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IN SILICO PREDICTING METABOLIC ACTIVATION OF METRONIDAZOLE

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Abstract: *Metronidazole is an antimicrobial drug with wide spectrum of activity. The aim of this work is to predict the probable metabolic activation of metronidazole in the liver (in vivo and in vitro rat) and to determine the protein and DNA binding of its metabolites by OECD QSAR Toolbox. The parent structure of metronidazole can bind to DNA (Radical mechanism via ROS formation and S_N1 mechanism of action) but it cannot bind to protein and experimental metabolic pathways of action were not observed for rat in vivo and in vitro. The generated metabolites after hepatic metabolic activation simulator for both conditions (in vivo and in vitro rat) are nine and seven, respectively. The reactive metabolites for both (in vivo and in vitro) have different mechanisms of action (Radical mechanism via ROS formation, S_N1, $A_N2$and S_N2) by DNA binding. Some reactive metabolites are with the following mechanism of action (Schiff base formation) by protein binding.*

Keywords: metronidazole, prediction, metabolism, liver, OECD QSAR Toolbox

INTRODUCTION

Metronidazole is a five-nitroimidazole derivative with antibacterial and antiprotozoal activity. The chemical structure of metronidazole is presented in Fig.1. Its wide spectrum of activity includes different Gram positive and Gram negative bacteria, protozoa and microaerophiles such as *Clostridium spp, Clostridium ramosum, Actinomyces spp, Propionibacterium propionica, Bacteroides spp, Prevotella spp, Porphyromonas spp, Fusobacterium spp, Entamoeba histolytica, Giardia lamblia, Trichomonas vaginalis, Helicobacter pylori* and etc. (Connor B. Weir & Le K. Jacqueline, 2020). This spectrum of activity is a reason for different lines of application of metronidazole: bacterial vaginosis, severe abdominal diseases, pelvic inflammatory diseases, dental infections, human and animal bites, diabetic foot infection (Sonja Löfmark, Edlund Charlotta & Nord Carl Erik, 2010). Metronidazole have been extensively explored as hypoxic cell radiosensitizers (Asquith J. C., Foster J. L. & Willson Robin, 1974) and as a tool for targeted cell ablation in development studies (Curado Silvia, Stainier Y. R. Didier & Anderson M. Ryan, 2008).

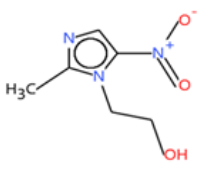


Fig.1. Structure of Metronidazole.

Metronidazole is a prodrug, which is weakly active before transformation into reactive intermediates with multiple targets. Mode of action of metronidazole is not related to any specific mechanisms to uptake such as transporters. It depends on metabolic activity providing an energized membrane (Müller M., Gorrell E. Thomas, 1983). It could be described in four main steps: entry of the drug into cell, reductive metabolic activation to cytotoxic intermediates, and interaction of the short-lived intermediates with intracellular targets components and nonenzymatic decomposition of unreacted intermediates into products (Connor B. Weir & Le K. Jacqueline, 2020).

The liver is a dynamic, heterogeneous organ highly regulating by physiological control (Hristova N., Dobrev D., Dimitrova D. & Mihaylova S., 2019). Metronidazole is extensively metabolized in the liver to two major oxidative metabolites: 1-(2- hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole6 and 1-aceticacid-2-methyl-5-nitroimidazole (Pendland S. L., Piscitelli S. C. & Schreckenberger C. Paul. (1994) are illustrated on Fig.2.

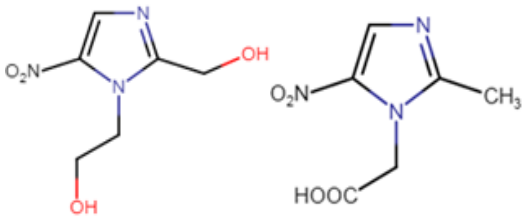


Fig. 2. Chemical structures of two main metabolites of metronidazole.

Despite the considerable progress in the understanding of the mechanisms of liver toxicity, in many cases, researchers are still unable to design no hepatotoxic drugs rationally; the effect of the dose administered is also a major obstacle in this respect. Presently, there are no universal *in vitro (*molecular or cellular) screening approaches that can be applied systemically for early identification of "hepatotoxic" molecular structures (Chan Katherine, 2008). The aim of this work is to predict the probable metabolic activation of metronidazole in the liver (in vivo and in vitro rat) and to determine the protein and DNA binding of its metabolites by OECD QSAR Toolbox.

