Recombinant Human Platelet-Derived Growth Factor: Biology and Clinical Applications

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The abilities of bone to remodel, fractures to repair, and bone grafts to incorporate are all fundamental reflections of the bone remodeling cycle. This process is characterized by the recruitment and differentiation of osteoblastic and osteoclastic cell populations, whose cellular activities are coordinated and regulated by an elaborate system of growth factors and cytokines. One of the crucial biological factors responsible for reparative osseous activity is platelet-derived growth factor (PDGF). The potent stimulatory effects of PDGF as a chemoattractant and mitogen for mesenchymal cells (including osteogenic cells), along with its ability to promote angiogenesis, have been demonstrated in a variety of preclinical models predicting maxillofacial, spine and appendicular skeletal, and soft-tissue applications. The biological profile of PDGF, including its ability to recruit osteoprogenitor cells, makes it particularly suited to address the skeletal defects that are seen with comorbid conditions such as osteoporosis, diabetes, and the effects of smoking. The clinical success and safety that have been demonstrated with use of recombinant human PDGF (rhPDGF) in the repair of periodontal defects have led to U.S. Food and Drug Administration (FDA) approval of rhPDGF for this indication. Ongoing pilot and pivotal trials in the United States and internationally will continue to clarify the promising role of PDGF in the treatment of challenging skeletal disorders.

PDGF and the Bone Remodeling Cycle

The skeleton has a robust, intrinsic capacity to regenerate during homeostasis and following injury. This remarkable regenerative process is characterized by the remodeling cycle, in which cell populations are recruited and differentiated for the purposes of bone resorption or bone formation. These activities are coordinated and regulated by an elaborate system of growth factors and cytokines, several of which are either now available or in promising stages of development for clinical application through recombinant technology. One of the crucial biological factors responsible for reparative osseous activity is PDGF. PDGF works by binding to cell-surface receptors on most cells of mesenchymal origin, and it stimulates the reparative processes in multiple tissue types. The potent stimulatory effects of PDGF as a chemoattractant and a mitogen, along with its ability to promote angiogenesis, position it as a key mediator in tissue repair. As a consequence of the recognized importance of PDGF in wound-healing and its orthopaedic therapeutic potential, a review on PDGF is timely. This article will highlight the biology behind PDGF, the preclinical history of PDGF in dentistry and orthopaedics, and the compelling dental and clinical orthopaedic studies of PDGF that have appeared in the literature.

Biology

PDGF Expression and Function in Bone-Healing

The family of PDGF polypeptide growth factors includes PDGF-A, B, C, and D, encoded by four genes located on different chromosomes. PDGF-A and PDGF-B can form both homodimers and heterodimers, whereas PDGF-C and PDGF-D exist as homodimers[1]. PDGF has a half-life of approximately thirty minutes when circulating in the blood[2-4], suggesting that local delivery of the growth factor will be critical to achieving clinical success.

Following injury and hemorrhage, bone repair is characterized by activation of the coagulation cascade and formation of a blood clot at the site of trauma (Fig. 1). Platelets aggregate and release their cytokine-laden granules, including varying amounts of PDGF-AB, PDGF-AA, PDGF-BB, and PDGF-CC, into the developing blood clot[5-8]. The PDGFs act early in the wound-healing cascade by initially attracting and activating neutrophils and macrophages[9-13], which are key cell

