UP446, analgesic and anti-inflammatory botanical composition

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disease. Long-term use of currently available therapies for RA produces adverse effects that limit dosage and duration; hence there is a need for safe and effective alternatives suitable for long term chronic use. UP446, a composition consisting primarily of baicalin from *Scutellaria baicalensis* Georgi (Family: Lamiaceae) and (+)-catechin from the heartwoods of *Acacia catechu* (Family: Mimosaceae), has been previously shown to reduce production of eicosanoids and leukotrienes through dual inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes and to decreased mRNA and protein levels of the proinflammatory cytokines, interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α. **Aim:** To evaluate the likelihood of UP446 in moderating arthritis and its associated symptoms in an experimental animal model of RA. **Materials and Methods:** A RA rat model was induced by injecting Freund's complete adjuvant into left and right hind paw and base of the tail. Animals were administered UP446 (50 mg/kg), ibuprofen (150 mg/kg mg/kg) or vehicle by oral gavage 30 min prior to arthritis induction and each day thereafter for 14 days. **Result:** Animals treated with UP446 showed 23.7, 31.9, 33.4, 29.3, and 33.1% reduction in pain sensitivity; 46.0, 36.7, 33.7, 34.8, and 33.4% reduction in ankle diameter on days 3, 5, 7, 9, and 13; respectively; compared to vehicle. Similarly paw edema was significantly reduced with an average of 30% for the first inflammatory reaction period (day 1–8) followed by 37.1 and 33.6% reduction on day 9 and 13. **Conclusion:** These data indicate potential benefit of UP446 in alleviating symptoms of RA and support human clinical evaluation of this botanical composition in patients with RA.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory autoimmune disease characterized by chronic inflammation of multiple joints associated with subsequent progressive, erosive destruction of articular bone and cartilage, mononuclear cell infiltration, pannus formation, and functional impairment. Long-term uses of disease modifying anti-rheumatic drugs for the treatment of RA causes serious toxicities when used at a dose that would result in maximum efficacy. Even though currently used nonsteroidal anti-inflammatory drugs (NSAIDs) are graded as good anti-inflammatory therapy, their usage as RA treatment have little potential for disease modification at the doses that are generally safe for prolonged use in humans due to their associated gastrointestinal side effects.[1] Similarly, though selective cyclooxygenase (COX)-2 inhibitors minimize gastrointestinal associated risks, their usages have been limited as a result of unwanted side effects on kidney and cardiovascular systems.[2]

Adjuvant- and collagen-induced arthritis in rats are the most widely used experimental animal models of inflammatory polyarthritis with clinical and pathological features similar to those of human RA.[3,4] Adjuvant-induced arthritis in rats is characterized by reliable onset of vigorous polyarticular inflammation, discernible bone resorption, and periosteal bone proliferation accompanied by cartilage destruction.[3-5] The adjuvant rat model is also commonly used to study disease pathogenesis and to validate therapeutic RA targets. When a complete adjuvant is used to induce arthritis in rats, two

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overlapping phases of inflammation are elicited. The initial primary reaction is an acute inflammation at the site of injection mediated through the COX-lipoxygenase (LOX) pathways and lasting through day-8 followed by a delayed secondary systemic cellular and humoral immune reaction occurring on days 9 through 14 in association with increased inflammatory cytokine levels, for example, tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), and increased nuclear factor (NF)-κB transcriptional activity.[6]

