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Protein tyrosine phosphate-1B (PTP-1B): a novel and challenging therapeutic target for type-2 diabetes and obesity

Deep Sharma*, Ranbir Singh
Himachal Institute of Pharmaceutical Education and Research, Nadaun, Himachal Pradesh, India

Abstract
Diabetes and Obesity are the major health problems in the modern world. Although a no. of drugs are available to control both of these problems but due to limitations of their side effects, there is need of search for the novel targets for the treatment of these problems. Protein tyrosine phosphatase -1B (PTP-1B) is a novel target of such type. It is implicated in both insulin and leptin signaling and is found to be negative regulator of both. Therefore it is an emerging therapeutic target to treat both diabetes and obesity. But due to some important issues like less selectivity and low bioavailability, the inhibitor development is a challenging job and therefore a safe, selective and efficacious PTP-1B inhibitor has yet to be identified. So many researches are currently in progress related to this aspect and attempts are made to avoid the problems associated with the safety and efficacy of PTP-1B inhibitors. Along with diabetes and obesity, PTP-1B is also implicated in some non-metabolic diseases like cancer. Therefore PTP-1B is an interesting novel therapeutic target that has attracted the interest of many researches toward itself.

Keywords: protein tyrosine phosphatase -1B (PTP-1B), Diabetes, Selectivity, TC-PTP

1. Introduction
Protein phosphorylation and dephosphorylation are two distinct but interrelated processes that regulate various signaling events in the body and play a major role in activation and deactivation of proteins thus maintain the homeostasis between different physiological processes such as growth, proliferation, differentiation, survival or apoptosis, as well as adhesion and motility. Protein phosphorylation is catalyzed by protein tyrosine kinases (PTKs) while protein dephosphorylation is catalyzed by protein tyrosine phosphatases (PTPs) that antagonize the PTK - mediated signalling. This article is concerned about protein tyrosine phosphatases-1B (PTP-1B), a member of Tyrosine Phosphatases family and its role in the regulation of Diabetes mellitus type-II and obesity [1]. There are at least 108 genes in human genome that code for evolutionary distinct families of Protein Tyrosine Phosphatases (PTPs) - the class I, class II, and class III Cys-based PTPs, and the Asp-based tyrosine phosphatases. All Cys-based PTPs are having a common catalytic mechanism based on a nucleophilic cysteine that forms a thiophosphate intermediate during catalysis [2]. Protein tyrosine phosphatase-1B (PTP-1B) is a major protein among the Cys-based PTPs and in humans, it is encoded by the *PTPN1* gene. PTP-1B is a negative regulator of the insulin and leptin signaling pathway. Resistance to these two hormones i.e. insulin and leptin are the hallmarks for type-2 diabetes mellitus and obesity respectively. Therefore, inhibition of PTP-1B activity or down-regulation of its expression should ameliorate insulin and leptin resistance. And hence it is considered as a promising potential therapeutic target particularly for treatment of type-II diabetes and obesity. Along with this, it is also implicated in development of and breast cancer and hence has emerged as a potential therapeutic target in this avenue as well [3]. The importance of the PTP-1B in diverse pathophysiology has attracted the intense interest of researchers toward itself as a new class of drug targets.

2. Diabetes mellitus
According to World Health Organization (WHO), Diabetes mellitus is a chronic metabolic disease that is caused by inherited and/or acquired deficiency in production of insulin by the beta cells of pancreas, or by the ineffectiveness of the insulin produced i.e. cells become insensitive to the insulin. This deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves.
Globally, as of 2010, an estimated 285 million people had diabetes, with type 2 making up about 90% of the cases. In 2013, according to International Diabetes Federation, an estimated 381 million people had diabetes. Greatest numbers of diabetic people are between 40-59 years of age. Its incidence is increasing rapidly, and by 2030, this number is estimated to almost double [4].
It is divided into two main types: Type-1 diabetes and type-2 diabetes.
Type-1 diabetes: Type 1 diabetes (previously known as insulin-dependent, juvenile or childhood-onset) is characterized by deficient insulin production and requires daily administration of insulin. The cause of type 1 diabetes is not known and it is not preventable with current knowledge.
Symptoms include excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger, weight loss, vision changes and fatigue. These symptoms may occur suddenly [5].

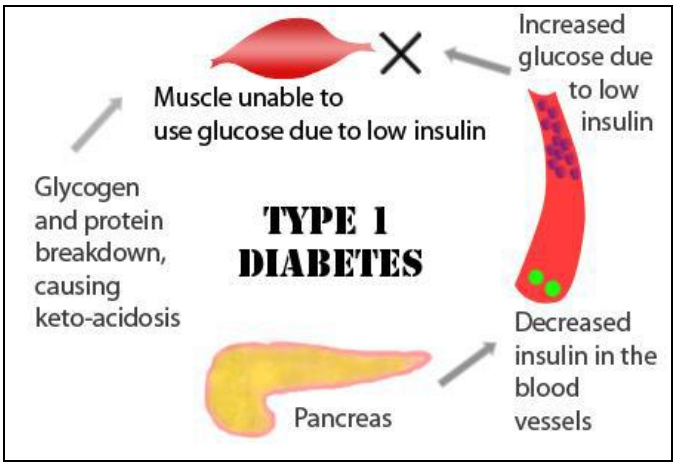

Fig 1: Pathogenesis of type-1 diabetes mellitus [5]

Type-2 diabetes

Type 2 diabetes (formerly called non-insulin-dependent or adult-onset) results from the body's ineffective use of insulin. Type 2 diabetes comprises 90% of people with diabetes around the world, and is largely the result of excess body weight and physical inactivity. Symptoms may be similar to those of Type 1 diabetes, but are often less marked. As a result, the disease may be diagnosed several years after onset, once complications have already arisen [6]. REVIEW

