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Research Article

Diabetes and oxidative stress: Dipeptidyl peptidase-4 inhibitors

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ABSTRACT

Diabetes mellitus is one of the commonmetabolic diseases increasing the mortality worldwide.As predicted by the World Health Organization, the number of people suffering from these diseases is rapidly increasing. Two important factors in the progression of the disease are obesity and reduced physical activity. The greatest cause of death among diabetic patients is the cardiovascular complications caused by the microvascular and macrovascular dysfunctions. Clinical studies and experimental evidence suggest that oxidative stress plays an important role in the pathophysiology of diabetes mellitus. Free radicals in diabetes are produced by oxidation of glucose, non-enzymaticglycation of proteins, and subsequent oxidative damage of glycosylated proteins and other macromolecules. High levels of free radicals and lipid peroxidation and malfunction of antioxidant defense mechanisms cause cell damage in various organs, leading to insulin resistance and other diabetes mellitus complications. Several studies have pointed the role of antioxidant therapy in diabetes. Dipeptidyl peptidase-4(DPP-4)inhibitors inhibit the degradation of incretin hormones by DPP-4 membrane-bound enzyme and play an important role in glucose homeostasis and insulin secretion. These inhibitors are new candidates for the treatment of diabetes. This study is aimed to review the roles of changes in biomarkers of oxidative stress and antioxidant defense systems in the progression of diabetes, and the antioxidative roles of DPP-4 inhibitors in the prevention of diabetic complications.

Keywords: Diabetes, DPP-4 inhibitors, Antioxidant, ROS

1. NTRODUCTION

Diabetes mellitus is one of the most common metabolic diseases which its incidence and prevalenceis progressively increasing worldwide. Diabetes mellitus, characterized by continuous hyperglycemia, is caused due to a defect in insulin secretion from the pancreatic beta cells (type I diabetes or insulin-dependent diabetes) or insulin function (type II diabetes or non-insulin dependent diabetes) or both. Type II diabetes mellitus is a multifactorial disease caused by the dysfunction of insulin receptors and flaw in insulin's actions in the peripheral tissues. Due to the need of vital tissues such as the brain to glucose, the body always keeps blood glucose levels within a narrow range[1]. In patients with type II diabetes, disruption in insulin sensitivity in the periphery as well as continuous high blood glucose levels lead to the disturbances in glucose homeostasis which

finally cause functional damages to various vital organs, including kidneys, heart, eyes and blood vessels[2].

Oxidative stress is a harmful condition to the body, in which an imbalance between the oxidative stress reactions and the antioxidant defenses is occurred[3]. This imbalance leads to the production of reactive oxygen species (ROS) in the body, which their role in the pathogenesis of diabetes has been validated by the clinical and experimental studies[4, 5]. Overproduction of ROS and oxidative stress insults during diabetes may be considered as a main pathogenic reason for the occurrence of diabetes-induced comorbidities such as stroke, nephropathy, retinopathy, and neuropathy. Oxidative stress results from the overactivationof lipid peroxidation, the destruction of glutathione metabolism and reduction in the levels of enzymatic (like superoxide dismutase) and non-enzymatic (like vitamin C) antioxidants[6, 7]. The cellular mechanisms of increased oxidative stress in diabetes include increased activation of protein kinase C pathways, increased NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) levels and activation of stress-related kinases, activation of other transcription factors, and generation of advanced glycation end products (AGEs)[8, 9].

Reduction of the burden of oxidative stress in diabetes is clinically important and various strategies have been proposed to reduce this burden. Weight lossand exercise training are considered as two effective strategies in reducing complications related to the oxidative stress during diabetes[10, 11]. Various hypoglycemic medications can also reduce the burdens of oxidative stress through scavenging and eliminating free radicals (directly) and/or through their hypoglycemic effects (indirectly). In general, vitamins, new herbal products containing compounds such as flavonoids and coumaric acid, and oral antidiabetic drugs such as sulfonylurea and metformin can be pointed out as the effective antioxidants in diabetes[12, 13].