EXPOSITION

Materials and methods

Compound. The parent compound metronidazole (https://www.sigmaaldrich.com/catalog/substance/metronidazole1711544348111?lang=en&region=BG) was supplied from a Sigma Aldrich and its chemical structure is shown in Figure 1.

Organisation for Economic Co-operation and Development (OECD) QSAR Toolbox. (Quantitative) Structure-Activity Relationships [(Q)SARs] are methods for estimating the properties of a chemical from its molecular structure and have the potential to provide information on the hazards of chemicals, while reducing time, monetary costs and animal testing currently needed. To facilitate practical application of (Q)SAR approaches in regulatory contexts by governments and industry and to improve their regulatory acceptance, the OECD (Q)SAR project has developed various outcomes such as the principles for the validation of (Q)SAR models, guidance documents, as well as the QSAR Toolbox (The OECD (Q)SAR Toolbox: https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm).

Liver metabolism (observed and simulator) – in vivo and in vitro for rat:

Observed rat in vivo metabolism. The observed (documented) metabolic pathways for 647 chemicals, extracted from the scientific literature, and associated with the in vivo biotransformations of xenobiotic chemicals in rodents (mostly rats) are stored in a database format that allows easy computer access to the metabolism information. This database includes structurally different chemicals of various functionalities such as aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, alcohols and phenols, carbonyl compounds, carboxylic acids and esters, nitro compounds, amines, organic sulphides, heterocyclic and, mostly, multi-functional chemicals etc. (The OECD (Q)SAR Toolbox: https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm).

In vivo rat metabolism simulator. The current in vivo rat liver metabolic simulator (transformation table) represents electronically designed set of 671 structurally generalized, hierarchically arranged abiotic and enzymatic transformation reactions, which are characteristic for the metabolism for in vivo experimental systems such as rodent (mostly rat). The principal applicability of this simulator is associated with the reproduction as well as the prediction of the metabolic activation reactions and pathways of xenobiotic chemicals, which may elicit in vivo genotoxicity effects (The OECD (Q)SAR Toolbox: https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm).

Observed rat liver S9 metabolism. The documented metabolic pathways for 261 chemicals observed with the use of in vitro experimental systems such as rodent (mostly rat) liver microsomes and S9 fraction are stored in a database format that allows easy computer access to the metabolism information. This database includes structurally different chemicals of various functionalities and fields of application such as aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, carboxylic acids and esters nitro compounds, amines, heterocyclic and multi-functional chemicals, etc. (The OECD (Q)SAR Toolbox: https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm).

Rat liver S9 metabolism simulator. The current in vitro rat liver metabolic simulator (transformation table) represents electronically designed set of 551 structurally generalised, hierarchically arranged biotransformation reactions, which are characteristic for the metabolism for in vitro experimental systems such as rodent (mostly rat) liver microsomes and S9 fraction. The principal applicability of this simulator is associated with the reproduction as well as the prediction of the metabolic activation reactions and pathways of xenobiotic chemicals, which may elicit in vitro genotoxicity effects such as bacterial mutagenicity and chromosomal aberrations (The OECD (Q)SAR Toolbox: https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm).

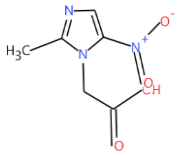
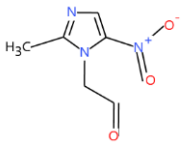
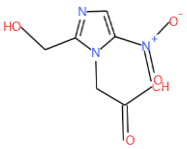
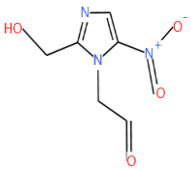
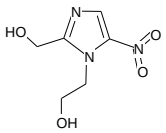
DNA binding by OASIS. The profiler is based on Ames Mutagenicity model part of OASIS TIMES system. The profiler consists of 85 structural alerts responsible for interaction with DNA analyzed in Ames Mutagenicity model. The scope of the profiler is to investigate presence of alerts within target molecules, which may interact with DNA (The OECD (Q)SAR Toolbox: https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm).

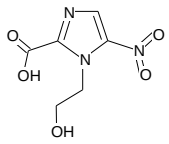
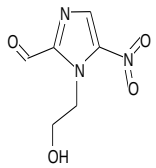
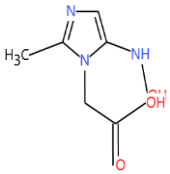
Protein binding by OASIS. The scope of the profiler is to investigate presence of alerts within target molecules responsible for interaction with proteins. The list of 112 structural alerts has been separated into 11 mechanistic domains. Each of the mechanistic domains has been separated into more than two mechanistic alerts. The profiling result outcome assigns a target to the corresponding structural alert, mechanistic alerts and domain (The OECD (Q)SAR Toolbox: https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm).

