JOURNAL OF EDUCATION, SCIENCE AND HEALTH – JESH
Revista de Educação, Ciência e Saúde
DOI: https://www.doi.org/10.52832/jesh.v3i2.192
Home page: https://bio10publicacao.com.br/jesh
e-ISSN: 2763-6119

MOLECULAR DOCKING: A VALUABLE TOOL FOR DETERMINING THE TOXICITY OF METHYL MERCURY TO HUMANS

DOCKING MOLECULAR: UMA FERRAMENTA VALIOSA PARA DETERMINAR A TOXICIDADE DO METIL MERCÚRIO EM SERES HUMANOS

ACOPLAMIENTO MOLECULAR: UNA HERRAMIENTA VALIOSA PARA DETERMINAR LA TOXICIDAD DEL METILMERCURIO EN LOS HUMANOS

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Recebido: 08/02/2023| A**provado**: 19/05/2023| **Publicado**: 09/06/2023

Abstract: Mercury, mainly in its form of methyl mercury (MeHg), is a heavy metal of high environmental and toxicological relevance, as it can compromise the physiology of organisms and generate various pathologies in animals. Even with several toxic effects known in animals, studies of toxicity in humans remain the most restricted among mammals, possibly due to ethical barriers. Despite the anatomical and physiological similarities between experimental and human models, divergences may occur, at the molecular level, regarding the patterns of interaction between toxic agents and target proteins. Thus, simulations in the computational environment, through molecular docking using exclusively human proteins, present themselves as an experimental advantage, bringing precision to results, saving time and financial resources, and contributing to the reduction of the use of animals in research. This study aimed to screen mercury toxicity through proteins related to human physiological systems using molecular docking methodology with AutoDock Vina. The binding energies, amino acid residues, and the number of residues were similar in all analyzed proteins, demonstrating the possible systemic toxicity of mercury due to the possibility of compromising the function of proteins related to the nervous, digestive, excretory, respiratory, and reproductive systems.

Keywords: Metals. Toxicity. Organism. Environmental.

Resumo: O mercúrio, principalmente em sua forma de metil mercúrio (MeHg), é um metal pesado de alta relevância ambiental e toxicológica, pois pode comprometer a fisiologia dos organismos e gerar diversas patologias nos animais. Mesmo com vários efeitos tóxicos conhecidos em animais, os estudos de toxicidade em humanos continuam sendo os mais restritos entre os mamíferos, possivelmente devido a barreiras éticas. Apesar das semelhanças anatômicas e fisiológicas entre modelos experimentais e humanos, divergências podem ocorrer, em nível molecular, sobre os padrões de interação entre agentes tóxicos e proteínas-alvo. Assim, simulações em ambiente computacional, por meio de *docking molecular* utilizando proteínas exclusivamente humanas, apresenta-se como uma vantagem experimental, trazendo: precisão de resultados, economia de tempo e recursos financeiros, além de contribuir para a redução do uso de animais em pesquisas. O objetivo deste estudo foi rastrear a toxicidade do mercúrio através de proteínas relacionadas aos sistemas fisiológicos humanos usando a metodologia de *docking molecular* com o AutoDock Vina. As energias de ligação, resíduos de aminoácidos e número de resíduos foram semelhantes em todas as proteínas analisadas, demonstrando a possível

toxicidade sistêmica do Mercúrio, devido à possibilidade de comprometer a função de proteínas relacionadas aos sistemas nervoso, digestivo, excretor, respiratório e reprodutor.

Palavras-chave: Metais. Toxicidade. Organismo. Ambiental.

Resumen: El mercurio, principalmente en su forma de metilmercurio (MeHg), es un metal pesado de alta relevancia ambiental y toxicológica, ya que puede comprometer la fisiología de los organismos y generar diversas patologías en los animales. Incluso con varios efectos tóxicos conocidos en animales, los estudios de toxicidad en humanos siguen siendo los más restringidos entre los mamíferos, posiblemente debido a barreras éticas. A pesar de las similitudes anatómicas y fisiológicas entre los modelos experimentales y humanos, pueden ocurrir divergencias, a nivel molecular, sobre los patrones de interacción entre los agentes tóxicos y las proteínas diana. Así, las simulaciones en ambiente computacional, a través del acoplamiento molecular utilizando exclusivamente proteínas humanas, se presenta como una ventaja experimental, trayendo: precisión de resultados, ahorro de tiempo y recursos financieros, además de contribuir a la reducción del uso de animales en investigación. El objetivo de este estudio fue evaluar la toxicidad del mercurio a través de proteínas relacionadas con los sistemas fisiológicos humanos utilizando la metodología de acoplamiento molecular con AutoDock Vina. Las energías de unión, los residuos de aminoácidos y el número de residuos fueron similares en todas las proteínas analizadas, lo que demuestra la posible toxicidad sistémica del Mercurio, debido a la posibilidad de comprometer la función de las proteínas relacionadas con los sistemas nervioso, digestivo, excretor, respiratorio y reproductivo.