The inhibitors of enzyme dipeptidyl peptidase-4 (DPP-4), also called gliptins, are a new class of drugs orally used in the treatment of diabetes and include anagliptin, sitagliptin, linagliptin, alogliptin, saxagliptin and vildagliptin[14, 15]. By preventing of the breaking down and inactivation of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide(GIP), gliptins improve insulin secretion from pancreatic beta cells in response to high blood glucose levels and thereby increase the levels of this hormone in the blood to higher than its physiological levels and also reduce glucagon secretion from pancreatic alpha cells [16, 17]. Incretins-related agents include GLP-1 analogs and DPP-4 inhibitors, inhibit the production of ROS by reducing the activity of NADPH oxidase, resulting in the activation of downstream signaling pathways like PI3K/Akt (Phosphatidylinositol 3-kinase/a serine-threonine kinase also known as protein kinase B) and cAMP/PKA (Cyclic adenosine monophosphate/protein kinase A)[18, 19]. Protective effects of GLP-1 against pathological conditions in organs such as the heart and endothelial cells have been demonstrated and many cellular pathways are suggested to be involved in this protection[20-22]. Almost all gliptins have antioxidative properties, some more, and some less. For example, studies have shown that the antioxidative effects of vildagliptin are more than other DPP-4 inhibitors like sitagliptin [23]. Vildagliptin therapy has reduced the oxidative and endoplasmic reticulum stress in pancreatic tissue by increasing the activities of superoxide dismutase (SOD) and catalase (CAT)[24].

In this review, we have addressed the roles of oxidative stress in the pathogenesis of diabetes as well as the antioxidative effects of DPP-4 inhibitors and related underlying mechanisms during diabetic conditions.

2. LESSONS FROM OXIDATIVE STRESS

The relationship between oxidative stress and lifestyle-relateddiseases has been revealed so far [25, 26]. Oxidative stress is a condition in which

the antioxidant system is overcome by the oxidants; this is secondary to the increased production of oxidants or defects in the antioxidant defense [27, 28]. Typically, each atom consists of a central molecule and a pair of electrons orbiting around it, but some atoms have free, unpaired electrons, known as free radicals, which are unstable and highly reactive [29]. High levels of free radicals along with defects in antioxidant defenses can damage cellular organelles, proteins, enzymes, and lipids [30]. During lipid peroxidation induced by high free radicals, free radicals separate electrons from the lipids and lead to the oxidative degradation of lipids.

Free radicals in the biological systems can be divided into two main groups [31]: reactive oxygen species (ROS) and reactive nitrogen species (RNS). Biological roles of free radicals are many and include activation of intracellular signaling pathways such as mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK)-related pathways [32]. ROS plays a role in the process of signalstransduction regulating cell activities [33]. Several intracellular and extracellular sources generate free radicals; intracellular sources include non-enzymatic production of ROS and oxidative phosphorylation in the mitochondrial pathway, especially in complexes I and III of the mitochondrial electron transport chain, and also in peroxisomes by cytosolic lipoxygenase enzymes as well as by NADPH oxidase, inflammatory cytokines, endoplasmic reticulum stress, destruction of lysosomes/endosomes and cytochrome P-459 dependent oxygenase and xanthine oxidase [34, 35].In addition, external factors such as ultraviolet light, radiation, toxins, hypoxia, hyperoxia, high-calorie diets, glucotoxicity, smoking, industrial products, and chemotherapy can all produce ROS in the body [35, 36]. Malondialdehyde (MDA) is the end product of lipid peroxidation and is measured as the index of increased oxidative stress [37]. Increased concentration of thiobarbituricacid-reactive substances (TBARS) also indicates lipid oxidation [38].

The body has different enzymatic and non-enzymatic antioxidative mechanisms to remove the effects of free radicals. Antioxidative systems counteract with the production of free radicals and neutralize their toxic effects and thereby protect the body organs against oxidative stress-related diseases [39]. Vitamins (A, C, E), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx), and glutathione reductase (GR) are commonly used antioxidants in the body[40]. Other antioxidants include alpha lipoic acid, coenzyme Q10, mineral antioxidants (zinc, magnesium, copper, and selenium), and cofactors such as folic acid and vitamins group B (B1, B2, B6, B12). All these antioxidants have a synergistic effects on each other to counterbalance free radicals [41]. Vitamin E suppresses the initiation of lipid peroxidation [42] and in combination with vitamin C inhibits the formation of hydroperoxides[43]. Studies in diabetic animals have shown that the activity of SOD, CAT and GPx decrease during diabetes as the concentration of TBARS (or MDA) increases [44].

