Salubrinal is a small synthetic compound that suppresses de-phosphorylation of eukaryotic translation initiation factor 2 alpha (eIF2α) and attenuates stress to the endoplasmic reticulum. It has been reported that salubrinal enhances osteoblastogenesis and suppresses osteoclastogenesis. We have recently reported that salubrinal also presents chondroprotective effects through downregulation of NFKB signaling, and it reduces arthritic inflammation by inhibiting dual-specificity phosphatase 2 (Dusp2). In this review, we summarize salubrinal’s beneficial effects on skeletal diseases and suggest its novel therapeutic possibilities. Journal of Nature and Science, 1(8):e151, 2015

eIF2α | salubrinal | endoplasmic reticulum | bone

Chemical structure of salubrinal
Salubrinal (C₃₁H₃₁Cl₄N₉O₄S; 479.8 Da) is a synthetic compound with an IUPAC name of (2E)-3-phenyl-N-[2,2,2-trichloro-1-[(8-quinolinylamino)thioxomethyl]amino]ethyl]-2-propenamide. As outlined in Fig. 1, it is synthesized with quinolinicinnamamide 1 as a starting material. The first reaction with anhydrous chloral forms a reactive intermediate 2. The second reaction transforms it into the amine via substitution chlorination with phosphorus pentachloride followed by treatment with ammonia. Lastly, amine 3 is reacted with 8-isothiocyanatoquinoline to produce salubrinal [1]. Salubrinal’s hydrophobic parameter, log(P), is 4.56 (pH 7.40) by Pallas 3.0, and its uptake in organs and cells is predicted relatively high.

Salubrinal’s action as anti-apoptosis and neuroprotection
Salubrinal inhibits protein phosphatase 1 (PP1) and suppresses de-phosphorylation of eukaryotic translation initiation factor 2 alpha (eIF2α). It is known as a suppressor of stress to the endoplasmic reticulum (ER) [2], which can be induced by a multitude of causes, including protein misfolding, UV, and viral infection [3, 4]. ER stress elevates the level of phosphorylated eIF2α (eIF2α-p) [5], and severe ER stress induces apoptosis and tends to worsen systemic disease states [6-8]. Based on its structure-activity relationship, it is suggested that all functional groups except for quinoline moiety are essential to induce protective activity against apoptosis by ER stress [1]. Interestingly, salubrinal in general acts as an anti-apoptotic agent [9-12], although at a high dose it may lead to apoptosis. For instance, salubrinal is reported to prevent glucocorticoid induced-apoptosis in osteoblasts and osteocytes [9]. Salubrinal also protects neurons against apoptotic death by downregulating eIF2α-ATF4-CHOP signaling [10]. Besides protection from apoptosis, salubrinal is reported to generate beneficial effects in animal models for neuronal disorders [13-16]. First, it alleviates neurological dysfunction after cardiopulmonary resuscitation in rats [13]. Second, it provides neuroprotective effects in a mouse model of traumatic brain injury [14]. Third, it inhibits the expression of proteoglycans and increases neurite outgrowth [15]. Lastly, it modulates sleep homeostasis and activates sleep- and wake-regulatory neurons [16].

Salubrinal’s stimulation of osteoblastogenesis and inhibition of osteoclastogenesis
In the skeletal system, osteoblasts and osteoclasts play dualing roles in bone remodeling. Osteoblasts are bone-forming cells derived from mesenchymal stem cells, while osteoclasts are bone-resorbing cells derived from myelomonocytic lineage [17]. Differentiation of osteoblasts and osteoclasts are, in part, regulated by transcription factors [18, 19] including activating transcription factor 4 (ATF4). ATF4 is not only one of the key transcription factors for osteoblast maturation but also an essential regulator in stress to the ER [8, 20]. In osteoblasts, ER stress decreases the level of eIF2α-p and stimulates the expression of ATF4 [8]. Salubrinal, on the other hand, increases eIF2α-p by blocking PP1 and enhances matrix deposition through upregulation of ATF4 and osteocalcin [21].

Osteoclast development also involves eIF2α [21-23]. Osteoclastogenesis is induced by administration of receptor activator of nuclear factor kappa-B (RANKL), and RANKL-driven osteoclastogenesis is suppressed by salubrinal. Salubrinal’s inhibitory action is consistent with its downregulation of nuclear factor of activated T cells c1 (NFATc1), which is a master gene for osteoclast differentiation [21-23]. Principal component analysis of genome-wide microarray data predicted transcription factors that followed the phenotypic response of salubrinal-driven suppression of osteoclastogenesis. Several of these predicted transcription factors were experimentally validated to conclude that salubrinal downregulates AP-1 proteins such as c-Fos and JunB [24] and suppresses RANKL-driven NFATc1 expression.

