Screening of virtual libraries for monoamine oxidase inhibitors

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Preface:

This dissertation is submitted in article format and contains an original research article. Those results not included in the article are presented in Chapters 3 and 4 of the dissertation. The article was submitted for publication to the academic journal Arzneimittelforschung/ Drug research. The author guidelines for the journal are included in annexure B. The research described in this dissertation was conducted by Ms. M. Barkhuizen at the North-West University, Potchefstroom campus.

Letters of agreement from the co-authors of the research article are included in annexure A.

Abstract:

The traditional view of drug design is that a single drug should interact with a single molecular target. As science progressed, there was an understanding that most drugs interact with more than one target and that multiple targets may be responsible for either adverse effects or additional therapeutic effects. The idea of polypharmacology, which suggests that the focus of drug design should shift from a single drug that interacts with a single target to a single drug that can have interactions with multiple targets and multiple therapeutic effects, revolutionized the drug discovery process. Discovering new drugs is a long and costly process with years of research and development and clinical trials required before the drugs reach the market for much needed therapeutic applications. By repurposing drugs that are already on the market for a new therapeutic target, the discovery process is accelerated significantly.

One such a target disease, for which there is a great need for new effective therapies, is Parkinson's disease (PD). PD is a progressive neurodegenerative disease that is caused by the death of dopaminergic neurons in the substantia nigra with the resulting loss of dopamine from the striatum. Degeneration in PD leads to varying degrees of motor difficulty and disability, along with other symptoms. Current therapies are focussed on symptomatic management and an improvement of the quality of life of patients, rather than on a cure.

There are several therapeutic targets that are currently used in the treatment of PD. One of those targets is the monoamine oxidase (MAO) enzymes, in particular the MAO-B isoform. The MAO enzymes are responsible for the metabolism of amine neurotransmitters, such as dopamine, and inhibition of MAO-B has proven to be an effective strategy to increase the dopamine levels in the brain. Clinically, selective MAO-B inhibitors are administered concurrently with levodopa (a precursor of dopamine) to increase the levels of dopamine derived from levodopa. This approach prolongs the beneficial effects of levodopa.

Because MAO-A is responsible for the breakdown of noradrenalin, adrenalin, serotonin and tyramine, non-selective and selective MAO-A inhibitors have therapeutic applications in other neurological and psychiatric disorders such as depression. MAO-A inhibitors, particularly irreversible inhibitors, are also notable from a toxicological point of view. Irreversible MAO-A inhibitors may lead to potentially dangerous effects when combined with serotonergic drugs and certain foods containing tyramine, such as cheeses and processed meats. Selective MAO-B inhibitors and reversible MAO-A inhibitors appear to be free of these interactions.

Based on the considerations above, this study aimed to identify clinically used drugs which also inhibit the MAO enzymes as a secondary pharmacological property. Such drugs may, in theory, be repurposed as MAO inhibitors for therapeutic use in the treatment of PD and depression. The identification of potential MAO-A inhibitory properties among clinically used drugs are of further importance since the irreversible inhibition of MAO-A may lead to dangerous effects when combined with certain drugs and foods.

To screen clinically used drugs for potential MAO-A and MAO-B inhibitory activities, a pharmacophore approach was followed. A pharmacophore model is a virtual 3D representation of the common steric and electrostatic features of the interaction between an enzyme and a ligand. By identifying hydrogen bond acceptor, hydrogen bond donor and hydrophobic interactions between a reference ligand and an enzyme, a model is created that can search databases for other molecules that would have similar interactions with the enzyme and arguably also act as ligands. This enables the screening of a large amount of molecules in a short amount of time. To assist in the identification of MAO inhibitors, pharmacophore models of the MAO enzymes were constructed using the known crystallographic structures of MAO-A co-crystallized with harmine, and MAO-B co-crystallized with safinamide. The Discovery Studio® software package (Accelrys) was used for this purpose.

In this study, virtual libraries of United States Food and Drug Administration (FDA) approved drugs and the United States Environmental Protection Agency (EPA) maximum daily dose databases were screened with pharmacophore models of MAO-A and MAO-B. Among the hits, 26 drugs were selected on the basis of availability and cost, and were subjected to *in vitro* bio-assays in order to determine their potencies (IC_{50} values) as inhibitors of recombinant human MAO-A and/or MAO-B. Among the drugs tested, 6 compounds exhibited inhibitory activity towards the MAO enzymes. Of the 6 compounds, pentamidine (IC_{50} = 0.61 µM for MAO-A and IC_{50} = 0.22 µM for MAO-B) and phenformin (IC_{50} = 41 µM for MAO-A) were selected for further analysis.

An examination of the recoveries of the enzymatic activities after dilution and dialysis of the enzyme-inhibitor complexes showed that both pentamidine and phenformin interact reversibly with the MAO enzymes. A kinetic analysis suggests that pentamidine acts as a competitive inhibitor with estimated K_i values of 0.41 µM and 0.22 µM for the inhibition of MAO-A and MAO-B, respectively. An analysis of the available pharmacokinetic data and typical therapeutic doses of phenformin and pentamidine suggests that the MAO inhibitory potencies (and reversible mode of action) of phenformin are unlikely to be of pharmacological relevance in humans. Pentamidine, on the other hand, is expected to

interact with both MAO-A and MAO-B at typical therapeutic doses. Because of its MAO-A inhibitory activity, pentamidine may thus, in theory, lead to a tyramine-associated hypertensive crisis when combined with tyramine-containing foods. However, pentamidine is unlikely to inhibit central MAO since it does not appear to penetrate the central nervous system to a large degree.

