Exploration of the mechanisms of Aflatoxin B1 toxicity and the targets of Oltipraz by reverse docking

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Abstract: Aflatoxin B1 toxicity is well known but the mechanism of this toxicity is still unclear. In addition, the target of the anti-aflatoxin chemopreventive drug Oltipraz remains to be identified. In this study, we employed computer aided reverse docking analysis to identify putative targets of Aflatoxin B1 (AFB) and Oltipraz. The results showed that the clinically known toxic effects of AFB are related to this molecule's strong binding affinity for key proteins involved in cell apoptosis, hormone metabolism, immune suppression, and digestive organ function. In addition, virtual binding assay indicated that Oltipraz neutralizes the toxicity of AFB by inhibiting its biotransformation enzymes. In conclusion, the technique of reverse docking may be used to identify the specific targets of AFB and Oltipraz, and our findings could significantly accelerate the mechanistic studies of the two molecules and provide guidance for the development of anti-AFB drugs.

Keywords: Reverse docking; Aflatoxin B1; Oltipraz

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1. Introduction

Aflatoxins are among the most potent natural hepato-carcinogenic products, which are produced mainly by the fungi *Aspergillus flavus* and *A. parasiticus*. These fungi infect food crops such as maize, peanuts and tree nuts[1], leading to a global exposure of about 4.5 billion people a year to aflatoxins through diet[2]. The International Agency for Research on Cancer (IARC) has classified "naturally occurring mixture of aflatoxins" as a Group 1 human carcinogen, and in this group Aflatoxin B1 (AFB) is the most toxic agent[3]. It has been reported that population attributable risk of aflatoxin-related hepatocellular carcinoma was estimated at 17% (14%–19%) overall, and higher in HBV+ (21%) than in HBV– (8.8%) populations[1].

As previously reported, exposure to aflatoxin leads to hepatotoxicity, which also affects the hematologic, immune, reproductive, and digestive systems[4]. Because of the serious potential outcomes of exposure to AFB, the mechanism of its toxicity has been an important research topic. It has been reported that the toxicity and carcinogenicity of AFB in human and animal tissues are mediated by the short-lived AFB_1 exo- and endo-8,9-epoxide (AFBO), which can react with DNA bases and amino acids in proteins, resulting in mutation and cytotoxicity[5]. In addition, AFB has also been reported to suppress immunity, inhibit the generation of hepatic glycogens and affect lipid metabolism. However, further studies are needed to fully understand the mechanisms that are involved.

Currently, as there are no therapeutic agents that can selectively block AFB toxicity after exposure, therapy is aimed at relieving symptoms for individuals exposed to AFB. For example, atropine is used to treat AFB poisoning that accompanies with emesis or abdominal pain. However, preventative treatment offers an alternative for people who are at high risk of exposure to AFB. Among potential prophylactic agents, Oltripra has attracted most interest in recent years, and a clinical trial was carried out in Qidong, China[6], where obvious protective effects were observed. In addition, dietary compounds such as sulforaphane and some flavonoids are also reported to have some protective effects[7]. However, although the drug Oltipraz was observed to have positive clinical effects, its mechanism of action remains to be elucidated. An additional consideration is that this drug is relatively expensive, which limits its long-term daily use. The identification of its target is therefore of great importance for the development of effective but less expensive analogues.

AFB's severe toxicity in conjunction with the limited therapeutic methods warrants further research to identify the molecular targets of AFB and Oltipraz. Proteomic methods can be used in the identification of targets, but they are generally time consuming and expensive. In this study, we applied a computer assisted method, using reverse docking to predict the potential targets of AFB and Oltipraz. Reverse docking is a process similar to virtual screening. The difference lies in the fact the virtual screening is intended to identify potential new ligands for a given target, whereas reverse docking is used to identify target candidates for a given small molecule. The reliability of reverse docking can be assessed by whether its data are in agreement with published experimental results. Moreover,