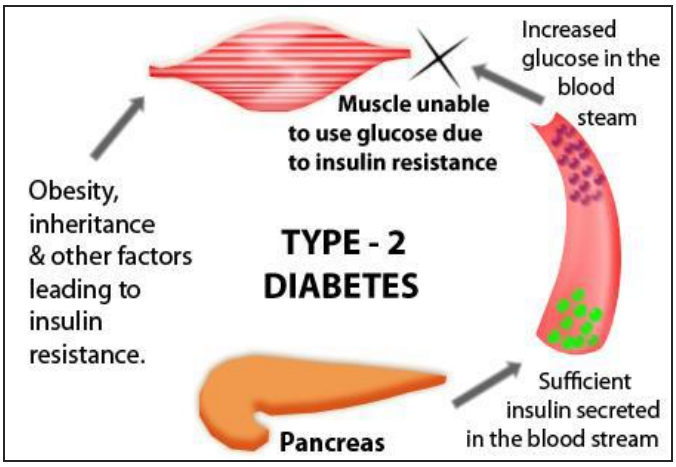

Fig 2: pathogenesis of type-2 diabetes mellitus [6]

Link between obesity and type 2 diabetes 'Diabesity [9]

The International Diabetes Foundation (IDF) says that, "Diabetes and obesity are the biggest public health challenge of the 21st century. The relationship between obesity and diabetes is of such interdependence that the term 'diabesity' has been coined [7]. Of the people diagnosed with type II diabetes, about 80 to 90 percent are also diagnosed as obese. This fact provides an interesting clue to the link between diabetes and obesity. Regulation of body mass is an essential parameter for the survival of a living being. Disorders of body mass control like obesity contribute to the pathogenesis of many important human diseases, including type II diabetes [8].

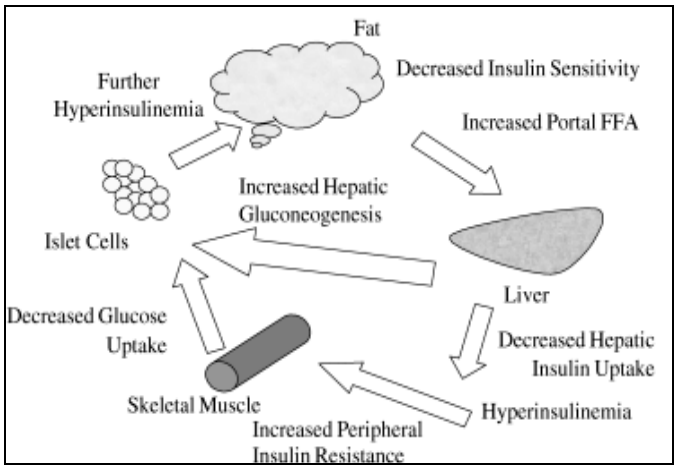

Fig 3: Link between obesity and type 2 diabetes

Diabetes mellitus-type 2 and Obesity are interrelated to each other. Obesity is the major side effect of a no. of antidiabetic drugs and this also explains the relation between these two. The relationship between obesity and type-2 diabetes mellitus has a polygenetic component and is associated with insulin resistance [9]. With the passage of time, abdominal fat makes fat cells to release 'pro-inflammatory' chemicals, which can disturb the function of insulin-producing pancreatic islet to respond to insulin. This is known as insulin resistance – which is a major trigger for type 2 diabetes [10]. So if a drug candidate act on a common pathway or common target of both of these problems then it will be very beneficial because both of these problems generate each other along with many other health problems. And one of these kinds of target is protein tyrosine phosphatase-1B (PTP-1B) enzyme inhibition of which solves both of these problems. The mechanistic pathway is discussed later in this article.

3. Protein Tyrosine Phosphatase-1B (PTP-1B) – A novel and promising therapeutic target for Diabesity (Diabetes type 2 and Obesity)

Protein phosphatases are classified by their substrate specificities into serine/threonine-phosphatase (STP), protein histidine-phosphatase (PHP), protein tyrosine-phosphatase (PTP) and dual-specific phosphatases (DSPs) which catalyze the dephosphorylation of both serine/threonine or tyrosine residues [11]. Among various members of the PTP superfamily, PTP1B has emerged as the best-validated drug target for the treatment of diabetes and obesity [12]. The enzyme PTP-1B which is also known as Tyrosine protein phosphatase non-receptor type 1 is the prototypic member of the PTP family. It is encoded by *PTPN1* gene in humans and located at the cytoplasmic phase of the endoplasmic reticulum. This enzyme was named from a pool of PTP activity resolved by ion-exchange chromatography and was purified from human placenta as a 37 kDa catalytic domain [13]. PTP1B is found to be involved in intracellular dephosphorylation of the insulin receptor (IR) and insulin receptor proteins (IRS) and hence cause the down regulation of insulin signaling. Similarly PTP-1B also dephosphorylates the Leptin receptor (ObR) and cause

its deactivation thus down regulate the following JAK-STAT pathway for the food uptake and energy homeostasis.

Studies revealed that the PTP-1B knockout mice showed enhanced insulin sensitivity, lower plasma glucose and insulin levels, and resistance to weight gain compared to the control mice when fed with high fat diets. The PTP1B deficient mice also have normal development and was found free from the typical adverse immune and neuronal development effects that are observed with most other phosphatase Knockout mice**.** [3] For example TC-PTP (T-cell protein tyrosine phosphatase) which is found to be most homologous phosphatase to PTP1B (74% homology in the catalytic domain and identical active sites). The TC-PTP Knockout mice die at 3 to 5 weeks agter birth because of impaired B cell and T cell functions. On the basis of these studies, PTP1B is currently considered one of the novel & best biological target for type -2 diabetes and obesity i.e. diabesity.

