REVIEW ARTICLE

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Animal models of steroid-induced osteonecrosis of the femoral head—a comprehensive research review up to 2018

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Abstract

Osteonecrosis of the femoral head (ONFH) is a significant cause of both pain and disability that often affects young adults during what ought to be their most productive age. Two broad categories of ONFH exist: traumatic and non-traumatic. Traumatic ONFH results from acute mechanical disruption of the femoral head's blood supply. Many factors that increase the risk of non-traumatic osteonecrosis have been identified. Steroid-induced osteonecrosis of the femoral head (SONFH) is the most common form of non-traumatic ONFH. Many hypotheses as to the pathogenesis of SONFH have been proposed, including intravascular thrombosis, abnormal fat metabolism, intramedullary adipocyte hypertrophy, and osteoporosis; however, the exact mechanism of SONFH is still not clearly understood. Animal models using rats, mice, rabbits, chickens, pigs, and emus have been used to study SONFH. Unfortunately, these models each have limitations. Therefore, it is necessary to establish a reproducible model that better simulates human disease. The present review is intended to summarize the currently available models, evaluative indicators, and application of current understanding to both the prevention and treatment of SONFH.

Keywords Steroid-induced osteonecrosis of the femoral head · Ischemic necrosis · Aetiology · Animal models

Introduction

Glucocorticoids are inexpensive, effective, and widely used for conditions both acute and chronic, including severe acute respiratory syndrome (SARS), asthma, rheumatoid arthritis (RA), systemic lupus erythematous (SLE), and organ transplantation. Despite their utility, glucocorticoids have been recognized as a major risk factor of ONFH, with ONFH occurring in up to 13% of patients on high doses (> 3 g cumulative) of glucocorticoids [1–5]. Femoral head collapse—the end stage of ONFH—requires total hip arthroplasty, a procedure that carries with it both morbidity and significant economic burden to the patient and the health care system at large. Preventing the stepwise accumulation of insulting events that lead to ONFH would allow for effective steroid treatment while avoiding femoral head collapse. Though multiple studies have established a causal relationship between steroids and ONFH, an ideal animal model has not yet been created. It is therefore essential to establish an animal model of SONFH and femoral head collapse that mimics the human clinical aetiology [6, 7]. The present review is intended to summarize the currently available models, evaluative indicators, and application of current understanding to both the prevention and treatment of SONFH.

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Animal models of SONFH

Rabbit

Rabbits are the most commonly used animal model for SONFH. They share mammalian characteristics with humans and are simple to acquire, cheap to maintain, and easy to breed. Many mechanisms for the pathogenesis of SONFH have been proposed, but no consensus yet exists as to the exact mechanism. Glucocorticoids directly inhibit differentiation of osteogenic progenitors to osteoblasts while simultaneously promoting adipocyte formation and deposition [8–10]. Liu et al. [8] found that impaired osteogenic differentiation associated with connexin43/microRNA-206 is implicated in SONFH. Fan et al. [9] found that hypoxia inducible factor prolyl hydroxylase inhibitor prevents SONFH in rabbits by promoting angiogenesis and inhibiting apoptosis. Li et al. [10] found that small interfering RNA (siRNA) that targets the PPARγ gene inhibits adipocytic differentiation of bone marrow mesenchymal stems cells (BMSCs) and prevents SONFH in rabbits.

The vascular milieu has been implicated in rabbit models of SONFH. Wu et al. [11] and Kang et al. [12] found that the increased levels of membrane microparticles and platelets seen in SONFH may be related to hypercoagulability, thrombosis, and inflammation in the microcirculatory environment. Meanwhile Guan et al. [13] showed that hypercoagulability potentiates thrombus formation in the presence of SONFH. In addition, vascular endothelial growth factor (VEGF), an important mediator of angiogenesis and overall vascular function [14], was found by Wang et al. [15] to be decreased in animals treated with glucocorticoids. Decrease VEGF and VEGF mRNA levels correlated with diminished femoral head blood flow, a precursor to ONFH.