Glutathione is a powerful antioxidant and protects the key components of the cell against reaction with oxygen-containing molecules such as hydroxyl radicals and peroxides [45]. Glutathione peroxidase is the generic name of a family of enzymes with peroxidase activity whose main biological role is to protect the organism against oxidative damages. The biochemical function of glutathione peroxidase is to reduce lipid peroxides to their corresponding alcohols and to reduce free hydrogen peroxide to the water. The lipid compositions of cells especially lipid membranes are sensitive to free radicals and in response to this reaction, they produce lipid peroxides[46]. Using glutathione, GPx enzyme reduces peroxides to alcohol and thus prevents the formation of free radicals. In fact, glutathione peroxidase catalyzes the reduction of hydrogen peroxide (H_2O_2) and a wide variety of organic

peroxides to the corresponding alcohol and water, using cellular glutathione [47]. SOD is a metalloprotein enzyme exists in almost all cells by which dismutation of superoxide anions (O2) is catalyzed to hydrogen peroxide (H_2O_2) and oxygen (O_2). Dismutation is a certain type of redox reaction in which a species is oxidized and reduced simultaneously and creates two different products. In general, the human brain, liver, heart, erythrocytes and kidney express a high concentration of SOD enzymes [48]. Catalase enzymes (CAT) have a tetrameric structure that exists in most cells, and mainly in the peroxisomes. During its catalytic activity, hydrogen peroxide (H_2O_2), which is one of the final products of oxidation in the metabolism of carbohydrates, is hydrolyzed into water and oxygen [49].

3. DIABETES AND ROLE OF OXIDATIVE STRESS

Diabetes mellitus is occurred due to the insufficiency of blood insulin levels and insulin functions, leading to the continuous hyperglycemia [50]. Along with hyperglycemia, other variables such as hyperlipidemia as well as increased oxidative stress play the important roles in the pathogenesis of diabetes mellitus [51]. Patients with diabetes mellitus type II are more at risk of cardiovascular mortality due to the highly association of this disease with accelerated atherosclerosis, obesity, lipid disorders and hypertension [52]. During diabetes, continuous hyperglycemia leads to the formation of free radicals, and these free radicals in turn cause auto-oxidation of glucose, non-enzymatic glycation of proteins, destruction of glutathione metabolism, changes in antioxidant enzymes activity, formation of lipid peroxides, reduction of ascorbic acid levels and oxidative destruction of glycated proteins. The high levels of free radicals simultaneous with defects in the endogenous antioxidant defense such as superoxide dismutase, glutathione and catalase activities, cause oxidative damageto the cellular vital organelles and macromolecules, and increased lipid peroxidation, leading to the development of insulin resistance in the periphery [53]. Increased production of mitochondrial superoxide subsequent to the hyperglycemia leads to the activation of the following five pathways of cellular damage, by inhibiting GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) enzyme activity [54-57]:

- Increased glucose entry to the polyol pathway
- Increased intracellular AGE formation
- Increased expression of the receptor for AGE and activation of its ligands
- Activation of PKC isoforms
- Activation of hexosamines pathway

In addition, in obese diabetic patients endoplasmic reticulum stress subsequentto a high fat diet results in the production of additional unused glucose in the liver (which finally leads to insulin resistance). Insulin resistance in fat tissues causes the liberation of FFA (free fatty acid) from stored triglycerides. Then, FFA oxidation in cells following the lack of insulin stimulates the production of malonyl coenzyme-A, and this is followed by the increased generation of superoxide by the mitochondrial electron transport chain [58-61].

4. ROLE OF ANTIOXIDANTS IN DIABETES

Given the role of oxidants and oxidative stress in the pathogenesis of diabetes, antioxidant therapy would be an effective method for the treatment of diabetes and prevention of its complications [62]. Increased endogenous peroxidation activity and reduced antioxidant mechanisms during diabetes emphasize the usefulness of antioxidative agents in decreasing the disease outcomes. Many pharmacological agents that are effective in reducing diabetes-induced mortality have antioxidativeinfluences[63].