PTP-1B structure and mechanism:

By a no. of structural studies, coupled with enzymatic and kinetic analysis from several laboratories, important insights have been provided into the mechanism of substrate recognition and catalysis [13]. The native protein consists of 435 amino-acid residues, of which amino acids 30–278 comprise the catalytic domain. The carboxy-terminal amino-acids (amino acids 299–435) residues are rich in proline, and are involved in targeting the enzyme to the cytoplasmic face of the endoplasmic reticulum.[14] The main structural features are the catalytic loop containing the catalytic residue Cys215, the WPD (tryptophan, proline, aspartic acid) loop and the secondary aryl-phosphate-binding site [15].

Active site

The active site of PTP1B contains a common structural modification of PTPs. The base of the catalytic site is defined by the 214–221 PTP signature motif (histidine(His)-Cys-Ser-alanine (Ala)-glycine (Gly)-isoleucine (Ile)-Gly-arginine (Arg) in PTP1B), a loop of eight amino-acid residues that forms a rigid, cradle-like structure that coordinates to the aryl-phosphate moiety of the substrate This loop also contains the active-site nucleophile Cys215 [16].

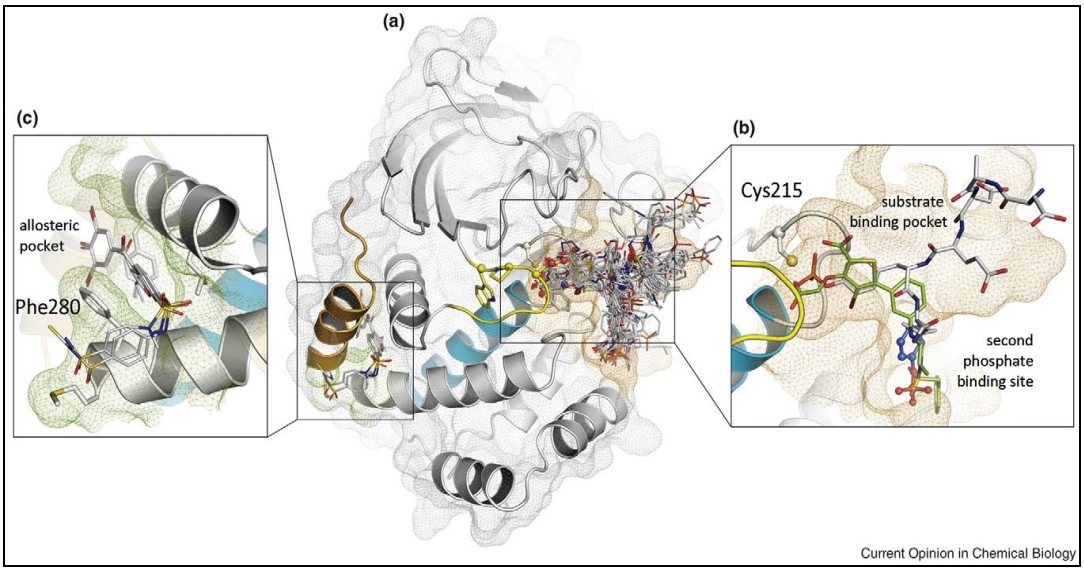

Fig 4: Structure of PTP-1B

Catalytic site

The first crystal structure of catalytic domain of PTP1B has revealed that the catalytic site exists within a deep cleft of the protein that is formed by three loops including –

- The WPD loop with the Asp181 residue.
- A pTyr loop with the Tyr46 residue.
- And a Q loop with the Gln262 residue.[17]

The pTyr loop and Tyr46 residue are located on the surface of the enzyme protein, and they help to determine the depth that a substrate can obtain within the cleft. This acts as a means of driving selectivity, as substrates containing smaller phospho residues cannot reach the site of catalytic activity at the base of the cleft. [19] Upon binding to the substrate, PTP1B undergoes a modification in structure in which the WPD loop (amino acids 79–187) closes around the substrate and thus introducing stabilizing pi stacking interactions between the aromatic rings of the phosphotyrosine (pTyr) substrate residue and the Phe182 residue on the WPD loop. As there are two conformations of PTP1B — open and closed —inhibitors could be designed to target either conformation [18].

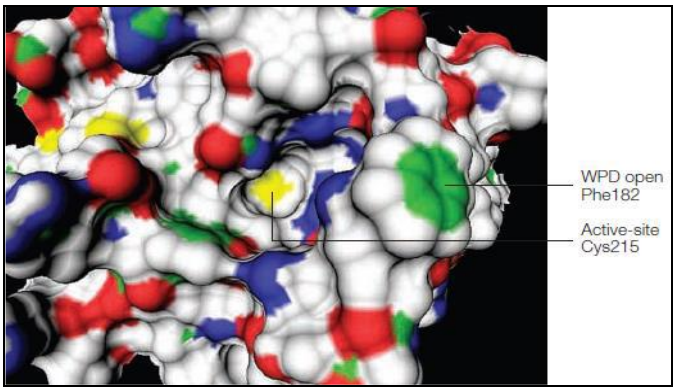

Fig 5: WPD open form

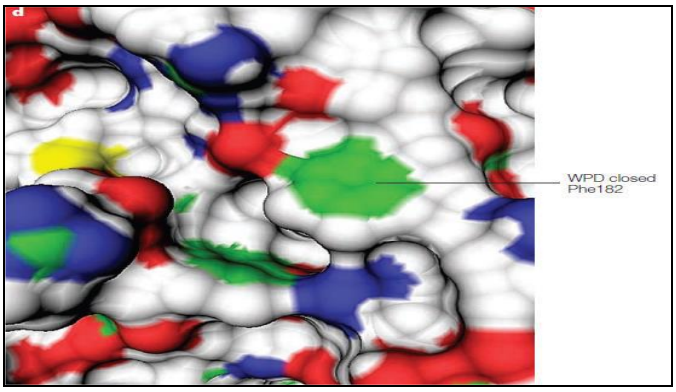

Fig 6: WPD close form.