Palabras-clave: Rieles. Toxicidad. Organismo. Ambiental.

1 INTRODUCION

Heavy metals can be easily found in the environment, entering food chains, and causing irreversible damage to organisms (Chen *et al.,* 2016). Among these heavy metals, mercury (Hg) is a toxic pollutant that has been steadily accumulating in various environmental compartments, such as water, air, soil, and sediment, due to urbanization and industrialization resulting from anthropic action (Veiga *et al.,* 1999).

Water, sediments, and soils are susceptible to mercury contamination, which can have detrimental effects on human health as well as plant and animal species (Kogularasu *et al*., 2018; Jeromiyas *et al*., 2019; Govindoamy *et al*., 2020). Mercury exposure in animals and humans occurs mainly through methylmercury (MeHg), which is highly toxic due to its ability to bioaccumulate and biomagnify within the food chain (Chan, 2019). In this context, biomagnification refers to the ability of living organisms to accumulate certain chemicals in a concentration greater than those found naturally. For instance, animals that consume contaminated food will accumulate the contaminant in their bodies (Freedman, 2021). Improperly discarding as little as 60-120 mg of mercury can contaminate at least three cubic meters of water, leading to significant harm to the soil, atmosphere, and human health (Guo *et al*., 2013; Pant & Singh 2014).

According to IBAMA (2021), fluorescent lamps contain mercury in two chemical forms: elemental mercury in vapor form and bivalent mercury adsorbed in the phosphor powder located within the tube ends or other components. The minimum amount of mercury vapor required to energize the lamp is 50 micrograms, approximately 0.5 to 2.5% of the total mercury present in the tube. Following the breakage of a fluorescent lamp, mercury vapor can continue to be released for several weeks (EPA, 2010). The release of 1 mg of Hg in a 500 m^3 room with no ventilation can be exceeded by up to 10 times the recommended exposure limit (Johnson *et al*., 2008).

In its natural forms, inorganic mercury can induce oxidative stress in living organisms (Jahan et al., 2019), immune response (Sun *et al*., 2018), and disrupt energy production (Lavoie & Summers, 2018). These

effects can result in neurotoxicity (Tan *et al.*, 2018), nephrotoxicity (Li *et al.*, 2020), and hepatotoxicity (Brandão *et al.*, 2015).

Several procedures and analytical techniques are reported in the literature for determining MeHg, such as processes based on acid leaching, gas chromatography, and voltammetric techniques (Suvarapu *et al.,* 2013). However, developing these methods for quantifying MeHg in biological samples is complex and involves multiple analytical steps (Farias *et al.*, 2009). Molecular docking is an emerging computational technique in predictive toxicology (Trisciuzzi *et al.,* 2018). Among the numerous advantages of the molecular docking approach, this technique can reduce the use of animals in toxicological tests, experimental time, and research costs.

Recent studies have highlighted the ability of molecular docking in toxicology research. For instance, Liu *et al.* (2018) emphasized the successful application of molecular docking in studying biodegradation mechanisms for environmental remediation. In addition, Jeong *et al.* (2019) demonstrate that this method can be applied to discover molecular initiation events (MIEs). Therefore, this research aims to verify mercury toxicity using proteins related to human physiological systems through molecular docking.

2 MATERIAL AND METHODS

2.1 Protein selection

The selection of protein targets for MeHg toxicity assessment involved conducting a literature search to identify proteins relevant to the physiological systems affected by mercury in humans. Subsequently, the Protein Database was utilized with a filter for the species *Homo sapiens* to narrow down the selection of targets.

Table 1 - Selected protein in physiological human systems.

PDB ID	System	Protein name	Function	Reference
1FYC	Digestive	Pyruvate dehydrogenase (PDH)	The E2 (E2p) component of the PDH multienzyme complex is the major autoantigen recognized by antibodies in patients with primary biliary cirrhosis. Immunodominant sites on E2p were located within the two lipoyl domains, where the essential cofactor lipoic acid is covalently linked.	Howard et al., 1998.
2F73		Fatty Acid-Binding Protein 1 (FABP1)	FABP1 protein participates in the metabolism of fatty acids in the cytoplasm, facilitates the transport, storage, and utilization of fatty acids and their acyl-CoA derivatives, and may exert a protective effect against lipotoxicity, facilitating their oxidation or incorporation into TGs and binding to free fatty acids of cytotoxics.	Kursula et al., in preparation.
2P31	Excretory, reproductive and respiratory	Glutathione Peroxidase 7 (GPX7)	The glutathione peroxidase family protects against oxidative stress, scavenging, and inactivating hydrogen peroxides and water lipids	Kavanagh; Oppermann, in preparation.

3GRS		Glutathione reductase (GSH)	or lipid hydroxyls in a glutathione-dependent reductive reaction. GPX7 has not been widely characterized to date, but it is reported to be cytoplasmic and widely expressed. GSH catalyzes the reduction of glutathione disulfide to the sulfhydryl form, an important cellular antioxidant.	Karplus; Schulz, 1987.
4B3E		Superoxide dismutase 1 (SOD1)	Its primary function is to catalyze the dismutation of superoxide into O_2 and H_2O_2. It reacts with H_2O_2, forming a solid copper-bound oxidizing species that can inactivate the enzyme or oxidize other substrates.	Strange et al., 2012.
5YHU	Nervous	Myelin regulatory factor (MYRF)	MYRF is a membrane-bound transcription factor. This protein is responsible for oligodendrocyte differentiation and myelination of the central nervous system. Followed by a self-cleavage by the intramolecular chaperone self-processing domain, the MYRF DNA-binding domain is released from the endoplasmic reticulum and is then translocated to the nucleus to regulate gene expression.	Chen et al., 2018.
6A9P		Glial fibrillary acidic (GFAP)	GFAP protein is an intermediate filament (IF) that plays essential roles in cell migration, mitosis, development, and signaling in astrocytes and a specific type of glial cell. It is overexpression and genetic mutations that lead to abnormal IF networks and accumulation of Rosenthal fibers, which result in the fatal neurodegenerative disorder (Alexander's disease).	Kim et al., 2018.

2.2 Molecular Anchoring

Protein structures (receptors) were obtained from Protein Data Bank PDB (PDB ID in Table 1) (Berman *et al*, 2000), and MeHg structure (ligand) was obtained from PubChem (PubChem ID: 6860) (Bolton *et al.*, 2008). The 2D structures were converted to 3D coordinates using the OpenBabel software (O'Boyle *et al.*, 2011). The generated 3D structure was then subjected to energy minimization using the Avogadro software (Hanwell *et al.*, 2012). Subsequently, the receptors were prepared by removing heteroatoms, ligands, and crystallographic water molecules. Polar hydrogen atoms were added, and Kollman charges were assigned. For the ligands, Gasteiger charges were assigned, and the number of torsional degrees of freedom (TORSDOF) in the binder was set using AutoDockTools 4 software for AutoDockVina (Trott & Olson 2010). The files were saved in the PDBQT format (which stores atomic coordinates, partial charges, and AutoDock atom types) (Morris *et al.*, 2009).

A "blind docking" strategy was performed, meaning that the docking algorithm was not provided with information about the binding site but could still locate it (Hetényi & Spoel 2002). For the docking simulations, grid boxes were configured to cover the entire protein, and the exhaustiveness were configured according to Table 2. Molecular docking simulations were performed using Autodock Vina (Trott & Olson 2010). Then, Free

Energy of Binding (FEB) of docked ligand-receptor was estimated in Kcal/mol. More negative FEB indicates greater stability of the ligand-receptor complex. The fit results were visually analyzed with PyMol (available for download https://pymol.org/2/). Protein-binder interactions were analyzed using LigPlot+ (Laskowski & Swindells, 2011).

Table 2 - Summary of Auto Dock Vina parameters:

PDB ID	Grid box size	Grid box center coordinates	Exhaustiveness
1FYC	x: 52; y: 40; z: 50	x: 7.343; y: 0.140; z: -2.815	500
2F73	x: 110; y: 40; z: 126	x: 18.247; y: -1.610; z: -48.419	500
2P31	x: 40; y: 40; z: 76	x: -8.239; y: -0.651; z: -23.636	500
3GRS	x: 68; y: 52; z: 60	x: 70.396; y: 54.006; z: 18.736	500
4B3E	x: 108; y: 126; z: 40	x: 157.867; y: 94.569; z: 52.191	500
5YHU	x: 40; y: 70; z: 74	x: 7.979; y: 34.210; z: 73.860	500
69AP	x: 58; y: 126; z: 114	x: -4.539; y: -270.977; z: 795.941	500

3 RESULTS AND DISCUSSION

Molecular docking is an in-silico method widely applied in drug discovery programs to predict the binding mode of a given molecule interacting with a specific biological target (Trisciuzzi *et al.*, 2018). This computational technique is emerging today in predictive toxicology for regulatory purposes, being successfully applied to develop classification models for predicting the endocrine disruptor potential of chemicals (Trisciuzzi *et al.*, 2018). One of the objectives of animal tests and clinical trials is to assess a drug candidate's toxicity and side effect. These tests and trials have consumed a large percentage of time and money spent on drug development, leading to efforts towards developing experimental techniques for molecular analysis and high-throughput screening of toxicological effects as an early assessment tool.

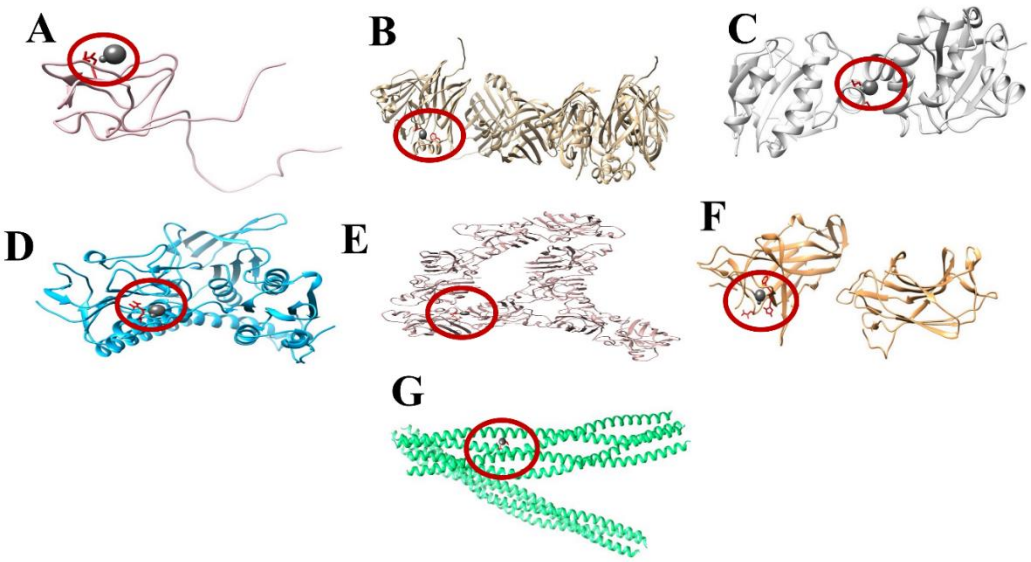
Computer programs have also been developed to predict toxicity and metabolism using statistically derived structure-toxicity/metabolism relationships (Chen & Ung, 2001). Wildlife and humans are exposed to Hg primarily through diet in methylmercury (MeHg) because MeHg bioaccumulates and biomagnifies potential and accumulates along the food chain (Chan, 2019). MeHg-induced toxicity involves multiple mechanisms, including oxidative stress, repression of protein translation, disruption of calcium homeostasis and mitochondrial energetics, and post-translational modification of proteins (Martins *et al.*, 2021). Accumulating evidence shows that these mechanisms are involved in several critical cellular processes and functions.

Once MeHg is absorbed, it enters the bloodstream. It is distributed quickly to various tissues and organs as it binds to cysteine in fluids mimicking methionine, which makes it easily transported across cell membranes by amino acid transporters (Clarkson, 1993). First, it is distributed to the liver, kidney, and spleen and later to muscle and the brain (Oliveira Ribeiro et al., 1999). MeHg is slowly metabolized to inorganic Hg and has also been shown to accumulate in various tissues and organs (Bridges and Zalups, 2010).

In this work, we use proteins exclusively present in different human physiological systems, which help to

understand the toxicity of mercury in this species (Fig. 1). However, most research published so far has determined mercury toxicity in other mammals, such as rats. Despite anatomical and physiological similarities between humans and specific experimental models, some divergences may occur (Datta *et al.*, 2020).

Figure 1 - Proteins related to various human physiological systems interacting with methylmercury (MeHg): Within a red circle, the two dark gray balls represent MeHg, and the red rods represent some of the amino acids in each protein directly involved in binding MeHg. A: Internal lipoyl domain of the human pyruvate dehydrogenase complex (PDH) in pink; B: Fatty acid binding protein 1 (FABP1) in beige color; C: Glutathione peroxidase 7 (GPX7) in gray; D: Glutathione reductase in blue color; E: Copper-zinc superoxide dismutase complexed in pinkish-brown color; F: human myelin gene regulatory factor (MYRF) DNA binding domain in orange; G: Domain 1B of human glial fibrillary acidic protein in green.