One of the powerful therapeutic classes of antioxidant for diabetes is transketolase activators, such as benfotiamine. When superoxide radicals increases, GAPDH activity is inhibited and, in diabetes, high concentrations of glycolytic intermediates reduce the activity of this enzyme. Among these glycolytic intermediates are fructose-6-phosphate and glyceraldehyde 3-

phosphate, which are also the final products of the transketolase reaction. Enzyme transketolase is a rate-limiting enzyme in the non-oxidative part of pentose phosphate pathway. Although the normal function of this enzyme is to shift the pentose phosphate pathway toward the production of glycolytic intermediates, it can also act in a reverse manner. In diabetes the concentrations of these two glycolytic intermediates are high and the enzyme transketolase can reduce their concentrations and thereby suppress the damage pathways caused by hyperglycemia [64].

The second class of therapeutic agents is poly (ADP-ribose) polymerase-1 (PARP-1) inhibitors that prevent AGE formation, PKC and NF-κB activation, and the hexosamine pathway activation [65]. The third therapeutic class is the enzymatic antioxidants, including CAT/SOD mimetics and functional mimics of GPx[66]. SOD-like medicines include metalloporphyrins, cyclic polyamines and nitroxides. Metalloporphyrins have protective effects on the development of T-cells involved in autoimmune diabetes [67, 68]. GPx-like mimetics such as ebselen or PZ51 possess antioxidant properties, and the oxidative stress developed by diabetes can be fully suppressed using these agents [69].

Ascorbic acid (vitamin C), glutathione (GSH), alpha-tocopherol (vitamin E), carotenoids, and flavonoids can be mentioned as non-enzymatic antioxidant agents [70]. Some flavonoid-like compounds such as caffeic acid phenethyl ester (CAPE) can improve the oxidative stress in heart tissue. Non-vitamin antioxidants include N-acetylcysteine (NAC), alpha lipoic acid (ALA), and coenzyme Q10. Coenzyme Q10 is a fat-soluble antioxidant that destroys the superoxide radicals and improves vascular endothelial function in diabetic conditions. Herbal medicines with antioxidant activity have the protective effects on diabetic rat'sorgans and these effects have been associated with removing oxygen free radicals and reducing expression levels of intracellular adhesive molecule-1 (ICAM-1)[71].

In a general overview, in addition to the above-mentioned findings, the medical treatment in diabetes mellitus approved by FDA include as the following [72]:

- Sulfonylureas: stimulate the release of insulin from pancreatic beta cells; such as clopramide, glipromide, glipizide, glyburide, tolazamide and tolbutamide
- Biguanides: reduce insulin resistance; such as metformin
- Thiazolidinedione: reduce insulin resistance; such as pioglitazone and rosiglitazone
- Meglitinides: are similar to sulfonylureas with shorter effects; such as repaglinide and nateglinide
- Alpha-glucosidase inhibitors: delay the absorption of glucose in the digestive tract; such as acarbose and miglitol
- Dipeptidyl peptidase-4 inhibitors: inhibit the DPP-4 enzyme; such as sitagliptin, saxagliptin, alogliptin, linagliptin, and vildagliptin
- Combined oral antidiabetic drugs: improve the effectiveness of certain drugs when used in combination; such as metformin combined with sitagliptin
- Incretin mimetics: act like incretin hormones in the body, increase insulin production and decrease its destruction rate; such as exenatide and DPP-4 inhibitors
- Amylin analogues: act like hormone amylin and increases insulin production in the body and decrease its destruction rate and reduce appetite; such as pramlintide [72-74].

These agents improve diabetes and reduce its complications through various pathways and mechanisms. For example, pioglitazone and metformin can activate the AMPK (5' adenosine monophosphate-activated protein kinase) and its pathway. The AMPK pathway decreases the ROS level through FOXO3 activation and up-regulates the thioredoxin expression [75, 76]. In addition, PPARγ (peroxisome proliferator-activated receptors)agonists such as thiazolidinediones or glitazones and PPARα activators such as fibrates antagonize the effects of angiotensin-2 in

theproduction of intracellular ROS. PPARγ alters the metabolism of lipids and PPARα reduces the fatty acids in plasma, leading to the reduction of TG (triglycerides) and LDL (low-density lipoprotein) levels [77].

