Review

There are many potential medical therapies for atraumatic osteonecrosis

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Abstract

Atraumatic osteonecrosis is a common complication of SLE and is seen in other connective tissue diseases, in patients treated with high doses of CSs, in HIV-infected patients and in alcoholic patients. Standard care is confined to analgesia, core decompression if the condition is early and affects the femoral head and joint replacement. However, consideration of the underlying biological mechanisms leads to the recognition of many potential therapies that might either prevent progression or, even, reverse the process if it is not yet too far advanced. These potential therapies merit detailed consideration. Critical points are that (i) histopathological evidence shows that the initial cellular event is apoptosis of osteocytes; and (ii) another requisite, as homeostasis requires that death and rebirth of osteocytes be balanced, is an accompanying inadequate proliferative capacity of osteoblasts. Thus, a logical approach to treatment includes measures that (i) reduce apoptosis of osteocytes and (ii) enhance proliferation of osteoblasts/pre-osteoblasts. Measures to reduce the ongoing apoptosis of osteocytes require reinforcing the effects of members of the Bcl-2 family (Bcl-2 itself and Mcl-1), the Wnt/catenin pathways (using an available sclerostin antibody) and HSPs (by application of local heat using US, deep wave diathermy or infrared), as well as administration of bisphosphonates and nitrates. Measures to enhance proliferation of osteoblasts/pre-osteoblasts include the use of stem cells, extracorporeal shock wave therapy, aspirin, the proteosome inhibitor bortezomib, melatonin and application of local heat. Use of VEGF would encourage proliferation of blood vessels and osteogenesis. Certain drugs that inhibit osteoblast proliferation should be avoided, including NSAIDs, serotonin reuptake inhibitors and thiazolidinediones.

Key words: atraumatic osteonecrosis, apoptosis of osteocytes, proliferative capacity of osteoblasts/pre-osteoblasts, Bcl-2 and Mcl-1, Wnt/catenin pathways, heat shock proteins, bisphosphonates, nitrates, proteosome inhibition, melatonin.

Introduction

Atraumatic osteonecrosis is a major complication in patients with SLE, other collagen vascular diseases and alcoholism; in patients infected with HIV and in patients treated for any reason with high doses of corticosteroids (CSs). The thesis presented here is that the primary and fundamental initiating mechanism for most cases of atraumatic osteonecrosis, excluding those whose immediate cause is interruption of blood flow, is either increased apoptosis of osteocytes or reduced proliferation of pre-osteoblasts/osteoblasts, or both. In the following discussion, the term osteonecrosis applies to all instances of atraumatic osteonecrosis except those cases caused by interruption of blood flow. Treatment options for osteonecrosis are largely limited to analgesics, core decompression if the condition affects the femoral head and replacement of the adjacent joint if seriously damaged by the process. The purpose of this presentation is to show that the molecular biological underpinnings of osteonecrosis suggest multiple other possible medical approaches to treatment, and that several of these treatments are immediately practical and warrant consideration for preventing progression or, even, reversing the pathological process. If the adjacent joint is already destroyed, efforts to prevent progression still have merit because osteonecrosis is often polyostotic. In brief, it is hoped that this presentation might expand the way we think about treating patients with osteonecrosis.
Although the treatments mentioned here are logical, it must be recognized at the outset that clinical trials involving meaningful numbers would be difficult to mount because the condition is uncommon. However, major centres might see several patients per year, and successful treatments in even a small number of patients might provide proof of concept for a multi-centre international clinical trial.

The suggested treatments depend on two critical elements of pathogenesis: (i) that the initial histopathological event in osteonecrosis is apoptosis of osteocytes; (ii) that homeostasis requires that death and rebirth of osteocytes be balanced. Thus another requirement if the process is to proceed to osteonecrosis is inadequate proliferative capacity of osteocyte precursors, which are bone marrow mesenchymal stem cells (BMSCs), pre-osteoblasts and osteoblasts. Therefore logical approaches to treatment include measures that (i) reduce apoptosis of osteocytes and (ii) enhance proliferation of osteoblasts and their precursors.

