ORIGINAL PAPER

MYOGLOBIN *vs.* HEMOGLOBIN BLOCKADE MODEL RELATED SMOKE GAS INHALATION - A COMPUTATIONAL ANALYSIS

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Abstract. *To identify the pattern of myoglobin compared to hemoglobin blockade by the combustion products contained in the fire smoke using computational chemistry tools. The myoglobin unavailability can be a determining factor of the severity and consequences of smoke poisoning, generating the inability of patients to attempt evacuate themselves, compromising myocardial function, leading to crush syndrome-like effects and increasing of multi-organ failure to the victims of mass burn casualties event. In this study, it was used quantum chemical calculations performed with the Gaussian program suite using DFT/B3LYP/6-311G level of theory to optimize molecular geometries, calculate the molecular electrostatic potential, and obtain the vibrational spectrum. These calculations were applied to the myoglobin and hemoglobin model, thus studying their binding to the essential components of toxic fire smoke. Comparing the structural descriptors – frontier molecular orbitals, energy difference, electric dipole moment (μ), was obtained the highest values as belonging to hydrogen cyanide (2.9), which may explain its increased reactivity, meaning strong interaction with both myoglobin and hemoglobin, followed by hydrochloric acid (1.03) and carbon monoxide (0.122). Within the framework of complex intoxication generated by the inhalation of fire smoke, myoglobin is blocked in a temporal manner and according to a very similar pattern to hemoglobin. It follows that the significant rhabdomyolysis found in these patients is due not only to hypoxemia but also to the primary unavailability of myoglobin, and myocardial damage is also multifactorial. Although slightly discussed, the calculation of different structural and geometric descriptors for hydrochloric acid reveals for hydrochloric acid high values, suggesting binding affinities comparable to those of carbon monoxide. Molecular modeling programs allow for new approaches and can identify parameters or areas of their reference that influence the management of patients intoxicated with fire smoke components.*

Keywords: *fire smoke inhalation; carbon monoxide intoxication; cyanide poisoning, myoglobin; computational chemistry.*

1. INTRODUCTION

The toxic nature of the smoke given off by a fire is known since ancient times, even though burns were considered the only feared consequence of fire for a long time. It took a

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long time to demonstrate that the sublethal effects of fumes can affect fire survival [1, 2], and very rarely was there solid and readily available pertinent data on which to base fire safety decisions [3]. It is difficult to inventory all the components of smoke from a fire. Experimental studies on the thermal degradation of materials have identified many products and isolated over 150 different molecules in the blood of deceased victims [4], mainly as a consequence of items in rooms that now have hazardous chemical compositions [5]. According to the classification of fires by the nature of combustible substances [6, 7] involved in the combustion process, cyanide can be liberated during the combustion of products of A class [8] (solid fuels the combustion of which occurs with embers formation, rubber and plastic products which do not melt with heat) and class B, (liquid or solid fuels burning in the melted state and plastics which melt quickly with heat) [9, 10].

During the past 50 years, synthetic polymers have been introduced in buildings in very large quantities. Many contain nitrogen or halogens, resulting in the release of hydrogen cyanide and inorganic acids in fire smoke as additional toxic threats [11-17]. As it is well known, domestic fires are, at the same time, the most common cause of cyanide poisoning according to American Association of Poison Control Centers [17, 18].

As a visible product of most combustion materials, smoke consists of unburned solid particles of the burning material, vapors and suspended gases, giving a characteristic color, smell, and taste. The color of the smoke varies depending on the burned material, from light blue, in the case of efficient combustion, to opaque black [19] during the combustion of high molecular weight hydrocarbons [20].

Many laboratory devices have been developed to measure the lethality of smoke [21, 22]. Still, none of them, reflects relevant fire conditions, nor have been tested in real fire situations to determine the effect of the smoke produced. Some of them use an algebraic equation (N-gas equation) to predict the deadly toxic potency at various concentrations of a small number of gases (CO, CO_2, HCN, HCl, HBr, low oxygen) released during combustion and based on data from rat exposure [23,24] to these types of gases, individually and/or in combination. However for flashover fires, the N-gas equation must be corrected for large amounts of carbon monoxide resulting from poor ventilation of the burned spaces. Therefore, the assumption that the irritant and asphyxiant effects of gases are immediate remains to be verified, and the combining equations of the flue gases to produce sublethal effects are generic and have not been validated.

On the other hand, computational chemistry uses methods of theoretical chemistry, integrated into efficient computer programs, to calculate molecular structures and properties because the many-body quantum analysis cannot be solved otherwise, much less experimentally [25]. Physicochemical descriptors represent valuable information regarding chemicals [26], which result from quantum chemical calculations computationally performed with various software, and the modeling of the structures of biological macromolecules allows in-depth study of molecular functional characteristics [27-30].

The study hypothesizes that the myoglobin blockage by the combustion products present in toxic smoke, although less studied than the one of hemoglobin, is a critical determinant aspect of the severity and consequences of smoke intoxication, causing the inability of patients to rescue themselves, compromising myocardial function, crush syndrome-like effect and increasing multi-organ failure in mass burn casualties situations. The first macromolecules to be shaped using computational chemistry were DNA double helix and myoglobin [31, 32], so, enabling the application of computational chemistry methods to study the conformations, structural models and binding energies of myoglobin and hemoglobin with various toxicants in fire smoke, are possible and can provide working tools for accurate clarification of the phases, poisoning mechanisms of making, not only

hemoglobin but also myoglobin [33], the factors affecting the processes, as well as the methods of reversing the toxicity, in complete safety for the patients.

2. MATERIALS AND METHODS

Regarding computational chemistry, although it is usually used to validate aspects experimentally observed, it can sometimes predict behaviors or interactions not yet specified or clinically expressed, without exposing the patient, using molecular geometries, and accurately measuring and/or calculating potentials of binding and bond energies, site configuration, responsible amino acids, and correlating these data with the biological activity of the molecules. In this vein, here, can be explored the hypothesis that the blockade of myoglobin and hemoglobin function, under the conditions of a complex toxic atmosphere, can be clarified by using theoretical chemistry methods specific to computational chemistry, determining for each of the main compounds of the smoke, the type and energy of bonds, the binding sequence, the stability, and reactivity specific to hemoglobin and respectively myoglobin.

Quantum chemical calculations were performed using Gaussian program suite at DFT/B3LYP/6-311G optimization to optimize molecular geometries, to calculate the molecular electrostatic potential and obtaining vibrational spectrum. Applying these calculations to the myoglobin and hemoglobin model, we were able to study their binding to the essential components of toxic fire smoke: carbon monoxide, carbon dioxide, hydrogen cyanyde, hydrochloric acid, substances whose concentrations in the victims' blood are of clinical interest [34], all of them being strong predictors of operational risk [35], mortality, long-term morbidity, and length of hospital stay [36-42].The calculation of the molecular geometry in the singlet ground state, the electronic structure and the total energy of the molecule, were based on the hybrid model of the change of correlation functional, B3LYP (Becke 3 Lee Yang Parr), which expresses the contribution of the correlation energies resulting from DFT formalism (dispersion free density functional theory) and change energy provided by Hartree-Fock models.

Molecular geometries have been shaped and optimized using the Gaussian program suite optimization (Fig. 1); utilizing these geometries made it possible to obtain data on the electronic structure of the studied substances (molecular electronic levels, electronic population, dipole moment, net electric charges of atoms, energy partitions by type of chemical bonds or interactions, bound order or free valence, etc.).

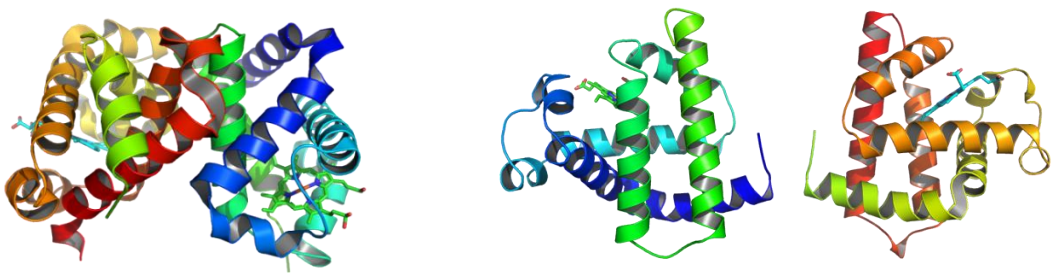


Figure 1. Optimized molecular structure of hemoglobin (left) and myoglobin (right).

2.1. LIGANDS PREPARATION AND DESCRIPTOR CALCULATIONS

Three-dimensional coordinates of all ligands was generated using the Gaussian program suite using DFT/B3LYP/6-311G level of theory. The molecular quantum calculations of molecular geometries were performed using the MOPAC 2016 program. The output data contains physico-chemical information about selected molecules.

