, data suggest that the mechanisms associated with the protective effects of CAG against bleomycin-induced lung fibrosis involve specific types of lung cells rather than all cell types in the lung (7). The mechanisms by which CAG works in specific cell types are likely related to the basic cellular proliferative ability; however, this issue has yet to be resolved.

Telomerase activation and recovery of damaged tissue. Telomere length is proportional to cell proliferation at the cellular level; that is, telomerase activation and elongation of telomeres can increase the proliferative ability of cells. The present study indicates that this hypothesis is correct. CAG can improve skin fitness, wound healing, and fur recovery in elderly mice and is a more remarkable agent for wound healing in both *in vitro* and *in vivo* assays than that of the other three main saponins from *Astragalus* species, including AG, cyclocephaloside I (CCI), and cyclocanthoside E (CCE). Of these compounds, CAG is the most effective at inducing human keratinocyte proliferation and migration at a concentration of 1 ng/ml. Further, a 5% preparation of CAG is the most effective for *in vivo* wound healing (14). CAG stimulates telomerase activity in human keratinocytes, which may contribute to the wound healing effects associated with CAG.

In addition to its wound healing properties, CAG promotes recovery from brain injuries, as shown in both cell and animal experiments. Neural stem cells are important for recovery from brain injuries such as hypoxic-ischemic brain injury. CAG increases proliferation and improves survival in neural stem cells and counters the effects of oxygen-glucose deprivation injury *in vitro* (15). Further, CAG reduces neuron apoptosis, improves nerve functional scoring, and reduces cerebral infarction volume in a cerebral ischemia reperfusion injury rat model *in vivo*. These functions are related to increasing expression of TERT (16,17). Furthermore, CAG promotes the renewal capacity of flight feathers in captive zebra finches, which are nonmammalian animals (18). In addition, CAG attenuates depression-like behavior in experimental mice. Telomere elongation and activation of telomerase activity are proportional to the tissue telomere-dependent recovery of cells and are potential treatment targets and indicators of tissue recovery.

Improved lipid metabolism. Lipids store energy; however, when lipids accumulate too much, it is harmful for health. CAG could ameliorate various biomarkers associated with lipid metabolism. CAG has been shown to reduce cytoplasmic lipid droplets in 3T3-L1 adipocytes at a low dose. At high doses, CAG prevents differentiation of 3T3-L1 preadipocytes.

Further, it was observed that CAG dose-dependently stimulates calcium influx in 3T3-L1 preadipocytes. Since elevated intracellular calcium plays a vital role in suppressing adipocyte differentiation, CAG may mediate the balance in lipid metabolism by activating calcium influx in 3T3-L1 preadipocytes (19).

Farnesoid X receptor (FXR) is a potential drug target for the treatment of non-alcoholic fatty liver disease (NAFLD), and CAG is a direct activator of FXR. Animal studies have shown that CAG reduces high-fat diet-induced lipid accumulation in the liver, accompanied by lowered blood glucose levels, serum triglyceride levels, and hepatic bile acids. CAG also ameliorates hepatic steatosis in methionine- and choline-deficient L-amino acid diet (MCD)-induced non-alcoholic steatohepatitis (NASH) mice. These results indicate that CAG ameliorates NAFLD via enhancement of the FXR signaling pathway (20). In addition, CAG increases TERT, c-Myc, and c-Jun expression in the liver and ameliorates liver lipid accumulation as well as glucose tolerance in 2-year old mice. CAG-ameliorated lipid metabolism may occur via multiple mechanisms, and CAG may be a multi-target monomer that binds with various receptors and exerts a variety of functions.

Anti-oxidative properties. The oxidative stress hypothesis is a generally accepted mechanism of senescence. Reactive oxygen species (ROS), including superoxide (O^{2-}), hydrogen peroxide (H_2O_2), and the hydroxyl radical (-OH) damage cell functions and are a primary cause of disease and cell senescence. In a D-galactose-induced senescence mouse model, CAG treatment up-regulated total antioxidant capacity (T-AOC) and superoxide dismutase (T-SOD) activity, increased hydroxyproline (HYP) stores, and down-regulated malondialdehyde (MDA) in the skin, heart, and liver. Further, CAG enhanced the antioxidant capacity in a non-dose-dependent manner (21). The anti-oxidant effects of CAG may be related to the hydroxyl found in the chemical structure of CAG. Since oxidative stress is a main reason for telomere attrition (22), the telomere-protective effects of CAG may be related to both its antioxidant and telomerase activation properties.

Anti-inflammatory properties. Inflammation is a very common and important basic pathological process. Body surface trauma, infections, common organ diseases, and frequently-occurring diseases (such as pneumonia, hepatitis, and nephritis) are all related to inflammation. ROS generation was suppressed in human umbilical vein EA.hy926 cells exposed to CAG in the setting of endoplasmic reticulum (ER) stress. Further, CAG attenuates phosphorylation of inositol-requiring enzyme 1 (IRE1)-α, suppresses thioredoxin-interacting protein/NLR family pyrin domain containing 3 (TXNIP/NLRP3) inflammasome activation, inhibits mitochondrial cell death, and reduces apoptosis in endothelial cells. Further, CAG enhances 5'AMP-activated protein kinase (AMPK) phosphorylation, an effect that is diminished by AMPK inhibitors, indicating a potential role for AMPK in the anti-inflammatory properties of CAG (23).

We discussed the role of CAG in the normal immune system above; however, CAG also plays a role in the activated immune system. The effects of CAG in a concanavalin (Con) A pan-activated lymphocyte model were reported by Sun *et al* (24). CD69 (an early-activated marker) and CD25 (a middle-activated marker), both of which are expressed on the surface of lymphocytes, are inhibited in lymphocytes pretreated with CAG and are activated via Con A treatment *in vitro*. Further, pretreatment with CAG blocks Con A-induced lymphocyte mitogenesis, induces cell-cycle arrest in the G0/G1-phase, and downregulates cytokine expression. In addition, Ca^{2+} release from intracellular ER storage and Ca^{2+} influx from the extracellular environment are inhibited in Con A-induced activated T-cells treated with CAG; these changes may be related to the anti-inflammatory properties of CAG (24). CAG could activate the immune system and inhibit activated immunity similar to that of feedback inhibition. It is possible that the structure of CAG resembles substances found in our body, and it exerts its functions in a similar way.

Efficacy base on clinical trial. The Patton protocol-1 provided 37 subjects with a comprehensive dietary supplement pack containing CAG for 12 months. This clinical trial concluded that CAG lengthens critically short telomeres and remodels the relative proportion of circulating leukocytes in cytomegalovirus-positive [CMV(+)] subjects toward a more 'youthful' profile, as seen in CMV(-) subjects (25). Furthermore, the supplement pack improved biomarkers of metabolic, bone, and cardiovascular health, such as fasting glucose, insulin, cholesterol, blood pressure, and bone mineral density (BMD). These effects are mostly attributable to CAG (26). A study reported by Harley *et al* (26), reported an increase in BMD in naturally aging rats; clinical trials have further verified this function. It is noteworthy that the Patton protocol-1 did not establish a control before initiating the study, which may have affected the trial results.

A random clinical trial conducted in 117 relatively healthy CMV (+) subjects 53-87 years old verified that TA-65 can significantly lengthen telomeres (4). Other TCM extracts, such as *Cynomorium songaricum* polysaccharides and *Epimedium brevicornu* flavonoids, can also activate telomerase or protect telomeres; however, CAG is the only compound that has been shown to activate telomerase in humans to date. Further, a randomized placebo-controlled study showed that CAG significantly improves the macular function of treatment subjects (27).

CAG activates human telomerase and has various positive functions. It is still unknown whether these different functions are related to each other and to CAG dosages and whether CAG functions differently in different groups of people.

3. Adverse reactions

Critically short telomeres will lead to cell senescence and apoptosis, and numerous diseases or maladies are related to short telomeres. Telomerase activators could attenuate this process. However, telomeres are elongated via the telomerase enzyme in more than 80% of tumors. As such, activated telomerase is a hallmark of cancer (28). As CAG is a telomerase activator, it is vital to understand the adverse reactions associated with CAG treatment regimens. No treatment-related mortalities were observed in rats ingesting 0, 40, 80, or 150 mg/kg/day CAG via oral gavage for 91 consecutive days in a subchronic study with a 4-week recovery period; further,

no adverse effects were observed. In male and female rats, the oral no-observed-adverse-effect level (NOAEL) of CAG was >150 mg/kg/day. In addition, CAG did not induce any toxic or genotoxic effect according to the result of bacterial reverse mutation assay, *in vitro* chromosome aberration assay and *in vivo* erythrocyte micronucleus assay (29). Administration of CAG for 4 months in adult female mice (25 mg/kg/day) did not increase the incidence of cancer. However, CAG competitively inhibited UDP-glucuronosyltransferase (UGT) 1A8 and noncompetitively inhibited UGT2B7. Herb-drug interactions between CAG and drugs that are mainly metabolized by UGT1A8 or UGT2B7 might occur *in vivo* when the plasma concentration of CAG is above 0.034 and 20.98 μM, respectively (30). Furthermore, the Patton protocol-1, a commercial health maintenance program that provided 7000 person-years of use with a comprehensive dietary supplement pack containing CAG over 5 years, reported no adverse events or effects attributed to administration of the dietary supplement pack (26).

According to current research results, CAG is relatively safe within a certain dose range and has no serious adverse reactions; CAG was determined to be generally recognized as safe (GRAS) by an independent expert panel of the Food and Drug Administration (FDA) on the 19th of November, 2014. CAG has been used as a medical food (31). However, telomerase activation has been observed in 80% of human tumors, suggesting that telomerase plays a nonnegligible role in tumor development (32). Thus, the potential adverse reactions of CAG should be noted. More studies regarding adverse reactions of CAG are necessary to fully understand how to use CAG rationally.

4. Pharmacokinetics

CAG is efficiently absorbed through the intestinal epithelium by passive diffusion, as shown by a study that investigated the intestinal absorption and metabolism of CAG using an *in vitro* models composed of human small intestinal mucosa (Caco-2 model) and liver microsomes. However, metabolites were found in the apical and basolateral sides of Caco-2 monolayers, suggesting that first-pass intestinal metabolism of CAG might occur upon passage through the intestinal epithelium. After incubation for 30 min, CAG underwent extensive metabolism in rat (17.4%) and human (8.2%) liver microsomes (33). An comparison of CAG metabolizing ability of different rat tissues showed that the metabolic capacity of heart, spleen, lung, and kidney was lower than that of the liver *in vivo* (34). Further, a significant inhibitory effect on the cytochrome P_{450} 3A4 (CYP3A4) subunit, as well as significant induction of the CYP2E1 subunit were observed after administration of CAG via continuous gavage (35). These results indicate that although CAG is efficiently absorbed through the intestinal epithelium, its oral bioavailability CAG would be limited by extensive first-pass hepatic metabolism.

5. Discussion

CAG naming. In the process of searching documents, we found that CAG (CAS number: 78574-94-4) is noted by diverse names in different documents. CAG is named (2aR,3R,4S,5aS,5bS,7S,7aR,9S,11aR,12aS)-3-((2R,5S)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-2a,5a,8,8-tetramethylhexadecahydrocyclopenta[a]cyclopropa[e]phenanthrene-4,7,9-triol, other synonyms include CAG, CA, astramembrangenin, cyclogalegigenin, GRN510, and TA-65. TA-65 was named by the T.A Science Corporation as a nutrition and health care product. A document reported that GRN510 is a novel and proprietary chemical entity derived from GRN665/TAT2. However, GRN510 is considered equivalent to CAG in PubChem (an open chemistry database); therefore, we considered GRN510 as equivalent to CAG in this article. For simplicity, we termed cycloastragenol as CAG in this report.

Advantages and disadvantages associated with CAG. CAG has multiple pharmacological effects, including activation of telomerase, improved lipid metabolism, anti-oxidation, and anti-inflammatory properties. Clinical trials have proven components involved in CAG functions. No serious adverse effects are associated with CAG within a certain dose range. However, the carcinogenic potential of CAG is the main factor preventing the use of CAG clinically. Clarification of the quantitative effects and efficacy associated with CAG in different individuals is needed.

Unknown information regarding CAG. While there is a large body of literature regarding CAG, there are still significant gaps in knowledge.

CAG stimulates calcium influx or inhibits calcium influx depending on the cell type and pathological state. Further, CAG has different pharmacological effects at diverse concentrations in 3T3-L1 preadipocytes. The underlying mechanisms regarding these effects have not yet been reported.

CAG has extensive pharmacological effects; however, the detailed underlying mechanisms for most of these effects rem ain unclear. A summary of CAG-associated effects and the corresponding mechanisms are show in Table I. Mechanisms underlying the extensive pharmacological effects associated with CAG are complex. We attempted to establish a hypothetical mechanism for the effects of CAG based on previous results (Fig. 3).

CAG could increase BMD and improve glucose metabolism in naturally aged rats. There is no literature describing whether CAG has anti-osteoporosis or anti-diabetic effects in corresponding animal models.

6. Conclusions and prospective

Conclusions. In this review, the current state of CAG research is detailed and elucidated, and the efficacy, pharmacokinetics, and adverse reactions of CAG are summarized. According to the present research results, CAG has extensive pharmacological effects, including telomerase activation, telomere elongation, anti-inflammation, anti-oxidation, anti-viral, anti-pulmonary fibrosis, anti-ischemic and hypoxic injury, and anti-lipid accumulation properties. Studies also suggest that CAG improves liver metabolic homeostasis, promotes wound healing, promotes feather growth, and improves certain health-span indicators both in humans and animals. However, more attention and further studies are needed to evaluate whether CAG has potential adverse reactions, and studies examining the detailed

Table I. CAG pharmacological effects and mechanisms.

