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Research Article

# The Role of Oxidant on Dipeptidyl Peptidase-4 Enzyme in Benign Prostatic Hyperplasian Through Computational Study

Suhargo Listijani*, Sumitro SB, Widodo

*Department of Biology, Faculty of Mathematics and Natural Science, Brawijaya University, East Java, Malang, Indonesia*

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**ABSTRACT**

Dipeptidyl Peptidase-4 (DPP-4) is an epithelial transmembrane protein of many cell types such as gastrointestinal tract, biliary tract, pancreas, kidney, thymus, uterus, prostate and capillary endothelial cells. DPP-4 also found in soluble form of serum and other body fluid. Soluble DPP-4 has not a cytoplasmic domain and transmembrane domain. In diabetes, hydrogen peroxide as oxidant was involved in soluble DPP-4 generation from transmembrane DPP-4. In Benign Prostatic Hyperplasia (BPH), there were decreasing of antioxidant such as superoxide dismutase, catalase and gluthathione peroxidase, so the antioxidants in those diseases unable to discharge the oxidants and there was an accumulation of oxidants. This study aims to evaluate the role of some oxidant on DPP-4 enzyme in BPH through computational approach. The oxidants such as $H_2O_2$, $OH^-$, HOCl, $NO^-$, $NO_2$ $ONOO^-$, 13-hydroperoxy linoleic acid and 13-hydroxy linoleic acid (13-HODE) were docked with dipeptidyl peptidase-4. The result showed that all the oxidants may interact with DPP-4 on hydrolase domain (Gln 508 – Pro 766) or on β-propeller domain (Arg 54 - Asn 497). But 13-HODE may interact with DPP-4 on the stalk of DPP-4 that were on Asp 34, Ala 35, Thr 36, dan Ala 37 residues. So it may cause DPP-4 shedding to produce soluble DPP-4.

**Keywords:** Catalase, DPP-4, gluthathione peroxidase, superoxide dismutase

## INTRODUCTION

Benign prostatic hyperplasia (BPH) is a prostate condition that characterized by increasing in epithelial and fibromuscular cell proliferation, then followed by forming large nodules. The large nodules compress the urethral canal, interferes the flow of urine, cause urine retention and urinary tract infection[1].

BPH was occured because of disrupting in cell proliferation regulation. Antimitogen is a factor for regulating cell proliferation. One substance that function as antimitogen is dipeptidyl peptidase-4 enzyme (DPP-4) or CD26. Dipeptidyl peptidase-4 is an epithelial transmembrane protein of prostate[2]. It has a cytoplasmic domain (amino acids 1-6), transmembrane domain (amino acid 7-28) and extracellular domain (amino acids 29-766). The extracellular domain consists of a β-propeller domain (Arg 54 - Asn 497) and α/β hydrolase domain (Gln 508 – Pro 766) with the active sites of catalizing in amino acid Ser 630, Asp 708 and His 740[3].

Dipeptidyl peptidase-4 also found in soluble form of serum and other body fluid[4]. Soluble DPP-4 has no cytoplasmic domain and transmembrane domain. The level of soluble dipeptidyl peptidase-4 increased in diabetes[5]. In diabetes, hydrogen peroxide as oxidant was involved in soluble DPP-4 generation from transmembrane DPP-4[6].

