INHIBITION OF DPP-IV: A NEW THERAPEUTIC APPROACH FOR THE TREATMENT OF TYPE 2 DIABETES

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Abstract: Drugs that inhibit dipeptidyl peptidase-4 (DPP-IV), a ubiquitous enzyme that rapidly inactivates both GLP-1 and GIP, increase active levels of these hormones and, in doing so, improve islet function and glycemic control in T2DM. DPP-IV inhibitors are a new class of anti-diabetic drugs that provide comparable efficacy to current treatments. Although they differ in terms of their chemistry, they are all small molecules which are orally available. They are effective as monotherapy in patients inadequately controlled with diet and exercise and as add-on therapy in combination with metformin, thiazolidinediones, and insulin. The DPP-IV inhibitors improve glycaemic control, reducing both fasting and postprandial glucose levels to lower HbA1c levels, without weight gain and with an apparently benign adverse event profile. Thus, the DPP-IV inhibitors are a promising new treatment option, especially for patients with early stage T2DM and more severe hyperglycemia.

INTRODUCTION

Diabetes mellitus is a metabolic disorder, which is considered as a major public health issue all over the world. Recent WHO calculations indicate that worldwide almost 3 million deaths per year are attributable to diabetes. By the year 2025, it is projected that about 333 million people will suffer from the disease, with type-2 diabetes mellitus (T2DM) representing approximately 90-95% of the diagnosed cases[1].

Type 2 diabetes mellitus (T2DM) is a chronic disease characterized by several pathophysiologic defects including insulin resistance, excess hepatic glucose production and progressive pancreatic β-cell dysfunction[2]. It can cause a number of complications such asperipheral vascular insufficiencies, neuropathy, retinopathy and end stage renal disease. Besides lifestyle intervention, treatment of T2DM consists of some oral anti-hyperglycaemic drugs and insulin. The existing agents are found to be associated with an increased risk of adverse events. Therefore, more effective therapies are needed to improve glycaemic control. Among the numerous possible targets, the development of **Dipeptidyl Peptidase-IV Inhibitors** appears to be one of the most attractive, rational agents for the treatment of T2DM.

DIPEPTIDYL PEPTIDASE-IV ENZYME:

DPP IV was first isolated from rat liver in 1966. It is a 766 amino acid membrane associated serine protease bound to the membrane by a transmembrane sequence of 22 amino acids[3]. It has a molecular weight of

110–150 kD per subunit and is a homodimeric class II protein. In humans, the enzyme activity is found in almost all organs and tissues[4]. Surface profile of the DPP IV enzyme is shown below (Figure 1)

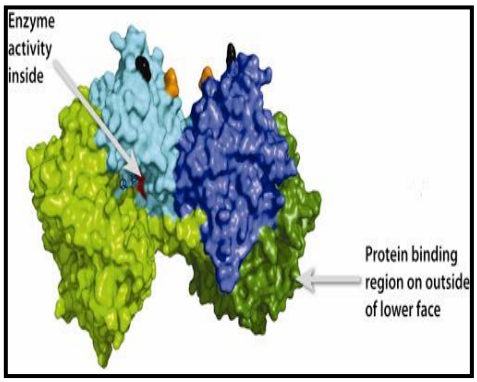


Fig.1 Surface profile of the DPP IV

The primary sequence of the protein has been assigned with different structural regions. Catalytically active residues of the enzyme are localized in the region of the α/β-hydrolase domain, the protein–protein interactions occur mainly within regions of the β-propeller domain[4]. (Figure 2)