RESULTS AND DISCUSSION

OECD QSAR Toolbox software (version 4.3) has been used for predicting possible metabolites of the metronidazole in the liver (in vivo and in vitro rat) and its DNA and protein binding. The parent structure of the metronidazole cannot bind to protein but it can bind to DNA (Radical mechanism via ROS formation (indirect) (Conjugated nitroalkenes and five-membered aromatic nitroheterocyclics and S_N1 (Nucleophilic attack after reduction and nitrenium ion formation (Conjugated nitroalkenes and five-membered aromatic nitroheterocyclics). In the liver metabolism simulator (in vivo rat), nine metabolites were generated. Results of toxic prediction of metronidazole in liver (in vivo rat metabolism simulator) and its protein and DNA binding are presented in Table 1 (only, active metabolites are given).

Table 1. Possible liver metabolic activation of metronidazole by QSAR Toolbox (in vivo rat metabolism simulator).

Number of metabolites	Structure of metabolite	DNA binding by OASIS (mechanism of reaction)	Protein binding by OASIS (mechanism of reaction)
1		1) Radical mechanism via ROS formation (indirect) (Conjugated nitroalkenes and five-membered aromatic nitroheterocycles) 2) S_N1 (Nucleophilic attack after reduction and nitrenium ion formation (Conjugated nitroalkenes and five-membered aromatic nitroheterocycles))	No alert found
2		1) Radical mechanism via ROS formation (indirect) (Conjugated nitroalkenes and five-membered aromatic nitroheterocycles) 2) S_N1 (Nucleophilic attack after reduction and nitrenium ion formation (Conjugated nitroalkenes and five-membered aromatic nitroheterocycles))	Schiff base formation with carbonyl compounds (aldehyde)
3		1) Radical mechanism via ROS formation (indirect) (Conjugated nitroalkenes and five-membered aromatic nitroheterocycles) 2) S_N1 (Nucleophilic attack after reduction and nitrenium ion formation (Conjugated nitroalkenes and five-membered aromatic nitroheterocycles))	Schiff base formation with carbonyl compounds (aldehyde)
4		1) Radical mechanism via ROS formation (indirect) (Conjugated nitroalkenes and five-membered aromatic nitroheterocycles) 2) S_N1 (Nucleophilic attack after reduction and nitrenium ion formation (Conjugated nitroalkenes and five-membered aromatic nitroheterocycles))	No alert found
5		1) Radical mechanism via ROS formation (indirect) (Conjugated nitroalkenes and five-membered aromatic nitroheterocycles) 2) S_N1 (Nucleophilic attack after reduction and nitrenium ion formation (Conjugated nitroalkenes and five-membered aromatic	No alert found

		nitroheterocycles))	
6		1) Radical mechanism via ROS formation (indirect) (Conjugated nitroalkenes and five-membered aromatic nitroheterocycles) 2) S_N1- Nucleophilic attack after reduction and nitrenium ion formation (Conjugated nitroalkenes and five-membered aromatic nitroheterocycles)	No alert found
7		1) Radical mechanism via ROS formation (indirect)(Conjugated nitroalkenes and five-membered aromatic nitroheterocycles) 2) S_N1 (Nucleophilic attack after reduction and nitrenium ion formation (Conjugated nitroalkenes and five-membered aromatic nitroheterocycles))	Schiff base formation with carbonyl compounds (aldehyde)
8		1)Radical mechanism via ROS formation (indirect) (N-Hydroxylamine); 2) SN1(Nucleophilic attack after nitrenium ion formation (N-Hydroxylamine); 3) A_N2 (Carbamoylation after isocyanate formation (N-Hydroxylamine)); 4)S_N2 (Acylation (N-Hydroxylamine)).	No alert found

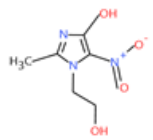
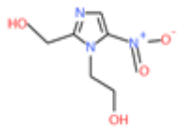
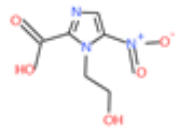
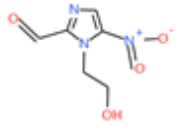
Eight metabolites of nine are reactive, i.e. alerts are found by DNA binding. The possible mechanisms of action of reactive metabolite are Radical mechanism via ROS formation (Conjugated nitroalkenes and five-membered aromatic nitroheterocyclics– seven metabolites and N-Hydroxylamines– 1 metabolite), SN1(Nucleophilic attack after reduction (Conjugated nitroalkenes and five-membered aromatic nitroheterocycles)-seven metabolites, SN1 (Nucleophilic attack after nitrenium ion formation (N-Hydroxylamine)-one metabolite), AN2 (mechanism of Carbamoylation after isocyanate formation (N-Hydoxylamines) and SN2(mechanism of Acylation (N-Hydoxylamines)).