In recent years, a number of different treatment options for ONFH have been proposed. Li et al. [16], He et al. [17], Yuan et al. [18], QI et al. [19], and Wu et al. [20] have reported that traditional Chinese medicine (such as Tao-Hong-Si-Wu Decoction and Gu fu sheng) can promote blood flow, the expression of endothelial cell growth factor and insulin-like growth factor, reduce the expression of tumour necrosis factor, and protect endothelial cells and prevent them from apoptosis. Given that apoptosis, endothelial growth, and blood flow disturbances have been implicated in ONFH, these traditional treatment regimens could potentially reduce the incidence of SONFH. Kang et al. [21] and Nishida et al. [22] found Hmg CoA reductase inhibitors (statins) inhibit the formation of fat and reduce blood lipid and free fatty acid concentrations, thereby reducing the risk of SONFH. In a follow-up study, Kang et al. [23] found that the synergistic effect of treatment with an anti-coagulant (enoxaparin) agent and a lipid-lowering (lovastatin) agent reduced the incidence of SONFH in rabbits. Similar results were found when Beckmann et al. [24] built a rabbit model of SONFH through a single intramuscular injection 20 mg/kg methylprednisolone, and found that a therapy group receiving subsequent enoxaparin had decreased rates of SONFH.

Vitamin E has recently become a substance of interest for the treatment and prevention of SONFH. It has been reported by Kuribayashi et al. [25], MIKAMI et al. [26], and JIA et al. [27] that vitamin E may prevent SONFH by inhibiting steroid-induced oxidative stress, alleviating DNA oxidative damage in bone marrow haematopoietic cells and therefore decreasing rates of apoptosis.

T. Asadal et al. [28] and Kang et al. [29] reported that direct injection of autologous bone marrow cells and strontium-doped calcium polyphosphate combined bone marrow mononuclear cells into femurs and prevents SONFH in rabbits. On the basis of these studies, Sun et al. [30, 31] and Xie et al. [32] found that the combination of bone marrow mononuclear cells or endothelial progenitor cells with surgical core decompression enhances neovascularization and bone regeneration in rabbit SONFH, which may provide a novel and effective therapeutic option for early SONFH. In addition, a variety of compounds including sodium ferulate [33], resveratrol [34], nitrate [35], recombinant human fibroblast growth factor microspheres[36], and hydroxysteroid dehydrogenase [37] have shown promise as preventative and therapeutic options for SONFH.

Rat

Rats have long been used as animal models of human disease. Easy to feed, and breed, they grow quickly and share both physiologic and metabolic characteristics with humans. Their moderate size makes their femoral head suitable for both imaging and pathology based studies [38]. Zhang et al. [39] injected methylprednisolone (MPS, 20 mg/kg) into rates weekly and found that MPS significantly damaged the blood supply to the femoral head. In contrast, they found that Vitamin K2 improved blood supply to the femoral head and prevented development SONFH in MPS treated rats.

Adipogenesis and fatty deposition are important causes of SONFH. Han et al. [40–42] reported successively in three articles that enhanced P-glycoprotein activity decreased the risk of SONFH by inhibiting adipogenesis in the femoral head. In these studies, rifampicin was shown to reduce SONFH risk by enhancing glycoprotein activity.

Nozaki et al. [43] studied the effect of lipid-lowering agents on SONFH. They induced adipocyte deposition by injecting 4 mg methylprednisolone acetate (MPLS) subcutaneously into the back of 38 stroke-prone spontaneously hypertensive rats (SHRSP), which led to adipocyte deposition in bone marrow and hyperlipidemia. Pravastatin treatment prevented SONFH in these rats by decreasing blood lipid levels, oxidative stress, and adipocyte formation. Jiang et al. [44] found that pravastatin treatment prevented SONFH in Wistar rats who had

received MPLS injections by enhancing angiogenesis in rat femoral heads, decreasing circulating lipids levels, suppressing PPARγ expression, and activating Wnt signaling pathway. Subsequently, Zhang et al. [45] found that SONFH was caused by apoptosis which was linked to the Wnt/β-catenin pathway. Yu et al. [46, 47] found that lithium or lithium chloride can stabilize osteogenic-adipogenic homeostasis by activating the β-catenin pathway and thereby preventing development of SONFH.

Oxidative stress has repeatedly been shown to contribute to SONFH in rate models. Nozaki et al. [43] reported that corticosteroids increase oxidative stress. The toll-like receptor 4 (TLR4) pathway was implicated in SONFH by Okazaki et al. [48] and Tian et al. [49], who found that corticosteroids SONFH by disturbing the immune response via TLR4 signaling pathway. Subsequently, Erkam Komurcu et al. [50] and Miyata et al. [51] found that coenzyme Q10 prevented SONFH by reducing oxidative stress. Pentosan, a semi-synthetic polysulfated xylan appears to reduce the incidence of ONFH in SHRSP by improving lipid metabolism and decreasing oxidative stress. A single study has called in to question among oxidative stress, TLR4, and SONFH. S. Okazaki et al. [52] found that TLR4 stimulation by lipopolysaccharide administration increased the incidence of SONFH, but concomitant oxidative stress via TLR4 signaling did not contribute to the development of SONFH.

In recent years, many attempts to elucidate the genetic contributors to SONFH have been undertaken. Tong et al. [53] performed gene array analysis on a rat model of SONFH, confirmed the microarray results by using a quantitative RT-PCR and immunohistochemistry (IHC) assays. In their SONFH group, 190 individual genes showed differential expression relative to healthy controls, with 52 being upregulated and 138 being downregulated. The majority of the genes in question were associated with oxidative stress pathways, signal transduction, apoptosis, lipid metabolism, and extracellular matrix proteins. The microarray analysis of gene expression in rat model by Kerachian et al. [54] concluded that rat SONFH may be mediated byalpha-2 macroglobulin (A2M), though subsequently Carli et al. [55] found that elevated serum levels of A2M were not predictive of early SONFH in rodents. The elevated A2M levels observed during glucocorticoid treatment suggest that it may play a role in the host reparative response; therefore, A2M's contribution to SONFH warrants further study.

Zhao et al. [56] have shown that microRNA-145 mediates SONFH through targeting the OPG / RANK / RANKL signaling pathway. In addition, Tao et al. [57], Chen et al. [58], Wu et al. [59], Shi et al. [60], CHEN et al. [61], and Dong et al. [62] have recently explored the prevention and treatment of SONFH through modulation of signaling pathways, Chinese medicine, gene expression, osteogenesis, inhibition of adipogenesis, and inhibition of apoptosis, respectively. The

aforementioned rat-model studies have not completely elucidated the aetiology or optimal treatment of SONFH, but point in the way toward future fruitful research avenues.

Mouse

Mice are affordable and easy to breed. They reproduce rapidly and possess a skeletal system grossly similar in development to humans [63]. However, the mouse femoral head is relatively small, which prevents reliable characterization of mouse ONFH on MRI and CT. This limits the utility of mouse models. Ryoo et al. [64] induced SONFH in female BALB / cAnN / CrljBgi mice through prednisolone treatment. Repeated administration of LPS to these mice showed development of SONFH more strongly associated with TNFα levels in the femoral head, rather than cytokine levels in immune tissues. Wang et al. [65] induced SONFH in C57BL/6J mice using MPS (21 mg/kg). They found 23 bone marrow miRNA expression variations in the experimental group, including 7 upregulated and 16 downregulated. The results of reverse transcriptase-quantitative polymerase chain reaction (RT-PCR) supported the results of their microarray assay, indicating that SONFH may cause miRNA changes in BMSCs.

Yang et al. [66] evaluated the effect of continuous glucocorticoid therapy versus discontinuous therapy in 14 different strains of mice and found that discontinuous treatment led to lower rates of SONFH despite comparable cumulative doses between the two groups. This suggests that prevention may be achieved, in part, through judicious dosing regimens.

Chicken

Chickens are small, cheap, and easy to raise birds. Though they do not share a mammalian heritage with humans, the bipedal gait of chickens places stresses across their hips more similar to human than those seen by small mammalian quadrupeds like mice and rats. Cui et al. [67] studied a chicken model of SONFH by selecting injecting 48 chickens with MPS (3 mg/kg) on a once weekly basis. Empty bone lacuna and bone marrow necrosis were seen after only six weeks of this regimen. A cohort of chickens injected treated with both MPS and lovastatin were observed to have less bone resorption and no bony necrosis. Chen et al. [68] looked at the molecular messengers associated with SONFH in chickens. They fed MPS sodium succinate (5.2 mg/kg) to 32 healthy female Leghorn chickens once weekly for eight weeks. Hyperlipidemia, thin trabecula, increased number of empty lacuna and adipocyte proliferation, and hypertrophia were observed in MPS chickens. These changes were accompanied by increased expression of RANKL and Smad7. Decreased levels of OPG, BMP2, TGFβ1, and Smad4 were seen in the treated mice. Huogo I Formula, a traditional Chinese medicine, has been shown to decrease rates of SONFH. Erken et al.

[69] used a similar model for SONFH, injecting MPS (3 mg/kg/week) intramuscularly into Leghorn chickens. They found that treatment with pentoxifylline decreased rates of SONFH.

While these studies have corroborated small mammal molecular mechanism and treatment regimens for SONFH, Xu et al. [70] performed an anatomic study of the blood supply to the chick femoral head. The study found that only the acetabular branch of the femoral artery, lateral retinacular artery, trochanteric artery, and the ascending branch of the middle femoral nutrient artery perfuse the chicken femoral head. The femoral circumflex artery, whose branches perfuse the majority of the human femoral head, merely perfuses the chicken thigh musculature. This difference in vascular anatomy do calls into question the degree to which the chicken model accurately mimics human disease.

Emu

Small mammals and chickens have most often been used to model SONFH. The convenience of working with small animals comes at a cost: hormone induction in these animals has not been found to cause true structural collapse of the femoral head, the final and most morbid stage of human SONFH. Emus, massive bipedal birds, do experience true femoral head collapse when osteonecrosis occurs [6]. The size, weight, and biomechanical similarities shared by humans and emus make this bird a useful model for studying SONFH [71–73]. Recognition of this fact has led an increasing number of research groups to study the emu model of ONFH. Conzemius et al. [71], Reed et al. [74], and Fan et al. [75] conducted studies on temperature-induced osteonecrosis of the emu femoral head. They found that cryogenic insults as well as vast temperature swing alternating between freezing and heating episodes led to eventual collapse of the femoral head. Zheng et al. [6] made the emu model directly applicable to SONFH with their study of LPS- and MPS-induced emu ONFH, finding that 70% of emus treated with the combination of toxin and steroid went on to femoral head collapse. Untreated emus did not develop ONFH or collapse.

While the similarities between end stage emu and human SONFH make the birds an attractive model organism, the birds are rare relative to the abundant traditional small lab mammal. Many animal research facilities have neither experience with, nor access to emus. As more studies establish the utility of the emu model, it would be prudent to pursue this model in the transition from small mammal to human clinic studies.

Other animal models

In recent years, animal models of ONFH have also been reported in large animals such as pigs [76], sheep [77], and dogs [78]. The larger size of these experimental animals makes access to and macro scale visualization of the femoral head possible. Therefore, surgery is usually performed, and the local application of alcohol or focal cryogen insults is used to induce osteonecrosis [76, 77]. However, a few large animal models that utilize steroids do exist. Drescher et al. [79] treated pigs with corticosteroids and observed contraction of femoral head lateral epiphyseal arteries in treated animals, but noted no evidence of ONFH in these pigs. In another report by Poignard et al. [80], 18 female pigs were used to creat ONFH by cryo-injury, the authors noted localized and sustainable subchondral necrosis of the femoral head, reproducing all stages of the human disease. Moreover, the good correlation between histological and MRI results indicates that the pig is a relevant model for human ONFH. Run et al. [78] established the ONFH model with 18 healthy adult dogs. In their study, group A was treated with MPS (10 mg/kg) combined with LPS (10 µg/kg), in group B, ONFH was induced by liquid nitrogen cryo-insult, and group C was a blank control group. They found that both the hormonal method and the liquid nitrogen method induced non-traumatic ONFH. They concluded, however, that the more reliable osteonecrosis level seen in their liquid nitrogen treated group made cryo-injury more suitable for the research of standardized treatment of existing ONFH. In the treatment of ONFH, Lebouvier et al. [81] found treatment of a natural pig ONFH by autologous BMSCs indicated a beginning of bone healing as early as two weeks with a complete healing after nine weeks. None of the above models have shown the reproducibility demonstrated by small mammal, chicken, and emu models of SONFH.

Selection of SONFH modeling methods

While steroid only models of SOFHN do exist, the research community has paid increasing attention the limitations of these studies. Clinically, chronic SONFH does occur without a pre-existing diseases state that necessitates steroid use. SLE, RA, chronic obstructive pulmonary disease, and organ transplantation along with acute bouts of SARS or asthma require steroid treatment. In order to better simulate the clinical setting of inflammation, the use of LPS or allogeneic serum in conjunction with steroid therapy has become more accepted as the most appropriate model of SONFH.

Glucocorticoid steroids alone modeling

The rate at which SONFH occurs in animal models has been shown to depend on the type of steroid use, dosage, and frequency of administration. All of these factors are important to consider when judiciously using steroids in the clinical setting. Zhao et al. [82] and Kang et al. [29] induced SONFH in male Japanese white rabbits with a single intramuscular injection of MPLS (20 mg / kg). The incidence of ONFH was 75%

and 70% after two weeks respectively. Wang et al. [83] reported that 16 rats were given intramuscular prednisolone acetate (12.5 mg/kg) twice a week for four weeks. The incidence of SONFH was 71.43%. Motomura et al. [84] compared four different single intramuscular doses of MPS single dose injection to induce SONFH rabbit model. After four weeks, the incidence of ONFH in the 1, 5, 20, and 40 mg/kg groups was 0, 42, 70, and 96%, respectively.

Glucocorticoid steroids + endotoxin modeling

LPS, also known as endotoxin, can cause fever, microcirculation disturbances, and even endotoxic shock and disseminated intravascular coagulation. When used in combination with steroids to simulate the inflammatory host state, it reliably initiates intravascular coagulation and eventual SONFH. Yu et al. [46] used SD rats in a hormone + LPS model. LPS (4 mg/kg) was intravenously injected two times with an interval of 24 hours, after 24 hours, MPS (60 mg/kg) was intramuscularly injected three times every 24 hours, and three weeks later, the histological examination revealed that the incidence of ONFH was 73.3% with an increased empty lacunae and morphological changes of osteocytes, haematopoietic cells, and adipocytes in bone marrow. Chen et al. [61] performed a similar study in Wistar rats. LPS (2 mg/kg) was intravenously injected two times with an interval of 24 hours, 24 hours later, MPS (20 mg/kg) was intramuscularly injected three times every 24 hours. After four weeks, the incidence of ONFH was found to be 66.7% with the increase of empty lacuna, pyknotic nuclei of osteocytes, and necrosis of bone marrow cells. Dong et al. [62] used SD rats in an even more reliable model of SONFH. LPS (20 μg/kg) was intravenously injected two times every 24 hours, and after 24 hours, MPS (40 mg/kg) was intramuscularly injected three over three days. Four weeks later, the incidence of ONFH was 83.3% with an associated decrease in bone mass, trabecular thickness, bone mineral density, and serum osteocalcin concentration.

Jia et al. [27] injected 4-month-old, healthy white rats with 100 μg/kg intravenous *Escherichia coli* endotoxin two times over 24 hours. These injections were followed by three times daily MPS (20 mg/kg) intramuscular injections. Four weeks later, histopathology of the femoral head showed typical ONFH with thin, broken, and disordered trabecula, hypertrophic fat cells, irregular arrangement of osteoblasts, and an increase in empty lacunae. At six weeks, histopathology showed osteocyte necrosis associated with karyopyknosis, karyolysis, and karyorrhexis. Still more bone lacunae vacuoles occurred, intramedullary vessels were compressed, vascular lumens became narrow. Fat emboli and thrombus formation were also observed.

Zheng et al. [6] used ten male emus at 24 months of age in their toxin + hormone model. LPS (8 μg/kg) was intravenously injected twice over four days. Subsequently, MPS (10 mg/kg) was intramuscularly administered three times every two days. In emus treated with this regimen, MRI showed a strong edema signal in the proximal femoral head starting at week two. This edema signal decreased gradually until the 12th week, at which point gait abnormalities were observed. Micro-CT scan showed a significant reduction of BMD, bone tissue, trabecular number, and thickness of the subchondral and femoral head central regions. By 24 weeks, the rate of femoral head collapse was 70%.

Glucocorticoid steroids + allogeneic serum modeling

Allogeneic serum can induce type III hypersensitivity, a process in which medium-sized antigen-antibody complexes deposit in the basement membrane of small blood vessels resulting in capillary congestion, oedema, necrosis, and local neutrophil infiltration. This inflammatory process is not dissimilar from that seen in many of the antibody mediated autoimmune diseases treated with steroids. The vasculitis induced by this process is complicated by the introduction of steroids—which inhibit not only immune cells (their intended therapeutic), but also the synthesis of collagen and elastic fibers, which can lead to avascular necrosis of the femoral head. Wang et al. [37] injected a sensitizing dose of horse serum (10 ml/mg) intravenously into rabbits and then injected 6 ml/kg of horse serum one daily for two days two weeks later. MPS (20 mg/kg) was then injected intraperitoneally twice weekly for two weeks. Two weeks later, MRI examination showed that rabbits in the treatment group had a larger joint cavity in the femoral head, a high level of T2WI signal in the metaphysis and bone marrow oedema. Hyperlipidemia and loss of femoral head cartilage coverage were also observed. Bony trabeculae were decreased and thinned, while bone marrow haematopoietic cells and osteocytes were decreased in number. This was all seen in the setting of shrunken capillary bed with numerous partially obstructed vessels.

In a similar study, Tian et al. [33] sensitized rabbits with horse serum (10 ml/kg) and then three weeks later injected a single dose of 7.5 mg/ml horse serum. After an additional two weeks, MPS (45 mg/kg) was injected intramuscularly once a day for three days. Observation at two weeks showed some early osteonecrosis, which was fulminant in 54% of the treated mice by week eight. Trabecular bone showed necrotic changes, with osteoclast and fibroblast invasion of the trabeculae tissue.

Identification of SONFH in animal models

The most commonly used method for identification of SONFH in experimental models is histopathology. The histopathology of osteonecrosis is characterized by the presence of sparse or fragmented trabeculae, disorganization of tissue, reduction of haematopoietic cells in the bone marrow, oedema

of adipocytes, and microvascular occlusion with thrombi or fat emboli [27]. This approach is reliable for small animals not amenable to conventional imaging studies, but has limited clinical application to practitioners who must often made this diagnosis based on plain film or MRI imaging.

Clinically, the course of SONFH is gradual. Early diagnosis and treatment limit the morbidity and economic burden associated with the disease. Chen et al. [85] showed that MRI is a highly sensitive method for the diagnosis of early ONFH, when articular cartilage and the bony interface between femur and acetabulum do not yet appear abnormal on plain radiographs. Later in the course of disease, in large animals like the emu, plain film radiographs show characteristic decreased bone density, cystic degeneration of the femoral head. Micro-CT can be applied to animal models of all sizes and defines bony architecture more clearly than plain radiographs. Recent studies have shown that angiography can also be used to identify the animal models of SONFH. Zhang et al. found fewer vessel-like structures in femoral heads by angiograph with associated, many vacuoles occupying a large area of the subchondral region [39].

Summary

In summary, scholars from across the globe have developed a variety of animal models of SONFH and focused their studies on pathogenesis, prevention, and treatment of SONFH. Despite all of this work, the ideal animal model has not yet been identified. While rodents have predominated early research, their four-legged gait and small body weight make application of their disease process imperfect. Larger animals, such as pigs and dogs ameliorate the weight discrepancy, but ambulate with biomechanics that differ significantly from humans. In fact, induction of true femoral collapse has proven difficult, despite its frequency in the human population suffering from SONFH. Bipedal animals such as chicken and emu walk in a similar manner to human beings and the weight of the emus more closely mirrors human body weight. These similarities allow for true femoral head collapse in emu models of SONFH. Significant differences in bloody supply to the femoral head between birds and mammals have been identified and could possibly change the stepwise pathology of the disease. The variety of induction methods (steroid only, steroid + toxin, and steroid + allogeneic serum) make comparing study results challenging, but likely reflect the varied inflammatory milieu present in humans who are treated with steroids for a variety of conditions.

The ideal combination of animal, induction regimen, and identification tool for the study of SONFH has not yet been established. Creating a replicating such an experimental model will allow for more effective study and treatment of

SONFH, leading to improved clinical outcomes at reduced cost to the individual and the health care system.

Compliance with ethical standards

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