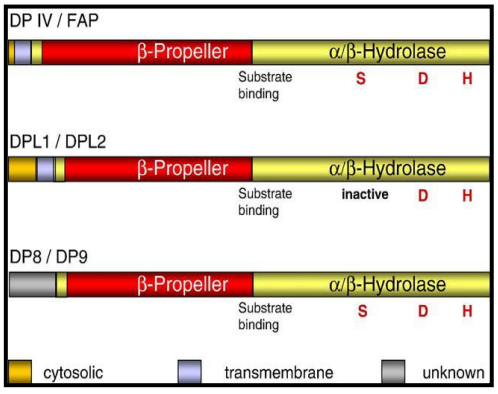


Fig.2 The human DPP- IV gene

SUBSTRATES FOR DIPEPTIDYL PEPTIDASE-IV ENZYME

DPP IV has multiple substrates. The incretin hormones like GLP-1 and GIP and a number of other bioactive peptides are potential substrates for DPP-IV. Neuropeptide Y, peptide YY, gastrin-releasing polypeptide, pituitary adenylate-cyclase-activating polypeptide, insulin-like growth factor-1, substance P, and various chemokines may be metabolized by DPP-IV. Looking at the substrates, it can be implied that DPP-IV is involved not only in glucose homeostasis but also in the regulation of other homeostatic mechanisms, such as blood pressure, neurogenic inflammation, and the immune system. However, whether these and other bioactive peptides are substrates for DPP-IV under physiological conditions is not yet established[5].

CHEMISTRY

The DPP-IV inhibitors comprise a diverse group of compound broadly divided into peptidomimetic (mimicking the dipeptide structure of DPP-IV substrates) and non-peptidomimetic. Peptidomimetic drugs include nitrile containing inhibitors like sitagliptin and vildagliptin whereas alogliptin (modified pyrimidinedione) and linagliptin (xanthine-based) are examples of the non-peptidomimetic inhibitors (Table 1).

These compounds are competitive reversible inhibitors displaying high affinity for DPP-IV enzyme. However, they interact differently with the DPP-IV enzyme. Drugs like sitagliptin, alogliptin and linagliptin form non-covalent interactions with residues in the catalytic site. In contrast, DPP-IV enzyme inhibition by saxagliptin and vildagliptin involves formation of a reversible covalent enzyme–inhibitor complex in which there is a slow rate of inhibitor binding and a slow rate of inhibitor dissociation. This results in the enzyme slowly equilibrating between the active and inactive forms. The catalytic activity is thus inhibited even after the free drug has been cleared from the circulation[6].

Inhibitor	Chemistry	Metabolism	Elimination Route
Sitagliptin	β-amino-acid-based	Not properly metabolised	Renal (approx. 80% unchanged as parent)
Vildagliptin	Cyanopyrrolidine	Hydrolysed to inactive metabolite	Renal(22% as parent, 55% as primary metabolite)
Saxagliptin	Cyanopyrrolidine	Hepatically metabolised to active metabolite	Renal(12-29% as parent,21-52% as metabolite)
Alogliptin	Modified pyrimidinedione	Not appreciably metabolised	Renal(> 70 % unchanged as parent)
Linagliptin	Xanthine-based	Not appreciably metabolised	Biliary(> 70 % unchanged as parent); <6% by kidney

Table 1: Chemistry, Metabolism and elimination of DPP-IV inhibitors[6]

INCRETIN HORMONES

Incretins are a group of gastro-intestinal hormones. They play an important role in reducing blood glucose levels before these levels elevate following meal ingestion. The two most important incretin hormones are **Glucose-Dependent Insulinotropic Polypeptide (GIP)** and **Glucagon-Like Peptide-1 (GLP-1).** The term 'incretin effect' is used to describe stimulated insulin secretion after oral glucose ingestion compared to iv glucose administration or isoglycaemic parenteral administration[7].

GIP is a 42-amino-acid peptide produced mainly by the K cells located predominantly in the duodenum. GLP-1, on the other hand, is produced by the L cells located predominantly in the lower part of the small intestine.

These hormones are released into the circulation minutes after ingestion of a meal. Carbohydrates and fat seem to be powerful stimulators of GIP and GLP-1[7,8].

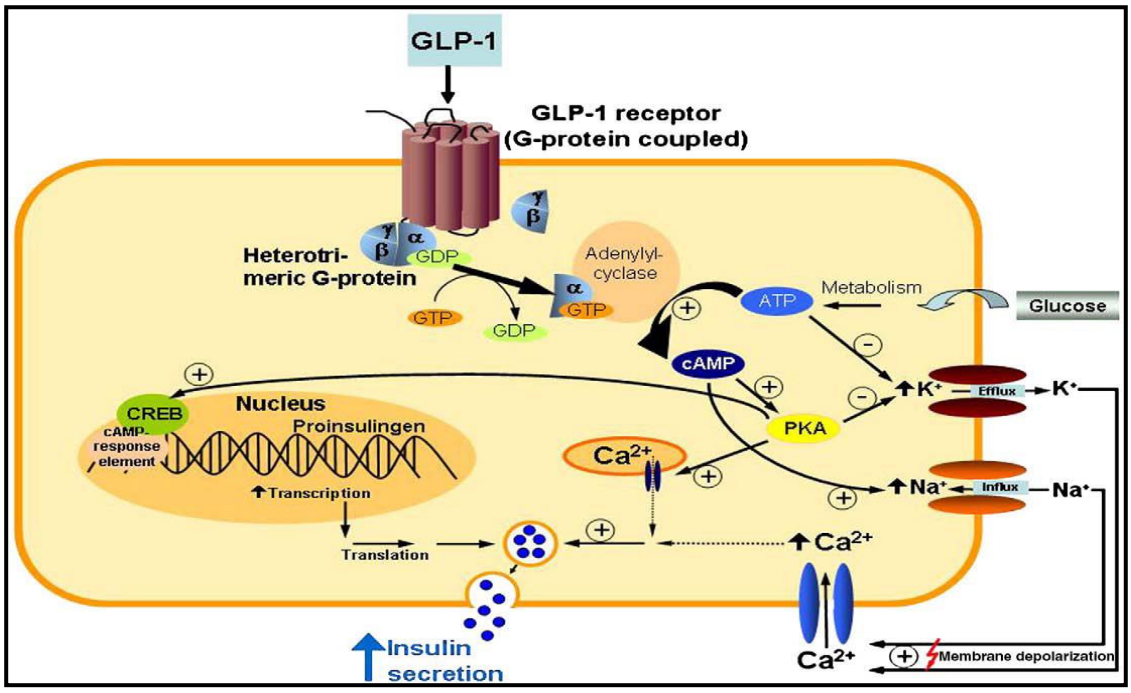


Fig 3.Mechanism of Insulin Secretion by the Incretin Hormones[9]

MECHANISM OF INSULIN SECRETION BY THE INCRETIN HORMONES TO IMPROVE GLYCAEMIC CONTROL IN T2DM

Incretin hormones, released from the gut, play a significant role in glucose homeostasis in healthy subjects. It has been estimated that the incretins are responsible for 50-70% of postprandial insulin release.

Both GIP and GLP-1 exert a marked effect on β-cells through their binding with specific G-protein coupled Receptor (GPCR) family. Binding to the specific receptors on β- cells results in increase cAMP and activation of protein kinase A (PKA). After binding to their specific receptors expressed on the islet β-cells, GIP and GLP-1 hormones transmit signals to the Gs protein which rapidly dissociates into α-s and β-γ subunits. Subsequently the β-γ subunits can stimulate adenylate cyclase (AC) resulting in conversion of ATP into cAMP. cAMP then activates the protein kinases A(PKA) to increase endoplasmic reticulum (ER) calcium release. The incretins synergize with glucose to stimulate insulin secretion by inhibiting the activity of K-ATP channels which are consistently thought to be cAMP/PKA-dependent. However, the mechanism of action of PKA on the K-ATP channels is not completely understood. The closure of K-ATP channels mediated by the incretins results in membrane depolarization and increases the Ca^{+2} influx through voltage-dependent Ca^{+2} channels(VDCCs). Finally, the whole process triggers exocytotic release of insulin-containing granules[7] (Figure 3).

Besides increasing insulin secretion, other physiological actions of GLP-1 and GIP are gradually being recognized. In α-cells, GLP-1 inhibits glucagon secretion, probably indirectly via somatostatin secretion and via its effect on insulin release. In the β cells,

GLP-1 and GIP have been shown to promote cell proliferation and survival. Some other anti-diabetic actions of the incretin hormones are as follows:

1) Inhibition of food intake and weight gain,

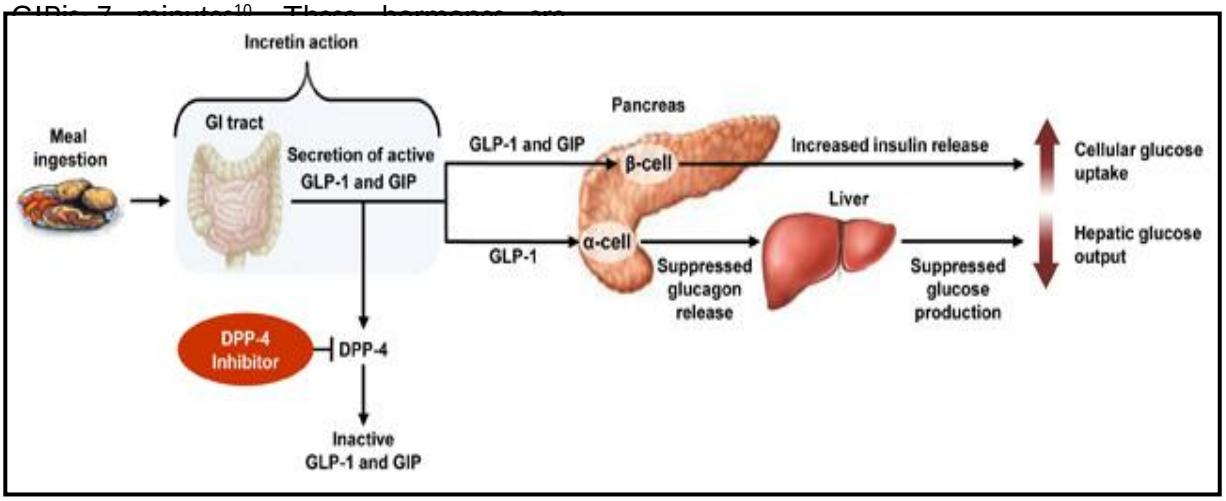
2) Retardation of gastric emptying which can attenuate the meal-associated increases in blood glucose,

3) Promotion of insulin-stimulated incorporation of fatty acids into triglycerides.

MECHANISM AND RATIONALE FOR DEVELOPMENT OF DPP- IV INHIBITORS

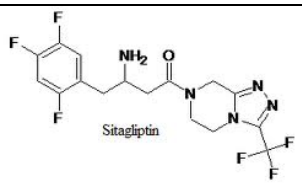
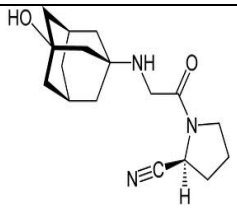
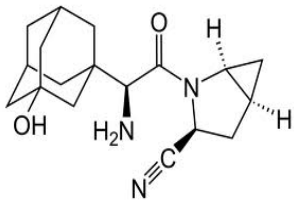
Post–meal ingestion, GLP-1 and GIP are released from the small intestine. The half-life of active GLP-1 is <2 minutes and that of GIP is 7 minutes[10]. These hormones are rapidly inactivated by DPP-IV. The truncation of these peptides by cutting the N-terminal dipeptide end results in biologically inactive peptides that are incapable of stimulating insulin secretion[10,11].

DPP-IV inhibitors act by blocking the DPP-IV enzyme (Figure 4). As a result, levels of GLP-1 which are low in people with type-2 diabetes become elevated. GLP-1 can thus act to stimulate glucose dependent insulin release from the pancreas[12]. DPP-IV inhibitors thus helped to lower blood glucose and reduce HBA1c when given orally to people with type-2 diabetes. The inhibition DPP-IV enzyme by DPP-IV inhibitors in order to prevent the degradation of the incretin hormones has become a promising therapeutic strategy[13].



DPP-IV INHIBITORS

DPP-IV inhibitors lowered HBA1c compared with placebo by 0.74% with similar efficacy as monotherapy or as add on therapy. Sitagliptin and Vildagliptin have not been compared directly but both appear to lower HBA1c by a similar amount as compared to placebo[16]. (Table 2)

DRUG (BRAND)	STRUCTURE	COMPANY	STATUS	Available strengths
Sitagliptin (Januvia)		Merck & Co	FDA Approved & Launched In 2006	25mg, 50mg, 100 mg tablets. Also available in combination with metformin (50/500 mg, 50/1000 mg) 100 mg once daily.
Vildagliptin (Galvus)		Novartis	EU Approved & Launched In 2008	50 mg tablets available. Also available as fixed dose combinations with metformin at doses of 50/500, 50/850 and 50/1000 mg)
Saxagliptin (Onglyza)		Bristol-Myers Squibb And Astra Zeneca	FDA approved in 2009, Recently approved in Europe for treatment of T2DM with moderate to severe renal insufficiency or mild hepatic insufficiency (2011)	2.5mg and 5 mg tablets.