Mechanism of PTP-1B enzyme activity

The Cys215 residue is very essential for the activity of PTP1B enzyme and similarly the cysteine residues are required for the activities of other members of the Class I PTP family [19]. The phosphatase activity of enzyme PTP-1B occurs via a two-step mechanism. The first step involves the dephosphorylation of the pTyr substrate and the enzyme intermediates are broken down during the second step [13].

- During the first step, there is nucleophilic attack by the sulphur atom of the thiolate side chain of the cysteine residue on the substrate phosphate, which is followed by the protonation of the tyrosyl-leaving group of the substrate by the side chain of a conserved acidic residue (e.g. Asp181 in PTP1B) that acts as a general acid. And this leads to formation of a cysteinyl - phosphate catalytic intermediate.
- In the second step, which is mediated by Gln262, which coordinates a water molecule, and Asp181, which acts as a general base, hydrolysis of the catalytic intermediate takes place so as to release the phosphate [13].

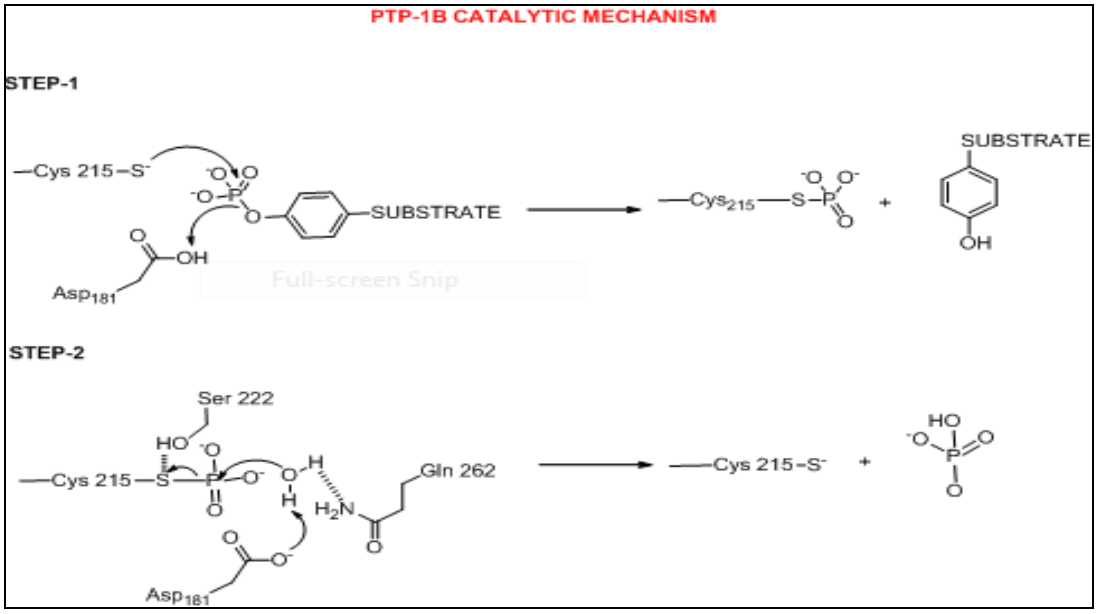

Fig 7: Mechanism of Enzymatic Activity of PTP-1B [15]

Role of PTP-1B in insulin and leptin resistance

PTP-1B is the enzyme that negatively regulates both the insulin as well as leptin signaling which are the hallmarks of the diabetes type 2 and obesity respectively. And therefore researchers found it as a promising target in Diabetes type 2 and obesity as well. Here we will see how PTP-1B act on its substrates and what is the cascade of events that take place following the action of PTP-1B.

Insulin signaling pathway of glucose transport

Insulin signaling is mediated by a complex and highly integrated network that begins with binding of insulin to its cell-surface receptor (IR). The insulin receptor (IR) belongs to a subclass of a large family of protein tyrosine kinases (PTKs). It is a trans membrane protein. It comprises two extracellular α-subunits and two trans membrane β-subunits. When binds to insulin, IR undergoes auto phosphorylation on several tyrosine residues located in the cytoplasmic portion of the β-subunits. Auto phosphorylation enhances IR kinase activity and then triggers several downstream signaling events including tyrosyl phosphorylation of IR substrate (IRS) proteins (IRS 1-4) and other adaptor molecules (e.g. Grb2 and Shc) that are linked to the activation of two main signaling pathways:

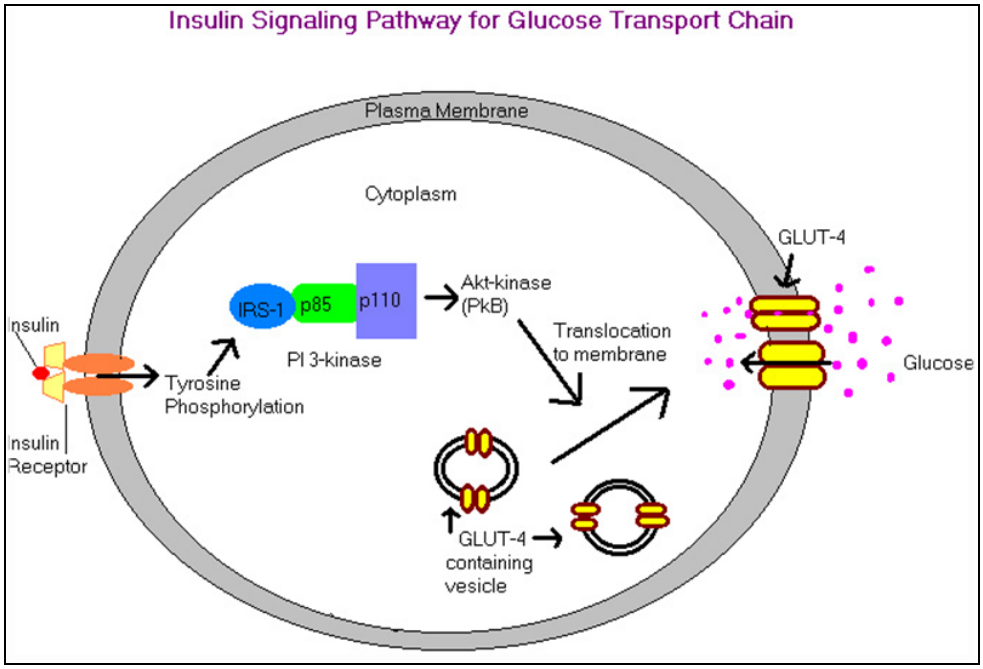

Fig 8: Insulin signaling pathway of glucose transport [22]

- The phosphatidylinositol 3-kinase (PI3K)–AKT/protein kinase B (PKB) pathway, which is responsible for most of the metabolic actions of insulin (e.g. translocation of the glucose transporter GLUT4, stimulation of glycogen synthesis).
- And the Ras–mitogen-activated protein kinase (MAPK) pathway, which regulates the expression of some genes and cooperates with the PI3K pathway to control cell growth and differentiation.

PTPs play a key role in the regulation of insulin action by dephosphorylation of the activated, auto phosphorylated IR and downstream substrate proteins such as IRS-1/2.

PTP-1B as a negative regulator of insulin signaling

Insulin resistance is one of the major cause of the type 2 diabetes. Insulin signaling is switched off by dephosphorylation of tyrosine residues on the activation loop of the IR and thus abolish its kinase activity. There are several protein tyrosine phosphatases which are proposed to play this role namely, PTPα, LAR, CD45, PTPε, SHP2, T-cell protein tyrosine phosphatase (TC-PTP) and PTP1B. These all have been observed to control insulin signaling *in vitro* usually when they are overexpressed in the cell lines but *in vivo* studies on knockout mice have shown that there are no obvious effects on insulin signaling for any of them except PTP1B [23]. Therefore PTP-1B has been taken to consideration as a novel therapeutic target against diabetes type 2 and obesity among the several types of PTPs.

By the use of the substrate-trapping method, Seely *et al.*, firstly demonstrated a direct association between the IR and PTP1B [24]. *In vitro* studies revealed that the microinjection of PTP1B protein into Xenopus oocytes blocked insulin-stimulated S6 kinase phosphorylation and inhibited the insulin-induced oocyte maturation.IR and IRS-1 are the substrates of PTP-1B. It has been also found that Osmotic loading of PTP-1B antibodies into hepatoma cells of rat increased insulin-stimulated IR auto phosphorylation, IRS-1 Tyr phosphorylation, PI3K activity, and DNA synthesis [23]. Moreover in the cells that over-expressing the enzyme PTP-1B, GLUT4 translocation to the cell membrane and glucose uptake were also reduced.

Interestingly from the *in vivo* studies it has been found that the PTP-1B deficient mice were remained insulin-sensitive and also resistant to the weight gain when subjected to a high-fat diet, while the amount of food consumed was not different. And along with this, levels of circulating triglyceride were also markedly reduced [24].

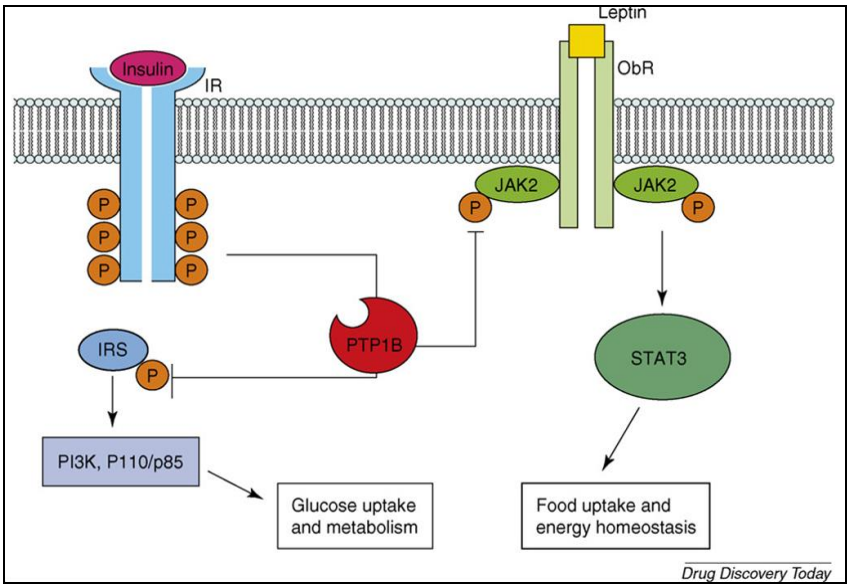

Fig 9: PTP-1B as a negative regulator of insulin and leptin signaling [1]

PTP-1B as a negative regulator of leptin signaling

As it is already discussed that the down regulation of insulin signaling due to PTP-1B is responsible for insulin resistance and therefore PTP-1B $^{-/-}$ or PTP-1B deficient shows increase in insulin sensitivity. But this does not completely explain the reduced weight gain on a high-fat diet. By numerous studies on that aspect, it has been observed that along with the Insulin signaling, PTP-1B, also down regulate the leptin signaling.

Leptin is an adipocyte-derived hormone which acts on the hypothalamus to decrease food intake and increase energy expenditure [19]. In obesity and type-2 diabetes, the circulating levels of leptin are usually elevated and resulting to the reduced sensitivity to leptin, i.e. leptin resistance [25].