The docking simulation demonstrated that the binding energies of the MeHg-protein complex were very similar. Therefore the toxic effect must be significant for all human physiological systems (Table 3). Lohren *et al.* (2015) state that organic mercury species exert their toxicity mainly in the central nervous system.

Table 3 - Free binding energy (FBE/Kcal/mol) of complex (protein-MeHg) and amino acids (AA) from proteins involved in binding for human systems proteins:

Protein	FBE	**AA**
Inner lipoyl domain from human pyruvate dehydrogenase (PDH) complex	**-1.7**	**4:** Gly 54; Ile 53; Glu 45; Thr 52
Fatty acid binding protein 1 (FABP1)	**-1.9**	**5:** Leu 28; Ser 56; Ile 29; Phe 15; Gly 32
Native human angiotensin converting enzyme-related carboxypeptidase (ACE2)	**-1.7**	**6:** His 540; Cys 542; Ser 411; Leu 410; Gln 526; Leu 539
Glutathione peroxidase 7 (GPX7)	**-1.6**	**5:** Phe 103 (A and B chains); Thr 107; Ser 102; Arg 106
Glutathione reductase	**-1.8**	**7:** Lys 348; Ser 360; Leu 349; Arg 352; Lys 361; Phe 318; Gln 319
Copper-zinc superoxide dismutase complexed	**-1.8**	**7:** Val 7; Val 148 (A and F chains); Cys 6; Asn 53; Asp 52; Gly 51
DNA-binding domain of human myelin-gene regulatory factor (MYRF)	**-1.9**	**8:** Gln 388; Phe 393; Phe 472; His 471; Asn 391; Cys 387; Lys 390; Lys 389
Human glial fibrillary acidic protein 1B	**-1.8**	**6:** Arg 173; Ala 176 (G and H

domain	chains); Tyr 172 (G and H chais); Glu 175

Abbreviations: Gly: Glycine; Ile: Isoleucine; Glu: Glutamic acid; Thr: Threonine; Leu: Leucine; Ser: Serine; Phe: Phenilalanine; His: Histidine; Cys: Cysteine; Gln: Glutamine; Arg: Arginine; Lys: Lysine; Val: Valine; Asn: Asparagine; Asp: Aspartic acid; Ala: Alanine; Tyr: Tyrosine.

Recent studies suggest that MeHg chronic exposure to even low levels of mercury can lead to various toxicities, including cardiovascular, reproductive, and developmental toxicity, neurotoxicity, nephrotoxicity, immunotoxicity, and carcinogenicity (Zahir *et al.*, 2005; Genchi *et al.*, 2017). Among the studied proteins, FABP1 and MYRF exhibited more negative FBE values, suggesting a greater potential for their functions to be compromised. FABP1 plays a crucial role in digestive physiology by participating in the cytoplasm's fatty acids metabolism, facilitating the transport, storage, and use of fatty acids. MYRF is essential for the functioning of the nervous system, as it acts as a transcription factor responsible for the differentiation of oligodendrocytes and myelination of the central nervous system. Based on these findings, the biological systems most affected by mercury can be the digestive and nervous systems, being the most urgent candidates for further investigation. Molecular docking has proven to be a valuable tool in directing mercury toxicology studies by enabling a more specific selection of target proteins.

4 CONCLUSION

This article investigated proteins related to different human systems to assess their potential interactions with mercury. The docking simulation revealed that the binding energies of the MeHg-protein complex were very similar, suggesting that the toxic effects of mercury could potentially impact multiple physiological systems in humans. Therefore, molecular docking proved a valuable tool in assisting toxicological studies, providing insights into the potential interactions between mercury and various proteins in the human body.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Author contributions

Estela Fernandes e Silva: Conceptualization; Methodology; Visualization; Writing–original draft; Eduarda Medran Rangel (corresponding author): Conceptualization; Methodology; Writing–original draft; Daiana Kaster Garcez: Conceptualization; Methodology; Writing–revision and editing; Karine Laste Macagnan: Conceptualization; Methodology; Writing–revision and editing; Adrize Medran Rangel: Conceptualization; Methodology; Writing–revision and editing; Louise Vargas Ribeiro: Conceptualization; Methodology; Writing–revision and editing; Paula Fernandes e Silva: Conceptualization; Methodology; Writing–revision and editing; Tainã Figueiredo Cardoso Conceptualization; Methodology; Writing–original draft; Writing–revision and editing; Timóteo Matthies Rico: Conceptualization; Methodology; Writing–original draft; Writing–reviewing and editing

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