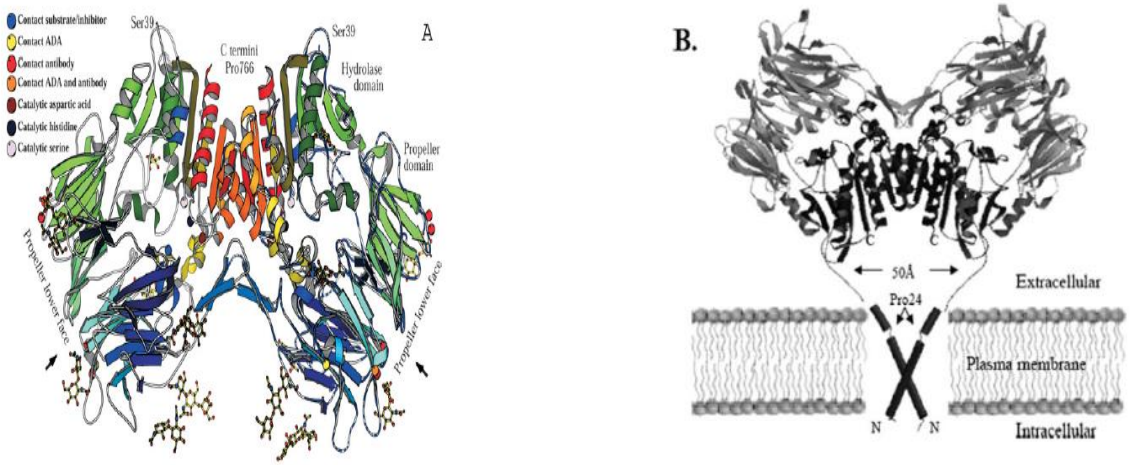
Oxidant is a free radical from fatty food, smoking, alkohol, environmental pollutants, hydrogen peroxide, ozone, toxin, ionization etc. Oxidants were derived from the end product of living system especially from metabolism process of mitochondria and endoplasmic reticulum. In normal condition, oxygen is reduced to produce water. In about 2% of electron from electron transport chain in mitochondria was passed, bound to oxygen and reduced to become superoxide radical ($O_2^-$). Superoxide can react with water to produce hydrogen peroxide ($H_2O_2$). $H_2O_2$ undergo Fenton reaction to form hydroxyl radical ($OH^-$). $H_2O_2$ can react with $Cl^-$ anion to give hypochlorous acid (HOCl) oxidant[7]. In benign prostatic hyperplasia, there is increasing of NO oxidant because BPH in inflammation condition. Superoxide can react with NO radical to give peroxynitrite (ONOO-) radical[8]. Superoxide radical also oxidize linoleic acid to become 13-peroxy linoleic (13-PODE) and then become 13-HPODE and 13-HODE. Linoleic acid is a main component of low density lipoprotein (LDL) triglyceride that was oxidized if there was oxidants accumulation[9]. Low density lipoprotein presents in extracellular matrix before binds in LDL receptor[10].

Under normal condition, living system can handle free radical by breakdown the compound through metabolism process or by antioxidant produced from healthy body. That antioxidants are superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx). In benign prostatic hyperplasia (BPH), there were decreasing of

*Author for Correspondence

Table 1: The interaction of dipeptidyl peptidase-4 and some oxidants

| Target | Ligand | Energy binding (kcal/mol) | Active Site Location |
|---|---|---|---|
| DPP-4 | $O_2^-$ | -2,96 | - Arg 356, Gly 355, Arg 382 |
| | $^{\bullet}OH$ | -2,14 | - Met 591, His 592, Ala 593, Ile 594, Asn 595 |
| | HOCl | -4,91 | - Ser 349, Gly 352, Trp 353, His 592 |
| | $H_2O_2$ | -5,03 | - Gly 355, Arg 356, Arg 382, Cys 551, Met 591 |
| | NO | -6,13 | - Trp 353, Gly 355, Arg 356, Arg382 |
| | $ONOO^-$ | -8,31 | - Gln 123, Trp 124, Glu 191, Tyr 195, Tyr 211 |
| | 13-HODE | -34,96 | - **Asp 34, Ala 35, Thr 36, dan Ala 37,** Pro531, Pro 532, His 533, Tyr 540, Ala 568, Ser 569, Thr 570, Asn 572 |
| | 13-HPODE | -47,80 | - Phe 208, Ser 209, Ala 210, Ser 212, Ala 213, Leu 214, Trp 215, Asp 302, Arg 358, Glu 361 |




Transmembrane DPP-4 Soluble DPP-4

Figure 1: Transmembrane and soluble dipeptidyl peptidase-4 (DPP-4) structure

antioxidant such as superoxide dismutase (SOD), catalase and glutathione peroxidase, so the antioxidants in those disease unable to discharge the oxidants and there was an accumulation of oxidants[11].

By computational analysis, it will be proven the ability of some oxidants ($H_2O_2$, $OH^-$, HOCl, $NO^-$, $NO_2$ ONOO-, 13-hydroperoxylinoleic acid (13-HPODE) and 13-hydroxy linoleic acid (13-HODE) to bind the particular site of DPP-4 (29-39 amino acid residue) thereby cause damaged in that site and then release of the extracelluler domain of DPP-4 to become soluble DPP-4.