Other therapeutic agents such as 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors (statins) have beneficial effect on diabetic-induced oxidative stress [78]. Also, ACE (angiotensin-converting-enzyme) inhibitors inhibit prooxidants' effects on angiotensin-2 and are effective in preventing the outcomes of type II diabetes [79]. Some studies have shown beneficial effects of combination therapy of zinc with metformin in reducing fasting blood glucose and glycosylated hemoglobin (HbA1c) levels in patients with diabetes, but some other studies have reached opposite conclusion about the effects of zinc in the treatment of type II diabetes [80]. One of the new classes of antidiabetic drugs with powerful impact is dipeptidyl peptidase-4 inhibitors, such as sitagliptin and vildagliptin, which their influences on the oxidative complications of diabetes are discussed below.

5. DPP-4 INHIBITORS AND MECHANISMS

Based on the classification of new drugs, inhibitors of the enzyme dipeptidyl peptidase-4 (CD26 or DPP-IV, DPP-4), also known as gliptins, are antidiabetic drugs currently prescribed widely for the treatment of type II diabetes. TheDPP-4 enzyme is a membrane-bound serine peptidase composed of 766 amino acid, and expressed in all body tissues, including vascular endothelial cells and immune cells, and also has a soluble form in the plasma. The enzyme is responsible for the degradation of proline subunit in incretin hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulin secretion peptide (GIP). GLP-1 and other similar incretin peptides play an important role in glucose homeostasis [81, 82]. By increasing the plasma levels of GLP-1, administration of DPP-4 inhibitor drugs for patients with type II diabetes increase their body's ability to better control of blood glucose level, leading to lower fasting blood glucose and HbA1C values. It has been reported that long-term treatment with DPP-4 inhibitor has led to the preservation of the number of pancreatic beta cells during type II diabetes through reducing apoptosis and increasing proliferation in preclinical research [83-85].

5.1 TYPES OF INHIBITORS

DPP-4 enzyme inhibitors include sitagliptin, (Januvia; MK-0431), alogliptin, linagliptin, saxagliptin (BMS-477118), teneligliptin, gemigliptin, anagliptin, omarigliptin, trelagliptin, and vildagliptin (Galvus;LAF-237) [86]. They are a very new class of oral antidiabetic medicine that inhibit DPP-4 enzyme to increase the level of active incretins especially GLP-1 and thereby increase the pancreatic beta cells' response to glucose and therefore enhance the insulin secretion and its sensitivity and also overwhelm improper glucagon secretion. They also improve the metabolism of lipoprotein and fat after meals and decrease fasting and postprandial blood glucose levels. They control blood glucose levels without causing chronic hypoglycemia. Recent studies have tested their protective effects on some tissues including hearts of rats, and the results showed that these drugs can significantly prevent complications of myocardial ischemic injury [87].

5.2 DPP-4 INHIBITORS IN THE CONTROL OF OXIDATIVE STRESS IN DIABETIC STATES

Incretin-related agents inhibit oxidative stress and have anti-apoptotic effects, besides their conventional hypoglycemic actions [88]. DPP-4 enzymes are highly expressed on T-cells, monocytes, and endothelial cells. The DPP-4 enzyme causes different signaling in T-cells, notably the TCR/CD3zeta *(*T-cell receptor *(*TCR*)*-CD3 zeta *complex*) chain signal, IL-2 (interleukin-2) production and T-cell proliferation [89]. Inflammatory reactions are critical forthe development of the oxidative stress and previous studies have reported the anti-inflammatory effects of the DPP inhibitors in patients with type

II diabetes. DPP-4 enzyme inhibitors may lead to the vascular endothelial protective effects by reducing blood glucose after meals (postprandial hyperglycemia), which is a pathogenic factor for the development of atherosclerosis [90]. In a study, sitagliptin has increased CD34, a marker of endothelial progenitor cells (EPCs) [91], In addition, linagliptin has inhibited the production of ROS, lipid peroxidation and TBARS reactions by AGEs in endothelial cells and thereby improved the endothelial dysfunction caused by uncontrolled diabetes [92].