Although the initiating cause(s) of RA is not known, elevated levels of proinflammatory cytokines such as TNF-α, IL-1 and IL-6, and activation of NF-κB are thought to be essential to disease pathophysiology and progression.[7,8] The UP446 botanical composition, which consists primarily of baicalin from *Scutellaria baicalensis* Georgi (Family: Lamiaceae) (Chinese skullcap) and (+)-catechin from *Acacia catechu* (Family: Mimosaceae) (black catechu), possesses activities suggestive of benefit in RA including: (i) Inhibition of COX and LOX;[9] (ii) normalization of COX-2, TNF-α, IL-1β, IL-6, and NF-κB gene expression in lipopolysaccharide (LPS)-induced human and animal cell lines;[10] (iii) inhibition of COX-2, 5-LOX, and inducible-nitric oxide synthase (iNOS) gene expression and blunted NF-κB binding activity in endotoxin-stimulated rat peritoneal macrophages.[11] The potential utility of UP446 is also supported by *in vivo* studies demonstrating: (i) Substantial inhibition in ear swelling induced by topical arachidonic acid application and restoration of joint function in mice inoculated intra-articularly with arachidonic acid;[9] (ii) potent analgesic effects in three animal pain models (carrageenan-induced paw edema, formalin test, and abdominal constriction assay);[12] (iii) equivalent efficacy to naproxen in improving quality of life in osteoarthritic patients;[13] and (iv) superiority to celecoxib in alleviating stiffness and functional impairment in a randomized, double-blind, placebo-controlled human clinical study in osteoarthritic patients.[14]

The present study was designed to evaluate the likelihood of UP446 in moderating arthritis and its associated symptoms in an experimental animal model of RA.

MATERIALS AND METHODS

Preparation of UP446: Detailed methods for preparing extracts enriched for baicalin and catechin from the roots of Scutellaria baicalensis Georgi (Family: Lamiaceae) and the heartwoods of Acacia catechu (Family: Mimosaceae), respectively, were disclosed in a USA patent.[15]

UP446 is the combination of a standardized baicalin extract from *Scutellaria* and a standardized catechin extract from *Acacia* with baicalin content of not less than 60% and catechin content of not less than 10%.

Extracts and UP446 were analyzed by high-performance liquid chromatography (HPLC) using a Phenomenex Luna 5 μm C-18, 250 mm × 4.6 mm with a C-18 SecurityGuard cartridge in a column oven at 35°C. The mobile phase had a flow rate of 1.0 ml/min and used an isocratic 1% phosphoric acid:acetonitrile ratio of 85%:15% for the first 7 min and then a new gradient to 10%:90% from 7 to 16.5 min and then an isocratic 1% phosphoric acid:acetonitrile gradient with a ratio of 85%:15% for 7.5 min. The flavonoids were detected using a UV detector at 275 nm and identified based on retention time by comparison with known standards [Figure 1].

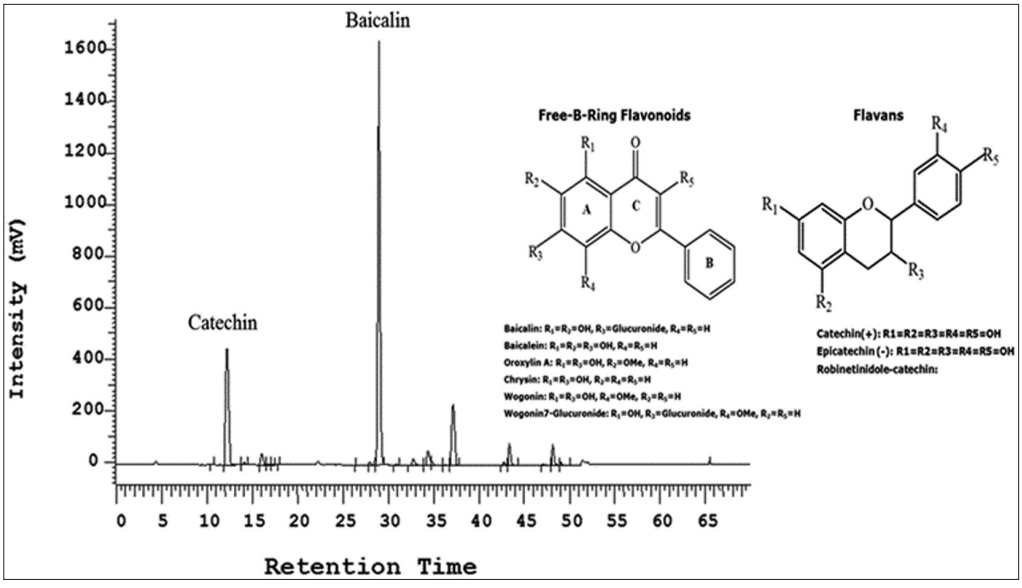

Figure 1: HPLC chromatogram and chemical structure of free-B-ring flavonoid (baicalin) and flavan (catechin). The flavonoids were detected using a UV detector at 275 nm and identified based on retention time by comparison with known flavonoid standards

