Possible involvement of advanced glycation end-products in bone resorption

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Abstract. Advanced glycation end-products (AGEs) are formed in long-lived matrix proteins by a non-enzymatic reaction with sugar. We recently demonstrated the presence of AGEs in amyloid fibrils of dialysis-related amyloidosis, one of the characteristic features of which is an accelerated bone resorption around amyloid deposits. This suggested a potential link of AGEs in bone resorption and led us to investigate whether AGEs enhance bone resorption. An immunohistochemical study using anti-AGE antibody revealed positive immunostaining for AGEs in bone tissues from elderly subjects. AGE-modified proteins were shown to stimulate monocyte/macrophage to secrete bone-resorbing cytokines such as interleukin-1β, interleukin-6 and tumour necrosis factor-α. AGE-modified proteins enhanced net calcium efflux in cultured neonatal mouse calvariae to a much greater extent than unmodified proteins. Furthermore, when mouse unfractionated bone cells containing osteoclasts were cultured on dentin slices, AGE-modified proteins increased the number of resorption pits formed by osteoclasts, whereas their normal counterparts or those modified with the early glycation products did not. These findings suggest that AGEs enhance bone resorption by osteoclasts. The modification of bone matrices with AGEs might, therefore, play a pathophysiological role not only in the remodelling of senescent bone matrix tissues, but also in diabetes-related amyloidosis or osteoporosis associated with diabetes and ageing.

Key words: advanced glycation end-products; bone resorption; interleukin 1β; interleukin 6

Introduction

Bone resorption is an essential component of normal bone remodelling which is controlled by both hormonal and local factors within a complex regulatory system [1]. Regulation of bone resorption is dependent upon the formation of new osteoclasts from precursors as well as the activation of mature osteoclasts. These activated osteoclasts initiate bone resorption under the control of both local and hormonal factors. Cytokines are important local mediators of bone resorption. The regulation of local cytokines, however, remains to be defined.

Advanced glycation end-products (AGEs) are the pigmented and fluorescent adducts which are formed by a non-enzymatic reaction between aldoses and long-lived proteins, called the Maillard reaction [2]. AGE proteins are thought to play a role in normal tissue remodeling, i.e. the removal and replacement of senescent extracellular matrix components. We recently demonstrated the presence of AGEs in amyloid fibrils of dialysis-related amyloidosis [3,4]. One of the characteristic features of this complication is an accelerated bone resorption around amyloid deposits that leads to subchondral erosion and bone cysts, detected radiologically, in patients undergoing dialysis for > 10 years [5,6]. Further studies from our group have demonstrated that AGE protein (β2-microglobulin; β2m), but not the normal counterpart, induces chemotaxis of monocytes and secretion of potent bone-resorbing cytokines such as interleukin 1β (IL-1β), tumour necrosis factor-α (TNF-α) and IL-6 from monocyte-derived macrophages [7–9]. These findings suggest a potential link of AGEs with bone resorption. This article discusses the possible involvement of AGEs in bone resorption.

Presence of AGEs in bone matrix proteins

An immunohistochemical study using anti-AGE

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antibody revealed positive immunostaining for AGEs in the trabecular bone tissue from elderly subjects (Figure 1). The extent of AGE modification in bone matrix proteins was also examined in vitro. Demineralized bone powder from newborn mice was incubated with 0.5 M glucose for 70 days in vitro and the amount of pentosidine, which is one of the AGE structures [10], was determined in the acid hydrolysate by HPLC. The pentosidine content in demineralized bone powder upon incubation with glucose (990 pmol/mg) was significantly greater than that upon parallel incubation without glucose (0.97 pmol/mg; Figure 2).

These findings indicate that AGEs are present in bone matrix proteins. This is not surprising since the AGE modification occurs in long-lived matrix proteins such as collagens, and type I collagen is a major component forming the bone matrix.

Secretion of bone-resorbing cytokines by AGEs

Several cytokines are known to induce bone resorption [1]. Both IL-1β and TNF-α are potent stimulants for monocytes/macrophages and osteoblasts to secrete IL-6, which in turn induces the differentiation of osteoclasts from precursors [11]. Furthermore, mature osteoclasts are activated to resorb bone by the action of a particular set of cytokines that are released from osteoblasts by IL-1β and TNF-α [12].

