International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 2 (1): 93-97, 2006
ISSN 1811-7775

PTP1b: A Promisimg and Challenging Target for Metabolic Disease

Yan-ling Zhang, James F. Tobin and David V. Erbe
Wyeth Research, Cardiovascular and Metabolic Diseases, Cambridge, MA, USA

Abstract: PTP1b is clearly involved in attenuating signaling by both insulin and leptin, prompting much effort towards the development of inhibitors of this enzyme as therapeutics to treat type 2 diabetes and obesity. In this mini-review, we discussed the physicochemical nature of the PTP1b active site and how this poses challenges for drug design.

Key words: Insulin resistance, protein-tyrosine phosphatases, leptin, signal transduction, PTP1b, insulin, diabetes

INTRODUCTION

Changes in diets and lifestyles throughout the world have led to considerable increases in the prevalence of obesity and type 2 diabetes[1,2]. These developments create an acute need for better pharmacologic treatments, with improved efficacy and reduced side effects. Of the more promising recent developments, the thiazolidinedione class of insulin sensitizers act through PPAR-γ agonism to improve the underlying insulin resistance that develops as part of the obesity-diabetes axis[3-5]. These drugs exert their therapeutic effect in part by decreasing lipotoxicity and as a consequence, improve insulin sensitivity. The main target tissue of PPAR-γ agonists is adipose tissue, where these agents act to induce the expression of genes involved in fatty acid metabolism and adipogenesis[6], which then results in improved insulin sensitivity in muscle and liver. However, these drugs have relatively moderate effects on glucose metabolism[7] and their side effects of weight gain and edema provide a clear opportunity for improved therapies[5,8,9]. This has prompted efforts to identify approaches to increase insulin sensitivity through other mechanisms.

Tyrosine phosphorylation and dephosphorylation of cellular proteins is of critical importance in the regulation of cellular responses to many stimuli[10]. The tyrosine phosphatase PTP1b appears to play a crucial role in regulating signaling by two hormones of profound importance to metabolism: insulin and leptin. Insulin regulates a number of pathways responsible for glucose uptake and lipogenesis and leptin regulates pathways controlling food intake and peripheral energy expenditure. Binding of insulin to its receptor activates the intracellular tyrosine kinase domain, leading to autophosphorylation as well as tyrosine phosphorylation of the Insulin Receptor Substrates (IRS). These then couple to the various downstream signaling cascades that mediate each biological response to insulin[11]. Attenuation of each signal occurs via dephosphorylation of these activated proteins. Leptin signals through its receptor to activate Jak2 which then phosphorylates the Stat3 (signal transducers and activators of transcription 3) protein. Following phosphorylation of Tyr-705, Stat3 forms a homodimer that translocates to the nucleus to mediate transcription of target genes[12]. Again, de-activation occurs through de-phosphorylation of the Jak-Stat signaling molecules.

The importance of PTP1b in attenuating both insulin and leptin signaling pathways was validated by the generation of PTP1b knockout mice. The phenotype of these mice drew the attention of the pharmaceutical industry towards this phosphatase as an attractive target for development of the next generation of insulin sensitizers[12-15]. Importantly, when compared to wild type controls, the knockout mice had lower plasma glucose, insulin and triglyceride levels as well as improved insulin sensitivity in glucose and insulin tolerance tests. This improved insulin sensitivity appears to result from prolonged phosphorylation of the insulin receptor and IRS proteins. In addition, the PTP1b knockout animals showed increased leptin sensitivity (from prolonged Jak2 dependent signaling), resulting in decreased adiposity, seen as a decrease in the size of the white adipose depots and resistance to weight gain on a high fat diet. Serum leptin levels were lower in the knockout animals while their metabolic rate was increased, a likely contributor to their resistance to weight gain.

