Extracellular Calcium and Inflammation

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Burn injury serves as an example of a condition with a robust systemic inflammatory response. The elevation of circulating interleukins (IL)-1β and -6 in children and adolescents with severe burn injury upregulates the parathyroid calcium-sensing receptor (CaSR), resulting in hypocalcemic hypoparathyroidism accompanied by urinary calcium wasting. This effect protects the body from the hypercalcemia that results from bone resorption, liberating calcium into the circulation. Extracellular calcium can exacerbate and prolong the inflammatory response by stimulating mononuclear cell chemokine production as well as the NLRP3 inflammasome of the innate immune system, resulting in increased IL-1 production by monocytes and macrophages. Interestingly, the CaSR upregulation in response to inflammatory cytokines disappears with age, potentially trapping calcium from bone resorption in the circulation, allowing it to contribute to increased inflammation and possibly increased calcium deposition in small arteries, such as the coronaries, as conditions with increased chronic inflammation, such as spinal cord injury, osteoarthritis, and rheumatoid arthritis have an incidence of cardiovascular disease and coronary artery calcium deposition significantly higher than the unaffected age-matched population.

inflammation | calcium-sensing receptor | burns | chemokines | NLRP3 inflammasome

1. Introduction

Inflammation results in the release of various cytokines from the body's immune cells, most notably interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)-α. In addition, various immune cells produce chemoattractant cytokines, called chemokines. These substances attract immune cells to a site of inflammation within the body, increasing the intensity and/or duration of the inflammatory response.

Burn injury is an example of a condition in which a robust systemic inflammatory response is manifested by elevated circulating concentrations of IL-1β and IL-6 by 3-fold and 100-fold, respectively [1].

The robust systemic inflammatory response is due to the destruction of skin as a barrier to infection, and burn patients are all presumed to be septic due to wound infection. In conjunction with relative immobilization and elevated endogenous glucocorticoids [2], resorptive bone loss is observed in children with over 40% of total body surface area burned. The result is a loss of 7% of total trabecular bone density of the lumbar spine over the first six weeks following burn injury and a 3% loss of total body bone mineral density, mainly cortical bone, over the first six months post-burn.

In the laboratory, in vitro studies of bovine parathyroid cells [3] and equine parathyroid cells [4] incubated with IL-1β and IL-6 [5] demonstrate the upregulation of the parathyroid calcium-sensing receptor (CaSR). The CaSR is found in many organs across the body, including kidneys [6], bone [7], intestine [8], and cardiovascular endothelium [9][10]. In the parathyroid, the CaSR is a G-protein-coupled membrane-bound receptor on the parathyroid chief cells that detects extracellular calcium and signals the cell the amount of PTH to be secreted into the circulation. The coordination of body CaSRs requires further investigation. The upregulation of the parathyroid CaSR has the effect of lowering the amount of circulating calcium necessary to suppress parathyroid hormone (PTH) secretion, leading to hypocalcemic hypoparathyroidism.

Another observation in the same sheep model of burn injury and over the same time frame, i.e., the first five days following burns, was that backscatter scanning electron microscopic study of iliac crest demonstrated scalloping, a hallmark of resorption, plainly visible by day 5 [11]. In addition, urine C-telopeptide of type I collagen (CTx), a biochemical marker of bone resorption, was elevated on day one. Notably, the coincidence of the cytokine-mediated upregulation of the parathyroid CaSR and the onset of bone resorption, stimulated by inflammation, immobilization, and increased endogenous steroid production, likely serves as a way to facilitate the excretion of excess calcium entering the circulation following acute bone resorption (see **Figure 1**).