2.2. TARGETS PREPARATION

The X-ray crystal structure of the targets was retrieve like target.pdb files from the major protein databases Protein data Bank [36] and optimized with the ModRefiner software [43]. The targets code are the following: hemoglobin (3WTG code, resolution 2.3 A) and myoglobin (2SPL code, resolution 1.7 A). The preparation of targets involves adding all the polar hydrogens, delete water and computing the Gasteiger charge.

2.3. DOCKING PROTOCOL

Molecular docking is an efficient computational method which can rapidly calculate the binding potential of a small molecule/drug candidate to a target. The molecular docking analysis was performed using the Autodock 4.2.6 software together with the molecular viewer and graphical support AutoDockTools. Docking simulations involved hemoglobin/myoglobin targets at physiological blood pH (7.4). In future studies, it is possible to simulate the type and energy of ligand-target binding in different degrees of blood acidosis/alkalosis. In the docking protocol the grid box was created using Autogrid 4 with 120×120×120 Å in x, y and z directions with 1 Å spacing from the target molecule center for proteic targets. For the docking process was used the Lamarckian genetic algorithm (Genetic Algorithm combined with a local search), with a population size of 150, maximum number of 2.5 x 106 energy evaluations, rate of gene mutation 0.02 and a number of 50 runs. The other docking parameters were used from defaults settings and all the calculations were performed in vacuum. The calculations were achieved in triplicate and expressed the results as averages. All Autodock results were exported in the PyMOL [44] and Accelrys Discovery Studio 4.2 Client molecular visualization system [45].

3. RESULTS AND DISCUSSION

3.1. RESULTS

The biological activity of chemical molecules occurs as a result of the interaction between toxic substance (L) and biological receptor (R), which can be synthetized as follow:

$$L+R\sim[L\text{-}R]\sim\text{biological response.}$$

The interaction is often "nonspecific", purely chemical, physical or physicochemical. For this reason, and for a more detailed picture of their interaction with biological substrate, was described the chemical structure of the substances by several parameters that form the "interface" for correlating chemical or biological activity with the chemical structure of the target substances.

Several structural descriptors were calculated contributing to the biological activity manifested by the studied compounds. Frontier orbitals, HOMO (the highest energy level in the molecule occupied by electrons) and LUMO (Lowest Unoccupied Molecular Orbital), expresses how a molecule will interact with a biological receptor through electron transfer processes involving the OMO layer (Occupied Molecular Orbitals) or by electron acceptance processes involving the UMO layer (Unoccupieed Molecular Orbitals) (Fig. 2).

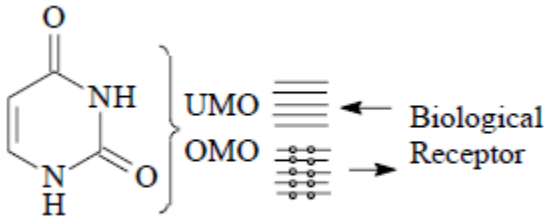


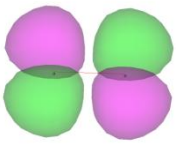
Figure 2. Ligand – acceptor interaction by electron transfer.

Interactions between toxic substance and the receptor can occur by electron transfer in both directions:

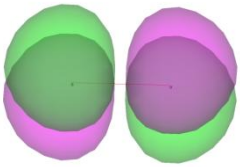
ligand (OC-MO) => receptor and ligand (UN-MO) <= receptor (Fig. 2).

Fig. 3 shows the frontier orbitals of the studied molecules, where green represents positive polarity, and purple negative polarity.

Oxygen

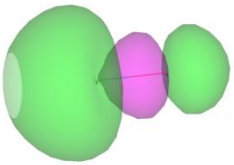


E_{HOMO} = -10.7335 eV

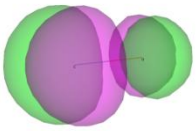


E_{LUMO} = -0.9821 eV

Carbon monoxide

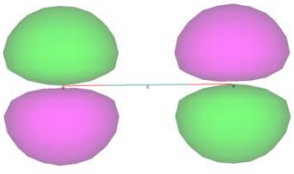


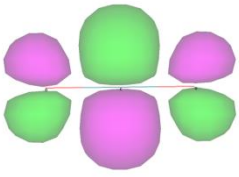
E_{HOMO} = - 13.0278 eV



E_{LUMO} = 1.000 eV

Carbon dioxide





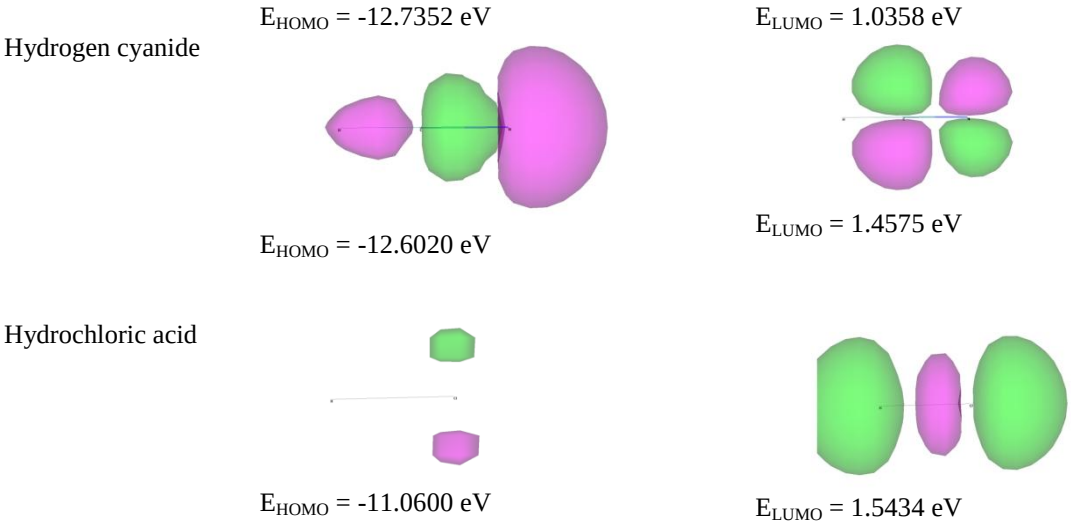


Figure 3. **Frontier molecular orbitals of the studied molecules.**

The study of molecular areas for the two types of molecular orbitals shows the contribution of atomic orbitals to their formation. The value of the E_{HOMO} descriptor allows an evaluation of the donor properties of a molecule, respectively, of its oxidation tendencies. Instead, the value of the E_{LUMO} descriptor allows the evaluation of the accepting properties of a molecule, respectively of its reduction tendencies. Thus, molecules for which E_{HOMO} has a maximum value are the most susceptible to electrophilic attack, and those for which E_{LUMO} has a maximum value are more susceptible to nucleophilic attack. The energy difference between HOMO and LUMO levels ($\Delta E = E_{LUMO} - E_{HOMO}$) is also a chemically important molecular descriptor that explains the stability of the molecule, a low value, indicating that the molecule is highly reactive. The most stable molecule is hydrogen cyanide, followed by carbon monoxide, hydrochloric acid, and carbon dioxide (Table 1) from the values presented in the Table. 1

Table 1. HOMO and LUMO descriptors for the main toxic smoke compounds

Substance	**ΔE [eV]**	**λ**	**η**
Oxygen	9.7514	5.8578	4.8757
Carbon monoxide	14.0278	6.0139	7.0139
Carbon dioxide	11.6994	5.8676	5.8497
Hydrogen cyanide	14.0595	5.5722	7.0297
Hydrochloric acid	12.6034	4.7583	6.3017

Absolute electronegativity (λ) and absolute hardness (η) [46, 47]. The sum and difference of the energies of frontier molecular orbitals are correlated with the chemical reactivity of molecules by the quantum descriptors of global reactivity:

$$\lambda = -E_{LUMO}+E_{HOMO}/2$$

$$\eta = E_{LUMO}-E_{HOMO}/2$$

The tendency of elements to donate or accept electrons is described by the electronegativity χ. Rigidity (hardness) is a quantity that describes the opposition of an atomic or molecular system to the variation in the electronic density in the system. Carbon monoxide has the highest electronegativity, so the tendency to donate – accept electrons (as can be seen in the Table 2), and hydrocyanic acid has the highest stiffness value.