These inhibitors reduce VCAM-1 (Vascular cell adhesion protein 1) and TNF-α (tumor necrosis factor alpha) activities, both can induce oxidative stress in the vascular system. Furthermore, a study showed the antiplatelet aggregation effect of sitagliptin, suggesting that these inhibitors also have antithrombotic potentials [93]. It has been also reported that vildagliptin, compared to sitagliptin, although had similar effects on fasting and postprandial blood glucose and HbA1c levels, it reduced MAGE activity and the level of proinflammatory cytokines, more potently. It also reduces significantly the level of nitrotyrosine, which is the product of the reaction between free radical $^{\cdot}O2$ and nitric oxide (NO) [94].

Studies have shown that DPP-4 inhibitors activate the CREB (cAMP response element-binding protein) transcription factor in the cAMP/PKA signaling pathway via enhancing GLP-1 activity. They also activate indirectly the epidermal growth factor receptor through the PI3K/Akt signaling, thereby delaying the aging process induced by free radicals in body cells, and particularly in vascular endothelial cells. In addition, GLP-1 has anti-apoptotic effects on pancreatic beta cells and cardioprotective effects against ischemia-reperfusion injury, both of which are accomplished through the cAMP/PKA and PI3K/Akt signaling pathway. DPP-4 inhibitors also play an important role in reducing the oxidative damages in DNA. GLP-1-related peptides activate the defense pathway against oxidative stress through two related genes, HO-1 and NOS-1. A study has shown that the expression of the HO-1 gene in endothelial cells which is regulated by CREB is significantly dependent on GLP-1 activity [95].

In their study, Yen-Ta Chen *et al*.concluded that treatment with sitagliptin enhances the expression of the antioxidant markers SOD and GPx and catalase in renal tissue [96]. El-Sahar et al. showed that sitagliptin has a neuroprotective effect following a cerebral stroke in the diabetic setting; they attributed this cerebral protection to the antioxidative effect of sitagliptin, as it could reduce the formation of lipid peroxidation and MDA while increased the glutathione (GSH) content in the hippocampus [97]. Similarly, Raia and colleagues observed that treatment with vildagliptin decreases TBARS levels in the brain [98]. In another study, it has been shown that treatment with sitagliptin in type II diabetes resulted in alower level of MDA in the serum, pancreas and heart [99]. Furthermore, it has been reported that sitagliptin can improve memory and increase neurogenesis in the hippocampus of rats with high-fat diet due to increased SOD expression and decreased oxidative stress [100]. In another study, sitagliptin has reduced cardiomyocytes apoptosis by increasing the levels of SOD and GSH-GPx [101]. The study of Danielle and colleagues showed that vildagliptin increases the activity of antioxidants catalase and SOD in the pancreas in type I diabetes [102]. More importantly, treatment of rats with vildagliptin during insulin resistance induced by high-fat diet reduced lipid peroxidation and MDA levels in the hearts [103]. Additionally, sitagliptin led to increased levels of SOD and decreased levels of MDA and GSH in lung tissue following allergic conditions [104]. Treatment with vildagliptin reduces the gene expression levels of liver phosphomevalonate kinase (MvK), medium-chain acyl-CoA dehydrogenase (Acadm) mevalonate (diphospho) decarboxylase (Mvd) and acyl-CoA synthetase-1 (Acsl1) in rats. These factors play important roles in cholesterol synthesis and fatty acid oxidation [82]. Finally,

Shinji *et al*. evidenced that linagliptin reduced MDA levels in the arterial injuries induced by diabetes [105].

6. CONCLUSION

Although controlling blood glucose levels is the best prevention strategy for the complications of diabetes, DPP-4 inhibitors could be considered as the very important therapeutic agents in controlling the development of diabetes and its complications, due to their significant antioxidative and anti-inflammatory potentials. Therefore, it is necessary to investigate other important therapeutic aspects of these drugs as well as the newer inhibitors of this class in the setting of diabetes in further studies.

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