Animals: Purpose bred male Wistar rats weighing 285–330 g were purchased form USDA approved laboratory animal vendor (Charles River Laboratories Inc., Wilmington, MA). Animals were acclimated upon arrival for a week before assigned randomly to their respective group. Rats (3/cage) were housed in a polypropylene cage and individually identified by numbers on their tail. Each cage was covered with wire bar lid and filtered top (Allentown, NJ). Individual cages were identified with a cage card indicating project number, test article, dose level, group, and animal number. The Harlan T7087 soft cob beddings was used and changed at least twice/week. Animals were provided with fresh water and rodent chow diet #T2018 (Harlan Teklad, 370W, Kent, WA) ad libitum and were housed in a temperature-controlled room (22.2°C) on a 12-h light-dark cycle. All animal experiments were conducted according to institutional guidelines congruent with the guide for the care and use of laboratory animals.

Adjuvant-induced arthritis model: Arthritis was induced by injection of Freund's complete adjuvant containing 5 mg/ml (w/v) suspension of heat-killed *Mycobacterium tuberculosis* in liquid paraffin into subplantar region of left and right hind paw and into the base of tail.[6,16-18] Rats were placed under inverted plexiglass cages on a wire mesh rack and allowed to acclimate for 20–30 min before each measurement was taken. Allodynia was evaluated by responsiveness to semi-flexible tips applied through the mesh floor perpendicular to the central plantar surface of the right hind paw. The tips were gradually applied with sufficient force to cause slight buckling of the filament against the paw. A positive response to the applied tactile pressure, noted by sharp withdrawal of the paw, was recorded automatically by an electronic Von Frey Anesthesiometer (2390 series Electrovonfrey, IITC, Woodland Hills, CA).[19] Mechanical allodynia was evaluated before arthritis induction, and day 3, 5, 7, 9, and 13 after induction. Paw edema was measured with the use of Plethysmometer (IITC, Woodland Hills, CA; Model 520) before arthritis induction and on day 3, 5, 7, 9, and 13 after induction. Ankle diameter was measured using Pocket Thickness Gage (7309, Mitutoyo Corp., Japan) before arthritis induction and on day 3, 5, 7, 9, and 13 after induction.

Animals (n = 12 per dose group) were administered ibuprofen (Sigma-Aldrich, St. Louis, MO; lot #037K1345) (150 mg/kg), UP446 (Unigen, Seattle, WA; lot #UV07080) (50 mg/kg), and vehicle control (propylene glycol) by oral gavage 30 min before administering the complete Freund's adjuvant injections and each day thereafter for 14 days.

Statistical analysis

Data were analyzed using Sigmaplot (Version 11.0). The results were represented as mean ± 1 standard deviation (SD). Statistical significance between groups was calculated by means of single factor analysis of variance followed by a paired t-test. P-values less or equal to 0.05 ($P \leq 0.05$) were considered as significant. When normality tests failed, data for nonparametric analysis were subjected to Mann-Whitney sum ranks for t-test and Kruskal-Wallis one way analysis of variance on ranks for ANOVA.

RESULTS

Cardinal signs of inflammation, hyperalgesia, swelling, and hyperemia were evident in all animals within 24 h of administering the complete Freund's adjuvant. Measurement of vital signs of inflammation in both hind limbs is more practical and accurate than a visual grading system[20] and also allows the effects of treatment groups to be expressed quantitatively. As a result, we measured the thickness of swollen ankle, hypersensitivity, and paw edema of each rat joint.

As seen in Figure 2, the positive control ibuprofen showed statistically significant reduction of pain by 21.1, 36.1, 45.6, 53.7, and 53.3% on days 3, 5, 7, 9, and 13; respectively; compared to vehicle control. Similarly, animals treated with a daily oral dose of 50 mg/kg UP446 showed a 23.7, 31.9, 33.4, 29.3, and 33.1% reduction in pain sensitivity on days 3, 5, 7, 9, and 13; respectively; when compared to the vehicle control animals. These percentage reductions were statistically significant at each time point analyzed compared to vehicle controls.

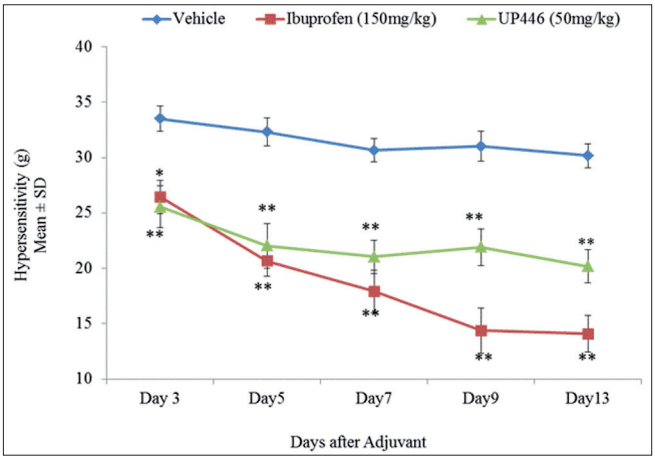

Figure 2: Anti-pain effect of UP446 in adjuvant induced arthritic rat. A rheumatoid arthritis rat model was induced by injecting Freund's complete adjuvant into subplantar region of left and right hind paw and base of the tail of Wistar rats (n = 12). Animals were administered UP446 (50 mg/kg), ibuprofen (150mg/kg), or vehicle by oral gavage 30 min prior to arthritis induction and each day thereafter for 14 days. Pain sensitivity was measured before adjuvant injection and thereafter on day 3, 5, 7, 9, and 13 after injection. Data are expressed as mean ± SD. *$P \leq 0.001$ vs vehicle; **$P \leq 0.0001$ vs vehicle

Administration of UP446 also showed a marked reduction in paw edema measurements that were observed in two phases. An average of 30% reduction in swelling was observed for the first inflammatory reaction period from day-3 to 5 after oral administration followed by a 37.1 and 33.6% reduction on day-9 and 13, respectively after treatment [Table 1], when compared to vehicle-treated diseased rats. These reductions were statistically significant at each time point.

Furthermore, as presented in Figure 3, the ankle diameter data substantiates the complementary effect of UP446 in reducing joint inflammation. Animals treated with a daily oral dose of 50 mg/kg UP446 showed 46.0, 36.7, 33.7, 34.8, and 33.4% reductions in ankle diameter on days 3, 5, 7, 9, and 13; respectively; as compared to the vehicle control animals. These percentage reductions were statistically significant at each time point analyzed compared to vehicle controls. The positive control ibuprofen treatment showed statistically significant 61.1, 16.6, 23.1, 18.9, and 21.8% reduction in ankle diameter on days 3, 5, 7, 9, and 13; respectively; compared to vehicle control.

DISCUSSION

As new treatment strategies for RA continue to evolve, countering the underlying cause remains the first line of treatment strategy.[21] In this frontier biological therapeutics that target the proinflammatory cytokine TNF-α has been highly successful.[22-24] It has been known that TNF-α has an early and crucial role in the cascade of proinflammatory cytokine production and subsequent inflammatory process.[25] TNF-α activates IL-1β and IL-6 and thereby cause induction of hyperalgesia, mediated through COX products like prostaglandin.[25,26] With the concept of TNF-α as the tip of proinflammatory network in early and long standing RA pathogenesis, efforts have been made to develop anti-TNF-α antibody that could defuse TNF-α activity in the last 2 decades. These biologics showed remarkable clinical benefit validating the hypothesis that TNF-α is a major pillar in the pathology of RA.[27] However, their usage is limited due to high costs, lack of oral activity, immune suppression, and adverse autoimmune reactions.[28-32] As a result, in recent years, natural products are increasingly gaining attractions in patients with RA for additional relief. In this respect, previously Altavil *et al.*,[11] in rat endotoxin-stimulated rat peritoneal macrophages and Tseng-Crank *et al.*,[10] in LPS-induced human and animal cell lines showed that UP446 can reduced and normalize TNF-α gene expression. Chou *et al.*,[33] have also reported that when baicalin was pre- or postadministered in rat with carrageenan-induced paw edema at 100 mg/kg, a significant reduction in TNF-α, IL-1β, and IL-6 as well as a significant increase in anti-inflammatory cytokine IL-10 were observed. It has also been reported that in LPS-stimulated primary macrophages, (+)-catechin showed inhibition of nitric oxide and TNF-α production.[34] Recently, inhibition of TNF-α expression in aorta of mice was also observed when catechin was given at 50 μg mixed with drinking water

