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Research Article

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The Role of Oxidant for Matrix Metalloproteinase Activation in BPH through Computational Study

L Suhargo[1*], SB Sumitro[2] and Widodo[2]

[1]Department of Biology, FST, Airlangga University, Surabaya, Indonesia
[2]Department of Biology, Brawijaya University, Malang, Indonesia

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ABSTRACT

Benign Prostatic Hyperplasia (BPH) occurs due to interference of cell proliferation regulation. Two factor effects cell proliferation regulation are mitogen and antimitogen. The mitogen is growth factor and the one antimitogen is CD-26 or Dipeptidyl Peptidase-4 (DPP-4). DPP-4 could inhibit cell proliferation by blocking basic-Fibroblast Growth Factor (b-FGF). The expression of Dipeptidyl Peptidase-4 decreases due to damage or enzyme shedding from cell membrane. One of the factor that contribute to the Dipeptidyl Peptidase-4 shedding is Matrix Metalloproteinase (MMP) especially MMP-1, MMP-2 and MMP-9. The increasing of cell proliferation in BPH was also caused by collagen degradation. MMP could degrade collagen. So the activation of MMP was important in BPH. By computational study it was examined the interaction of ROS (Hydrogen Peroxide, Hydroxyl radical, Nitric Oxide and Superoxide) with MMP (MMP-1, MMP-2 and MMP-9). It was used Pathdock and Firedock program. Protein Structure of MMP could be obtained from UNIPROT and the structure of oxidant could be obtained from NCBI Pubchem Compound. The result showed that ROS could release propeptide domain of MMP-1,MMP-2 and MMP-9, then activate MMP.

Keywords: Matrix metalloproteinase; Kolagen; Reactive oxygen species; Dipeptidylpeptidase-4 (DPP-4)

INTRODUCTION

Benign Prostatic Hyperplasia is a prostate condition that characterized by hyperplasia of epithelial and fibromuscular tissue in the transition and periurethral zone. Prostate is an organ that sorrounds the urethra. When the prostate got bigger, it blocked urethra and caused problem with urinating. Prostate hyperplasia also caused infection of bladder, forming stone in bladder and impaired renal function [1].

Cell hyperplasia occurs due to interference of cell proliferation regulation. Two factors that regulated cell proliferation were mitogen and antimitogen. The mitogen was a growth factor and the one antimitogen is CD-26 or Dipeptidyl Peptidase-4 (DPP-4). In Benign Prostatic Hyperplasia, there was transmembrane Dipeptidyl Peptidase-4 (tDPP-4) enzyme in epithelial cell membrane of prostate gland. Transmembrane DPP-4 could inhibit cell proliferation by blocking basic Fibroblast Growth Factor [2]. The expression of Dipeptidyl Peptidase-4 decreased due to damage or enzyme shedding from cell membrane and become soluble DPP-4. Soluble DPP-4 could degrade extracellular matrix (ECM) and form a spatial, so proliferating cells could migrate and formed bigger glands or more glands [3]. One factor that contribute to Dipeptidyl Peptidase-4 shedding is Matrix Metalloproteinase (MMP) [4]. Matrix metalloproteinase is an extracellular matrix enzyme. MMP especially MMP-1,MMP-2 and MMP-9 that caused DPP-4 shedding. Matrix metalloproteinase also degrade the component of extracellular matrix such as collagen. Collagen degradation by MMP or by soluble DPP-4 could form a spatial that important to induce cell proliferation in BPH. This MMP enzyme was synthesized in inactive form because it has propeptide domain that inhibits catalytic domain. If propeptide domain was released from that enzyme, so the enzyme will be active to

catalyze substrate [5]. Matrix metalloproteinase could be activated in increasing of oxidative stress [6]. So the oxidant could release propeptide domain**.**

In Benign Prostatic Hyperplasia, the oxidant increased and the antioxidant decreased. There was inflammation in BPH that produce free radicals such as superoxide, Hydrogen peroxide, hydroxyl radical and Nitric Oxide [7]. Free radical could influence matrix metalloproteinase. By computational study, we want to know the interaction between matrix metalloproteinase with oxidant and to determine the active site of oxidant in matrix metalloproteinase, so we could predict whether oxidant could release propeptide domain matrix metalloproteinase or not and to predict which one oxidant could do best.

EXPERIMENTAL SECTION

Matrix Metalloproteinase Model

This researches used the 3D structure of MMP-1, MMP-2 and MMP-9, that were derived from Uniprot. The 3Dstructure of MMP-1, MMP-2 and MMP3 could be seen in Figure 1. The 3D structure of oxidants (H_2O_2, NO, *OH and O_2^-) were obtained from NCBI Pubchem. Matrix metalloproteinase-1 had 32-466 amino acid, matrix metalloproteinase-2 had 31-660 amino acid and matrix metalloproteinase-9 had 1-707 amino acid.