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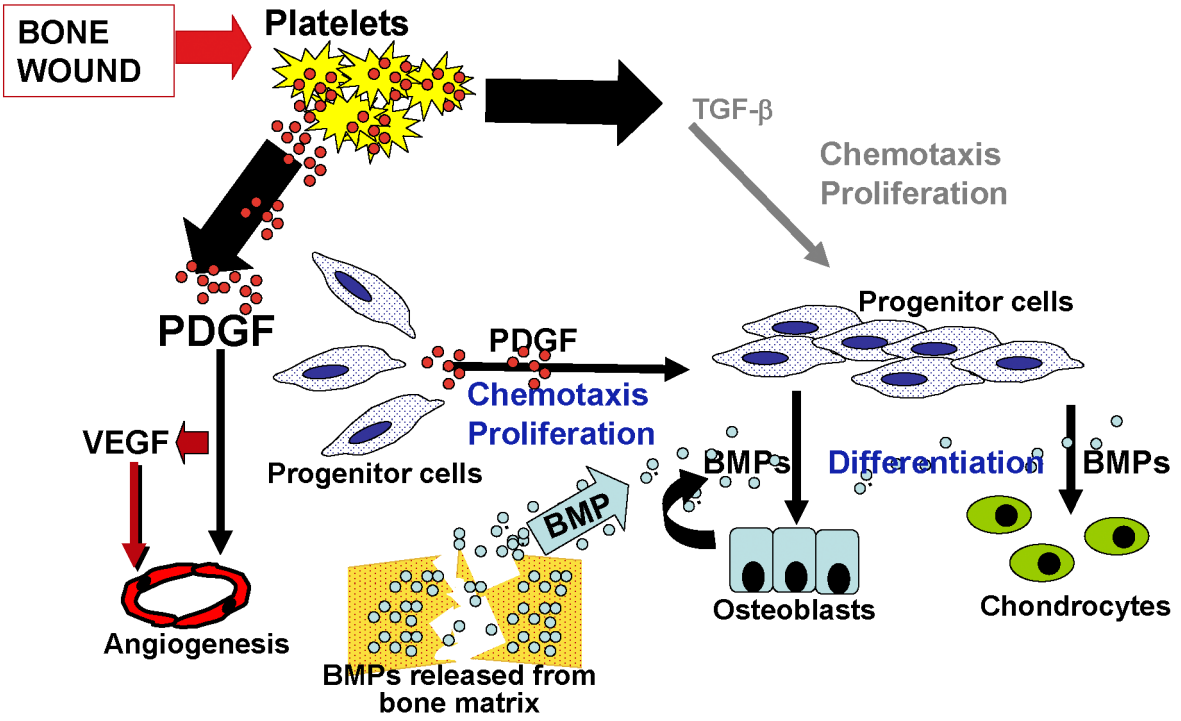

Fig. 1

Platelet-derived growth factor (PDGF): Mechanism of action and bone regeneration. As a consequence of injury, alpha granules containing PDGF are jettisoned by platelets for the purpose of angiogenesis, chemotaxis, and mitogenesis. In addition, PDGF up-regulates vascular endothelial growth factor (VEGF), further enhancing angiogenesis. Transforming growth factor-beta (TGF-β) also appears to play a role in chemotaxis and cell proliferation during wound-healing. The attraction of osteoprogenitor cells (chemotaxis) and their increase in number (mitogenesis) provide a pool of osteo-regenerative cells that will respond to the bone morphogenetic proteins (BMPs). BMP is a differentiating factor. Consequently, BMPs and PDGF are primary and powerful co-regulatory controls for healing and regeneration of bone. (Reprinted, with permission, from: Hollinger JO, Hart C, Gruber R, Doll B. Protein therapeutics and bone healing. In: Lynch SE, Wisner-Lynch LA, Nevins M, Marx RE, editors. Tissue engineering: applications in oral and maxillo-facial surgery and periodontics. 2nd ed. Chicago: Quintessence; 2008. p 5.)

mediators of early tissue repair. These cells then serve as an ongoing source of PDGFs and other growth factors that are responsible for the formation of granulation tissue, which is the next step in endochondral bone repair. PDGFs also exert mitogenic and chemotactic activity on osteogenic cells derived from calvaria[14-16], periosteum of long bones[17], trabecular bone[18,19], and bone-marrow stromal cells[20-22].

Chemotaxis and mitogenesis of a variety of mesenchymal-derived cells, including fibroblasts, osteoblasts, chondrocytes, and smooth muscle cells, are also accomplished by the local release of PDGF into the wound-healing milieu. Additionally, the ability of PDGF to recruit mesenchymal precursor cells, with their subsequent differentiation into osteoblasts, is particularly noteworthy in the setting of compromised bone-healing, such as that which occurs with diabetes mellitus. A decrease in cellular proliferation within the fracture callus and reduced levels of PDGF transcripts have been demonstrated in diabetic rats[23]. Furthermore, platelets from diabetic patients have been reported to contain less PDGF than those from individuals without diabetes[24]. In light of the beneficial effect of PDGF in the treatment of soft-tissue diabetic ulcers[25], we expect a similar beneficial role for PDGF therapy for bone repair in diabetic patients.