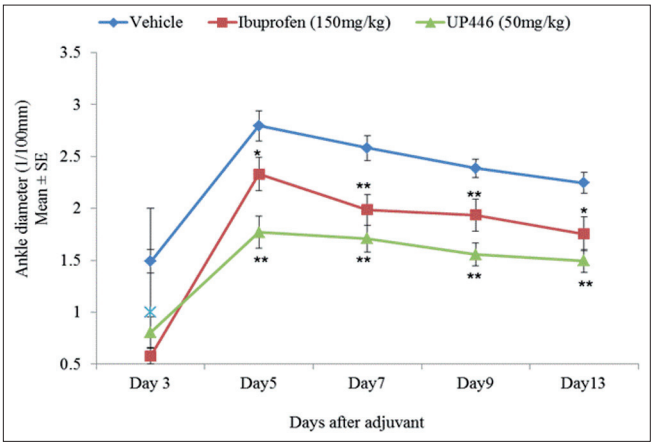

Figure 3: Effect of UP446 in adjuvant induced arthritic rat. A rheumatoid arthritis rat model was induced by injecting Freund's complete adjuvant into subplantar region of left and right hind paw and base of the tail of Wistar rats (n =12). Animals were administered UP446 (50 mg/kg), ibuprofen (150 mg/kg) or vehicle by oral gavage 30 min prior to arthritis induction and each day thereafter for 14 days. Ankle diameter was measured before adjuvant injection and thereafter on day 3, 5, 7, 9, and 13 after injection. Data are expressed as mean ±SD. *$P \leq 0.01$ vs vehicle; **$P \leq 0.0001$ vs vehicle

Table 1: Effect of UP446 in adjuvant induced arthritic rat

Groups	Dose (mg/kg)	Paw edema (ml)				
		Day 3	Day 5	Day 7	Day 9	Day 13
Vehicle	0	0.72 ± 0.13	0.79 ± 0.14	0.68 ± 0.12	0.70 ± 0.14	0.61 ± 0.14
Ibuprofen	150	0.49 ± 0.20* (32.4)	0.65 ± 0.22 (18.4)	0.54 ± 0.14* (20.6)	0.48 ± 0.21* (31.4)	0.39 ± 0.17* (36.9)
UP446	50	0.50 ± 0.17** (31.7)	0.54 ± 0.16** (31.6)	0.47 ± 0.12** (31.6)	0.44 ± 0.13** (37.1)	0.41 ± 0.11** (33.6)

A rheumatoid arthritis rat model was induced by inoculating Freund's complete adjuvant into subplantar region of left and right hind paw and base of the tail of Wistar rats (n = 12). Animals were administered UP446 (50 mg/kg), ibuprofen (150 mg/kg), or vehicle by oral gavage 30 min prior to arthritis induction and each day thereafter for 14 days. Paw edema was measured before adjuvant injection and thereafter on day 3, 5, 7, 9, and 13 after injection. Data are expressed as mean ±SD. *P ≤ 0.001 vs Vehicle; **P ≤ 0.0001 vs vehicle. Data in parentheses are percent reduction of vehicle

daily for 30 days.[35] This gives the notion that the results observed in the present study in alleviating RA-associated symptoms could be partially attributed to anti-TNF-α activity of baicalin and catechin in UP446.

Over the years, augmented reports have been generated to support involvement of chemokines and their receptors in the pathogenesis of RA. As chemokines play a pivotal role in for the recruitment, localization, and retention of inflammatory cells in inflamed synovium and hence result in bone and cartilage destruction, preventing this phenomenon could cause moderation of the inflammatory process. Studies have shown that baicalin can inhibit the binding of chemokines to leukocytes or cells transfected to express specific chemokine receptors.[36-38] Therefore, another possibility for UP446 to reduced severity of arthritis could be by binding to chemokines and limit their biological function which would have otherwise assisted in more inflammatory cells to migrate to the synovium and cause serious damage to the already inflamed joint.