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reverse docking is also convenient to perform. The concept of inverse docking was established in 2001. The first computer program capable of reverse docking was developed in the University of Singapore in 2001[8]. Jiang's group at the Shanghai Institute of Materia Medica subsequently developed the free online server TarFishDock with the intention of improving the use of this method. Shortly after, they developed another free online server PharmMapper for use in reverse docking. In 2011, Jackson's group at the University of Leeds developed another server for this purpose called Reverse Screen 3D. Target identification includes many distinct algorithms. In cases where 3D structures of the targets of interest are already available, instead of identifying target, the research focuses on finding the best interaction mode between potential targets and probe molecules.

In the present study, three online servers (PharmMapper[9], ChemMapper[10–12], and Reverse 3D[13]) were used for reverse docking evaluation of AFB and Oltripraz. The targets that were used for screening were from TargetBank, DrugBank, BindingDB and PDTD. The results were obtained online.

2. Experimental method

2.1. Ligand preparation

The 2D structure of Aflatoxin B1 and Oltipraz was generated by ChemDraw Ultra 12.0 (CambridgeSoft, Cambridge, MA, USA). H-bond and force field were applied, and energy was minimized before performing reverse docking procedures.

2.2. Computational pipeline

Three online reverse docking servers including PharmMapper (http://lilab.ecust.edu.cn/pharmmapper/), ChemMapper (http://59.78.96.61:8080/chemmapper/) and ReverseScreen3D (http://www.modelling.leeds.ac.uk/ReverseScreen3D) were utilized in this study.

To ensure the reliability of our results, different servers and target banks were used. Targets which ranked high in different servers were selected, and comprehensive analysis was carried out according to clinical symptoms resulting from AFB toxicity. Binding results were analyzed with regards to cellular functions in apoptosis, hormone metabolism, clotting mechanisms, and tissue specific toxicity.

3. Results and discussion

Targets that ranked high using different servers were considered having the potential for binding with AFB, and these were selected and are listed in Table 1. The likely validity of these results was supported by the fact that some identified targets were consistent with results of previous researches. For example, the known AFB interactions with serum albumin and other enzymes related to coagulation were also identified by this computer technique. Comprehensive analysis was carried out according to clinical symptoms of AFB toxicity with regard to apoptosis, hormone metabolism, immune suppression and tissue specific toxicity.

3.1. Mechanism of the AFB induction of apoptosis

Many proteins related to cell cycle control and apoptosis were identified by reverse docking, including chk1 (Checkpoint kinase-1), cdk2 (Cyclin-dependent kinase 2), pim-1 (Proto-oncogene serine/threonine-protein kinase Pim-1). Chk1, a serine/threonine-protein kinase, is an important cell cycle regulator. It is activated through phosphorylation in response to DNA damage, and can negatively regulate cell cycle progression and allow cells to evade apoptosis[14]. The target cdk2 is another serine/threonine-protein kinase, which also plays an important role in cell cycle control. It mainly acts at the G1-S transition to initiate DNA synthesis and increase cell proliferation through phosphorylation of its substrates and interaction with cyclins[15,16]. Pim-1 is a proto-oncogene which also has serine/threonine kinase activity that is involved in cell survival and cell proliferation.

Table 1. AFB targets predicted by reverse docking

Target name	PharMapper rank	ChemMapper rank	Reverse 3D rank	Found in Oltripraz's top 100 hits
Caspase-1	63	79	–	
Cell division protein kinase 2	49	89	63	√
Serine/threonine-protein kinase Chk1	50	12	66	
Serine/threonine-protein kinase Pim-1	96	2	56	
Progesterone receptor	46	>100		
Androgen receptor	29	>100	37	√
Estrogen sulfotransferase	80	–	2	
Estrogen receptor	62	47	3	
Sex hormone-binding globulin	61	88	11	
Prothrombin (thrombin alpha)	41	–	57	√
Glutathione-requiring prostaglandin D synthase	45	–	45	
Phospholipase A2, membrane associated	57	16	23	
Prostaglandin G/H synthase 2	–	55	70	
Macrophage migration inhibitory factor	>100	20	15	
Cytochrome P450 family	60	7	5	√
Estradiol 17-beta-dehydrogenase 1	43	–	4	