## MATERIALS AND METHODS

All studies were carried out using Pymol (for visualize 3D molecular structure) and Patchdock (for docking DPP-4 enzyme with oxidant) and LigPlot program (to determine the active site of the oxidant in protein). All program were run in Windows operating sytem, 2G RAM, Quad core processor.

The 3D structures of oxidants were derived from NCBI PubChem. All structures were saved as .pdb file format for input to Patchdock program. The 3D structure of DPP-4 was come from Uniprot program for the fasta of DPP-4 and then by 3D Jigsaw program, the 3D structure of DPP-4 could be visualized. The sequences of DPP-4 amino acid used are from 10 to 766 amino acid for transmembrane DPP-4 model. As the receptor is dipeptidyl peptidase-4 enzyme (DPP-4) and as the ligands are some of the oxidants that usually increase in particular diseases such as diabetes and liver chirossis. The oxidants used for docking with DPP-4 are $H_2O_2$, OH, HOCl, $NO^-$, $NO_2ONOO^-$, 13-hydroperoxy linoleic acid (13-HPODE) and 13-hydroxy linoleic acid (13-HODE)

The protein and oxidant were docking by Patchdock program that running online. The results were sent by email. The results have many solutions number from 1 to 1000, then using Firedock program, the refine best solutions will be chosen, and there will be only 10 best solutions. The solution was visualized by Pymol program then to determine the active site of oxidant in DPP-4, the result with Pymol was continued with Ligplot program, so it can be known the active site of oxidant on DPP-4.

## RESULTS AND DISCUSSION

The objective of the study was to determine the oxidants which have the best ability to bind to the particular sites of DPP-4 (29 - 39 amino acid). If DPP-4 in that site has interaction with oxidant, then oxidant will damage the amino acids and it would be released as o oxidant ($O_2^-$, OH, HOCl, $H_2O_2$, $NO^-$, $ONOO^-$, 13-HPODE and 13-HODE) with enzyme DPP-4 (Table. 1)