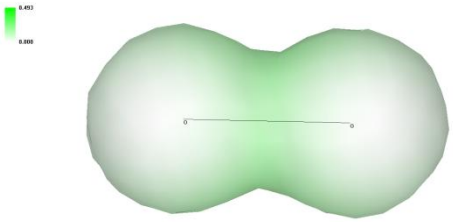
Electric dipole moment (μ), is a molecular parameter resulting from calculations that reflects the partial separation of the electric charge into the molecule. This molecular descriptor is predictor of the chemical reactivity of molecules, beaing a measure of the molecular system polarization. Table 2 shows the dipole moment values for the studied molecules [48].

Table 2. The values of the dipole moment for the main components of the smoke

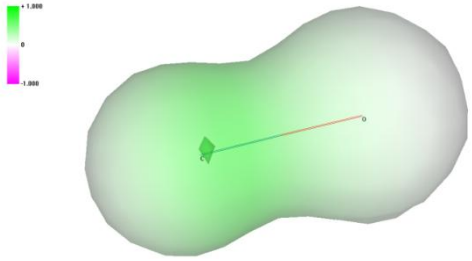
Substance	M [g/mol]	Dipol moment [D]
Oxygen	32	0
Carbon monoxide	28	0.122
Carbon dioxide	44	0
Hydrogen cyanide	27	2.9
Hydrochloric acid	36.5	1.03

Therefore, the results that the highest value of the dipole moment belongs to hydrogen cyanide, which may explain its increased reactivity, that is, the strong interaction with both myoglobin and hemoglobin, followed by hydrochloric acid and carbon monoxide [49].

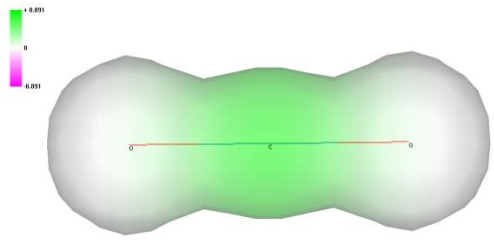
The medicinal substance – biological receptor interaction, an interaction that gives rise to a corresponding biological response, is possible only for a certain shape of the molecule and a certain distribution of electron density in the molecule that generates electron-rich regions.



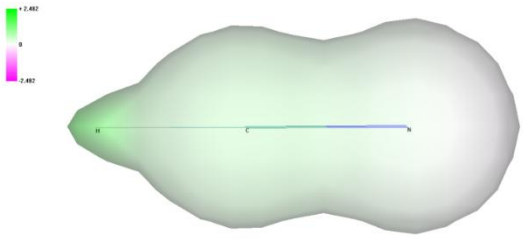
Molecular electrostatic potential surface of oxygen



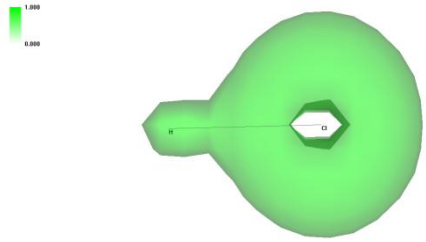
Molecular electrostatic potential surface of CO



Molecular electrostatic potential surface for carbon dioxide



Molecular electrostatic potential surface for hydrogen cyanide



Molecular electrostatic potential surface of hydrochloric acid

Figure 4. **Molecular electrostatic potential surface for main smoke compounds.**

Based on the molecular property called electrostatic potential, the three-dimensional map of the electron density was obtained, the different values of the electrostatic potential are marked by different colors (Fig.4). It can be used for the quantitative assessment of lipophilicity because it characterizes the polarity of a certain region on the van der Waals surface of the molecule. Regions with high values of the potential strongly attract water molecules, and regions with low values do not attract them, and can be considered hydrophobic.

The three-dimensional model (Fig. 4) that indicates the spatial distribution of electrons in chemical molecules, highlights the following: the size and shape of the molecules, which exclude steric hindrance, allow a better interaction; there are reactive centers on the molecular surface that correspond to the most negative values of the molecular electrostatic potential.

Table 3. **Connolly descriptors value of the main compounds of smoke**

Substance	**CSAA [Å^2]**	**CSEV [Å^3]**
Oxygen	49.05	31.35
Carbon monoxide	57.74	40.19
Carbon dioxide	49.06	31.36
Hydrogen cyanide	61.10	43.45
Hydrochloric acid	54.50	37.64

Among the molecular descriptors that effectively characterize the shape of the ligand molecule - shape that plays an essential role in the ligand (medication) - biological receptor interaction - are Connolly surface area accessible to the solvent (CSAA) and Connolly volume of solute excluded from the solvent (CSEV). From data presented in Table 4 is observed that the highest value of CSAA both in the case of myoglobin and that of hemoglobin belongs to hydrocyanic acid, followed by carbon monoxide, which explains the severity of smoke poisoning and the incomplete reversal when oxygen is administered, even with high flow.

Automated docking is widely used for prediction of biomolecular complexes in structure/function analysis and in molecular design. Dozens of effective methods are available, incorporating different trade-offs in molecular representation, energy evaluation, and conformational sampling to provide predictions with a reasonable computational effort. AutoDock combines an empirical free energy force field with a Lamarckian Genetic Algorithm, providing fast prediction of bound conformations with predicted free energies of association [50]. The force field evaluates binding in two steps. The ligand and target start in an unbound conformation. In the first step, the intramolecular energetics are estimated for the transition from these unbound states to the conformation of the ligand and target in the bound state. The second step then evaluates the intermolecular energetics of combining the two components in their bound conformation. The force field includes six pair-wise evaluations (V) and an estimate of the conformational entropy lost upon binding (ΔS_{conf}) [50]:

$$\otimes G = (V_{boundL-L} - V_{unboundL-L}) + (V_{boundP-P} - V_{unboundP-P}) + \left(V_{boundP-L} - V_{unboundP-L} + \otimes S_{conf}\right)$$

where L refers to the “ligand” and P refers to the “protein” in a ligand-protein docking calculation. Each of the pair-wise energetic terms (Table 4) includes evaluations for dispersion/repulsion, hydrogen bonding, electrostatics, and desolvation.

Table 4. The value of the ligand-target binding energy [kcal/mol]

Ligand	HMG	MGB
Oxygen	-0.99	-0.98
Carbon monoxide	-2.84	-2.81
Carbon dioxide	-0.1	-0.19
Hydrogen cyanide	-2.89	-2.87
Hydrochloric acid	-2.33	-2.31

From the ligand-target binding energy value (kcal/mol) it can see that there are no significant differences between the ligand-hemoglobin/ ligand/ myoglobin stability. This argues the assumption that the unavailability of myoglobin occurs independently and in addition to the blocking of hemoglobin by cyan compounds and carbon monoxide, representing a complementary pathway of muscle destruction and myocardial suffering, which paraclinical investigations frequently identify (increased biomarkers of myocardial necrosis and muscle, myocardial contractility impairment, left ventricular ejection fraction decrease) [51-53].

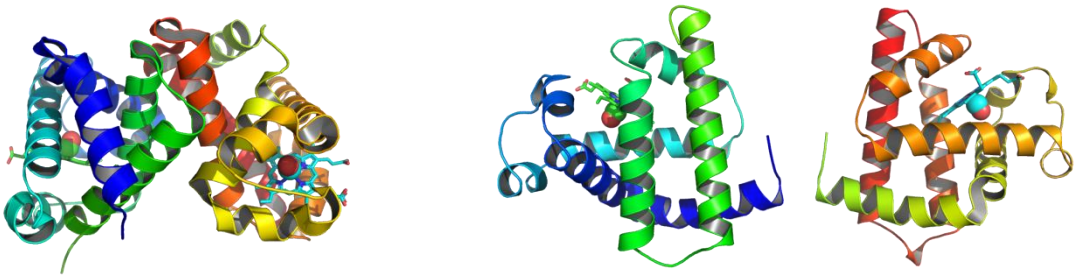


Figure 5.1 3D Target-ligand interaction map.