**Discussion**

**Initial histopathology of osteonecrosis shows death of osteocytes**

The terms avascular and atraumatic were applied in the early literature because the histopathology resembles the osteonecrosis caused by arterial injury from fracture of the femoral neck. The term osteonecrosis is itself misleading, as there is apoptosis of osteoblasts and osteocytes, but necrosis of bone is not *sine qua non*; histological necrosis affecting marrow comes later. Osteoblasts were lost by day 3 in traumatic osteonecrosis; by days 3 and 4, osteocyte nuclei were lost, and there was necrosis of blood vessels in marrow, and by day 10, marrow necrosis was complete [1]. Other authors saw similar loss of osteocytes in atraumatic osteonecrosis [2–4], and it is relevant, as osteonecrosis is seen with increased frequency in HIV-infected patients, that heat-inactivated HIV or recombinant gp120 induced apoptosis of human osteoblasts in culture [5]. In early atraumatic osteonecrosis there was either medium or strong staining for VEGF and fibroblast growth factor-2 (FGF-2), showing that these growth factors appear as early responses [6].

Timing the onset is more accurate when osteonecrosis is induced in animals. After administration of methylprednisolone to rabbits, the changes seen before severe damage to femora were apoptosis of both osteoblasts and osteocytes, whereas no specimen showed necrosis of marrow cells or vascular change [7]. In humans with osteonecrosis due to CSs, apoptosis of osteocytes is also prominent [8]. Moreover, a dearth of blood vessels is an early feature of osteonecrosis [9].

**Initial histopathology of osteonecrosis also shows regeneration of osteoblasts**

Within 24 h of the injury there was proliferation of fibroblasts, which later increased [1]. Fibroblasts in marrow, under the influence of FGF-2 [10], are precursors of osteoblasts [11]; their response is inadequate, otherwise there would be sufficient reconstitution of osteocytes, healing and no clinically evident osteonecrosis. Indeed, osteoblasts taken from the proximal femur of patients with osteonecrosis showed reduced proliferative capacity [12].

Thus the earliest anatomical changes at the onset of the osteonecrosis are apoptosis of osteocytes and the rapid, but inadequate, appearance of reparative pre-osteoblasts and new vessels. Based on these observations, there are two rational approaches to treatment: (i) antagonize apoptosis of osteocytes and (ii) enhance the regeneration of osteoblasts and new vessels.

**Antagonizing apoptosis**

The mechanisms underlying apoptosis are complex and reviewed in detail elsewhere [13–15]. Important anti-apoptosis participants include members of the Bcl-2 family, Bcl-2 itself and Mcl-1; the Wnt/catenin pathways; and HSPs. Bcl-2 promotes the differentiation, activation and survival of osteoblasts: osteocytes from Bcl-2 knockout mice showed a >4-fold increase in apoptosis as compared with wild-type osteocytes [16]. Mcl-1 was up-regulated in myeloid progenitor cells by GM-CSF [17] and by IL-5 [18]. Thus it might be useful to explore the therapeutic use of GM-CSF and IL-5 in patients with osteonecrosis. The important Wnt/catenin signalling pathway is involved in the control of osteoblastogenesis [19] and accomplishes this by activating kinase signalling that up-regulates Bcl-2 [20]. Peroxisome proliferator-activated receptor γ (PPARγ) signalling plays a role: repression of PPARγ signalling in BMSCs via both the canonical and non-canonical Wnt-β-catenin pathway-induced osteoblastogenesis, whereas stimulation of PPARγ by thiazolidinedione anti-diabetic drugs promoted adipogenesis [21] at the expense of osteoblastogenesis [22]. Mice that are heterozygous for PPARγ, i.e. PPARγ<sup>−/−</sup>, have increased osteoblastogenesis, and in culture, cells that are PPARγ<sup>−/−</sup> spontaneously differentiate into osteoblasts [23]. DHEA induced β-catenin signalling in prostate cancer cells [24] and merits similar investigation in osteoblasts. On the other hand, sclerostin, which is produced almost exclusively by osteocytes, antagonizes Wnt signalling and thus also antagonizes osteoblastogenesis [25]. A sclerostin antibody (AMG785) was developed for human use; 85 days after a single dose, DEXA at the lumbar spine increased by 5.3% [26]. A trial to assess the efficacy of this agent in patients with osteonecrosis should be considered. Inhibition of secreted frizzled-related protein-1, another Wnt antagonist, activates β-catenin signalling in bone and stimulated bone formation [27].