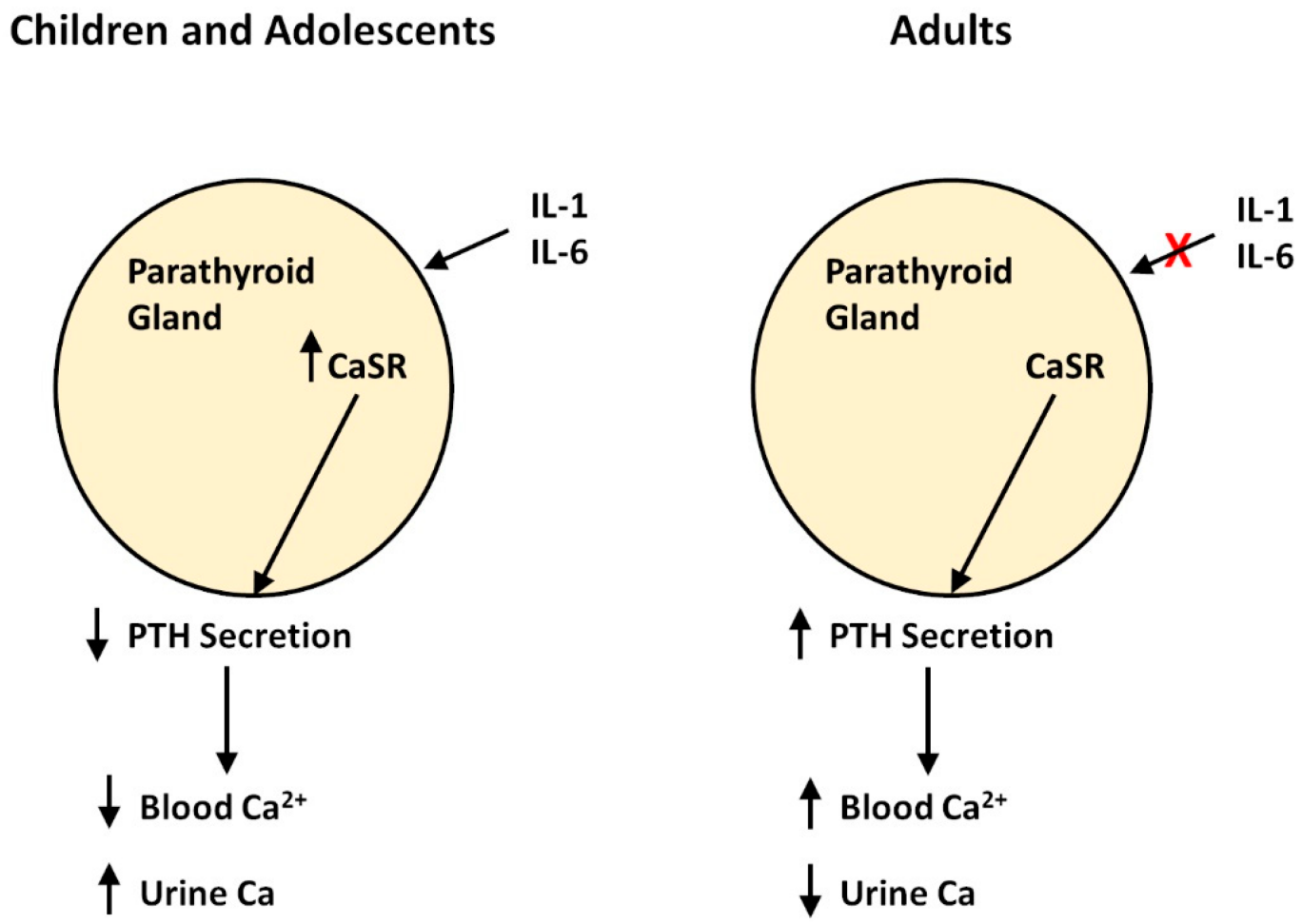

Figure 1. Parathyroid gland response to pro-inflammatory cytokines in children and adolescents and in adults following burn injury. In children and adolescents, the cytokines upregulate the membrane-bound G-protein coupled calcium-sensing receptor, causing a reduction in the amount of circulating calcium necessary to suppress parathyroid hormone secretion by the gland. The result is hypocalcemic hypoparathyroidism with increased urinary calcium excretion. In adults, this ability for the calcium-sensing receptor to upregulate in response to inflammatory cytokines appears to be lost.