Moreover, irrefutable evidences have been documented that, NF-κB plays a major role in the regulation of inflammatory genes. In RA, active NF-κB plays a pivotal role in both, at the initiation and maintenance of chronic inflammation. Some have suggested that suppression of activation of NF-κB or targeting NF-κB inhibitors to specific tissues or cell-type could mitigate severity of bone and cartilage destruction.[39-42] For instance, previously Xue *et al.*,[43] have showed that when rats with acute pancreatitis were treated with 50 mg/kg of baicalin intraperitoneal, a significant inhibition of activation of NF-κB was observed. Similarly, a significant inhibition in activation of NF-κB was also reported when IL-1β or TNF-α activated human mast cell line-1 (HMC-1) were treated with baicalein (metabolite of baicalin) at concentration of 30 μM.[44,45] On the other hand, an increase in anti-inflammatory cytokine IL-10 level was found significantly increased when a polyseptic C57BL/6J mice were treated with baicalin at 100 mg/kg intraperitoneally.[46] Substantiating these findings, blunting[11] and normalization of expression[10] of NF-κB were reported as a result of administrating UP446. Thus, arthritis mitigation observed in the present study could partially be explained by the ability of UP446 to inhibit cellular gene expression regulated by transcription factor NF-κB.

Knowing the fact that UP446 is active in inhibiting arachidonic acid metabolism, COX, LOX, iNOS, cytokines (ILs and TNF), and NF-κB;[9-10] it was not surprising to see significant progressive decreases in thickness of both ankles, improved pain resistance, and suppression of paw edema in animals treated with UP446 compared to vehicle-treated diseased rats. These marked inhibitions in pain and swelling were observed both in the primary and the secondary inflammatory reactions in the course of adjuvant-induced arthritis pathology when UP446 was administered orally at a dose of 50 mg. In support of our data, Krakauer *et al.*,[47] have demonstrated that, besides reducing mRNA and protein expression of IL-1β, IL-6, and TNF-α, when baicalin was incubated at 100 μg/ml with human peripheral blood mononuclear cells, it showed a 98% inhibition in staphylococcal exotoxins-stimulated proliferation of T-cells. This may further justify disease modifying activity of UP446 in the second phase of immunological reaction that occurred after day-9 of treatment.

Similarly, Kubo *et al.*,[48] have showed that baicalin suppresses the secondary lesion in adjuvant-induced arthritis in rats. In their study, rats were given 100 mg/kg of baicalin orally for 27 days where inhibition of edema was observed after day-11 of treatment. The authors presumed that the anti-inflammatory activity of baicalin could be attributed to inhibition of delayed-type allergic reaction or activation of components. In a similar study, when catechin (as low as 60 mg/kg) was given orally to adjuvant-induced Sprague-Dawley rats, a significant suppression in secondary inflammatory paw edema, hypersensitivity, and polyarthritis index as well as inhibition in production of IL-1, TNF-α, and prostaglandin E2 was observed.[49]

CONCLUSION

To sum up, in addition to its dual COX-LOX inhibition activity, various reports have shown impact of UP446 to decrease expression of pro-inflammatory cytokines TNF-α and IL-1β and/or inhibiting activation of transcription factor NF-κB. In the present study, UP446, a defined bioflavonoid composition of primarily baicalin and catechin, has showed a significant improvement in the major cardinal signs of arthritis which includes reduction in pain sensitivity, paw edema and ankle diameter. Though specificity, potency, and long-term use needs further clinical evidence; UP446, analgesic and anti-inflammatory agent of botanical origin, could potentially be used as medical foods and dietary supplements to manage the symptoms associated with RA.

AUTHOR CONTRIBUTIONS

MY conceived and designed, carried out study, data calculation, statistical analysis, data interpretation, and drafted/edited the manuscript. MP assisted in conducting the study. QJ and LB conceived the study, participated in its design, interpreted data, and edited the manuscript. All authors read and approved the final manuscript.

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