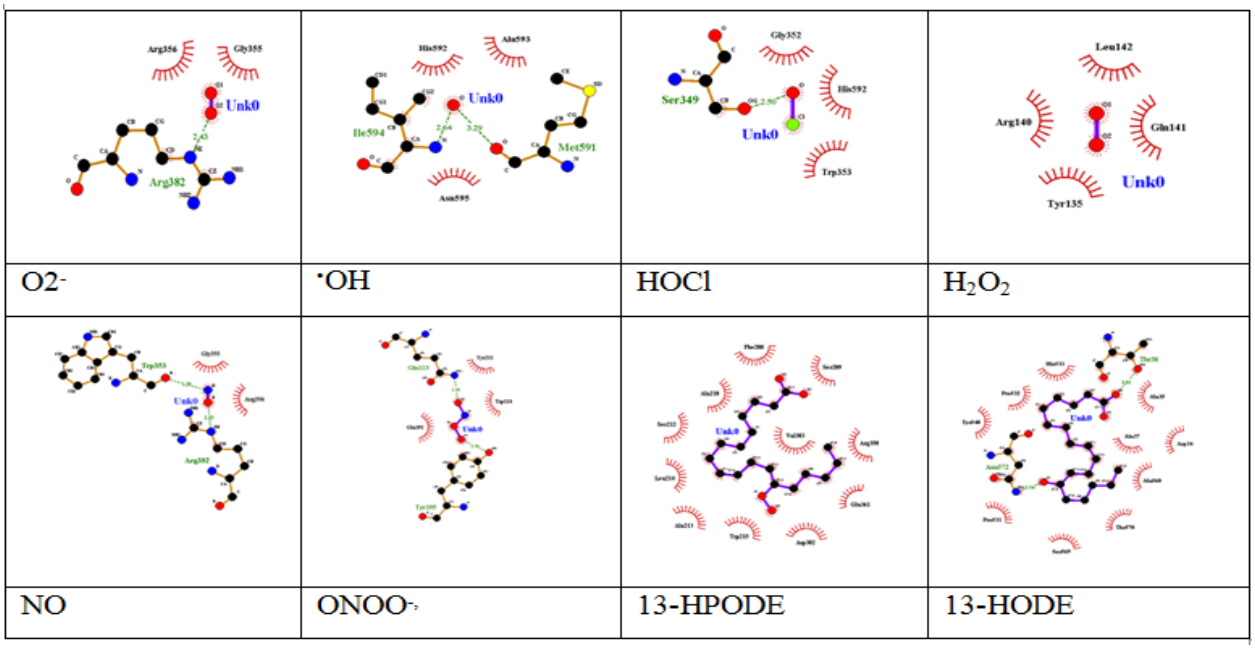



Figure 2: The active sites of DPP-4 interaction with all Oxidant ($O2^-$, •OH, HOCl, H2O2, NO, $ONOO^-$, 13-HPODE and 13-HODE)

Oxidants that had the best interaction with dipeptidyl peptidase in active site 29-39 amino acid was 13-hydroxylinoleicacid/13-hydroxyoctadecadienoic acid (13-HODE). The oxidant, 13-hydroxylinoleic acid/13-hydroxyoctadecadienoic acid (13-HODE), binds with DPP-4 on Threonin 36 by hydrogen bond, and binds with aspartic acid 34, Alanine 35 and Alanine 37 by hidrophobic bond. The oxidant 13-HODE was stealing electron from alanine 37. So there will be alkoxyl radical of alanine 37 and it will lead to cleavage between alanine 37 and aspartic acid 38. It will also be released as a soluble DPP-4 with 38-766 amino acid. The cleavage may also occur between alanine 37 and threonine 36 and it will be released as soluble DPP-4 with 37-766 aminno acid.

Soluble DPP-4 will bind to adenosine deaminase (ADA) and it will activate matrix metalloproteinase (MMPs). MMPs degrade collagen, and the growth factor that bind with collagen will be released, bind to the receptor in cell membrane and increase cell proliferation[2].

DPP-4 interaction with the 13-HODE also having the active site on hydrolase domain are at Pro 531, Pro 532, His 533, Tyr 540, Ala 568, Ser 569, Thr 570 and Asn 572. It means that the oxidant could make damage in hydrolase domain, so it could decrease the enzyme activity or could damage the protein because there is degradation at that site. Compound 13-HODE was detected in human prostate adenocarcinoma specimens and prostate cancer. It was involved in cell proliferation and differentiation[12].

Another oxidant have the active site on β-propeller (Arg 54-Asn 497) and a C-terminal α/β-hydrolase domain (Gln 508-Pro 766). The active sites of binding hydroxyl radical, hydrogen peroxide and hypochlorous acid with DPP-4 were in hydrolase domain of DPP-4, so they could damage that domain and effect the catalytic function or breakdown the protein. The oxidants like superoxide, NO, $ONOO^-$ and 13- HPODE bind with DPP-4 on β-propeller domain, so they could damage that domain or breakdown the protein. β - propeller domain function in dimeric formation of DPP-4, so if there is damage in that domain, the DPP-4 structure will be monomer. Monomeric and dimeric structure effects on the catalizing ability of enzyme. Dimeric structure of enzyme is more potential in the catalizing ability than monomeric structure[3].

DPP-4 function to degrade Stromal Derived Factor-1α (SDF-1α)[5]. The level of stromal derived factor-1α increased in aging man and aging is a risk factor for Benign Prostatic Hyperplasia incident[13]. If there is damage in the DPP-4 in hydrolase domain or propeller domain that effect in DPP-4 catalytic function, so SDF-1α could not be degraded by DPP-4 and cell proliferation increased.

## CONCLUSION

In benign prostatic hyperplasia (BPH), all the oxidants ($O_2^-$, OH, HOCl, $H_2O_2$, $NO^-$, $ONOO^-$, 13-HPODE and 13-HODE) may interact with DPP-4 on hydrolase domain (Gln 508 – Pro 766) or on β-propeller domain (Arg 54 - Asn 497). But 13-hydroxy linoleic acid (13-HODE) may interact with DPP-4 on the stalk region of DPP-4 that were on Asp 34, Ala 35, Thr 36, dan Ala 37 residues. So it may cause DPP-4 shedding to produce soluble DPP-4.

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