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Mini Review

Targeting adipocyte apoptosis: A novel strategy for obesity therapy

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ABSTRACT

Obesity is an increasing world problem that may cause several metabolic complications including insulin resistance, hyperlipidemia, hypertension, and atherosclerosis. Development of therapeutic drugs for obesity has been proven difficult. Current strategies for weight reduction are inhibition of food intake through the central nervous system or blocking the absorption of lipids in the gut. These therapies have many side effects, so new treatments are urgently needed. Fat loss could also be achieved through a decrease in the size and number of adipocytes through apoptosis. Apoptosis is a normal phenomenon of cell death for the purpose of maintaining homeostasis. Induction of apoptosis is a reasonable way to remove adipocytes in obese patients. It is reported that several adipokines and natural products play roles in induction of adipocyte apoptosis. Here we review the recent progress of the roles and mechanisms of adipocyte apoptosis induced by leptin, tumor necrosis factor-α (TNF-α), and natural compounds.

1. Introduction

Obesity is a worldwide problem with rapidly increasing prevalence [1], attracting public health attention especially in Western countries [2]. Obesity is as a result of the excess calories being stored as triglyceride in adipose tissue and ectopically in other tissues [3–5], and is associated with insulin resistance, type 2 diabetes, cardiovascular disease, hypertension, hyperlipidemia, non-alcoholic steatohepatitis, stroke, and certain cancers [4,6,7]. Prevention and treatment of obesity will prevent or greatly benefit the treatment of these related diseases. However, several therapeutic drugs to treat obesity have been withdrawn from the market in the last decade [8]. Currently there is only one approved drug, orlistat, for long-term use in the treatment of obesity and new therapeutic approaches are urgently required for treatment of obesity [6].

Obesity is characterized by the increase in adipocyte size and number. Therefore, targeting adipose tissue has become a new strategy for obesity treatment, and includes suppression of adipogenesis or adipose tissue mass, obstruction of lipid accumulation in adipocytes, and adipocyte deletion by apoptosis [9–12].

Apoptosis is characterized by plasma membrane blebbing, cytoplasm condensation, DNA fragmentation, and phagocytosis of the apoptotic body by macrophages [13], all regulated by the anti-apoptotic and pro-apoptotic proteins. Apoptosis is necessary for maintaining homeostasis by removing dangerous and unnecessary cells [14–16]. For example, anti-cancer drugs inhibit the growth of carcinoma cells and other overgrown cells through the activation of apoptosis. However, adipocytes are resistant to apoptosis because of high levels of Akt/protein kinase B and the anti-apoptotic factor Bcl-2. Adipocytes could be removed through apoptotic mechanisms in some pathological conditions, such as in patients with tumor cachexia and HIV-infected patients receiving antiretroviral therapy. Therefore, the induction of apoptosis in adipocytes could be an attractive method to reduce the adipocyte number.

2. Adipokines

As an endocrine organ, adipose tissue secretes abundant adipokines that affect the regulation of fat weight and homeostasis. These proteins build a multitudinous array of adipose targets for drugs to treat and prevent obesity [17]. Since the discovery of leptin in 1994, many attempts have been made to use it in anti-obesity therapy [18,19]. There is evidence that administration of leptin can induce adipocyte apoptosis [20].

2.1. Leptin

Leptin, a product of the obese(ob) gene, includes 167 amino acid peptides [21], is produced and secreted by adipocytes, and mediates adipose tissue mass by elevating thermogenesis and restricting food intake through both central and peripheral mechanisms [22,23]. Recent study has suggested that after intra-cerebroventricular (ICV) administration of leptin in rats, adipose tissue showed a rapid decrease and features of apoptosis. Interestingly, the apoptosis was not observed in control and pair-fed rats and in other tissues

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of leptin-treated rats [24]. Peripheral infusion of leptin is efficacious in inducing adipocyte apoptosis in mice [25]. High-fat feeding has a gender-specific role with only males, on reduced responsiveness to leptin-induced adipocyte apoptosis. Leptin does not act directly on adipocytes to induce apoptosis. The mechanism of this process is still uncertain.

Many efforts have been made to discover the mechanisms of leptin involvement in adipocyte apoptosis. It has been reported that leptin induces the expression of angiopoietin-2 in adipose tissue without an accompanying elevation in vascular endothelial growth factor (VEGF), and leads to apoptosis in adipose endothelial cells [26]. Qian and colleagues have found that PPAR-γ mRNA levels showed a 70–80% increase after ICV administration of leptin, suggesting that PPAR-γ may be involved in leptin-induced adipocyte apoptosis [27]. This result was supported by the finding that thiazolidinedione (TZD), an agonist of PPAR-γ, can evoke adipocyte differentiation and apoptosis in white adipose tissue from rodents, contributing to prevention of adipocyte hypertrophy [28]. Loss of adipose tissue through leptin-induced apoptosis may present an effective mean of prevention and treatment of obesity.

2.2. TNF-α

Patients with cancer, AIDS, and chronic obstructive lung disease may have cachexia, characterized by loss of body mass and adipose tissues. In these patients, the inflammatory cytokine TNF-α, also called *cachexin*, was significantly increased. There is evidence that TNF-α may play a role in the loss of adipocytes.

TNF-α is synthesized and produced by adipocytes and plays a crucial role in regulation and control of adipose mass and adipocyte number via apoptosis mechanisms [29,30]. A study on brown adipocytes suggests that TNF-α induces the apoptosis of differentiated brown fat cells, and this effect is possibly adjusted mainly by the P55 TNF-α receptor subtype [31]. A further study has shown that brown adipocyte apoptosis in obese mice is abated with the absence of TNF-α P55 receptor [32]. During TNF-α-induced anti-adipogenesis experiment, TNF-α-induced DNA fragmentation was significantly augmented in the β-catenin knockdown preadipocytes, suggesting that a β-catenin signaling pathway may be involved in TNF-α regulation of 3T3-L1 preadipocyte apoptosis [33]. TNF-α also induces and enhances apoptosis in human preadipocytes and adipocytes [34]. A recent study shows that TNF-α reduces the number of mature adipocytes, but not preadipocytes, through the induction of apoptosis resulting in C/EBP and PPAR-γ-mediated suppression of NF-κB [35].

3. Natural compounds

Several compounds purified from plants might be effective methods for inducing adipocyte apoptosis.

3.1. Phenols

Phenols, which are commonly found in plants, have pharmacological and biochemical effects on anti-inflammatory and anti-cancer therapy [36–38]. Recent studies suggest that phenols also have anti-obesity effects. Curcumin, the major polyphenol in turmeric spice, has been shown to cause 3T3-L1 adipocyte apoptosis [39]. Green tea (GT) and GT components, particularly catechins, could induce preadipocyte differentiation and adipocyte apoptosis [40,41]. Green tea treatment also showed a high apoptotic rate in visceral adipose tissue in rats that was closely related to augmented aromatase expression, and circulating concentration of 17β-estradiol, and plasma concentration of testosterone [42]. Resveratrol, a naturally occurring phytoalexin derived from red wines and grape juice, induced apoptosis in 3T3-L1 adipocytes [43]. Resveratrol also induced the apoptosis of rat primary adipocytes by increasing the expression of Sirt1, Cytochrome *c*, cleaved Caspase 9, and cleaved Caspase 3 that are related to cell apoptosis [44]. Combined treatment of resveratrol, genistein, and quercetin enhanced apoptosis in pre- and lipid-filled mature murine adipocytes and in early- and mid-phase maturing and lipid-filled mature human adipocytes more than the responses to genistein, quercetin, and resveratrol when used separately [45]. Further, combination of resveratrol and genistein has a stronger effect on induction of adipocyte apoptosis [46]. Therefore, the enhanced effects due to combination of two or more natural compounds may have more potential than the individual compounds in preventing obesity.

3.2. Flavonoids

Flavonoids have been shown to have a broad spectrum of biological effects, including anti-inflammatory, anti-cancer, antioxidation, and cardiovascular maintenance effects [47]. In recent years, flavonoids have been shown to play a role in anti-obesity activity via the induction of apoptosis. Xanthohumol and isoxanthohumol could induce apoptosis in 3T3-L1 adipocytes through the activation of mitochondrial pathway and caspase-3/7 [48]. Recent study has shown that xanthohumol enhanced apoptosis in both preadipocytes and mature adipocytes. In addition, xanthohumol-treated preadipocytes show the increase of NF-κB expression that is associated with regulation of numerous cells apoptosis, differentiation, and proliferation [49,50]. Furthermore, xanthohumol plus honokiol reinforced induction of apoptosis in 3T3-L1 mature adipocytes via the increase of cleaved PARP and Cytochrome *c* release, and inhibition of Bcl-2 protein levels. In addition, they could activate PTEN and inhibit AKT signaling [51]. Polymethoxy flavones (PMFs), which exist exclusively in *Citrus* genus, have a fascinating and broad spectrum of bioactivity consisting of inducing apoptosis activity in various cell types [52–54]. In mature 3T3-L1 adipocytes, PMFs could induce apoptosis via activation of Ca^{2+}-dependent calpain and Ca^{2+}/calpain-dependent caspase-12. And apoptosis-inducing activity of hydroxylated-PMFs is significantly higher, which may be attributed to their stimulating effect on the production of reactive oxygen species (ROS) [55]. Recent study has shown that (2*S*,3*S*)-aromadendrin-6-C-β-D-glucopyranoside (AG), a novel flavonol isolated from *Ulmus wallichiana*, also evokes apoptosis of differentiated 3T3-L1 adipocytes [56].

Isoflavones from soybean products have several biological effects, including estrogenic and hypolipidemic activities. For example, genistein has been shown to reduce body weight via induction of apoptosis of adipose tissues in ovariectomized mice [10]. Administration of genistein and daidzein has also shown increased apoptosis through the alteration of the apoptosis-related proteins including augmentation of Bad and reduction of pAkt protein [57]. The interaction between genistein and 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) (vitamin D) evoked apoptosis in mature 3T3-L1 adipocytes via the increase of VDR (vitamin D receptor) [58]. These data suggest the potential of flavonoids as a therapy for obesity.

4. Other natural compounds

In addition to phenols and flavonoids, several other natural compounds have been proven to have anti-obesity activity. Esculetin (6,7-dihydroxy-2*H*-1-benzopyran-2-one) a coumarin derivative found in various plants, has been shown to enhance adipocyte apoptosis and induce apoptosis in the late stage of differentiation [59]. Ajoene, from garlic, induces apoptosis of 3T3-L1 adipocytes through generation of hydrogen peroxide, activation of mitogen-activate protein kinases, cleavage of DNA, translocation of AIF (apoptosis-

inducing factor), and reduction of PARP-1 [60]. Guggulsterone (GS), the active substance in guggulipid, may exert anti-obesity effects by evoking apoptosis in mature adipocytes through the increase of caspase-3 activity and release of Cytochrome *c* from mitochondria. And *cis*-GS is more potent than *trans*-GS in the induction of adipocyte apoptosis [61]. Several anti-obesity components purified from *Rubia cordifolia* L. have been reported, including mollugin and 2-carbomethoxy-2,3-epoxy-3-prenyl-1,4-naphthoquinone (CMEP-NQ), which belong to anthraquinones and naphthoquinones. In 3T3-L1 preadipocytes treated by mollugin and CMEP-NQ have shown the appearance of apoptosis, including mitochondrial membrane potential loss, and subsequent activation of caspases cascade including caspase-9, -3, and -7, resulting in PARP cleavage [12,62,63].

From the data above, the natural compounds show the ability to evoke apoptosis in adipocytes, which may have a major role in anti-obesity therapy.

5. Biochemical components

In addition to natural compounds, there are biochemical components for preventing obesity by inducing apoptosis. For example, preadipocyte differentiation may be augmented by fatty acids in the presence of adequate hormone cocktails. Palmitate, a long-chain saturated fatty acid, can provoke apoptosis in mouse 3T3-L1 and rat primary preadipocytes in the absence of adipogenic stimuli, which due to create multiple cellular stresses [64]. Docosahexaenoic acid (DHA, C22:6), a ($n-3$) polyunsaturated fatty acid in fish oil [65], and 1,25-dihydroxyvitamin D_3, the metabolic product of vitamin D, have been reported to have anti-obesity activity due to their roles in stimulating adipocyte apoptosis [66,67]. Clenbuterol, a selective β2-AR agonist, has been proven to induce white adipose tissue apoptosis in mice [68]. It has also been demonstrated that activation of β2-adrenergic receptor by clenbuterol evokes apoptosis in adipocytes though lipolysis stimulation [69]. Therefore, biochemical compounds may present an additional option for body fat loss through apoptosis.

Development of anti-obesity drugs remains difficult, but targeting adipocyte apoptosis may provide an opportunity for the treatment and prevention of obesity. Both natural products and biological products have apoptosis-inducing activities. Adipokines such as leptin and TNF-α are important anti-obesity drug candidates. Some natural compounds also induce apoptosis and may play a major therapeutic role in weight-reduction treatment. Before pharmaceutical interventions can be marketed, there are problems to overcome. First, provoking apoptosis in adipose tissue physiologically is likely to be harmful to other tissues, just as chemotherapy drugs induce apoptosis in cancer cells but also cause off-target effects on other organs and tissues. Developing a drug that specifically targets adipose tissue is a challenge. Secondly, targeting adipocytes may cause the cells to release lipids into blood or induce the storage of lipids in other tissues such as liver and muscle. An ideal apoptosis-inducing anti-obesity drug should avoid such side effects. Finally, the apoptosis-inducing agents may cause DNA damage and increase carcinogenesis. The anti-obesity drugs should be safe for long term use.

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