Author, year	Object of study	Effect of CAG	Mechanism	Tool or method of studying mechanism	(Refs.)
Fauce *et al*, 2008	CD8(+) T lymphocytes from HIV-infected human donors	Enhanced antiviral functions	Increase telomerase activity	Telomerase template antagonist-GRN163L	(13)
Fauce *et al*, 2008	CD8(+) T lymphocytes from HIV-infected human donors	Increase telomerase activity	ERK/MAPK pathway	MAKP inhibitor, ERK inhibitor	(13)
Molgora *et al*, 2013	Healthy human CD4 and CD8 T cells	Increase telomerase activity	MAPK pathway	MAKP inhibitor(PD98059)	(11)
Zhao *et al*, 2015	Endothelial cell	Ameliorated endothelial inflammation and reduced cell apoptosis	AMPK pathway	AMPK inhibitor	(23)
Sun *et al*, 2017	Activated lymphocytes	Anti-inflammation	Inhibited Ca^{2+} overload	Flow cytometry	(24)
Bernardes de Jesus *et al*, 2011	MEF *Terc*$^{+/-}$	Lengthened telomeres, reduced critically short telomeres and DNA damage	Increase telomerase activity	Gene knockout	(6)
Yung *et al*, 2012	Human embryonic kidney HEK293 fibroblasts	Increased telomerase activity	Src/MEK/ERK pathway	Selective inhibitors and dominant negative mutants	(8)
Le Saux *et al*, 2013	TRET+/- mice	Inhibited fibrosis and prevented senescent cell accumulation	Increased telomerase activity	Telomerase inhibitor-GRN163L	(7)
Wang *et al*, 2014	3T3-L1 preadipocytes	Reduced cytoplasmic lipid droplets	Stimulated calcium influx	Calcium mobilization assay	(19)
Gu *et al*, 2017	High-fat diet mice	Improved hepatic steatosis	Activated farnesoid X receptor signaling	PCR, WB, Molecular docking, gene knockout	(20)
Bernardes de Jesus *et al*, 2011	2-year old mice	Improved hepatic lipid accumulation	Increased telomerase activity via c-Myc and c-Jun	PCR, IHC	(6)
Ip *et al*, 2014	Human neonatal keratinocytes	Improved wound healing	Increased telomerase activity	RQ-TRAP assay	(3)
Meng *et al*, 2017; Yi *et al*, 2015; Gao, 2015	Hypoxic-ischemic brain injury	Countered hypoxic-ischemic brain injury	Increased telomerase activity	Telomerase inhibitor	(15-17)
Reichert *et al*, 2014	Zebra finches	Improved flight feather renewal capacity	Increased telomere length	qPCR	(18)
Cao *et al*, 2012	D-galactose-induced senescent mouse model	Enhanced antioxidant capacity	CAG anti-oxidant	Corresponding detection method	(21)
Salvador *et al*, 2016	Relatively healthy cytomegalovirus-positive subjects	Lengthened telomeres	-	Oral CAG	(4)

Table I. Continued.

Author, year	Object of study	Effect of CAG	Mechanism	Tool or method of studying mechanism	(Refs.)
Dow and Harley, 2016	Patients	Improved macular function	-	Oral CAG	(26)
Ip *et al*, 2014	PC12 cells and primary neurons	Induced telomerase activity and CREB activation followed by tert and Bcl2 expression	CAG function related to CREB activation	Blockade of CREB expression via RNA	(3)

HIV, human immunodeficiency virus; ERK, extracellular-signal-related kinase; MAPK, mitogen-activated protein kinase; AMPK, 5'adenosine monophosphate-activated protein kinase; PCR, polymerase chain reaction; WB, western blot; IHC, immunohistochemistry; TRAP, telomeric repeat amplification protocol; CREB, cAMP response element-binding protein; CAG, cycloastragenol.

Figure 3. Hypothetical mechanisms associated with CAG. Arrow heads represent stimulatory modifications. Solid lines are direct stimulatory modifications based on the literature. Dashed lines indicate stimulatory modifications; grey dashed lines indicate possible stimulatory modifications. CAG may activate telomerase through pathway 1, 2, 3 and 4, and then exert various effects. Through pathway 5, CAG directly stimulates the FXR to improve hepatitis. Through pathway 6, CAG indirectly stimulates AMPK to improve inflammation. CAG, cycloastragenol; FXR, farnesoid X receptor; AMPK, 5'adenosine monophosphate-activated protein kinase; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase kinase; JAK, Janus kinase; STAT, signal transducer and activator of transcription; TERT, telomerase reverse transcriptase.

underlying mechanisms associated with CAG are required. In addition, further multicenter clinical studies are required for specific diseases such as steatohepatitis, AIDS, pulmonary fibroses, wound healing etc.

Prospective. We anticipate that researchers will conduct more studies on CAG to evaluate its efficacy and adverse reactions, as well as understand how to use CAG rationally to enhance its effects and reduce adverse reactions so that CAG can be used for the clinical treatment of corresponding diseases. We believe that development of these clinical uses will benefit people, which is the purpose of this article.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LZ performed the literature review and mapping. YL and YYg made suggestions and edited the manuscript. YYu wrote the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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