Salubrinal-driven prevention of cartilage degradation in osteoarthritis
Osteoarthritis (OA) is the most common joint disease, resulting from multiple factors, including traumatic injury and obesity [25]. Matrix metalloproteinase 13 (MMP13) is one of the most influential collagenases in the destruction of cartilage tissue [26], in which salubrinal is able to attenuate its expression and activity. In vitro assays demonstrate that in TNFa and IL1β-treated chondrocytes activation of nuclear factor kappa B (NFKB) signaling is suppressed by salubrinal [27]. In a mouse model of surgically-induced OA, it has been reported that administration of salubrinal alleviates cartilage degradation by downregulating phosphorylated NFKB [28]. Other studies also report that salubrinal acts as an inhibitor of NFKB signaling and attenuates β-amyloid-induced neuronal death and microglial activity [29].

Salubrinal’s beneficial effects on inflammatory arthritis
In macrophages, the basal level of phosphorylated eIF2α was higher in old mice than young mice [30]. Our recent study demonstrated that salubrinal suppresses lipopolysaccharide (LPS)-stimulated inflammatory responses in macrophages [31]. Salubrinal is reported to reduce orofacial inflammatory pain [32], and it alleviates colitis through suppression of proinflammatory cytokines [33].

Conflict of interest: No conflicts declared.

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We also have reported that salubrinal attenuates inflammatory cytokines such as IL1β, Cox2, IL2, TNF, and IL13 in macrophages, T lymphocytes, and mast cells [31]. Using gene expression microarray data, potential targets of salubrinal were identified by their correlation with the observed phenotype in two cell lines. Of the predicted genes, a potential target, dual-specificity phosphatase 2 (Dusp2), which plays a critical role in the progression of inflammatory arthritis, such as in rheumatoid arthritis, was identified and validated. Dusp2 is highly expressed in activated immune cells, and inflammatory responses are reduced in the K/BxN model of rheumatoid arthritis by deleting Dusp2 [34]. In our study, salubrinal acts as a Dusp2 inhibitor and suppresses inflammatory responses in a mouse model of anti-collagen antibody-induced arthritis [31].

**Salubrinal’s action through eIF2α and NFκB**

Besides salubrinal, guanabenz is another agent that is known as an inhibitor of the de-phosphorylation of eIF2α [35]. These two agents inhibit PP1 by interacting with different subunits of PP1: PP1 alpha for salubrinal and GADD34 for guanabenz. Both agents enhance osteoblastogenesis and suppress osteoclastogenesis through regulating the level of eIF2α-p. However, one of the clear differences between these agents is that salubrinal presents chondroprotective effects through downregulating NFkB signaling, but guanabenz does not [28]. In addition, guanabenz and not salubrinal is known as an agonist of α2 adrenergic receptor. Further studies are necessary to identify salubrinal’s potential binding partner(s) that modulate NFkB signaling.

**Other therapeutic possibilities with salubrinal**

It is possible that salubrinal may present efficacy to other skeletal diseases we have not yet tested. It has recently been reported using a mouse in vivo model that salubrinal is able to attenuate tumor growth and migration by downregulating the activity of Rac1 GTPase [36]. Since salubrinal suppresses osteoclast differentiation, salubrinal may not only suppress proliferation of breast cancer cells but also protect bone from metastasis. Other therapeutic possibilities may include the prevention of bone loss for patients with diabetes and spinal cord injury-induced osteoporosis.

**Conclusions & Future Perspectives**

Salubrinal has been shown to present various beneficial effects in mouse models of osteoporosis, osteoarthritis, inflammatory arthritis, and breast cancer (Fig. 2). Two major functions in bone remodeling are enhancement of bone formation through upregulation of ATF4 and suppression of bone resorption through downregulation of NFATc1 and AP-1 proteins. Salubrinal also has chondroprotective effects by reducing NFkB signaling and MMP13 activity. Furthermore, it induces anti-inflammatory responses by suppressing Dusp2 and attenuates tumor growth and migration by blocking Rac1 GTPase. Collectively, salubrinal may provide a novel therapeutic possibility for multiple skeletal diseases including breast cancer and bone metastasis. We envision that administration routes, bioavailability, appropriate doses, and patient-related factors such as sex, age, and genetic makeup should be evaluated for each of the potential applications through pharmacokinetics and pre-clinical studies.

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