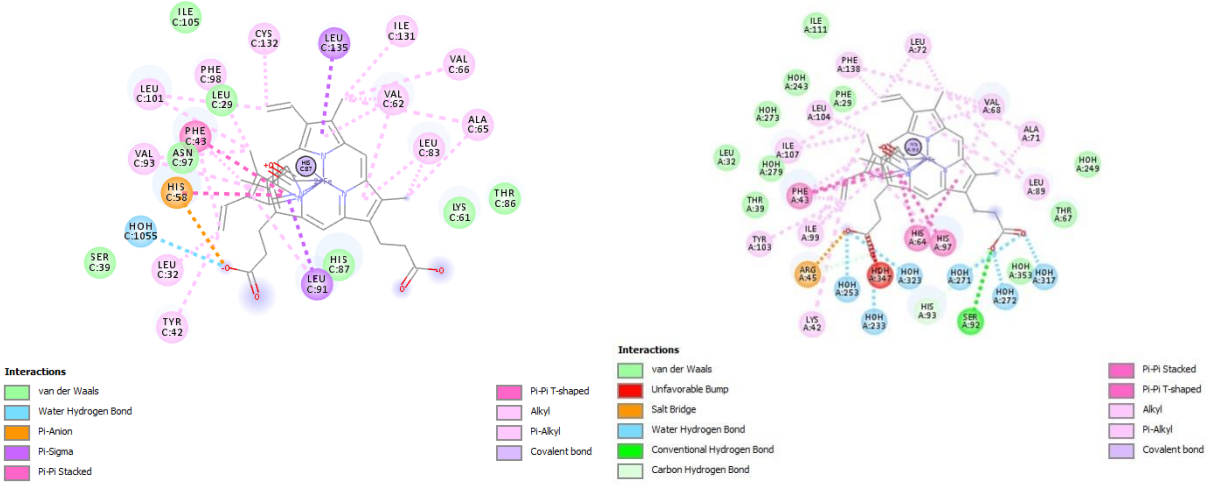


Figure 5.2 2D Target-ligand interaction map.

In addition, by using Autodock 4.2.6 redocking as robust method, it was obtained a very low RMSD values (all of them lower that 0.1 Å). Correlating the value of ligand descriptors with the stability of ligand-target complexes, it can observe that the dipole moment, Connoly coefficients, frontier orbitals are predictors in anticipation of the severity of intoxication [54], intricate mechanisms respectively direct muscle damage and hypoxic secondary damage [55]. 3D and 2D - target-ligand interaction map for carbon monoxide are shown in Fig. 5.1 and Fig. 5.2 (hemoglobin - carbon monoxide left, and myoglobin - carbon monoxide right). Although the binding of hemoglobin-carbon monoxide and myoglobin-carbon monoxide is different, the stability of the ligand-target complexes is similar (Table 4).

3.2. DISCUSSION

The impossibility of reproducibility of major events in general, all the more so of the reconstitution of the toxic atmosphere, the conditions of initiation, propagation and maintenance of mass fires, and the conditions of temperature and pressure, even in high-performance laboratories and simulators, lead to significant difficulties to perform typical clinical trials in emergency department or prehospital, on mass burns incidents. In addition, retrospective observational studies are extremely unlikely to enroll a substantial number of patients, with similar current and background pathologies profiles of comparable ages under ethicallly binding conditions, so that they become scientifically relevant, all the more so when these are victims of catastrophic events [56].

The benefit of integrating modern molecular research concepts, practices and techniques into elucidating the intimate mechanisms of fire smoke poisoning through computational chemistry-specific models and techniques [57, 58] may be to obtain relevant information in real-time without the use of living organisms.

There are currently protocols for dealing with different types of emergencies in special circumstances [59], including some poisoning and burns. however, there is still no consensus on specific smoke poisoning targeting and there is no standardisation of integrated prehospital and emergency department management of fire smoke poisoning in patients from civil, industrial, domestic or deliberate events, with a high volume of burn patients [60-62]. The fact that myoglobin is particularly blocked and unavailable during smoke poisoning is an important aspect that may influence the therapeutic approach and risk stratification trough muscle and myocardial damage severity. The findings on the affinity of hydrochloric acid for both myoglobin and hemoglobin bring to light the irritating effects of hydrochloric acid but also its cellular toxicity, in particular on myoglobin and oxygen transport.

The results of computational modelling may therefore be tactical reasoning tools for the re-standardisation of smoke poisoning management mainly for substances whose chemical determination by emergency laboratory analysis is not widely available or does not provide the capital, relevant, relevant information required in emergency clinical practice, sufficiently rapidly [63] to be decisive in improving prognosis.

4. CONCLUSIONS

It can state that molecular modeling programs allow the adoption of new approaches and identification of parameters (pressures and concentrations of oxygen, humidity, temperature), or their reference areas, that may influence and support improvement of the management of patients intoxicated with components of smoke. Within the framework of complex intoxication caused by inhaling toxic smoke, myoglobin is blocked in a temporal manner and follows a very similar pattern to hemoglobin. Although little discussed in the literature, the calculation of different characteristic structural and geometric descriptors for hydrochloric acid shows high values, suggesting binding affinities comparable to those of carbon monoxide. It can conclude that the significant rhabdomyolysis found in these patients is due not only to hypoxemia but also to the primary unavailability of myoglobin, and myocardial damage should also be multifactorial.

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