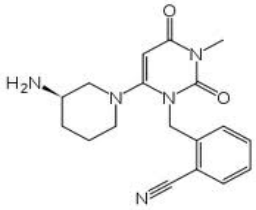
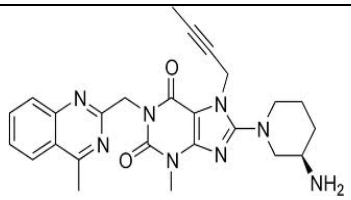
Alogliptin (SYR-322)		Tadeka Pharmaceutical Company	FDA application for the product is currently under review	25mg, 12.5 mg, 6.25mg. Also available in combination with pioglitazone and metformin.
Linagliptin (Trajenta)		Boehringer-Ingelheim &Eli Lilly Co	FDA approved in 2011 & launched	5mg tablets. Also available in combination with metforin/ sulfonylurea.

Table 2: DPP-IV Inhibitors [14]

PHARMACODYNAMIC AND PHARMACOKINETIC PROPERTIES OF THE DIFFERENT DIPEPTIDYL PEPTIDASE-IV INHIBITORS

Several DPP-IV inhibitors are on the market or in trials. They are all orally available and well absorbed (i.e. significant DPP-IV inhibition is observed as soon as 15 min after administration), and have high affinity for DPP-IV. They all potently inhibit plasma DPP-IV to similar levels, but with different IC50 ranging from 1 nM for linagliptin, 19 and 24 nM for sitagliptin and alogliptin, to 50 and 62 nM for saxagliptin and vildagliptin, respectively. Their half-life is different, leading to different dosing amount and frequency of dosing[7].

TOLERABILTY, SAFETY AND EFFICACY OF DIPEPTIDYL PEPTIDASE- IV INHIBITORS

DPP-IV inhibitors, have good tolerability and are associated with very few side effects. Both vildagliptin and sitagliptin are safe and well-tolerated as monotherapy or as combination therapy.Side effects associated with vildagliptin were found to be mild and more importantly, hypoglycemia was not reported. No drug-related serious adverse events were reported after 12 weeks of vildagliptin monotherapy or in combination with metformin. The overall incidence of adverse events was similar to that reported for the placebo-treated subjects.Preliminary reports emerging from clinical trials with sitagliptin of up to 12 weeks duration also showed good tolerability, with adverse events being described as transient and mild in intensity. Experience with other inhibitors in phase II and III trials also appears favourable. DPP-IV inhibitors also have no significant adverse

gastrointestinal side effects, such as abdominal pain, nausea and diarrhoea.No electrocardiogram abnormalities were observed during treatment with DPP-IV inhibitors. While non-selective DPP-IV inhibition and selective DPP-8/9 inhibition was found to be associated with severe, even lethal toxicities in pre-clinical species, selective inhibition of DPP-IV does not appear to cause problems [9,18].

POTENTIAL FOR DRUG–DRUG INTERACTIONS of DPP-IV INHIBITORS

Available data suggests that there is no great propensity for the DPP-IV inhibitors to be involved in any clinically relevant drug–drug interactions with other commonly prescribed medications including metformin, pioglitazone, rosiglitazone, glyburide and simvastatin. These agents can be co-administered with the DPP-IV inhibitors without the need for dose adjustment of either drug[6].

ADVERSE EFFECTS OF DPP-IV INHIBITORS

DPP-IV inhibitors are generally well-tolerated, and no increase in adverse events were noted compared to placebo or other comparatives, but again slight differences may exist between the different molecules of this class.

DPP-IV is present on the cell membrane of T-lymphocytes known as CD26. Here, it acts by activating intracellular signalling pathways to simulate T-cell proliferation. In pre-clinical models, DPP-IV deficiency resulted in modest abnormalities in immune response, decreased CD4+ T-cell number, and reduced production of interleukin (IL)-4 while IL-10 was increased. The peptidase activity of DPP-IV has not been associated to immune function. However, Saxagliptin administration was found to lead to a modest reduction in lymphocyte count within the normal range. DPP-IV inhibition may also affect human progenitor cell and haematopoietic stem cell migration. To date, no adverse events related to immunological effects have been reported in humans but additional long-term trials are needed before to conclude on their safety profile specifically with regards to immunological issues. Also, no risk of adverse gastrointestinal events such as nausea, diarrhoea or abdominal pain was reported.[6,7].