PDGF Isoforms and Signaling

The PDGF molecules signal through two cell-surface receptors, termed PDGF-R alpha and PDGF-R beta, which are capable of forming homodimers as well as heterodimers[26]. The different isoforms of PDGF have different binding specificities to the two receptors. PDGF-R alpha/alpha dimers bind PDGF-AA, AB, BB, and CC; alpha/beta dimers bind PDGF-AB, BB, CC, and DD; and beta/beta dimers bind PDGF-BB and DD. Different cell types will respond more or less strongly to the different PDGF isoforms depending on the level of expression of the two receptors. PDGF-BB is considered the universal PDGF isoform because of its ability to bind to all known PDGF receptor isotypes.

PDGF Cell Targets

Osteogenic progenitor cells respond to PDGF ligand-binding by activation of Src tyrosine kinases[27-29] as well as activation of the AKT protein kinase and Grb2-mediated extracellular-

regulated kinase-signaling[29]. As a consequence, PDGF is able to increase the pool of osteogenic cells at the injury site, acting as both a chemotactic agent and a mitogen. The subsequent differentiation of these cells into osteoblasts or chondrocytes is directed by the bone morphogenetic protein (BMP) family[30,31], hedgehog proteins[32,33], and activation of the Wnt-signaling pathway[34].

PDGFs exert indirect effects on bone regeneration by increasing the expression of angiogenic molecules such as vascular endothelial growth factor (VEGF)[35], which is an important molecule in bone regeneration[36], and hepatocyte growth factor[37] as well as the proinflammatory cytokine interleukin-6[38]. PDGF-BB, locally applied, will destabilize blood vessels, purportedly due to pericytes or vascular smooth muscle cells following the PDGF chemotactic gradient[39]. As a consequence, blood vessels contiguous to the healing wound are able to "sprout," and a filamentous web of neovasculature homes into the granulation tissue. When PDGF-BB is coadministered with VEGF and basic fibroblast growth factor, corneal and ischemic limb revascularization is observed[40,41]. The mechanism involves the upregulation of PDGF receptors alpha and beta by basic fibroblast growth factor, leading to improved survival of endothelial cells, increased proliferation of smooth muscle cells, and subsequent stabilization of newly formed capillaries[41]. Moreover, PDGF-BB can increase VEGF expression in mural cells, which in turn target endothelial cells and induce a potent angiogenic response[42,43].

PDGFs can modulate the responsiveness of osteogenic cells to BMPs by increasing the expression of the BMP inhibitory protein gremlin (but not noggin)[44,45] and enhancing insulin-like growth factor (IGF) signaling[46]. The responsiveness of osteogenic cells to PDGFs can also be regulated by the inflammatory cytokine interleukin-1, which inhibits PDGF-R alpha expression in MG-63 cells[47-49] and human osteoblastic cells[50].

Preclinical Studies

Periodontal Models

In human periodontal lesions, homodimers of PDGF-AA and PDGF-BB have been detected in the epithelium and in fibrin clots during wound-healing[51]. Data indicate that gingival epithelium may be a rich source of PDGF-AA and PDGF-BB for periodontal repair, while expression of PDGF receptors is increased in areas of tissue damage as a consequence of tissue injury[51]. Analysis of protein extracts from human gingival biopsies demonstrated that the concentration of PDGF-AB was approximately three times higher than normal in extracts isolated from inflamed sites[52]. As a consequence of this human data, a rat model of periodontitis (including diabetic and nondiabetic cohorts) was exploited[53]. Data indicated increased levels of PDGF-BB in normal rats but not in diabetic rats, suggesting that the PDGF-BB-driven repair process is suppressed under diabetic conditions[53]. The mitogenic responsiveness of periodontal cells to local application of PDGF-BB was confirmed in a dog model[54]. In fenestration defects in alveolar bone, recombinant human PDGF-BB (rhPDGF-BB) applied to root surfaces increased proliferation of the periodontal ligament, cementoblasts, osteoblasts, perivascular cells, and endothelial cells[54].