Leptin signaling is mediated via leptin (Ob) receptor by the stimulation of the JAK–STAT signal transduction cascade. JAKs (Janus kinases) are the cytosolic tyrosine kinases which phosphorylate the STAT (signal transducer and activator of transcription) proteins. Several in-vitro studies have demonstrated that enzyme PTP-1B is a negative regulator of the leptin signaling pathway.

In a mouse hypothalamic cell line, leptin stimulation caused phosphorylation of STAT3 and when of PTP1B is Over-expressed in these cells, it resulted in a dose-dependent decrease in JAK2 and STAT3 Tyr phosphorylation and also a decrease in the expression of various genes (e.g. SOCS3) that are induced by leptin was found. Like *In vitro* outcomes, also a no. of *in-vivo* studies have implicated PTP1B as a negative regulator of leptin signaling. PTP-1B $^{-/-}$ mice show resistant to weight gain as compared to normal mice when fed with high-fat diet keeping the amount of food same.

Development of PTP-1B inhibitor

As discussed above, PTP-1B is a novel target for the treatment of type 2 diabetes and obesity, therefore it is much essential to develop potent inhibitors of the same. In past, several researches have been done to develop PTP-1B inhibitors and also this research is continuously in process till the date. But unfortunately it has proved very difficult to identify a safe, selective and effective PTP-1B inhibitor. The two main challenges in the development of potential therapeutics to target PTP-1B are selectivity over other similar PTPs for example TCPTP, and less bioavailability (due to low cell permeability) [20]. Various strategies have been employed for the design of PTP-1B inhibitors. They are namely:

- The library approach
- The 'linked-fragment' approach
- The conformation-assisted approach [12]

The library approach

The library approach was used to identify highly potent and selective PTP-1B inhibitors that are capable of bridging and simultaneously associating with both the active site and an adjacent peripheral site [13].

The library contains:

- A biasing pTyr to ensure association with the active site.
- A structurally diverse set of 23 linkers that tether the pTyr moiety.
- A structurally diverse set of eight aryl acids, which were designed to associate with the peripheral sub site, positioned near the active site [28].

Compound 1 is found to be the most potent and selective PTP-1B inhibitor identified to date (Ki = 2.4 nM) – it exhibits, with one exception, a 1000- to 10 000-fold selectivity against a panel of other PTPs [29]. The sole exception is TC-PTP, which is 74% identical to PTP-1B. Tenfold selectivity in favour of PTP-1B was observed against TC-PTP. [28] Mutagenesis and structural analysis of the interactions between PTP-1B and compound 2 (a derivative of compound 1) phosphonodifluoromethy. phenylalanine occupies the active site, whereas the distal 4-phosphonodifluoromethyl phenylacetyl group makes both van der Waals and ionic contacts with Secondary aryl-phosphate-binding site [29]. The results show that, This, in turn, indicates that the library approach can be a general and effective method to acquire potent and selective PTP-1B inhibitors.

The 'linked-fragment' approach

A 'linked fragment' approach was employed to develop potent and selective PTP-1B inhibitors that can engage both the active site and the second aryl phosphate-binding site [30]. This second aryl phosphate-binding site lies within a region (Arg24 and Arg254) that is not conserved among the PTPs. In this approach, NMR was used to identify small molecules that bind to the active site. The identified hits were optimized based on crystal structures of the complexes. Using a separate NMR screen, small molecules that occupy the second aryl phosphate-binding site were also identified. Compounds 3 (Ki = 22 nM; two fold selectivity compared with TC-PTP) [34] and 4 (Ki = 18 nM; fourfold selectivity compared with TC-PTP) were both

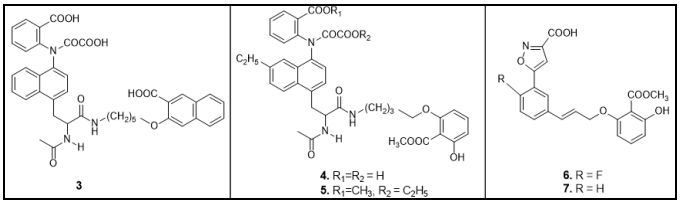

Fig 10: Design of inhibitor based on linked fragment approach

Obtained using this approach. Compound 4 is not cell permeable owing to the presence of two negative charges. Prodrug 5 was synthesized, and the carboxylic acids were functionalized by their esters, to demonstrate cellular activities [36]. Compound 6, with a less charged pTyr mimetic and a more rigid linker, displayed a 30-fold selectivity compared to TC-PTP [37]. In addition to its excellent selectivity, compound 6 also exhibited moderate cell permeability, probably because of the decreased charge. The fluorine at the ortho-position can also enhance cell permeability because compound 7, with a proton at this position, showed significantly lower cell permeability. These examples demonstrate that the linked fragment approach can be an effective strategy to obtain potent and selective PTP- 1B inhibitors.

The conformation-assisted approach

Unique conformations of PTP-1B are targeted by using the structure-based modelling for inhibitor development with both high affinity and selectivity.

A series of benzotriazole, phenyldifluoromethyl phosphonic acids were synthesized as non peptidic PTP-1B inhibitors. Many of these compounds showed good inhibitory activity, at the sub-mM level, for PTP-1B but none of them had selectivity compared with TC-PTP.