Six of nine metabolites are not reactive and for three metabolites were found alerts by protein binding. The possible mechanism of action of reactive metabolites is Schiff base formation with carbonyl compounds (aldehydes).The metabolites number 2, 3and 7 have possibility for protein binding by Schiff base formation. It is due to newly formed carbonyl group in the probable metabolites. Nucleophilic groups of proteins and nucleic acids are target sites of their actions. Covalent binding to biological macromolecules can explain hepatotoxicity.

Schiff' bases are formed from the condensation of primary amines and active carbonyl group (aldehyde and ketone) by nucleophilic addition forming an imine. In this case, the probable metabolites 2, 3and 7 are possible to form Schiff base with the newly formed aldehyde group.

Results of toxic prediction of the metronidazole in liver (in vitro rat metabolism simulator) and its protein and DNA binding are presented in Table 2 (only, active metabolites are given).

Table 2. Possible liver metabolic activation of metronidazole by QSAR Toolbox in vitro rat liver metabolic simulator.

Number of metabolites	Structure of metabolite	DNA binding by OASIS (mechanism of reaction)	Protein binding by OASIS (mechanism of reaction)
1		1)Radical mechanism via ROS formation (indirect) (Conjugated nitroalkenes and five-membered aromatic nitroheterocycles) 2)S_N1 (Nucleophilic attack after reduction (Conjugated nitroalkenes and five-membered aromatic nitroheterocycles))	No alert found
2		1)Radical mechanism via ROS formation (indirect) (Conjugated nitroalkenes and five-membered aromatic nitroheterocycles) 2)S_N1(Nucleophilic attack after reduction (Conjugated Nitroalkenes and Five-membered Aromatic Nitroheterocycles))	No alert found
3		1)Radical mechanism via ROS formation (indirect) (Conjugated Nitroalkenes and five-membered Aromatic Nitroheterocycles) 2)S_N1(Nucleophilic attack after reduction (Conjugated Nitroalkenes and Five-membered Aromatic Nitroheterocycles))	No alert found
4		1)Radical mechanism via ROS formation (indirect) (N-Hydroxylamine) 2)S_N1(Nucleophilic attack after nitrenium ion formation(N-Hydroxylamine) 3) A_N2 (Carbamoylation after isocyanate formation (N-Hydroxylamine)) 4) S_N2(Acylation (N-Hydroxylamine))	No alert found
5		1)Radical mechanism via ROS formation (indirect) (Conjugated Nitroalkenes and five-membered Aromatic Nitroheterocycles) 2)S_N1(Nucleophilic attack after reduction (Conjugated nitroalkenes and five-membered aromatic nitroheterocycles))	Schiff base formation with carbonyl compounds (aldehyde)

The data analysis shows that five of seven metabolites are reactive. They have ability for DNA binding by different reaction mechanisms. The possible mechanisms of binding are Radical mechanism via ROS formation (Conjugated nitroalkenes and five membered aromatic

nitroheterocycles)-four metabolites and N-Hydroxylamine-one metabolite), S_N1 (Nucleophilic attack after reduction-four metabolites and after nitrenium ion formation-one metabolite), AN2 (mechanism of Carbamoylation after isocyanate formation) and S_N2(Acylation (N-Hydroxylamine)).

CONCLUSION

The generated metabolites after hepatic metabolic activation simulator for both conditions (in vivo and in vitro rat) are nine and seven, respectively. The reactive metabolites for both (in vivo and in vitro) have different mechanisms of action (Radical mechanism via ROS formation, S_N1, $A_N2$and S_N2) by DNA binding. Some reactive metabolites can bind to protein by Schiff base formation. The mechanism of interaction with different biomolecules have implications for the antimicrobial, mutagenic, and radio sensitizing actions of this drug. The observed data can be applied for further researches of metronidazole liver metabolic transformation.

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(The OECD (Q)SAR Toolbox: https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm).