



The role of autophagy in steroid necrosis of the femoral head: a comprehensive research review

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Abstract

Steroid-induced osteonecrosis of the femoral head (ONFH) has the incidence of 9–40% in patients receiving long-term treatment and is mainly involved in the middle and young people. It is mostly bilateral, with a wide range of necrosis and high disability rate, which brings disaster for patients and families. The experimental study shows that autophagy participates in the pathological process of steroid ONFH and is closely related to apoptosis, and the interaction between autophagy and bone cells is related to the dose of hormones. Moreover, autophagy also affects the interaction between osteoblasts and osteoclasts in ONFH. In the present review, we have discussed the role of autophagy in the pathological process of the steroid-induced ONFH.

Keywords Osteonecrosis · Femoral head · Autophagy

Introduction

Osteonecrosis of the femoral head (ONFH) is caused by the interruption or damage of the blood supply of the femoral head, resulting in the death of the osteocytes and bone marrow components, and leads to a change in the structure of the femoral head and the collapse of the femoral head, then cause of pain and dysfunction of the joint. There are two types of the

cause of the necrosis of the femoral head: traumatic and non-traumatic. Moreover, steroid-induced osteonecrosis of the femoral head accounts for a large proportion of femoral head necrosis caused by non-traumatic factors [1]. And long-term application of glucocorticoids will increase the incidence of osteonecrosis of the femoral head [2, 3]. Furthermore, intra-articular injection of glucocorticoids can increase the possibility of ONFH [4].

At present, some scholars believe that the use of glucocorticoids will induce the apoptosis of osteoblasts and osteocytes and prolong the life of osteoclasts to reduce bone density [5–7]. The final stage of osteonecrosis of the femoral head is due to its poor vascular function which leads to the apoptosis of the bone cells. But other study shows that the apoptosis of bone and osteoblasts plays an important role in the development of osteonecrosis in the early stage of osteonecrosis [8].

The increase of bone cell apoptosis in steroid-induced ONFH is also related to the interstitial fluid, the formation of bone blood vessels, and the decrease of bone strength [9]. In addition, some hypotheses suggest that steroid-induced osteonecrosis is due to the increase of bone marrow adipocytes, resulting in increased intraosseous pressure and reduced bone perfusion of blood, as well as intravascular fat embolism and hypercoagulable state lead to reduced blood supply in femoral head [10]. Due to the lack of evidence for fat embolism in ONFH, more scholars prefer the apoptosis of bone cells, which plays a major role in osteonecrosis of the femoral head [11–14]. In addition, some scholars have found that

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glucocorticoids can interfere with the level of VEGF mRNA and reduce the formation of bone blood vessels [9]. This discovery plays a great role in the understanding of vascular factors in osteonecrosis of the femoral head. In this article, we have summarized the progress of autophagy in the pathogenesis of steroid-induced ONFH.

Autophagy is a self-protection mechanism in eukaryotic cells

Autophagy is a process for the lysosomes in eukaryotic cells to provide nutrients to the cells by degradation of the damaged cell organelles and proteins in the cells. It is a protective mechanism of the cells in the condition of harmful external stimuli or cell starvation, which plays a key role in maintaining balance and homeostasis in the cell [15]. On the one hand, autophagy can protect cells from apoptosis by removing oxidative damage organelles; on the other hand, excessive autophagy can destroy cell components. Therefore, autophagy can not only protect cell survival, but also lead to cell death [16]. According to the different pathways of substance entering lysosome, autophagy can be divided into three different groups: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). As the most studied macroautophagy, we call it autophagy in this article. The process of autophagy includes the formation of the dividing membrane, the formation of autophagy, the transport and binding of autophagy, and the decomposition of autophagy. At the initial stage, the induction of autophagy and the formation of autophagic membrane are mainly [17] (Fig. 1). The formation of autophagic membrane first requires the formation of autophagic precursors, which is the main regulating point during autophagy, and the Beclin1-VPS34 complex is the core complex in the formation of autophagic precursors in mammals [18]. Moreover, Atg14L and Rubicon may regulate autophagy by regulating Vps34 activity [19]. In addition, antiapoptotic Bcl-2 protein can inhibit the function of autophagy gene Beclin1 by binding to the BH3 domain of Beclin1, thus inhibiting the level of autophagy in the cell [20] (Fig. 1). In yeast cells, the corresponding Beclin 1, Atg6, and III phosphatidylinositol 3-kinase (PtdIns3K) complexes also play an important role in the formation of precursor of autophagosome. The lengthening and assembly of the outer autophagic membrane require two conjugates—ATG5-ATG12 and LC3-PE—ubiquitin like binding [21]. Tumor-inhibiting factor UVRAG not only regulates the interaction of Beclin 1 and Vps34 in vesicle formation stage, but also plays an important role in the maturation stage of vesicles. UVRAG directing the so-called binding protein (the protein attached to the autophagosome and its target) directing the autophagic membrane, thus promoting the fusion with the lysosome by activating the Rab7 [22]. In the cell low nutrition

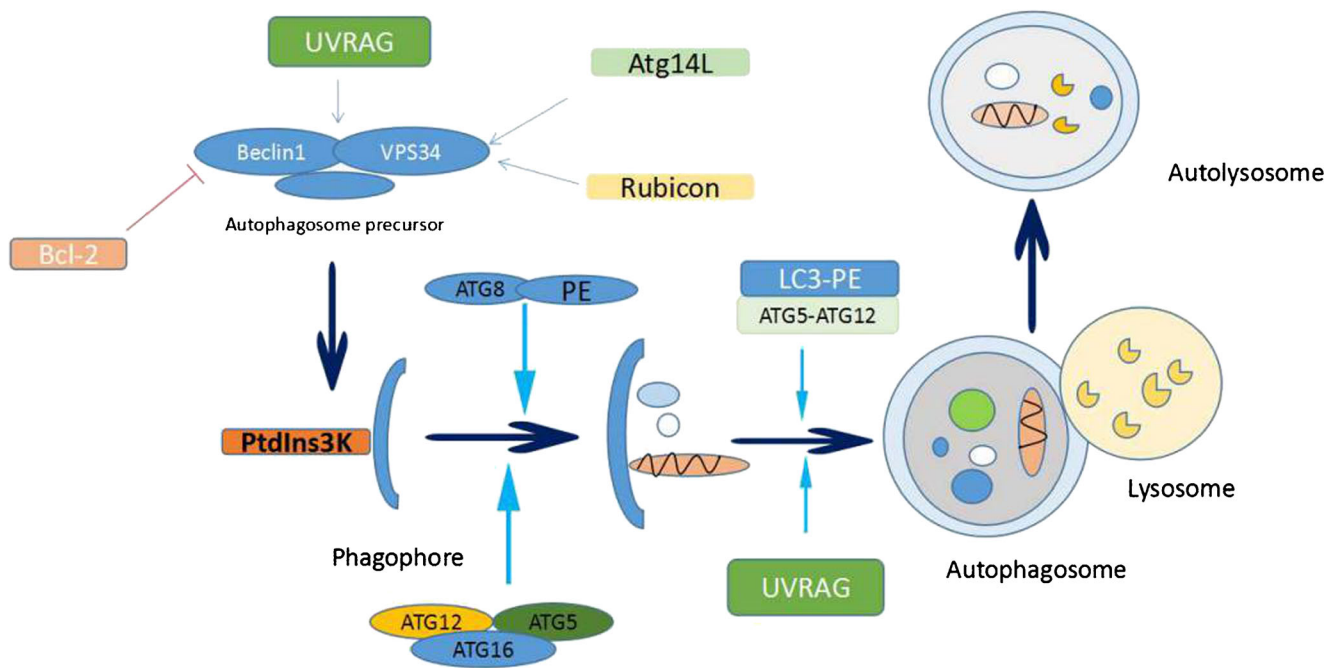
state, the small molecules produced by the degradation of the discarded substances in the cells will be transported back to the cytoplasm and used for the synthesis of nutrients and the maintenance of cell function.

Rapamycin (mTOR) pathway is the main regulation of mammalian autophagy: Rapamycin can inactivate TORC1 and stimulate autophagy under the condition of adequate nutrition. Cell stress leads to a downregulation of the mTOR1 activity of induced autophagy, and mTOR negatively regulates autophagy by causing phosphorylation of Atg13 to reduce the interaction with ULK1 and inhibit the formation of the tripolymer complex required for the formation of autophagic bodies [23]. Beclin1 is a specific gene for autophagy in mammals, mainly conforming to type III phosphatidylinositol 3, to regulate other Atg-encoded proteins and to locate in the structure of autophagic precursors, thus regulating autophagy activity [24]. But Bcl-2/Bcl-x can compete with Beclin-1 to inhibit autophagy [25]. P53 signaling pathway can increase autophagy by inhibiting mTOR and Bcl-2/Bcl-x [26] and phosphorylating Beclin-1 and lysosome proteins [27].

Vascular factors of glucocorticoid-induced osteonecrosis of the femoral head

Many experiments have shown that corticosteroids directly damage the endothelial cells, causing vascular contraction, coagulation fibrinolysis, and femoral head thrombosis, thereby reducing the blood circulation of the trabecular bone and ultimately leading to ONFH [28, 29]. Vasoconstriction may be due to corticosteroids that enhance the permissive action of catecholamines in blood vessels through glucocorticoid receptors, which play an important role in controlling vascular smooth muscle tension, and glucocorticoids can inhibit the production of vasodilators, such as carbon monoxide and prostacyclin [30]. In addition, steroids can regulate other vasoactive mediators to promote the expression of vasoconstrictor, such as endothelin-1 [31]. High-dose glucocorticoid (GC) also reduces the activity of tissue plasminogen activator (t-PA) and increases the level of plasma plasminogen activator inhibitor-1 (PAI-1) antigen, which increases the potential of GC to promote crur, and may lead to inhibition of angiogenesis, bone repair, and the metabolism of nitric oxide [32].

Glucocorticoids can cause the decrease of blood flow in the femoral head, resulting in endothelial cell injury, which eventually leads to plaque erosion and thrombosis. Related studies found that as one of the most angiogenesis-related factors, VEGF plays an important role in angiogenesis during wound healing [33]. Endothelial VEGF can prevent endothelial damage under hypoxic conditions and sustains endothelial cell viability and survival through signaling initiated in an intracellular compartment [34]. The self-renewal, multidirectional differentiation, and low immunogenicity of mesenchymal



The process and regulation of autophagy

Fig. 1 The process and regulation of autophagy. The formation of autophagic membrane requires autophagic precursors, and the Beclin1-VPS34 complex is the core complex in the process of mammalian autophagy precursors. UVRAG can regulate the autophagy process by regulating the binding of Beclin 1 and Vps34. Moreover, Atg14L and Rubicon may regulate autophagy by regulating Vps34 activity. The lengthening of the autophagic membrane requires the binding of two

conjugates of ATG5-ATG12 and LC3-PE. Moreover, ATG12-Atg5-Atg16 complex and Atg8-PE complex are involved in regulating the maturation process of autophagic membrane. UVRAG guidance for the fusion of autophagosome membrane and lysosome. Then, the autophagosome is fused with the lysosome. Finally, the substance degrades in the autolysosome and provides nutrients for the cells

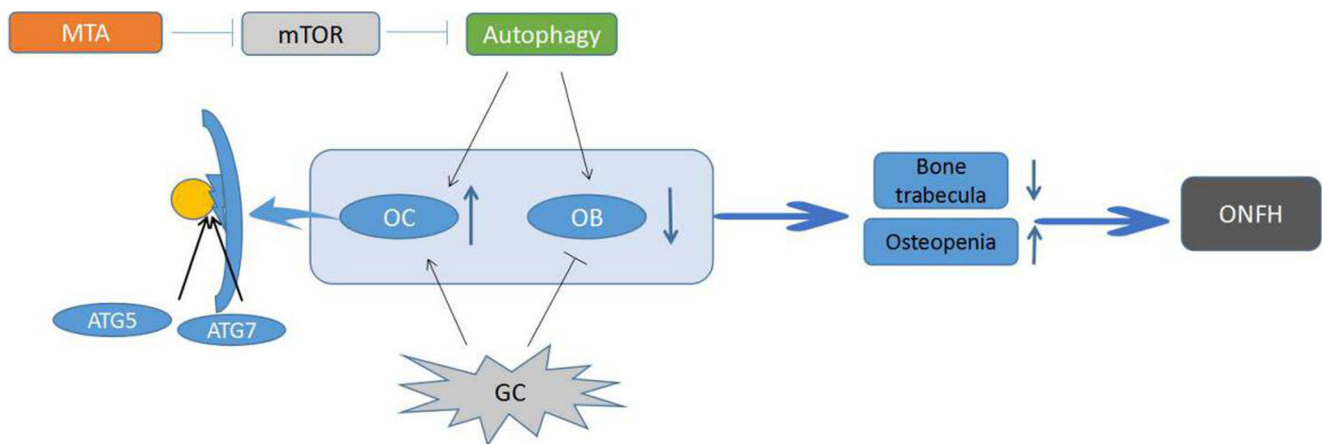
stem cells (MSCs) have been considered as a good source of adult stem cells and have been used in many clinical applications [35, 36]. Under inflammatory or hypoxic conditions, autophagy can protect MSC from apoptosis by AMPK/mTOR pathway [37]. Autophagy at the injured site can increase the regeneration ability of vascular endothelial cells after MSCs culture, and autophagy promotes the production of VEGF by phosphorylated ERK1/2 pathway to further promote angiogenesis [38]. Obviously, we can see that glucocorticoid has a negative effect on the blood flow in the femoral head and blood vessels, and autophagy has a protective effect on the blood vessel wall.

Autophagy in osteoblasts and osteoclasts

Steroid-induced avascular necrosis of the femoral head is due to the apoptosis of bone cells and osteoblasts that lead to the reduction of bone trabecula and formation, and the fracture of the trabecular bone under the weight of the bone, and the delay of the reconstruction and repair of the femoral head leads to the collapse of the femoral head [39] (Fig. 2). The core of this mechanism is bone fragility and bone loss. A recent report

shows that the proteins necessary for autophagy, including Atg5, Atg7, Atg4B, and LC3, are important for the production of osteoclast crinkle boundaries and the secretory function of osteoclasts and in vitro and in vivo bone resorption [40]. Otherwise, FIP200 is an integral part of the ULKs-Atg13-FIP200 complex and acts in the downstream of the mammalian rapamycin target (mTOR) in the formation of autophagy, which is essential for the induction of autophagy in mammalian cells [41]. Moreover, Liu et al. have found that the absence of FIP200 inhibits autophagy in osteoblasts that leads to bone loss and bone strength reduction, which defines the positive role of autophagy in osteoblast differentiation and bone development [42]. Osteoclasts absorb bone through the ruffled border which produced by fusion of bone-related plasma membrane and lysosome secreted [43]. Autophagic protein—Atg5, Atg7, and Atg4B/LC3—can regulate the fusion of lysosomes and phagosomes [44, 45]. The localization of the liposomal LC3 to the osteoclast ruffled border is Atg5 dependent, and the Atg5-Atg12 conjugation and the Atg4B are the necessary conditions for the high efficient lysosome localization and bone absorption [46].

The mechanism of bone remodeling is strictly controlled, in which osteoblasts (OB), osteoclast (OC), and bone cells are



Autophagy in osteoblasts and osteoclasts

Fig. 2 Autophagy in osteoblasts and osteoclasts. Osteoblasts and osteoclasts are the main participants of bone remodeling. GC directly inhibits the proliferation and differentiation of osteoblast lineages, reduces the maturation and activity of osteoblasts, and induces osteoblast apoptosis in the body. Moreover, GC acts

directly on osteoclasts to reduce the apoptosis of mature osteoclasts. Atg5 and Atg7 are necessary for lysosome secretion at the ruffled border. The decrease of osteoblasts and the increase of osteoclasts will lead to the decrease of bone trabecula and osteopenia. MTA can inhibit osteoclast production by reducing autophagic pathway for bone repair

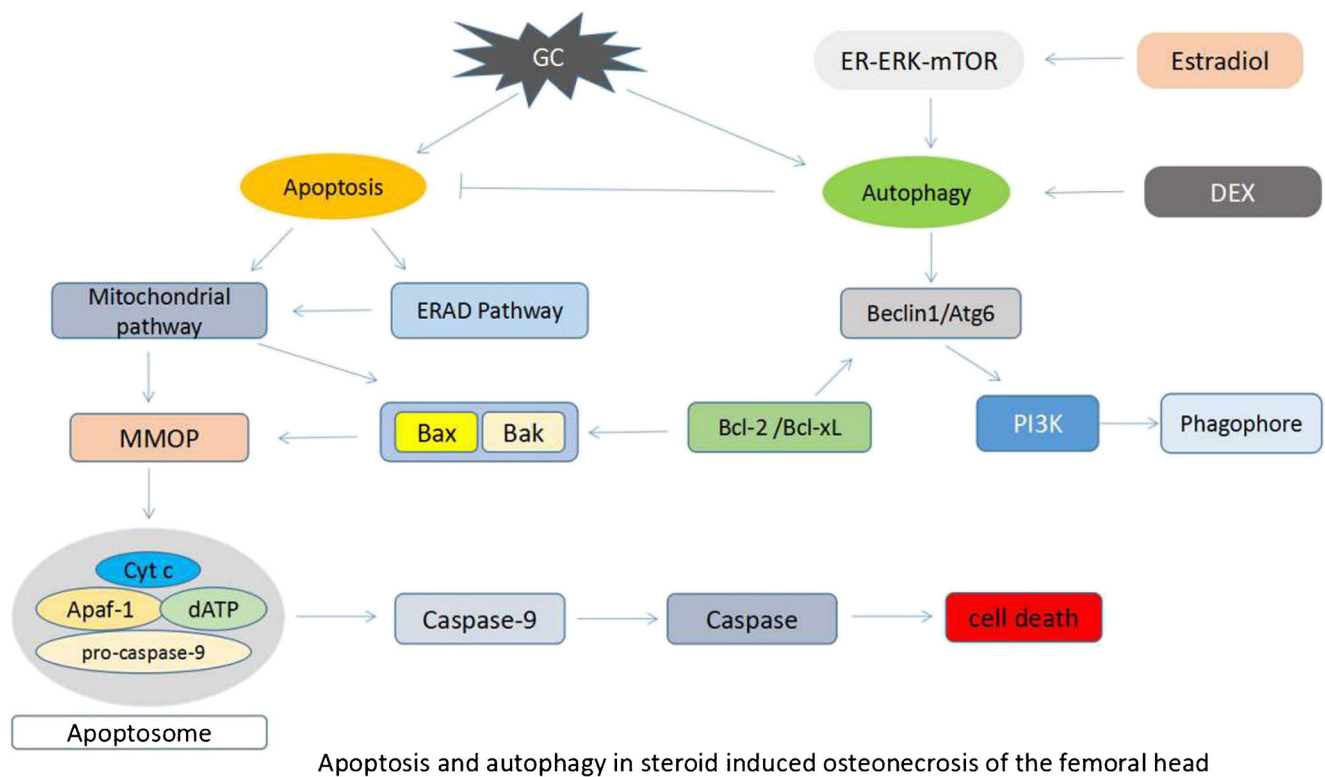
the main participants. GC directly inhibits proliferation and differentiation of osteoblast lineage cells [47], reduces osteoblast maturation and activity [39], and induces osteoblast and osteocyte apoptosis *in vivo* [48] (Fig. 2). The main function of osteoblasts is to produce and mineralized bone matrix, and Marie et al. found that mineralization is associated with autophagy induction, and autophagic inhibition leads to the decrease of OB cell mineralization, which is related to autophagic protein ATG7 and Beclin1 [49]. Recent studies have found that GC acts directly on osteoclasts to reduce the apoptosis of mature osteoclasts [50]. The activation of autophagy through rapamycin promotes the formation of multicore osteoclasts and increases the gene expression of osteoclast, and MTA can inhibit osteoclast formation by reducing autophagic pathways for bone repair [51]. Dexamethasone can reduce the number of normal metabolic bone cells, and this effect increases when autophagy is suppressed, and Xia et al. have found that osteocytes can reduce the negative effects of Dex on bone cells by increasing autophagy [52].

Apoptosis and autophagy in steroid-induced osteonecrosis of the femoral head

It is well known that programmed cell death is an active process in a multicellular organism in order to regulate the development of the body, maintain the stable internal environment, and be controlled by the gene. Apoptotic programmed cell death is the most common form of cell death in organisms. At present, most scholars believe that the mechanism of steroid-induced osteonecrosis of the femoral head is inseparable from the apoptosis-induced by ischemia [11, 53]. When

the body cells use glucocorticoids for a long time, lysosomes will recover the organelles extensively and may further cause apoptosis or cell death. There are mitochondrial pathways and endoplasmic reticulum pathways in the pathway of apoptosis, and the endoplasmic reticulum pathway also activates the mitochondrial pathway [54] (Fig. 3).

A variety of stimulants can activate internal apoptotic pathways, causing mitochondrial outer membrane permeabilization (MOMP), and MOMP can lead to rapid cell death by releasing cytochrome c and other apoptotic proteins [55]. Among them, the released cytochrome c interacts with Apaf-1, pro-caspase-9, and dATP to form the apoptotic body, which activates caspase-9 and activates the effector—caspase—to cause cell death (Fig. 3). MOMP is regulated by the Bcl family proteins, which are divided into apoptotic BH1234 proteins, such as Bcl-2, Bcl-xL, Mcl-1, and apoptotic BH123 proteins (such as Bax, Bak) based on the homologous domain, of which Bax and Bak are on the endoplasmic reticulum [56]. GC can induce autophagy and apoptosis of bone cells and is related to the dose of GC. Autophagy is activated by low dose of GC, while high dose of GC causes apoptosis [57]. As mentioned above, Atg6/Beclin1 is part of the III type PI3 kinase complex required for autophagy precursors, and interference with Beclin 1 can prevent autophagy induction. Bcl-2/Bcl-xL can not only bind and interfere with apoptotic protein Bax and Bak, but also inhibit autophagy by binding to Beclin 1, and this interaction is important in the regulation of autophagy induced by starvation [58]. The interaction between Beclin 1 and Bcl-2/Bcl-xL is achieved through the BH3 domain in Beclin 1 [59]. In addition, the study found that autophagy induced by dexamethasone (Dex) protects cells from apoptosis, and autophagy is an important regulator of osteoblast



Apoptosis and autophagy in steroid induced osteonecrosis of the femoral head

Fig. 3 Apoptosis and autophagy in steroid-induced osteonecrosis of the femoral head. Low doses of glucocorticoids can induce autophagy in bone cells, and high doses of glucocorticoids can cause apoptosis. There are two ways of apoptosis: mitochondrion pathway and endoplasmic reticulum pathway. The endoplasmic reticulum pathway also activates the mitochondrial pathway. Multiple stimulants can activate the intrinsic apoptotic pathway and induce mitochondrial outer membrane permeability (MOMP), and MOMP can release cytochrome c.

apoptosis through its interaction with Bax/Bcl-2 and maintains the osteoblast function of the cells after GC exposure; this indicates that autophagy is a survival mechanism in Dex-treated cells to reduce the incidence of apoptosis and contribute to cell survival [60]. And researchers have found that PTH can induce autophagy to protect bone cells from Dex damage [61], but the specific mechanism is not yet clear. In addition, estradiol has recently been found to increase the level of autophagy through ER-ERK-mTOR pathway to prevent osteoblast apoptosis [62]. Therefore, apoptosis and autophagy in osteonecrosis of the femoral head may be intersect and jointly regulate the pathological process of ONFH.

Conclusion

To sum up, autophagy is a self-protection mechanism for osteonecrosis of the femoral head, and autophagy and apoptosis are involved in the pathogenesis of steroid necrosis of the femoral head, in which the level of autophagy is related to the dose of hormone. However, scholars still argue about the mechanism of the pathogenesis of steroid necrosis of the

The released cytochrome c interacts with Apaf-1, pro-caspase-9, and dATP to form an apoptotic body, which activates caspase-9 and activates the effector caspase to cause cell death. Bcl-2/Bcl-xL can not only bind to pro-apoptotic protein Bax and Bak, but also further regulate MOMP, and it also inhibits autophagy by combining with Beclin 1. PTH can induce autophagy to protect the survival of bone cells due to Dex damage. Estradiol can increase the level of autophagy in the cell through the ER-ERK-mTOR pathway to avoid apoptosis of osteoblasts

femoral head and the interaction of autophagy and apoptosis. This needs to be further studied in vitro experiment, so as to study the effect of autophagy on the necrosis of the femoral head and provide a new idea for the treatment and prevention of the necrosis of the femoral head.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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