Docking MMP With Oxidant

These researches were done using Pymol and Chimera 1.9 for visualizes 3D molecular structure. For interaction between matrix metalloproteinase with oxidant, it was used Patchdock and Firedock. The active site of oxidants was determined with LigPlot program. All programs were run using Windows operating sytem, 2G RAM, Quad core processor. The matrix metalloproteinase was docking by on line Patchdock program and then followed by on line Firedock program to know the best result. The result of interaction between the MMP and oxidant were sent by email and were visualized with Pymol program. The best result from Firedock program was used to know the active site of oxidant to MMP by LIGPLOT program.

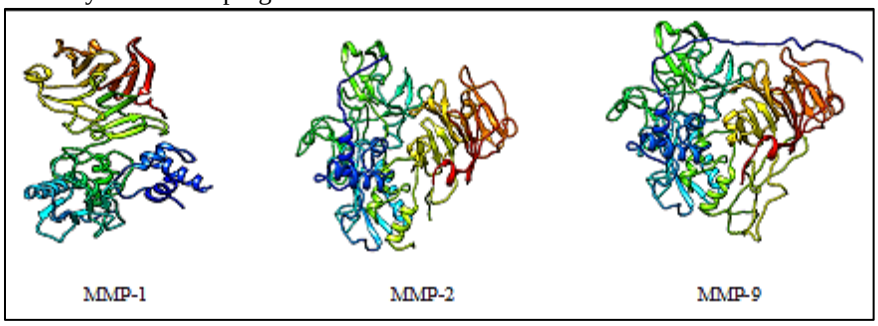

Figure 1: Matrix metalloproteinase structure (MMP-1, MMP-2 and MMP-9)

RESULTS AND DISCUSSION

The purpose of this study was to determine whether oxidant could activate matrix metalloproteinase (MMP) or not. Matrix metalloproteinase such as MMP-1, MMP-2, MMP-9 could be shedding DPP-4 from cell membrane by degrading it to be soluble DPP-4 [4]. Then soluble DPP-4 could induce spatial that important to induce cell proliferation in Benign Prostatic Hyperplasia (BPH). Spatial control is recognized by the cytoskeletal tension of cells. The cytoskeletal is a filament and tubule in the cytoplasm that extends from the nucleus to the plasma membrane. Cytoskeletal includes microfilaments, microtubules and intemediate filaments. Microfilaments consist of actin protein, microtubules composed of tubulin and intermediate filaments composed of varying cell-dependent protein types. Matrix metalloproteinase was synthesized as a zimogen that was inactive by fibroblast. To activate that zimogen, propeptide domain should be released from zimogen [5]. MMP domain that function for catalyzing was 100-269 amino acid and 270-469 amino acid for MMP-1, 110-660 amino acid for MMP-2 and 94-707 amino acid and 107-707 amino acid for MMP-9.

Increasing of oxidant (ROS/Reactive Oxygen Species) in Benign Prostatic Hyperplasia also effected matrix metalloproteinase activation [6]. Reactive Oxygen Species (ROS) was generated from superoxide (O_2-). Superoxide was form by reduction of Oxygen with NADPH oxidase enzyme, or by uncoupled endothelial nitric oxide synthase (eNOS) and mitochondrial electron transport chain (ETC). Superoxide was dismutated by Superoxide Dismutase

(SOD) to form H_2O_2. By Fenton reaction, Fe^{2+} with Hydrogen Peroxide formed Fe^{3+}, Hydroxyl ion and Hydroxyl radical. Nitric Oxide was synthesized by iNOS enzyme in inflammation condition in Benign Prostatic Hyperplasia [7]. Activation of MMP could be done by releasing domain that had Cystein amino acid. The location of Cystein was in 92 amino acid in MMP-1, in 102 amino acid in MMP-2 and in 98 amino acid in MMP-9. When Cystein binded zinc, there was water exclussion around the enzyme , so the substrate couldn't bind to the enzyme and catalyzing process didn't happen [8]. Cystein had sulphydryl group that could form a covalent bond with enzyme's substrate, and catalyzing process was done If that sulphydril group bind with zinc, so it couldn't bind to the substrate [9]. Activation of MMP could be done in increasing of oxidant. Oxidant could attach to matrix metalloproteinase and oxidized MMP. Then oxidant could modified the structure and function of MMP such as activate its proteolytic function [10]. It meant that the oxidant attach to the enzyme at the site that could release the zimogen (propeptide that had cystein amino acid), so it could activate matrix metalloproteinase. The result showed that Nitric Oxide was the most potent oxidant for activating MMP-1 and MMP-9 , but for activating MMP-2, superoxide was the most potent oxidant. Table 1 showed the interaction between oxidant (H_2O_2, NO, $^*OH$, O_2-) and MMP (MMP-1, MMP-2 and MMP-9). By computational studied was known that oxidant NO easily interacted with MMP-1 and MMP-9 because the interaction NO and MMP-1 or MMP-9 needed the smallest energy compared with other oxidant. In the interaction of oxidant with MMP-2, oxidant superoksid more easily interacted with MMP-2.