Perhaps of equal importance, both labs that originally generated the PTP1b deficient mouse strains reported that

Corresponding Author: David V. Erbe, Wyeth Research, 200 Cambridge Park Drive, T4007K , Cambridge, MA 02140, USA
Tel: 617 665 5430 Fax: 617 665 5419

these mice developed and reproduced normally, without any evidence of cellular hyperplasia or tumor formation[13,14]. Evidence that PTP1b plays a role in regulation of signaling through many receptor tyrosine kinases had led to the concern that its inhibition would be tumorigenic. In addition to insulin and leptin, PTP1b has been shown to negatively regulate signaling through PDFG, EGF and IGF-I by de-phosphorylating their activated receptors and growth hormone signaling is enhanced (due to increased Jak2 phosphorylation) in the PTP1b knockout animals[16]. However, in a recent study, cells lacking PTP1b displayed decreased Ras signaling (through increased p120RasGAP expression and p62Dok phosphorylation), that may help explain the lack of tumors in the knockout mice[17].

The benefits of PTP1b inhibition do not appear to be simply dependent on developmental changes in the knockout animals, since adult mice treated with PTP1b anti-sense oligonucleotides show increased insulin-dependent signaling, resulting in blood glucose normalization and improved insulin sensitivity[18,19]. Additionally, cynomologous monkeys treated subcutaneously with PTP1b-specific antisense oligonucleotides also showed changes in insulin sensitivity[20]. With a 50% decrease in PTP1B mRNA levels in the muscle and fat of treated monkeys, fasting insulin levels were lowered, as were insulin levels during a glucose tolerance test, although blood glucose was unchanged. Taken together with the data from the knockout mice, these data indicate that targeting PTP1b could provide compounds that improve insulin sensitivity without the weight gain seen with current PPAR-γ activators, through improved insulin and leptin action leading to normalized glucose levels and reduced adiposity in diabetic patients.

Identification of potent, orally active, small molecule inhibitors of PTP1b has been elusive due to the principal challenge of designing a drug-like inhibitor to a very polar tyrosine phosphatase active site[21,22]. The PTP1b catalytic site is located within a crevice on the molecular surface of the enzyme, with the base of the site formed by residues from His-214 to Arg-221, corresponding to the conserved motif characteristic of protein tyrosine phosphatases. Four of these residues (Gly-218 to Arg-221) occur within a GxGxxG sequence motif. For PTP1b, the active site cysteine is a thiolate anion at physiological pH that is stabilized by a salt bridge to Arg-221 and by one alpha-helix dipole[23]. The surface of this active site pocket is lined with polar residues – in fact, this site contains four highly coordinated water molecules in crystals of the apo protein[24]. Not surprisingly, many of the current potent small molecule inhibitors of PTP1b include a phospho-tyrosine mimetic as a headpiece for binding to this catalytic site. From the co-structure of PTP1b with phosphotyrosine-containing peptides, one sees that its interactions with pTyr are mainly through hydrogen bonds and salt-bridges with main chain nitrogens of Ser-216 to Arg-221 and the guanidinium side chain of Arg221, forming six hydrogen bonds and two salt bridges with the three terminal phosphate oxygens of the substrate. The pTyr phenyl ring is then sandwiched between the aromatic side chains of Tyr-46 and Phe-182 of PTP1b[25]. Similar interactions are seen in co-structures with a commonly used pTyr mimetic, F2Pmp (phosphonodifluoromethylphenylalanine). Here, the difluoromethylene group replaces the phenolic oxygen in pTyr. The two fluorine atoms at the benzylic position are known to be important for high affinity binding, with the added affinity believed to result from extra hydrogen bonding between the fluorine atoms and an enzyme-bound water molecule that is coordinated by the main chain nitrogen of Phe-182 and the amide side chain of Gln-266[26]. Other di-acid head pieces have also been shown to bind the PTP1b active site mainly through hydrogen bonds and salt bridges. For example, the oxalylamino group of Oxalyl-amino-Benzoic Acid (OBA) utilizes several of the same interaction points as pTyr, accepting hydrogen bonds from the main chain amide nitrogens of Gly-220 and Arg-221 in the PTP signature motif and forming a salt bridge with the guanidinium side chain of Arg-221. A salt-bridge is also observed between the ortho-carboxylic acid of OBA and Lys-120[27].

In addition to these studies with x-ray co-structures, thermodynamic studies also suggest that hydrogen bonds and salt-bridges are the dominant interactions in pTyr mimetic binding to PTP1b. Inorganic phosphate itself acts as a small inhibitor to mimic the oxyanion in pTyr. The binding of inorganic phosphate to PTP1b is dominated by a favorable enthalpic term with a ΔH value of -2.1 kcal mol^{-1}, while the entropic contribution to binding is only +0.2 kcal mol^{-1}. This indicates that hydrogen bonding or ionic interactions play a major role in binding inorganic phosphate to the active site of PTP1b. The binding of the phosphate analog, arsenate yields a even larger ΔH term (-7.4 kcal mol^{-1}) with a moderately unfavorable $T\Delta S$ value (-2.4 kcal mol^{-1})[28]. Moreover, the ΔH and $T\Delta S$ terms for the binding of the phospho-tyrosine mimetic vanadate to the Yersinia PTPase (with a similar active site to PTP1b) are -10 and -2 kcal mol^{-1}, respectively indicating that vanadate binding is also an enthalpically favorable but entropically unfavorable reaction[29]. Thus, binding of small pTyr mimetics to PTP1b is driven primarily by enthalpy, an observation that is consistent with the highly polar nature

of these interactions. Importantly, the binding of some active-site based, nanomolar di-acid small molecule inhibitors is also driven by enthalpy, even in the face of an unfavorable entropic contribution (our unpublished data). These small molecule binding results contrast with those for peptide binding to PTP1b. The binding of the hexapeptide DADEPmpL to the enzyme is driven by both enthalpy and entropy, although binding of a tripeptide is more enthalpy driven and less driven by the entropic contribution[30]. Together with the x-ray co-structure data, these results describe a catalytic active site on PTP1b that is optimized for binding to a highly charged substrate. In total, nearly 50 enzyme complexes of PTP1B with substrates or inhibitors have been reported. With Arg-221 at the bottom of the active site, Gln-262 and Lys-120 at each side and the nearby Asp-181, compounds that bind well here have also been highly charged.

One of the current strategies for reducing dependence on binding to the active site has been to use the channel to the second aryl-phosphate binding site of PTP1b to gain additional specificity versus other phosphatases with improved potency[31]. However, much like the active site itself, there is a high density of basic residues located on the enzyme surface of this site as well. The second aryl-phosphate binding site is a shallow groove on the protein surface connected via a channel to the catalytic pTyr-binding cleft. The dominant residues in this site are Arg-24 and Arg-254, whose side chains form salt bridges with the phosphate group of pTyr. In addition to these direct interactions with the phosphate, Arg-24 and Arg-254 interact indirectly with the phosphate group of pTyr via two ordered water molecules[31]. Consequently, the charge and the shape of this second site on PTP1b dictates that compounds that bind here with reasonable potency are also likely to be highly hydrophilic. Multiple weak interactions must be used for binding with limited opportunity for potent van der Waals driven binding[22]. Thus, it would be expected that targeting this second site will do little to change the over all hydrophilicity of inhibitors.

As a consequence of these limits posed by the polar active site of PTP1b, progress in drug design has been slow[22,32-34]. Although potent PTP1b inhibitors have been reported, these again are large, highly polar compounds whose binding is relatively inefficient in terms of binding energy per non-hydrogen atom. In fact, a recent review of such compounds noted that most PTP1b inhibitors have physicochemical properties that are inconsistent with oral absorption and delivery to target tissues, with polar surface areas exceeding 160 A^2, molecular weights in excess of 500 and many rotate-able bonds[22]. Additionally, efforts towards a drug to inhibit PTP1b have been thwarted by the reactive nature of the active site cysteine. Reversible oxidation of this residue appears to regulate PTP1b activity *in vivo* and this susceptibility makes the enzyme prone to oxidation by minor contaminants[32]. In addition to oxidation, a number of other mechanisms give rise to PTP1b inhibitors that are not classically behaved starting points for drug discovery[35,36]. In fact, the only compound from a PTP1b discovery effort to make it to clinical trials thus far (ertiprotafib) has been shown to derive its efficacy from additional mechanisms, including agonism of PPAR family members[37].