It exerts its oncogenic activity through the regulation of MYC transcriptional activity, the regulation of cell cycle progression and inhibition of pro-apoptotic proteins (BAD, MAP3K5, FOXO3), and to the suppression of apoptosis[17]. The identification of multiple targets involved in the cell cycle and apoptosis pathway indicates the AFB toxicity is related to apoptosis. And its molecular mechanism is probably through inhibiting the activities of chk1, cdk2 and pim-1. A study carried out in 2011 supports this conclusion. Rustemeyer et al. found that dietary exposure of pigs to aflatoxin resulted in changes in expression of apoptosis-related genes in the liver, including changes in expression of Pim-1[18].

3.2. Mechanism of the interference with hormone metabolism by AFB

It has been reported that AFB has reproductive toxicity and can induce breast cancer and ovarian cancer[19]. However, the mechanism has not been identified. In three different sets of reverse docking results, many enzymes and receptors related to hormone metabolism showed high scores, including androgen receptor, estrogen receptor, and progesterone receptor. Proteins regulating hormone levels also showed high scores such as Estradiol 17-beta-dehydrogenase 1 and sex hormone-binding globulin, which respectively regulate the level of sex hormone and function as a carrier of androgen that regulates sex hormone plasma concentration of steroid hormones. In addition, the latter showed a tissue specific distribution. The isoform 1 and 2 of sex hormone-binding globulin is distributed in the liver and testis[20,21]. Homeostasis of hormone levels is vital for the health of the reproductive system, and our reverse docking results suggest that AFB reproductive toxicity is related to its interaction with these proteins leading to the interference with hormone metabolism and subsequent diseases. The similarity of the AFB structure with the reproductive steroids permits the potential targeting of these proteins.

3.3. Mechanism of AFB immune suppression

It has been reported that AFB can damage the immune defense system by suppressing both humoral immunity and cellular immunity[22], but the underlying mechanism needs clarification. Reverse docking results showed that many immune related proteins are interacting with AFB, including glucocorticoid receptor, macrophage migration inhibitory factor and Ig gamma-1 chain C region. Some inflammatory mediators were also captured by reverse docking. Macrophage migration inhibitory factor is a pro-inflammatory cytokine involved in the innate immune response to bacterial pathogens, and it can inhibit the anti-inflammatory activity of glucocorticoids[23]. Thus AFB likely causes immune suppression by increasing the anti-inflammatory activity of glucocorticoids, suppressing antibody activity, decreasing the synthesis of inflammatory mediators and suppressing the activity of macrophage migration inhibitory factors.

3.4. Mechanism of AFB organ toxicity

AFB is toxic to several organs, including the liver, kidney and gastrointestinal tract. Based on our reverse docking data, AFB organ toxicity has several characteristics. First, reverse docking shows that AFB can interact with the CYP450 family, which has also been demonstrated by previous researches. AFB could be metabolized to form AFB-DNA adducts, which have higher hepatotoxicity[7]. Second, our results showed AFB can interact with enzymes that are located only in the liver such as glucokinase, glycogen synthase kinase-3 beta and protein-glutamine gamma-glutamyl transferase E (some of these are not shown in Table 1). Interactions with these tissue specific targets can cause organ toxicity. As for the kidney, our reverse docking results show AFB may damage renal function by targeting adrenergic receptor and carbonic anhydrase 2. In addition, its interaction with trypsin and gastrotropin may be the mechanism of its gastrointestinal toxicity.