HSPs, which are produced when cells are stressed by a variety of stimuli, including heat or cold, protect against apoptosis. Application of heat both *in vitro* and *in vivo* is protective, and the degree of protection correlates with the level of HSP that is induced. Thus, in mice transgenic for overexpressing HSP72, pre-heating before experimentally caused coronary occlusion led to a significant
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Bisphosphonates not only cause osteoclast apoptosis but also prevent osteocyte and osteoblast apoptosis [29] by a mechanism that includes induction of HSP90 expression [30]. Thus the use of bisphosphonates is considered rational in the therapy of osteonecrosis, and this was reported as successful in one study [31] but as unsuccessful in another [32]. It was shown that crosstalk between T lymphocytes and BMMSC, mediated by IFN-γ and TNF-α, is key in determining regeneration of bone by BMMSC [33]. Aspirin both reduced the concentrations of IFN-γ and TNF-α and restored bone formation mediated by BMMSC [33]. Several biologic agents that reduce the effects of TNF-α are commercially available and so, of course, is aspirin.

Nitric oxide mediates the pro-survival effect of oestrogen on osteocytes [34]. Women randomized to receive isosorbide mononitrate had less bone resorption and more bone formation compared with placebo [35]; nitroglycerin ointment gave similar findings [36]. Of several potential pharmacological options for early osteonecrosis, nitrates are among the simplest and least toxic.

Two case reports have shown resolution of mandibular osteonecrosis, owing to bisphosphonate treatment, using teriparatide [37, 38]. In each case, local ulcers healed after several months of teriparatide injections. Intermittent administration of PTH stimulates bone formation by delaying osteoblast apoptosis, thereby increasing the number of osteoblasts; this requires runt-related transcription factor (Runx2)-dependent expression of anti-apoptotic genes like Bcl-2 [39]. Observations from genetically modified mice suggest that the anabolic effect of intermittent PTH also requires insulin-like growth factor-1 (IGF-1), FGF-2 and perhaps Wnt [39].

Enhancing regeneration of osteocytes

Regeneration of osteocytes begins at the level of BMMSC, pre-osteoblasts and osteoblasts. Fibroblasts (precursors of pre-osteoblasts) taken from the femur of patients with osteonecrosis had reduced proliferative capacity [12], and fibroblast colony-forming units in iliac crest bone marrow of patients with osteonecrosis were reduced 8-fold compared with healthy marrow donors [40]. HSPs not only protect against apoptosis but may also enhance proliferation. They act as molecular chaperones involved in protein folding and stabilization and as modulators of proteins in signalling cascades. Exposure of human bone marrow stem cells to 39°C for 96 h increased the expression of HSP70 by 29% and DNA synthesis by 58% [41]. Heating the bone of rabbits in vivo led to an increase in trabecular bone formation [42]. As indicated above, local application of US, deep wave diathermy or infrared are non-toxic therapeutic approaches to the initial phase of osteonecrosis in humans.

It will be reasonable to consider adiponectin when this molecule becomes available for clinical use, as this hormone stimulates osteoblast proliferation and differentiation [43, 44], and it also suppresses osteoclastogenesis [44]. Adiponectin induces cyclooxygenase-2 (COX-2) [45], which mediates osteogenesis [41].

A substantial body of literature shows that there are melatonin receptors on osteoblasts, and that melatonin promotes osteoblast differentiation and osteogenesis [46-50]. The molecular mechanism by which melatonin influences osteogenesis has been investigated. In a pre-osteoblast cell line, levels of melatonin as low as 10 nM increased gene expression of bone sialoprotein (an extra-cellular matrix protein expressed during osteoblast differentiation), osteopontin, osteocalcin and ALP [47]. It was also shown that melatonin induces the expression of Runx2 and BMP-2 [49], activates the WNT-β-catenin pathway [50] and antagonizes the pro-apoptotic molecule BAX [48]. Melatonin is readily available without prescription.