2. Extracellular Calcium and Inflammation

It is not clear why chemokines such as Regulated on Activation Normal T and Secreted (RANTES) and Monocyte Inhibitory Protein (MIP)-1 α were strongly stimulated, whereas Monocyte Chemotactic Protein (MCP-1) was equally strongly inhibited by extracellular calcium [12], although it is possible that the sequence and timing of appearance in the blood of the various chemokines are important to the inflammatory response. The implication of these findings is that extracellular calcium stimulates or suppresses certain chemokines, which can serve to attract more inflammatory cells to the areas of existing inflammation, thus intensifying and possibly prolonging the inflammatory response in burn patients. These observations were reinforced by the work of Rossol et al. [13], who demonstrated that extracellular calcium could stimulate the nod-like receptor (NLR)-P3 inflammasome of the innate immune system via the CaSR on monocyte membranes to stimulate monocytes and macrophages to produce more IL-1, further intensifying the inflammatory process. Subsequently, this group also demonstrated that circulating calcium and phosphate are converted by the serum protein Fetuin A to colloidal calciprotein particles in order to prevent ectopic calcification. These undergo pinocytosis by monocytes, a function of increased circulating calcium-triggering monocyte CaSR upregulation prior to the pinocytosis. This action triggers the activation of the NLRP3 inflammasome, resulting in the production of IL-1β [14]. Thus, it would appear that the calcium entering the circulation following bone resorption is capable of intensifying and prolonging the inflammatory response to burn injury and perhaps other conditions. Furthermore, Olszak et al. [15] demonstrated in vitro that extracellular calcium could increase the monocyte expression of various chemokine receptors, thus building even a stronger case for extracellular calcium stimulating the various components of the inflammatory response. Further evidence implicating calcium in the inflammatory response comes from Michalick and Kuebler [16], who point out that transient receptor potential vanilloid (TRPV)-type 4, or TRPV4, a mechanosensitive calcium channel, is involved in neutrophil activation and chemotaxis. TRPV4 has also been implicated in macrophage activation leading to lung injury following mechanical ventilation. These issues have also been reported in a rat model of immobilization and mechanical ventilation [17] in which it was shown that the pharmacologic immobilization of rats was accompanied by marked trabecular bone loss, thus liberating calcium into the circulation and possibly contributing to the inflammation described by Michalick and Kuebler [16].

3. Preservation of the Hypocalcemic Response in Small Burns

The data on the longitudinal decrease in bone density following burns were originally published in 1995 [18] and again in 2005 in conjunction with a study of antiresorptive agents and bone density [19] and were applicable to children with burns of ≥40% total body surface area. In the first of these two publications [18], the researchers studied a cohort of children with burns of approximately 20% of total body surface area. Those children did not suffer significant resorptive bone loss and had no reduction in their bone mineral density. Therefore, there is a threshold effect regarding the metabolic response to burn such that the inflammatory response at 40% body surface area burn is sufficient to cause resorptive bone loss, but at 20% body surface area burn, even combined with the effects of immobilization on bone loss, there is no clinically significant bone loss, as determined by bone densitometry. From these data, the researchers can infer that the additional amounts of calcium entering the circulation from bone resorption in the latter group was negligible. Therefore, the researchers attempted to determine whether blood ionized calcium levels remained normal in children with small burns. Accordingly, the researchers obtained blood ionized calcium concentrations from the records of 190 anonymized children with burns ranging from 1% to 20% total body surface area. Throughout hospitalization, their mean ionized calcium concentration was 1.04 ± 0.08 (SD) mM, range 0.73–1.31, and normal range 1.12–1.37. The mean values were 8% below the lower limits of normal for age, while only 16.8% of the 190 pediatric patients with small burns had mean circulating ionized calcium concentrations within the normal range. In no setting of pediatric burn injury is the circulating ionized calcium normal. Since burn size plays a role in resorptive bone loss and reduced bone density, the hypocalcemic response to burns as small as 1% of total body surface area suggests that the upregulation of the parathyroid CaSR in response to acute inflammation is the prime purpose of the reactive hypocalcemia rather than this response being uniquely related to resorptive bone loss induced by immobilization or endogenous glucocorticoid production. Unfortunately, neither serum PTH concentrations nor quantitative urinary calcium data were available in this anonymized population. In other words, even in small burns, the initial inflammatory response in children is sufficient to upregulate the parathyroid CaSR without the addition to the circulation of calcium from resorbing bone. In more severe burns, resorbed calcium entering the circulation would theoretically add to the urinary calcium load in pediatric burns. However, the researchers do not have sufficient data at this time to document this statement.

4. Developmental Disappearance of Calcium-Sensing Receptor Response to Inflammatory Cytokines

Despite the apparent uniformity of findings in burn patients under the age of 19, studies in adults yield an entirely different picture of ionized calcium and PTH response to severe burn injury. In the researchers' patients [20], and in the adult burns reported in the literature [21][22], severely burned adult patients were normo- or mildly hypercalcemic and euparathyroid or mildly hyperparathyroid. In the researchers' patients, the mean blood ionized calcium concentration was 1.08 ± 0.03 (SD) mM in children with the normal range being 1.12 to 1.37 [23], while in adults with similarly sized burns, the blood ionized calcium concentration was 1.15 ± 0.06 mM, with normal range 1.00 to 1.15 mM. Similarly, serum PTH concentration in burned children was 7 ± 3 pg/mL, with a normal range of 15 to 55, whereas in adults, the mean PTH for similarly sized burns was 114 ± 96 pg/mL, with the normal range being 10 to 65. The mechanism explaining this difference is not apparent. However, it would appear likely that the ability of the

parathyroid calcium-sensing receptor to respond to inflammatory cytokines by the upregulation and consequent development of hypocalcemic hypoparathyroidism is lost with age, sometime after the onset of puberty. By age 19, there is still no change in the childhood response pattern of parathyroid CaSR upregulation in response to inflammatory cytokines, thus likely causing the age at the loss of response of the parathyroid CaSR to inflammatory cytokines to be at least in the early 20s. This age approximates the time of acquisition of peak bone mass, and the relationship between these two developmental milestones requires further investigation. Likewise, sexual dimorphism was not apparent in the analysis of the results of circulating ionized calcium and PTH concentrations in children and adolescents past the onset of puberty, through the ages of 18–19 years.

References

1. Klein, G.L.; Herndon, D.N.; Goodman, W.G.; Langman, C.B.; Phillips, W.A.; Dickson, I.R.; Eastell, R.; Naylor, K.; Maloney, N.; Desai, M.; et al. Histomorphometric and biochemical characterization of bone following acute severe burns in children. Bone 1995, 17, 455–460.
2. Klein, G.L.; Bi, L.X.; Sherrard, D.J.; Beavan, S.R.; Ireland, D.; Compston, J.E.; Williams, W.G.; Herndon, D.N. Evidence supporting a role of glucocorticoids in short-term bone loss in burned children. Osteoporos. Int. 2004, 15, 468–474.
3. Nielsen, P.K.; Rasmussen, A.K.; Butters, R.; Feldt-Rasmussen, U.; Bendtzen, K.; Diaz, R.; Brown, E.M.; Olgaard, K. Inhibition of PTH secretion by interleukin-1 beta in bovine parathyroid glands in vitro is associated with an up-regulation of the calcium-sensing receptor mRNA. Biochem. Biophys. Res. Commun. 1997, 238, 880–885.
4. Toribio, R.E.; Kohn, C.W.; Capen, C.C.; Rosol, T.J. Parathyroid hormone (PTH) secretion, PTH mRNA and calcium-sensing receptor mRNA expression in equine parathyroid cells and effects of interleukin (IL)-1, IL-6 and tumor necrosis factor alpha on equine parathyroid cell function. J. Mol. Endocrinol. 2003, 31, 609–620.
5. Canaff, L.; Zhou, X.; Hendy, G.N. The proinflammatory cytokine, interleukin-6, up-regulates calcium-sensing receptor gene transcription via Stat1/3 and Sp 1/3. J. Biol. Chem. 2008, 283, 13586–13600.
6. Riccardi, D.; Brown, E.M. Physiology and pathophysiology of the calcium-sensing receptor in the kidney. Am. J. Physiol. Renal. Physiol. 2010, 298, F485–F499.
7. Goltzman, D.; Hendy, G.N. The calcium-sensing receptor in bone- mechanistic and therapeutic insights. Nat. Rev. Endocrinol. 2015, 11, 298–307.
8. Tang, L.; Cheng, C.Y.; Sun, X.; Pedicone, A.J.; Mohamadzadeh, M.; Cheng, S.X. The extracellular calcium-sensing receptor in the intestine: Evidence for regulation of colonic absorption, secretion, motility and immunity. Front. Physiol. 2016, 7, 245.