3.5. Identification of targets by Oltipraz

Reverse docking of Oltipraz identified several enzymes that metabolize drugs, including members of the cytochrome P450 family, glutathione S-transferase (GST), glutathione reductase (GSR) and the sulfotransferase family cytosolic 2B member 1. As noted above, CYP450 participates in hepatic biotransformation of AFB; GST is reported to catalyze the reaction of AFBO and GSH to form AFB-mercapturic acid which can be excreted via urine; sulfotransferase interacts with hydroxylated AFB and the resultant complex has reduced toxicity[24,25]. It is thus likely that Oltipraz exerts its chemoprevention effect against AFB toxicity by inhibition of CYP450's activity, increasing GST activity, activating GSR to increase the production of GSH, and aiding sulfotransferase detoxification function. Of particular interest, some targets identified by Oltipraz docking also reached high scores in AFB docking results (as listed in Table 1). This result suggests that the effect of Oltipraz may also be due to antagonizing the interactions of AFB with these targets.

In conclusion, reverse docking has been used to predict the potential targets of AFB and reveal the potential mechanisms of AFB toxicity. We also applied this method in the same manner to identify the targets of the AFB prophylactic agent Oltipraz, and to reveal its pharmacological mechanism. As mentioned above, the rationality of the method is confirmed by the consistent results with published research. More importantly, this study revealed novel molecular mechanisms about AFB's toxicity and Oltipraz's detoxification activity. In addition to the published targets, we discovered several novel potential targets of AFB, which are chk1, cdk2, androgen receptor, estrogen receptor, estradiol 17-beta-dehydrogenase 1, sex hormone-binding globulin, glucocorticoid receptor, macrophage migration inhibitory factor, Ig gamma-1 chain C region, glucokinase, glucokinase, and glycogen synthase kinase-3 beta. Besides, another novel toxic mechanism

revealed by the reverse docking was that AFB may have neurotoxicity by targeting several key enzymes involved in neurotransmitters' metabolism, like amine oxidase, catechol *O*-methyltransferase, beta-secretase 1, etc. But because no neurotoxicity of AFB was published, this part was not discussed further. Novel targets of Oltipraz were also discovered by reverse docking, like glutathione reductase (GSR) and the sulfotransferase family. They are also involved in the metabolism of AFB and Oltipraz may interfere with their activities. But they were not revealed by other studies yet. Besides, we discovered several novel targets of AFB may also be novel targets of Oltipraz, like chk1 and androgen receptor. Altogether, these results offer new targets for the development of more effective therapeutic strategies to combat AFB toxicity. However, these results are all based on computer prediction and biological experiments will be needed to further test and verify the conclusions.

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反向分子对接应用于黄曲霉素B1毒性机制及药物Oltipraz靶点的探究

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摘要: 本研究旨在探究黄曲霉素B1的作用靶点, 以探讨其致毒机理, 并分析药物Oltipraz的减毒机制, 为更好地防治黄曲霉素中毒提供依据。本研究采用反向分子对接的方法, 使用了3个在线反向分子对接服务平台预测黄曲霉素B1, Oltipraz可能的作用靶点, 综合分析所得结果, 结合黄曲霉素中毒的临床表征对结果进行分析。黄曲霉素B1的对接结果表明AFB可能通过作用于细胞凋亡、激素代谢、免疫调节等过程中的相关蛋白扰乱了机体的正常功能, 以及通过作用于组织特异性的蛋白造成肝、肾、胃等器官毒性。另外, 药物Oltipraz的对接结果表明, Oltipraz可能一方面通过抑制AFB产生毒性的生物转化过程, 另一方面通过拮抗多个黄曲霉素可能作用的靶点产生减毒作用。本研究用反向分子对接的方法系统而有效地预测了黄曲霉素B1和药物Oltipraz的靶点, 为研发更有效的抗黄曲霉毒素的药物提供了一定依据。

关键词: 反向分子对接; 黄曲霉素B1; 奥替普拉