ADVANTAGES OF DPP-IV INHIBITORS OVER ORAL ANTI-DIABETIC DRUGS (OAD) (Table 3)

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CRITERIA	ORAL ANTI-DIABETIC DRUGS	DIPEPTIDYL PEPTIDASE-IV INHIBITORS
Weight	Thiazolidinediones and sulfonylureas produce weight gain.	DPP-IV inhibitors are weight neutral

Gastrointestinal Adverse Events	Metformin has gastrointestinal side effects.	DPP-IV inhibitors are well tolerated gastrointestinally.
Hypoglycemia	Sulfonylureas, in particular, are associated with an almost 3-fold elevated risk of hypoglycemia compared with metformin.	DPP-IV inhibitors are associated with a very low risk of hypoglycemia.

Table 3: Differentiating factors between oral anti-diabetic drugs and the DPP-IV inhibitor group[19]

FUTURE RESEARCH

DPP IV is a cell membrane protein which is present ubiquitously in many tissues. Therefore, long term effects of this enzyme on the immune system is of a major concern[17]. It is important therefore that studies of longer duration be carried out to assess the effects of long term DPP-IV inhibition on the immune system. Careful post marketing surveillance is also required. It is also recommended to set up long term high-end studies to look into the coronary heart disease risk posed by this new class of drugs. Also, natural plant resources are being screened and tested in-vitro for DPP – IV inhibitory activity. These herbal extracts/phytoconstituents when combined or used as alternatives to conventional drugs are expected to exert beneficial effects. Some of these extracts/phytoconstituents have shown promising DPP-IV inhibitory activity and can be used as lead molecules for further development [20, 21].

A NEW GENERATION OF DPP-IV INHIBITORS

Plans for development of second generation of DPP-IV inhibitors have already been taken up by research groups at pharmaceutical companies. They are looking to develop potent and highly selective DPP-IV inhibitors which will sustain the incretin levels for a longer period of time by prolonging DPP –IV enzyme inhibition at relatively lower doses taken once a day[22,23].

Short-term inhibitors of DPP-IV at meal times may benefit specific patient groups and have a potential role in the prevention of T2DM are also being investigated.

Research on DPP-IV like enzymes is coming of age, and it is hard to predict what direction it will take. DPP inhibitors with narrow or broad specificity may find applications beyond diabetes in the treatment of fibrotic skin disease and immune disorders, in leukapheresis and stem cell transplantation and in the prevention of ischemia or reperfusion damage.

CONCLUSION

DPP-IV inhibition is a novel and promising therapy for type-2 diabetes. It is orally active, it is safe and well tolerated, and it results in a sustained robust and clinically significant improvement in glycaemia both in monotherapy and in combination therapy.

DPP-IV inhibition works through preventing the inactivation of the incretin hormone GLP-1 through stimulation of insulin secretion and reduction in glucagon secretion,with a potential also of increasing the β-cell mass. Besides improvement in glucose metabolism, DPP-IV inhibition also

improves prandial lipid metabolism. The pathophysiologically relevant mechanisms of action of DPP-IV inhibition, its efficacy and tolerability, and its oral availability suggest that this novel approach will be of great value in the arsenal for treatment. Its place in therapy remains to be explored in more detail.

Because DPP-IV inhibition is safe, efficient and orally active, it may be established as a first-line treatment as monotherapy in subjects in whom metformin is contraindicated, or in subjects with adverse events from metformin. Potentially, however, DPP-IV inhibition will be of greatest value as a first-line treatment in combination with metformin. DPP-IV inhibition may also have advantages over existing treatment in long-term therapy of more advanced stages of the disease, provided that the beneficial effect on islet mass is also evident in humans.

Finally, DPP-IV inhibition will be a strong candidate as a first-line treatment for impaired glucose tolerance, when this condition will be pharmacologically treated. However, the establishment of DPP-IV inhibition as a first-line treatment requires more studies evaluating its long-term durability and safety and its action in subsets of patients.

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