AGE-modified bovine serum albumin or βm are known to stimulate monocytes/macrophages to secrete bone-resorbing cytokines such as IL-1β [7,8,13], TNF-α [7,8,13] and IL-6 [9]. As shown in Figure 3, AGE-modified βm stimulated monocyte-derived macrophages to secrete cytokines, but the normal counterpart did not induce any detectable cytokines from macrophages.

Neeper et al. recently isolated the cDNA clone of the receptor for AGEs, called RAGE, from a bovine lung cDNA library [14]. RAGE is a new member of the immunoglobulin superfamily of cell surface molecules and selectively interacts with AGE proteins. RAGE is shown to be expressed on various kinds of cells, including monocytes/macrophages [15]. We recently found that the macrophage secretion of cytokines caused by AGE-modified βm was inhibited by excess soluble RAGE, an extracellular domain of RAGE [T. Miyata, A. M. Schmidt and D. Stern, unpublished observation]. This suggests the involvement of RAGE in the interaction between AGEs and monocytes/macrophages.

AGE-modified βm was also found to stimulate the mouse osteoblastic cell line, MC3T3 E1, to secrete IL-1β [S. Sprague and T. Miyata, unpublished observation]. It is inconclusive at present whether or not IL-1β secretion from osteoblasts caused by AGEs is mediated by RAGE.

These findings, taken together, suggest that AGEs
Enhanced bone resorption by AGEs

Previously, we have demonstrated that incubating neonatal mouse calvariae with β2m stimulates bone resorption [16]. It has been further demonstrated that this increase in bone resorption is mediated, in part, by stimulation of IL-1β [17]. β2m isolated from patients is a mixture of heterogeneous molecular adducts derived from various types of modification—deamidation, Amadori products and AGEs [8]. As AGE proteins are a major stimulator of cytokines and β2m-induced bone resorption is mediated by cytokines, we evaluated whether AGE modification of β2m was responsible for its bone-resorbing activity. Calvariae were incubated in either control medium or medium supplemented with $10^{-6}$ M β2m or AGE-modified β2m and net calcium flux was determined. Compared with control, purified normal β2m caused only a mild increase in calcium release from bone, whereas the AGE-modified β2m induced an 8-fold increase in calcium efflux (Figure 4). This marked increase in calcium efflux demonstrates the importance of AGE modification in mediating the β2m-induced bone resorption.

The effect of AGEs on osteoclast-induced bone resorption utilizing the pit formation assay with an unfractonated bone cell culture system containing mature osteoclasts from newborn mice was then evaluated. A 4 day culture of tartrate-resistant acid-phosphatase-positive multinucleated cells on dentin slices in the presence of AGE-modified β2m significantly increased the number of resorption pits formed by osteoclasts, compared with control cells cultured in the medium alone (Figure 5). However, there was no statistically significant difference in the number of resorption pits between control cells and those cultured with normal β2m. It remains unknown whether or not AGEs can activate osteoclasts directly. An alternative possibility is that osteoclasts are activated by the action of a particular set of cytokines that are released from monocytes or osteoblasts by AGEs. This contention is supported by the observation that AGEs stimulate monocytes/macrophages or osteoblasts to secrete bone-resorbing cytokines, as described above. Further study is necessary to elucidate a molecular mechanism of bone resorption enhanced by AGEs.
Taking into consideration the observation that AGEs in the matrix tissues are markedly elevated in diabetic patients and old subjects compared with healthy young subjects [18,19], the present findings suggest a potential link of AGEs in osteoporosis associated with diabetes and ageing. More study is necessary to clarify this issue.

Conclusion

It is suggested that AGEs enhance bone resorption of osteoclasts, implicating a pathophysiological role of AGEs not only in the remodelling of senescent bone matrix tissues but also in dialysis-related amyloidosis or osteoporosis associated with diabetes and ageing.

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References