One of these inhibitors, compound 8, was cocrystallized with PTP-1B. The central carbon of 8 has four functional substituents. In the crystal structure, one of the phenyldifluoromethyl phosphonic acids binds the PTP1B active site; the benzotriazole group interacts with the Arg47 and adjacent residues; the benzene ring is important for the overall rigidity of whole molecule, which was found to be crucial for the inhibitory activity; and the other phenyldifluoromethyl phosphonic acid points to the second aryl phosphate site. Based on this structural information, the second phenyldifluoromethyl phosphonic acid group was modified. A longer and more rigid bi-phenyl linker was chosen, so that the phophonic acid could reach the second aryl phosphate binding site to increase binding affinity. To gain selectivity, the crystal structures of PTP-1B and TC-PTP were compared, to identify residues that are unique to PTP-1B.

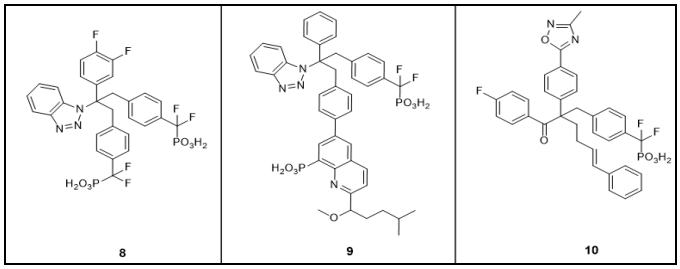

Fig 11: Design of inhibitor based on conformation assisted approach

One of the residues is Phe52 in PTP-1B, whereas the corresponding position in TCPTP is Tyr54.[42] Methoxylisobutylmethyl quinoline was then appended to the bi-phenyl linker (compound 9) to interact with Phe52. Interestingly, compound 9 has a sevenfold selectivity compared with TC-PTP. X-ray crystal structure of 9 with PTP-1B confirmed the interactions between the methoxylisobutylmethyl group and Phe52. [43] A similar approach was used to target Leu119 in PTP- 1B (Val121 in TC-PTP) to generate compound 10. The oxadiazole group in 10 was responsible for a tenfold selectivity compared with TCPTP.

Challenges in development of PTP-1B Inhibitor - Considerations for drug design:

The development of PTP1B inhibitors has received increased attention of a no. of pharmaceutical industries. But the task is not so easy because of some hurdles in the path of development of the inhibitor for PTP-1B. So the drug should be designed by keeping certain considerations in mind. The main three challenges in front of researchers and considerations for drug design are as follows:

- Selectivity
- Bioavailability
- Substrate specificity

Selectivity

Selectivity is one of the major issues in the development of PTP-1B inhibitors as drugs [1]. Generally, PTPs have less than 40% homology to each other. An exception for PTP1B is the enzyme TC-PTP (T-Cell Protein Tyrosine Phosphatase), which has 74% homology that co-inhibition of TC-PTP (along with PTP-1B), leads to serious adverse effects like hematopoietic defects and immune defects including over inflammatory reactions etc. Mice lacking the TC-PTP gene die within 3–5 weeks after birth from defects in hematopoiesis and immune function [22]. The active sites of PTP-1B and TC-PTP are nearly superimposable on each other, although the second aryl phosphate binding sites are different, but differ in those areas that are not considered as the major binding determinants. Researchers have achieved high selectivity over PTPs other than TC-PTP, but only a few compounds showed moderate selectivity over TC-PTP. This has been done either by targeting the second binding area or by inhibiting an allosteric site and preventing the formation of the active form

of enzyme by blocking the mobility of the catalytic loop [22]. Hence, it is highly desirable, and probably critical, to design PTP1B inhibitors that show significant selectivity compared to TC-PTP.

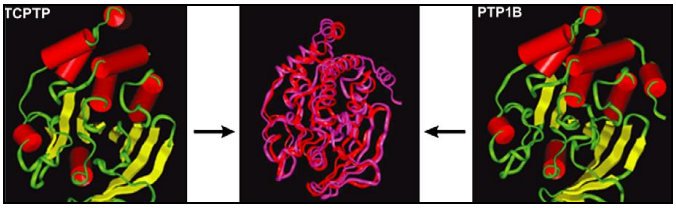

Fig 12: Overlapping x-ray crystal structures of TCPTP and PTP1B. The overlapping of protein tyrosine phosphatase 1B (PTP1B, purple) on T-cell protein tyrosine phosphatase (TCPTP, red) shows the high level of similarity, which poses a problem for developing selective inhibitors [22].

4. Bioavailability

Bioavailability is another important issue or challenge in the development of PTP1B inhibitors. The PTP1B catalytic site contains the common structural motif of PTPs and is highly charged. Consequently many of the inhibitors described are highly charged, thus limiting bioavailability due to a low capacity to cross the plasma membrane [22]. Several strategies have been applied to improve the cell permeability and/or bioavailability of PTP1B inhibitors that will be highlighted later in this review [1].

Substrate specificity

Salmeen *et al.*, defined the molecular basis of specificity in the interaction between PTP1B and the activation loop of the IR [46]. Of particular importance was the presence of tandem pTyr residues. Thus, PTP1B displays approximately a 70-fold higher affinity for bisphosphate-containing peptides relative to monophosphate derivatives. This finding has stimulated the search for novel PTP-1B inhibitors [22].

5. Strategies for improving selectivity and bioavailability of PTP-1B Inhibitors.

There are several approaches that are currently in practice to avoid or to overcome the difficulties of development of effective and safe inhibitor of PTP-1B. Some of them are discussed in brief as follow:

- Targeting allosteric sites for improved selectivity
- Targeting allosteric sites for improved bioavailability
- Charge reduction
- Increasing hydrophobicity
- Prodrug delivery

Targeting allosteric sites for improved selectivity

A secondary allosteric site has recently been described for PTP-1B, and several small-molecule inhibitors that occupy this site stabilize an inactive conformation of PTP-1B. Unlike the pTyr binding active site, the allosteric site is not well conserved and possesses substantially less polar. Thus, targeting the allosteric site might present an alternative strategy for developing selective inhibitors with acceptable pharmacological properties. Compounds 19, 20 and 21 are examples of allosteric inhibitors reported for PTP-1B. Compound 19 (IC50 = 350 mM) was identified through a screen of a non-pTyr-like compound library. Elaboration at the sulfonyl end of 11, with additional aromatic rings, afforded compounds 12 (IC50 = 22 mM) and 13 (IC50 = 8 mM) with improved potency and selectivity. Interestingly, compound 20 showed a six fold selectivity compared with TC-PTP, and compound 21 exhibited cellular activities (increasing the phosphorylation level of IR and IRS-1) at 250 mM.