9. Klein, G.L.; Enkhbaatar, P.; Traber, D.L.; Buja, M.; Jonkam, C.C.; Poindexter, B.J.; Bick, R.J. Cardiovascular distribution of the calcium-sensing receptor before and after burns. Burns 2008, 34, 370–375.
10. Schepelmann, M.; Yarova, P.L.; Lopez-Fernandez, I.; Davies, T.S.; Brennan, S.C.; Edwards, P.J.; Aggarwal, A.; Graça, J.; Rietdorf, K.; Matchkov, V.; et al. The vascular Ca2+-sensing receptor regulates blood vessel tone and blood pressure. Am. J. Physiol. Cell. Physiol. 2016, 310, C103–C204.
11. Klein, G.L.; Xie, Y.; Qin, Y.-X.; Lin, L.; Hu, M.; Enkhbaatar, P.; Bonewald, L.F. Preliminary evidence of bone resorption in a sheep model of acute burn injury: An observational study. J. Bone Miner. Metab. 2014, 32, 136–141.
12. Klein, G.L.; Castro, S.M.; Garofalo, R.P. The calcium-sensing receptor as a mediator of inflammation. Semin. Cell Dev. Biol. 2016, 49, 52–56.
13. Rossol, M.; Pierer, M.; Raulien, N.; Quandt, D.; Meusch, U.; Rothe, K.; Schubert, K.; Schöneberg, T.; Schaefer, M.; Krügel, U.; et al. Extracellular Ca2+ is a danger signal activating NLRP3 inflammasome through G protein-coupled calcium sensing receptors. Nat. Commun. 2012, 3, 1329.
14. Jager, E.; Murthy, S.; Schmidt, C.; Hahn, M.; Strobel, S.; Peters, A.; Stäubert, C.; Sungur, P.; Venus, T.; Geisler, M.; et al. Calcium-sensing-receptor-mediated NLRP3 inflammasome response to calciprotein particles drives inflammation in rheumatoid arthritis. Nat. Commun. 2020, 11, 4243.
15. Olszak, I.T.; Poznansky, M.C.; Evans, R.H.; Olson, D.; Kos, C.; Pollak, M.R.; Brown, E.M.; Scadden, D.T. Extracellular calcium elicits a chemokinetic response from monocytes in vitro and in vivo. J. Clin. Investig. 2000, 105, 1299–1305.
16. Michalick, L.; Kuebler, W.M. TRPV4—A missing link between mechanosensation and immunity. Front. Immunol. 2020, 11, 413.
17. Gugala, Z.; Cacciani, N.; Klein, G.L.; Larsson, L. Acute and severe trabecular bone loss in a rat model of critical illness myopathy.J. Orthop. Res. 2022, 40, 1293–1300.
18. Klein, G.L.; Herndon, D.N.; Langman, C.B.; Rutan, T.C.; Young, W.E.; Pembleton, G.; Nusynowitz, M.; Barnett, J.L.; Broemeling, L.D.; Sailer, D.E.; et al. Long-term reduction in bone mass after severe burn injury in children. J. Pediatr. 1995, 126, 252–256.
19. Klein, G.L.; Wimalawansa, S.J.; Kulkarni, G.; Sherrard, D.J.; Sanford, A.P.; Herndon, D.N. The efficacy of the acute administration of pamidronate on the conservation of bone mass following severe burn injury in children: A double-blind, randomized controlled study. Osteoporos. Int. 2005, 16, 631–635.
20. Klein, G.L.; Herndon, D.N.; Rutan, T.C.; Sherrard, D.J.; Coburn, J.W.; Langman, C.B.; Thomas, M.L.; Haddad, J.G., Jr.; Cooper, C.W.; Miller, N.L.; et al. Bone disease in burn patients. J. Bone

Miner. Res. 1993, 8, 337–345.

21. Klein, G.L.; Benjamin, D.A.; Herndon, D.N. Calcemic response to burns differs between adults and children: A review of the literature. Osteoporos. Sarcopenia 2017, 3, 170–173.
22. Rousseau, A.F.; Foidart-DeSalle, M.; Ledoux, D.; Remy, C.; Croisier, J.L.; Damas, P.; Cavalier, E. Effects of cholecalciferol supplementation and optimized calcium intakes on vitamin D status, muscle strength and bone health: A one-year pilot randomized controlled trial in adults with severe burns. Burns 2015, 41, 317–325.
23. Klein, G.L.; Nicolai, M.; Langman, C.B.; Cuneo, B.F.; Sailer, D.E.; Herndon, D.N. Dysregulation of calcium homeostasis after burn injury in children: Possible role of magnesium depletion. J. Pediatr. 1997, 131, 246–251.