Table 1: The binding energy (Kcal/mol) and the active site location between receptor and ligand

Receptor	Ligand	Binding Energy (Kcal/mol)	Active Site Location
MMP-1	H2O2	-1,83	Tyr 237, Thr 241, Ser 239
	NO	-3,19	Ser 243, Gly 244, Ser318
	OH	-0,91	Leu 226, Met 236
	O2-	-2,96	Ile 253, Gln 257, Arg 262
sMMP-2	H2O2	0,54	Ala 196, Asn 109, Phe 113
	NO	-1,33	Gly 103, Asn 104, Pro 105, Asn 111
	OH	0,85	Gln 91, Asn 92, Glu 95
	O2-	-2,70	Gly 103, Asn 104, His 193
MMP-9	H2O2	-2.29	Arg 98, Cys 99, Leu 188, Val 398
	NO	-2.94	Arg 98, Cys 99, Pro 421, Met 422, Tyr 423,
	OH	-0.32	Arg 98, Cys 99, Gly 100, Leu 187, Leu 188, Ala 189
	O2-	-1.53	Ala 150, Arg 446

With Ligplot Program, the interaction between matrix metalloproteinase could be shown in Figure 2. Interaction between Nitric Oxid (NO) with MMP-1 with binding energy -3,19 kcal/mol, had active site in Serine 243, Glycine 244 and Serine 318. Matrix metalloproteinase had catalytic activity at 270-469. And MMP-1 had Cystein at amino acid 92. So if oxidant NO attact MMP-1 at 243 or 244 amino acid [11,12], the results of cleavage enzyme were the enzyme with 244-469 amino acid or the enzyme with 245-469 amino acid. So the enzyme had catalytic activity and had released Cystein that function to inhibit catalytic activity of the enzyme. It mean that NO could activate MMP-1. In MMP-9, NO could attach at Cystein 99, and if there is cleavage of the enzyme, so the result was the enzyme with 100-707 amino acid, Cystein was released and the enzyme had the catalytic activity (107-707 amino acid). In MMP-2, Superoxid could attach Glycine 103 and Asparagine 104, so the result after enzyme cleavage were the enzyme with 104-660 amino acid or with 105-660 amino acid, and Cystein 102 had been released. Then the Cystein of MMP-2 and MMP-2 could be released dan that enzymes could be activated.

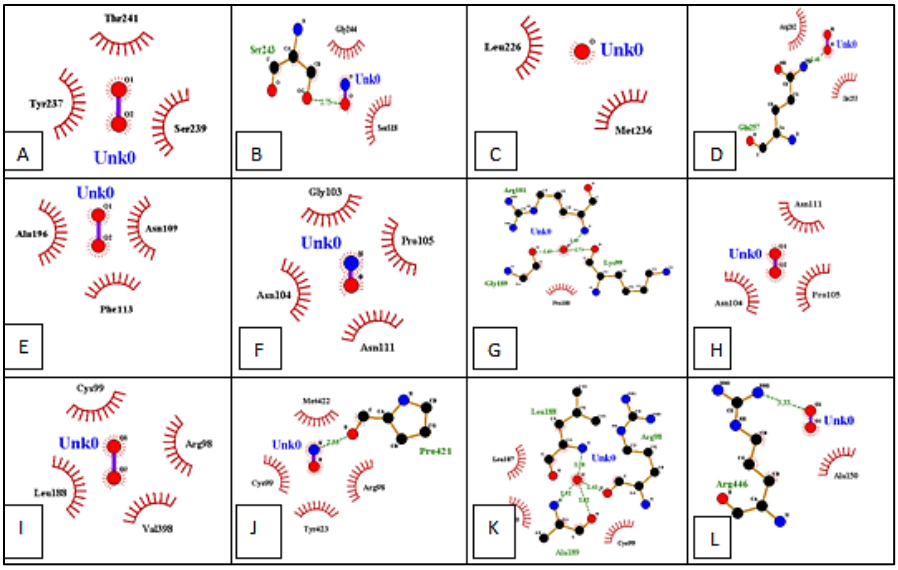

Figure 2: The site active location of interaction between matrix metalloproteinase with oxidant (H2O2, NO,*OH, $O2^{-}$. A,B,C,D for MMP-1, E,F,G,H for MMP-2 and I,J,K,L for MMP-9

CONCLUSION

Nitric Oxide could activate matrix metalloproteinase-1 and matrix metalloproteinase-9 and superoxide could activate matrix metalloproteinase-2. Other oxidant such as hydrogen peroxide and hydroxyl radical also could activate matrix metalloproteinase with greater energy. Activating MMP was done by releasing fragment of enzyme having Cystein as regulator of enzyme activity.

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