Several types of delivery systems have been investigated for PDGF in periodontal models. A gel, in which rhPDGF-BB and IGF-I were combined, has been used to apply the combination of the two growth factors to root surfaces in a dog model[4,55]. The outcome with this formulation was an increase in bone and cementum[4,55-57]. Similar findings were observed in nonhuman primates[58]. Furthermore, use of an rhPDGF-BB and IGF-I combination increased osseointegration of dental implants[3,56] and bone regeneration of peri-implant buccal dehiscence defects, again in canine models[57].

PDGF-BB has promoted regeneration of periodontium (alveolar bone, periodontal ligaments, and cementum) in cynomolgus monkeys[58]. In horizontal class-III furcation defects in teeth in beagle dogs, the combination of rhPDGF-BB and guided bone-regeneration therapy led to bone fill of 80% at eight weeks and 87% at eleven weeks, compared with 14% and 60%, respectively, with guided bone-regeneration therapy alone[59]. Additionally, rhPDGF-BB treatment increased periodontal ligament formation from 5% to 20% with no evidence of fibrosis[59].

In a dog study, the combination of rhPDGF-BB and a bovine cancellous block was evaluated in a chronic alveolar ridge-defect model[60]. The bovine bone block, treated with either rhPDGF-BB or buffer, was held in place by the use of titanium implants. Four months following the surgical procedure, the rhPDGF-BB treatment led to repair of the defect and replacement of the implanted bovine tissue with host bone, while the buffer-treated bovine bone block had minimal impact on tissue repair.

Orthopaedic Models

In a tibial fracture repair model, rhPDGF-BB in 20 mM sodium acetate buffer or acetate buffer alone was combined with a beta-tricalcium phosphate-collagen matrix and delivered to the injury site in eighty ovariectomized female rats at eighteen months of age, thus mimicking an osteoporotic and geriatric condition[61]. Measurement of the torsional strength of fractured tibiae at five weeks following injury showed that the vehicle-treated bones were significantly weaker ($p \leq 0.05$) than the contralateral nonfractured tibiae within each animal. In contrast, rhPDGF-BB-treated tibiae were equivalent in strength to the nonfractured controls, demonstrating the benefit of rhPDGF-BB treatment to accelerate fracture repair in a model of compromised healing.

In another orthopaedic study, ovariectomy-induced osteoporotic rats were treated with either rhPDGF-BB or vehicle alone by tail vein injection, three times per week for six weeks, in the presence or absence of alendronate therapy[62]. At the end of the treatment period, bone mineral density of the spine was decreased by 5% in the ovariectomized vehicle-treated rats but was increased by 9% in animals treated with either rhPDGF-BB alone or alendronate alone. In contrast, the rhPDGF-BB and alendronate combination increased bone mineral density

of the spine by 18%. Furthermore, quantitative computerized tomography of axial and appendicular bones indicated significant enhancement in bone mass ($p \leq 0.05$). Histologically, the rhPDGF-BB-treated rats had a substantial increase in overall osteoblast numbers and lining osteoblasts, without a change in osteoclast number when compared with untreated animals. Biomechanically, rats treated with rhPDGF-BB had significantly enhanced vertebral-body compressive strength and femoral shaft torsional stiffness ($p \leq 0.05$). The combination of alendronate with PDGF further increased these indices.

RhPDGF-BB delivered in a collagen gel was administered to rabbits to treat tibial osteotomies[63]. The authors reported an increase in callus density and volume, as measured radiographically, about the rhPDGF-BB-treated osteotomies compared with that seen about the osteotomies that were treated with collagen gel only. Tibiae treated with collagen alone were significantly weaker biomechanically than non-osteotomized tibiae ($p \leq 0.05$). In contrast, rhPDGF-BB-treated tibiae demonstrated increased strength and were not significantly different from the nonoperatively treated contralateral tibiae. Histologically, rhPDGF-BB produced more robust and advanced osteogenesis, on both the endosteal and periosteal surfaces, than the collagen gel alone. The investigators concluded, from the radiographic, biomechanical, and histological data, that locally administered rhPDGF-BB delivered with an injectable collagen gel to tibial osteotomies enhanced functional fracture repair and stimulated osteogenesis significantly ($p \leq 0.05$).