Scientists from Sunesis recently published a novel way to circumvent the problem of designing drug-like inhibitors to the phosphotyrosine binding site of PTP1b, through identification of an allosteric small-molecule binding site on the enzyme[38]. Compounds that bound to this site displayed classic non-competitive inhibition: affecting the catalytic rate of the enzyme (V_{max}) without inhibiting substrate binding (K_m). Their mechanism was further revealed through co-crystals structures with PTP1b. These showed that this allosteric site exists within 20 angstroms of the peptide-binding groove in a pocket formed by helices α3 and α6 of PTP1b, behind the conserved WPD loop (Trp-179, Pro-180, Asp-181) that must close on the active site as part of the catalytic step. Inhibitors that bound to this site block interactions and side chain movements necessary for loop closure, locking PTP1b in the open form and thus affecting the catalytic rate but not interfering with substrate binding to the active site pocket. Importantly, the nature of this novel site is quite distinct from the catalytic pTyr binding site with extensive hydrophobic interactions seen with the bound inhibitors. Key residues for compound binding are also not conserved among other PTPs. These authors hypothesize that the inherent structural adaptability of this site could provide opportunities for flexibility in drug design not afforded by the active site. However, the best compounds revealed in this initial study are relatively large (MW ~ 700 g/mole using ~ 40 non-hydrogen atoms) with only uM potency. Thus, the inherent ease of designing drug-like, efficient binders to this novel site remains to be established.

CONCLUSIONS

Drugs targeting PTP1b provide a unique opportunity to treat the underlying insulin resistance in type 2 diabetic patients. Unlike many other approaches to this problem, small molecule PTP1b inhibitors have the potential to directly affect both insulin and leptin signaling by prolonging the activation of their receptors and downstream signaling molecules. Importantly, small

molecule PTP1b inhibitors may provide a distinct advantage over PPAR-γ agonists for the treatment of type 2 diabetes by promoting increased insulin sensitivity without an increase in body weight. Although, PTP1b presents an exciting validated target for intervention in diabetes and obesity, drug development for this target is still in the early stages. It is clear that the limits placed on these efforts by the nature of the active site dictate that a successful approach must balance potency with permeability. To do this requires designing molecules that maximize potency and selectivity in the most efficient way, while allow the resultant leads to remain small and drug-like. Alternative approaches such as drugs that modulate the recently identified exosite on PTP1b may also prove fruitful. The possibilities for improved treatment of both diabetes and obesity provides continued motivation for efforts to develop drugs that act through inhibition of this enzyme.

REFERENCES

1. Zimmel, P., K.M. Alberti and J. Shaw, 2001. Global and societal implications of the diabetes epidemic. Nature, 414: 782-787.
2. Flier, J.S., 2004. Obesity wars: Molecular progress confronts an expanding epidemic. Cell, 116: 337-350.
3. Willson, T.M., P.J. Brown, D.D. Sternbach and B.R. Henke, 2000. The PPARs: From orphan receptors to drug discovery. J. Med. Chem., 43: 527-550.
4. Rosen, E.D. and B.M. Spiegelman, 2001. PPAR-γ: A nuclear regulator of metabolism, differentiation and cell growth. J. Biol. Chem., 276: 37731–37734.
5. Inzucchi, S.E., 2002. Oral antihyperglycemic therapy for type 2 diabetes: Scientific review. JAMA., 287: 360-372.
6. Mudaliar, S. and R.R. Henry, 2001. New oral therapies for type 2 diabetes mellitus: The glitazones or insulin sensitizers. Ann. Rev. Med., 52: 239-259.
7. Yki-Jarvinen, H., 2004. Thiazolidinediones. N. Engl. J. Med., 351: 1106-1118.
8. Nesto, R.W., D. Bell, R.O. Bonow, V. Fonseca and S.M. Grundy *et al.*, 2003. Thiazolidinedione use, fluid retention and congested heart failure. Circulation, 108: 2941-2948.
9. Diamant, M. and R.J. Heine, 2003. Thiazolidinediones in type 2 diabetes mellitus: Current clinical evidence. Drugs, 63: 1373-1405.
10. Hunter, T., 2000. Signaling-2000 and beyond. Cell, 100: 113-127.
11. Saltiel, A.R. and C.R. Kahn, 2001. Insulin signaling and the regulation of glucose and lipid metabolism. Nature, 414: 799-806.
12. Cheng, A., N. Uetani, P.D. Simoncic, V.P. Chaubey and A. Lee-Loy *et al.*, 2002. Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. Dev. Cell, 2: 385-387.
13. Elchebly, M., P. Payette, E. Michaliszyn, W. Cromlish and S. Collins *et al.*, 1999. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1b gene. Science, 283: 1544-1548.
14. Klaman, L.D., O. Boss, O.D. Peroni, J.K. Kim and J.L. Martino *et al.*, 2000. Increased energy expenditure, decreased adiposity and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1b-deficient mice. Mol. Cell. Biol., 20: 5479-5489.
15. Zabolotny, J.M., K.K. Bence-Hanulec, A. Stricker-Krongrad, F. Haj and Y. Wang *et al.*, 2002. PTP1B regulates leptin signal transduction *in vivo*. Dev. Cell, 2: 489-495.
16. Gu, F., N. Dube, J.W. Kim, A. Cheng, M. de Jesus Ibarra-Sanchez, M.L. Tremblay and Y.R. Boisclair, 2003. Protein tyrosine phosphatase 1b attenuates growth hormone-mediated jak-stat signaling. Mol. Cell. Biol., 23: 3753-3762.
17. Dube, N., A. Cheng and M.L. Tremblay, 2004. The role of protein tyrosine phosphatase 1B in Ras signaling. PNAS, 101: 1834-1839.
18. Zinker, B.A., C.M. Rondinone, J.M. Trevillyan, R.J. Gum and J.E. Clampit *et al.*, 2002. PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose and improves insulin sensitivity in diabetic mice. PNAS, 99: 11357-11362.
19. Gum, R.J., L.L. Gaede, S.L. Koterski, M. Heindel and J.E. Clampit *et al.*, 2003. Reduction of protein tyrosine phosphatase 1B increases insulin-dependent signaling in ob/ob mice. Diabetes, 52: 21-28.
20. Bhanot, S., M. Butler, S. Booten, R. Mckay, S. Murray, B.P. Monia, T.W. Beck and A.A. Levin, 2002. Effects of a novel PTP1B antisense inhibitor (ISIS 113715) in cynomologous monkeys. Diabetes Mellitus: Molecular Mechamisms, Genetics and New Therapies, Keystone, CO.
21. Tonks, N.K., 2003. PTP1B: From the sidelines to the frontlines. FEBS Lett., 546: 140-148.
22. Van Huijsduijnen, R.H., W.H.B. Sauer, A. Bombrun and D. Swinnen, 2004. Prospects for inhibitors of protein tyrosine phosphatase 1B as antidiabetic drugs. J. Med. Chem., 47: 4142-4146.
23. Barford, D., A.J. Flint and N.K. Tonks, 1994. Crystal structure of human protein tyrosine phosphatase 1B. Science, 263: 1397-1404.
24. Pedersen, A.K., G.H. Peters, K.B. Moller, L.F. Iversen and J.S. Kastrup, 2004. Water-molecule network and active-site flexibility of apo protein tyrosine phosphatase 1B. Acta Cryst., D60: 1527-1534.

25. Jia, Z., D. Barford, A.J. Flint and N.K. Tonks, 1995. Structure basis for phosphotyrosine peptide recognition by protein tyrosine phosphatase 1B. Science, 268: 1754-1758.
26. Sun, J.P., A.A. Fedorov, S-Y. Lee, X.L. Guo, K. Shen, D.S. Lawrence, S.C. Almo and Z.Y. Zhang, 2003. Crystal Structure of PTP1B complexed with a potent and selective bidentate inhibitor. J. Biol. Chem., 278: 12406-12414.
27. Iversen, L.F., H.S. Andersen, S. Branner, S.B. Mortensen, G.H. Peters and K. Norris *et al.*, 2000. Structure-Based design of a low molecular weight, nonphosphorus, nonpeptide and highly selective inhibitor of protein-tyrosine phosphatase 1B. J. Biol. Chem., 275: 10300-10307.
28. Zhang,Y.L. and Z.Y. Zhang, 1998. Low-affinity binding determined by titration calorimetry using a high-affinity coupling ligand. Anal. Biochem., 261: 139-148.
29. Wang, F., W. Li, M.L. Emmelt, C.L. Hendrickson, A.G. Marchall., Y.L. Zhang, L. Wu and Z.L. Zhang, 1998. Conformational and dynamic changes of yersinia protein tyrosine phosphatase induced by ligand binding and active site mutations revealed by H/D exchange and electrospray ionization fourier transform ion cyclotron reassurance mass spectrometry. Biochemistry, 37: 15289-15299.
30. Zhang,Y.L., Z.J. Yao, M. Sarmiento, L. Wu, T.R. Burke and Z.Y. Zhang, 2000. Thermodynamic study of ligand binding to protein-tyrosine phosphatase 1B and its substrate-trapping mutants. J. Biol. Chem., 275: 34205-12.
31. Puius, Y.A., Y. Zhao, M. Sullivan, D.S. Lawrence, S.C. Almo and Z.Y. Zhang, 1997. Identification of a second aryl phosphate-binding site in protein-tyrosine phosphatase 1B: A paradigm for inhibitor design. Prococeeding of National Academy of Science, 94: 13420-13425.
32. Tobin, J.F. and S. Tam, 2002. Recent advances in the development of small molecule inhibitors of PTP1b for the treatment of insulin resistance and type 2 diabetes. Curr. Opin. Drug Dicov. Dev., 5: 500-512.
33. Johnson, T.O., J. Ermolieff and M.R. Jirousek, 2002. Protein tyrosine phosphatase 1B inhibitors for diabetes. Natl. Rev. Drug Disc., 1: 696-709.
34. Moller, N.P.H., L.F. Iversen, H.S. Andersen and J.G. McCormack, 2000. Protein tyrosine phosphatases (PTPs) as drug targets: Inhibitors of PTP-1b for the treatment of diabetes. Curr. Opin. Drug Discov. Dev., 3: 527-540.
35. Doman, T.N., S.L. McGovern, B.J. Witherbee, T.P. Kasten, R. Kurumbail, W.C. Stallings, D.T. Connolly and B.K. Shoichet, 2002. Molecular docking and high-throughput screening for novel inhibitors of protein tyrosine phosphatase-1B. J. Med. Chem., 45: 2213-2221.
36. McGovern, S.L., E. Caselli, N. Grigorieff and B.K. Shoichet, 2002. A common mechanism underlying promiscuous inhibitors from virtual and high-throughput screening. J. Med. Chem., 45: 1712-1722.
37. Erbe, D.V., S. Wang, Y.L. Zhang, K. Harding and L. Kung *et al.*, 2005. Ertiprotafib improves glycemic control and lowers lipids via multiple mechamisms. Mol. Pharm., 67: 69-77.
38. Weismann, C., K.J. Barr, J. Kung, J. Zhu and D.A. Erlanson *et al.*, 2004. Allosteric inhibition of protein tyrosine phosphatase 1B. Natl. Struct. Mol. Biol., 11: 730-737.