BMMSCs are a multipotent population of cells that can differentiate into muscle, bone, fat and other cell types in a context-specific manner. Bortezomib, a clinically available proteasome inhibitor, was administered to mice and induced BMMSCs to undergo osteoblastic differentiation, in part by modulation of the bone-specific transcription factor Runx2 [51]. Other studies showed that bortezomib enhanced bone formation by increasing BMP-2 production and inhibiting expression of the Dkk1 gene [52]. Proteasome inhibitors less toxic than bortezomib are in preclinical development.

Enhancing the regeneration of blood vessels

VEGF and NGF are inadequate in HIV-infected patients with osteonecrosis and probably pivotal to pathogenesis because both growth factors are osteogenic [53, 54]; they should be examined as possible therapies for osteoporosis and osteonecrosis. In addition to both of them being osteogenic, VEGF antagonizes apoptosis. The literature concerning the effect of NGF on apoptosis shows varying results, but NGF induces pAKT, which enhances pro-survival factors CREB and IKK and inhibits pro-apoptosis factors BAD, Forkhead and caspase-9.

Drugs to avoid in patients with atraumatic osteoporosis

Thiazolidinediones, which activate peroxisome proliferator-activated receptors, have an inhibitory effect on osteoblasts [55]; on the other hand, PPARγ knockout mice showed delayed secondary ossification [56]. Until this paradox is resolved, thiazolidinediones should be avoided in diabetic patients with osteonecrosis. Also inhibitory to osteoblasts are serotonin reuptake inhibitors [57, 58]; as tricyclic antidepressants and lithium do not have this disadvantage, they should, if clinically appropriate, be used as substitutes for serotonin reuptake inhibitors in osteoporotic patients with depression. It is noteworthy that the number of osteoblasts and bone volume increased when lithium was administered to mice in doses that gave blood levels of lithium even lower than those that are therapeutic in humans with bipolar disorder [59]. As indicated above, COX-2 stimulates osteogenesis; for that reason NSAIDs,
particularly those with dominant specificity for COX-2, should be avoided in patients with osteonecrosis. Finally, as some HIV protease inhibitors (but not RT inhibitors) are anti-angiogenic [60, 61], may be either pro- or anti-apoptosis [62, 63] and adversely affect osteoblast gene expression and osteogenesis [64, 65], their use should be avoided in HIV-infected patients with early osteonecrosis.

Other emerging ideas

Hyperbaric oxygen has been used on the theory that it might reduce oedema and therefore intra-osseous pressure [66], or that the signalling that leads to apoptosis is oxygen sensitive [67]. A study was made in which patients who had symptomatic osteonecrosis were treated with hyperbaric oxygen; they had normal plain radiographs but had either a positive bone scan or positive MRI, and subchondral lesions at least 4 mm thick and 12.5 mm long were treated with hyperbaric oxygen [66]. The mean duration of hip pain before treatment was 4 months (range 1-8 months). Daily sessions, in total 100, used 100% oxygen at 2-2.4 atmospheres in a pressure chamber for 90 min. Of the 12 patients treated, 9 (75%) developed normal MRI appearance, as compared with only 15% reported in an untreated group of 72 patients with osteonecrosis of similar stage. Hyperbaric oxygen was also successfully used in 16 patients with osteonecrosis of the jaw associated with bisphosphonate use; at follow-up, 50% of the patients were in remission [66].