Clinical Studies

Periodontal Indications

Periodontium is composed of a series of tissues, including gingival epithelium and its underlying connective tissue, cementum that lines the tooth root, alveolar bone, and the periodontal ligament (the narrow band of dense, fibrous connective tissue that connects the tooth root to the alveolar bone). Chronic inflammation is the main cause of catabolic processes in the periodontium, and these processes lead to periodontal disease. If chronic inflammation remains untreated, there will be loss of periodontal structures, a condition that occurs in 87% of adults over seventy years of age[64]. Approximately 2.1 million periodontal surgical procedures are performed annually in the United States for the treatment of this disorder. Recombinant human PDGF-BB has been demonstrated to be effective and safe for use in regenerating periodontal tissues and is approved by the FDA for this indication (GEM 21S; BioMimetic Therapeutics, Franklin, Tennessee).

Success with GEM 21S in periodontics reflects the chemotactic and mitogenic action of rhPDGF-BB on cell types involved in periodontal repair that respond directly to the rhPDGF-BB, including mesenchymal stem cells, gingival fibroblasts, osteoblasts, periodontal ligament cells, cementoblasts, and vascular smooth muscle cells[5,9-13].

Additionally, rhPDGF-BB is pro-angiogenic in that it acts in synergy with endogenous VEGF to stimulate neovascularization at the defect site[35,42,43]. As a result, rhPDGF-BB will stimulate multiple wound-healing actions critical for the repair of periodontal defects.

In a preliminary clinical study[65], thirty-eight human subjects with bilateral osseous periodontal lesions were treated with rhPDGF-BB combined with IGF-I in a gel delivery system. At nine months after treatment with the rhPDGF-BB and IGF-I combination, alveolar bone formation increased by 2.08 mm ($p < 0.05$) in vertical bone height with a corresponding 42.3% filling of the defect site by new bone. In contrast, control-treated subjects had an increase of 0.75 mm in bone height and only an 18.5% filling of the defect with new bone. Neither local nor systemic safety issues were reported with rhPDGF-BB treatment[66].

Administration of rhPDGF-BB in combination with allograft demonstrated significant healing effects ($p \leq 0.05$) for the treatment of intraosseous defects in nine patients with advanced periodontal disease[67,68]. PDGF-BB/allograft treatment reduced pocket depth by 6.4 mm while stimulating a gain of 2.1 mm in overall bone height. The significance of this outcome was the regeneration of the periodontal complex, which includes alveolar bone, ligament, and gingiva. Moreover, treatment of furcation defects with a combination of PDGF-BB and allograft similarly improved clinical outcome, with pocket-depth reduction of 3.4 mm and a gain in clinical attachment of 4.0 mm. Histological analysis of intra-osseous and furcation defect sites indicated regeneration of cementum, periodontal ligament, alveolar bone, and blood vessels, with no root resorption, ankylosis, inflammation, or adverse tissue responses[67,68]. The clinical benefit of the combination treatment of PDGF-BB and allograft was a regenerative outcome of complex functional tissues. Importantly, there were no resorptive or lytic sequelae.

A prospective, blinded, and randomized controlled clinical trial assessed the safety and effectiveness of rhPDGF-BB (300 μg/mL) combined with beta-tricalcium phosphate for the treatment of advanced periodontal osseous defects[69]. Eleven clinical centers enrolled 180 subjects, each requiring surgical treatment of a 4-mm or greater intraosseous periodontal defect. Treatment with recombinant human PDGF-BB/beta-tricalcium phosphate caused a significant gain of clinical attachment level over beta-tricalcium phosphate treatment alone after three months (3.8 mm versus 3.3 mm; $p \leq 0.05$). The rhPDGF-BB-treated sites also had significantly greater linear bone gain (2.6 mm versus 0.9 mm) and percent defect fill (57% versus 18%) at six months than did the sites that received the beta-tricalcium phosphate with buffer ($p \leq 0.05$). No significant device-related adverse events were observed with the rhPDGF-BB treatment. The compelling treatment outcome from the clinical trial was the regeneration of periodontal tissues in response to treatment with rhPDGF-BB/beta-tricalcium phosphate. The percent defect fill of 57% and the linear bone gain validated the regenerative benefits that were achieved during the six-month study period.