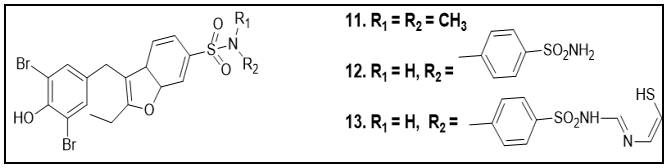

Fig 13: Allosteric site PTP-1B inhibitor

Targeting allosteric sites for improved bioavailability

Allosteric modulation of PTP1B activity with small molecules might provide a promising approach to overcome the potential challenges of targeting the active site. Allosteric inhibitors, mentioned previously, are more likely to be cell permeable because they normally do not have negative charges. In addition to compounds 11-13, compound 14 might serve as another example of an allosteric inhibitor. Compound 22, with an IC50 of 1.6 mM, is a reversible non-competitive inhibitor of PTP1B, suggesting that it can bind to a pocket other than the active site. As a non-charged inhibitor, compound 22 shows good cellular activity [1].

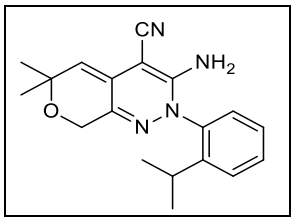

Fig 14

Charge reduction

The most straight forward approach is to reduce the number of negative charges, so that a less-charged derivative might be able to penetrate the cell membrane. One example is compound 15. Its analogue, 16, was first identified as an effective inhibitor of PTP-1B, but the two negative charges limit its cell permeability. Interestingly, when one of the carboxylic acids was replaced by a tetrazole group, the resulting compound, 16, gained cellular activity without loss in inhibitory activity.

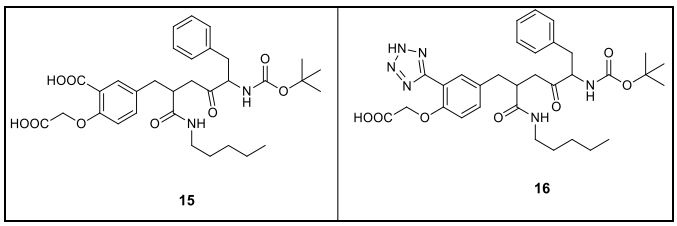

Fig 14: Less charged derivatives of PTP-1B inhibitor

Increasing hydrophobicity

Another approach to increase cell permeability is to enhance the hydrophobic character of the compounds. Because most pTyr mimetics have negative charge(s), they usually are hydrophilic. As more hydrophobic components are introduced, the compounds become more lipophilic, with favourable membrane partition coefficients. For example, although compound 9 contains four negative charges, it is active in a cell-based assay, possibly owing to the presence of multiple aromatic rings.

Prodrug delivery

The prodrug approach has been widely used to deliver compounds containing one or more carboxylic acid group(s). The corresponding methyl or ethyl esters are called prodrugs, and they are much easier to pass through the cell membrane. Once inside the cell, the prodrugs are hydrolyzed to regenerate the original inhibitors. Compound**5** is an example of a prodrug for a carboxylic acid based PTP-1B inhibitor.

6. Some alternative approaches to inhibit PTP-1B:

The development of small molecule competitive inhibitors of PTP-1B as therapeutic agents has been hampered by their unfavorable pharmacokinetic properties. Alternative strategies such as RNA-based therapy and allosteric inhibition of PTP-1B might provide novel mechanisms for therapeutic intervention.

Non-metabolic actions of PTP-1B – Other therapeutic implications

An interesting and potentially clinically relevant recent discovery provides another basis to develop PTP1B inhibitors. Thus, PTP1B has been implicated as an oncogene in the case of breast cancer. [22] Two recent papers revealed an important role for PTP1B as a positive regulator of the Erb2 (HER2/neu) protein tyrosine kinase. This PTK is over-expressed in about 25% of human breast cancers, where it is associated with a poor prognosis. Crossing transgenic mice expressing activated forms of ErbB2 with PTP1B KO mice caused delayed tumor development and decreased the incidence of lung metastases. Hence, PTP1B inhibitors may ultimately offer new treatment for breast cancer.

7. Conclusion

In conclusion, the search for PTP-1B inhibitors has been marked by both progress and pitfalls. Inhibition of PTP1B is predicted to be an excellent novel therapy to target obesity and type-2 diabetes, but identifying a specific, safe, selective and orally bioavailable agent has proved challenging. Currently, an efficient, safe and selective compound is not available. Two remaining obstacles include identifying compounds that are less highly charged and that display greater selectivity over TC-PTP. And therefore various researches are in progress worldwide to overcome the problems associated with it and we can expect that these researches will succeed and PTP-1B inhibitors with high safety and efficacy will be available very soon in future.

Research Agenda for future

It is already discussed in much detail that how the PTB-1B inhibition is essential and useful in diabetes and obesity and is proved to be a validated therapeutic target for the same. In concern to the PTP-1B inhibition, researches are already in progress to ensure safety and efficacy of inhibitors. But along with this, now the agenda for further research should be that the effects of PTP-1B inhibition should be further characterized, on physiological and pathophysiological states apart from insulin resistance, obesity and diabetes, such as cancer.

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