Extracorporeal shock wave therapy (ESWT) was shown to enhance the growth of bone marrow stromal cells and their production of both TGF-b1 and ALP, as well as the formation of bone nodules [68]. In 29 hips of 23 patients with osteonecrosis, ESWT caused improvement in 79% as compared with 29% of controls treated with core decompression [69]. In a later study of 17 patients with bilateral hip osteonecrosis, the same group compared ESWT on one side with hip arthroplasty on the other. Both procedures resulted in favourable pain and function scores; the 17 ESWT patients included 13 who had grade ≥1 at baseline but only 5 at follow-up 17 months later [70]. Patients with the earliest stage of osteonecrosis have the best results from ESWT [71]. In light of the hypothesis that impaired production of VEGF is central to the occurrence of osteonecrosis [54], it is noteworthy that ESWT caused up-regulation of VEGF in rabbits’ osteonecrotic femoral heads [72]. Likewise, Zaidi et al. [73] investigated reasons why osteonecrosis is not a cardinal feature of ACTH-producing adenomas where glucocorticoid excess is profound and found, in vitro, that ACTH-induced differentiation of osteoblasts was dependent on ACTH-stimulated osteoblast production of VEGF. In other work the same group showed that ACTH binds directly to osteoblasts and induces synthesis of collagen [74].

The use of stem cells to repopulate the apoptotic osteoblasts and osteocytes is another highly promising approach that is concordant with the thesis of this presentation. Hernigou and Beaujean [75] treated 116 patients (189 hips) who had early stage osteonecrosis with decompression and autologous bone marrow grafting. A total of 51% of the patients had osteonecrosis caused by steroids, alcohol or iatrogenic; in 34%, sickle cell disease was the cause. Marrow aspirates were concentrated in a cell separator; red cells, leucocytes and plasma were removed, leaving a suspension that contained the stem cells, which were then reinjected through the greater trochanter as in conventional decompression. The average injection was 25 x 10^6 of cells that could be cultured to form fibroblast colonies (fibroblasts are precursors of osteoblasts). After 7 years of follow-up in 136 patients who were in early stages 1 or 2, 116 remained in those two stages. A similar approach by other authors, involving 97 patients with early stage osteonecrosis, showed progression in only 2 of the 53 receiving BM MSCs but in 10 of 44 hips treated with core decompression [76]. Likewise, in a rabbit model of osteonecrosis induced by steroids, implantation of bone marrow-derived mononuclear cells (which include stem cells) enhanced healing [77].

Semaphorins (SEMs) are axonal growth cone guidance molecules. The possibility that SEMAs mediate the observed associations of osteopenia, osteonecrosis and peripheral neuropathy [53, 54] is raised by some of the studies, showing that SEMA may affect both bone and neurons. The SEMAs listed by Yazdani and Terman as influencing both bone and neurons include SEMAs 3A, 3C, 3D, 4C, 5B, 6B and 6C [78]. SEMA3A increases osteoblastic bone formation and inhibits osteoclast differentiation [79]. SEMA7A is expressed during osteoblast differentiation and migration [80]; moreover, SEMA7A enhances outgrowth of neuronal axons [81].

Conclusions

The current limited options for treating osteonecrosis do not reverse it, although evidence supports that core decompression is helpful in the early stages of femoral head osteonecrosis [82]. Recognition that either increased apoptosis of osteocytes or reduced proliferation of osteocyte precursors, or both concurrently, are fundamental and early cellular mechanisms of osteonecrosis suggests several possible rational therapies for those cases in which damage to the structure of the adjacent joint has been minimal. Besides, atraumatic osteonecrosis is polyostotic in up to 25% of patients, and the polyostotic process is not always concurrent, so the systemic medical treatments suggested here are appropriate even if one joint has already been destroyed by osteonecrosis. Additionally, if in future the culture of stem cells becomes routine, their local use might also become routine. It would be difficult, perhaps impossible, to attain adequate numbers for clinical trials in patients with symptomatic atraumatic osteonecrosis, which has a prevalence in the general population of only 0.01-0.04% [83, 84] but ~1% in HIV-infected patients [53], and 4.4% when in asymptomatic form, in patients infected with HIV [85]. It must be emphasized that the purpose here is not to advocate any particular one of the therapies mentioned above without
clinical trial but to point to rational possible approaches to saving an undestroyed joint adjacent to osteonecrosis, as well as to preventing progression or even allowing reversal of the early phase of osteonecrosis.

**Rheumatology key messages**

- Atraumatic osteonecrosis depends on osteocyte apoptosis and inadequate osteoblast regeneration.
- Various therapies are potentially available that might decrease osteocyte apoptosis or enhance osteoblast regeneration.

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