Orthopaedic Indications

Autogenous bone graft is recognized as the gold standard for use in a wide variety of surgical procedures for the treatment of fractures and nonunions and in securing osseous fusion

when a graft material is deemed necessary as an adjunct to the bone-healing process[70]. However, the time, cost, and morbidity involved in obtaining autogenous bone graft are well documented[71,72]. Recombinant human PDGF-BB combined with a beta-tricalcium phosphate matrix avoids the problems associated with the use of bone autograft. Because of its biology, preclinical performance, and early clinical success, it is under evaluation for use as an alternative to autogenous bone graft in several orthopaedic indications.

A twenty-patient randomized, controlled pilot study, recently conducted at three U.S. sites, evaluated the effectiveness of rhPDGF-BB in a beta-tricalcium phosphate matrix (GEM OS1, BioMimetic Therapeutics) as a bone-graft material in hindfoot and ankle fusions in comparison to the effectiveness of an autogenous bone graft control[73]. At both the six-week and twelve-week time-points following surgery, an independent radiographic evaluation of osseous bridging, based on computed tomography scans, demonstrated that the bridging obtained with GEM OS1 was at least equivalent to that obtained with autogenous bone graft. Functional (American Orthopaedic Foot and Ankle Society Score) and pain (visual analog scale) scores were also equivalent at both of these time-points between the two groups, with a >85% fusion rate for both groups at twenty-four weeks[73]. These data, collected as part of an FDA-approved Investigational Device Exemption (IDE) study, have allowed for expansion of this approach into an FDA-approved pivotal trial study, which will include approximately 400 patients at twenty-eight sites in the United States. Accrual for this study began in mid-2007.

Another foot-and-ankle fusion study, involving sixty patients, is ongoing at three centers in Canada[73] for the purpose of evaluating the performance of rhPDGF-BB plus beta-tricalcium phosphate (GEM OS1) with use of standardized radiographic and clinical assessments[74]. In reporting the preliminary results, the authors found the GEM OS1 bone-graft substitute to be at least as effective as autogenous bone graft, despite a challenging patient population. Of the patients reported, 33% were undergoing revision of a failed prior surgical treatment, a risk factor normally associated with slow and/or poor bone-healing. Additional risk factors that were associated with poor outcomes in the treated population included smoking, diabetes, and obesity. Preliminary results from the GEM OS1-treated patients demonstrated computed tomographically measured fusion rates (i.e., osseous bridging) of 42% at six weeks and 70% at twelve weeks after surgery[73]. These outcomes are similar to those reported for autogenous bone graft in foot and ankle fusions[75].

A single-investigator study in Sweden evaluated rhPDGF-BB in a beta-tricalcium phosphate matrix as an adjunct to the standard technique of open reduction and external fixation of distal radial fractures in elderly patients (average age, sixty-five years)[76]. Early results obtained with the use of computed tomography scans showed more rapid bone filling of the resultant defect at both three-week (44% versus 11% bone fill of more than 50%) and six-week (100% versus 56% bone fill of more than 50%) time-points for the group treated with rhPDGF-BB as compared with the group treated with open reduction and external fixation only. Function and grip-strength scores were judged to be equivalent at these early time-points. No serious device-related adverse events were reported in these studies.

Summary

RhPDGF-BB, as a consequence of its biological properties, is being considered as a therapy for an increasing number of musculoskeletal indications. RhPDGF-BB is a key regulatory molecule in bone homeostasis, repair, and regeneration. It is chemotactic and mitogenic for osteoblasts and undifferentiated osteoprogenitor cells, and it upregulates cytokines that are crucial to osseous and soft-tissue healing and regeneration. On the basis of compelling evidence from preclinical studies and a growing number of clinical investigations, rhPDGF-BB appears to be safe and effective in enhancing the repair of musculoskeletal and maxillofacial disorders. It is noteworthy that the safety profile of PDGF-BB has been well established, as demonstrated by the FDA approval of both GEM 21S, for use in the repair of periodontal bone defects, and Regranex (Johnson and Johnson Wound Management-Ethicon, Somerville, New Jersey), an rhPDGF-BB-containing formulation for repeat topical application to treat nonhealing lower-extremity diabetic ulcers. Moreover, the biology of PDGF is such that this molecule may prove to have profound musculoskeletal clinical advantages for patients with compromised wound-healing, such as those with diabetes mellitus, who should benefit greatly from the recruitment and proliferation of osteoprogenitor and other reparative cell types. ■

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