In an attempt to gain further insight into the mode of binding to MAO, pentamidine and phenformin were docked into models of the active sites of MAO-A and/or MAO-B. An analysis of the interactions between the enzyme models and the ligands were carried out and the results are discussed in the dissertation.

The results of this study show that the pharmacophore model approach may be useful in identifying existing drugs with potential MAO inhibitory effects. The search for new therapeutic MAO inhibitors, that can be used in the treatment of certain neurological disorders, including PD and depression, may be accelerated by employing a virtual screening approach. Such an approach may also be more cost effective than the *de novo* design of MAO inhibitors.

Keywords:

Monoamine oxidase, repurposing, Parkinson's disease, virtual screening, toxicology, enzyme inhibition

Uittreksel:

Die tradisionele beskouing van geneesmiddelontwerp was dat ’n enkele geneesmiddel net met ’n enkele molekulêre teiken interaksie ondergaan. Soos wat die wetenskap gevorder het, het die gedagterigting ontwikkel dat die meeste geneesmiddels met meer as een teiken interaksies het en dat meerdere teikens verantwoordelik mag wees vir beide die terapeutiese en newe-effekte. Die idee van polifarmakologie, wat voorstel dat die fokus van geneesmiddelontwerp behoort te verskuif vanaf ’n enkele geneesmiddel wat net met ’n enkele teiken ’n interaksie het, na ’n enkele geneesmiddel wat met verskeie teikens interaksies het om verskeie terapeutiese effekte te bewerkstellig, was verantwoordelik vir ’n revolusie in die geneesmiddelontdekkingsproses. Om nuwe geneesmiddels te ontdek is ’n lang en duur proses wat jare se navorsing, ontwikkeling en kliniese toetse vereis voordat geneesmiddels die mark bereik. Die geneesmiddelontdekkingsproses kan versnel word deur geneesmiddels wat alreeds op die mark is vir bestaande interaksies her aan te wend vir ’n nuwe terapeutiese teiken.

Een so ’n teikensiekte, waarvoor daar ’n groot nood vir nuwe, effektiewe behandelings is, is Parkinson se siekte (PD). PD is ’n progressiewe neurodegeneratiewe siekte wat veroorsaak word deur die afsterwe van dopaminergiese neurone in die substantia nigra en die gepaardgaande verlies aan dopamien. PD lei tot verskillende grade van motorgestremdheid en ander simptome. Huidige terapieë fokus op simptomatiese behandeling en ’n verbetering in lewenskwaliteit, eerder as op genesing.

Daar bestaan verskeie terapeutiese teikens wat tans gebruik word vir die behandeling van PD. Een van dié teikens is die monoamienoksidase-ensieme (MAO), veral die MAO-B-isoform. Die MAO-ensieme is verantwoordelik vir die afbreek van amien-neuro-oordragstowwe, soos dopamien, en die inhibisie van MAO-B is ’n effektiewe strategie om die dopamienvlakke in die brein te verhoog. Selektiewe MAO-B-inhibeerders word klinies gebruik saam met eksogeen toegediende levodopa (’n voorganger van dopamien) om die vlakke van dopamien wat uit die levodopa uit verkry word, te verhoog. Die benadering verleng die voordelige effekte van levodopaterapie.

MAO-A is verantwoordelik vir die metabolisme van noradrenalien, adrenalien, serotonien en tiramien. Beide nie-selektiewe en selektiewe MAO-A-inhibeerders word gebruik in die behandeling van neurologiese en sielkundige afwykings soos depressie. MAO-A-inhibeerders, veral onomkeerbare inhibeerders, is ook belangrik vanuit ’n toksikologiese oogpunt, want onomkeerbare MAO-A-inhibeerders kan gevaarlike interaksies hê indien dit met serotonergiese geneesmiddels en kossoorte wat tiramien bevat, soos kase en verwerkte

vleis, gekombineer word. Selektiewe MAO-B-inhibeerders en omkeerbare MAO-A inhibeerders toon nie dié interaksies nie.

Bogenoemde stellings in ag geneem, is met hierdie studie gepoog om klinies bruikbare geneesmiddels wat reeds in gebruik is, te identifiseer wat, benewens hulle primêre werking, ook die MAO-ensieme mag inhibeer as 'n sekondêre farmakologiese eienskap. Sulke middels kan teoreties heraangewend word as MAO-inhibeerders vir gebruik in die terapie van PD en depressie. Die identifisering van potensiële MAO-A-inhiberende eienskappe van middels wat reeds gebruik word, is ook belangrik omdat die onomkeerbare inhibisie van MAO-A tot gevaarlike interaksies met sekere geneesmiddels en voedsel mag lei.

'n Farmakofoorbenadering is gevolg om deur geneesmiddels wat klinies gebruik word, te sif vir middels wat moontlik MAO-A en MAO-B mag inhibeer. 'n Farmakofoormodel is 'n virtuele 3D-voorstelling van die algemene ruimtelike en elektrostatiese eienskappe van die interaksie tussen 'n ensiem en 'n ligand. Deur waterstofbinding ontvanger, waterstofbinding skenker en hidrofobiese interaksies tussen 'n verwysingsligand en 'n ensiem te identifiseer, word 'n model geskep wat gebruik kan word om deur ander databasisse te soek vir ander molekules wat soortgelyke interaksies met die ensiem sal hê en moontlik ook as ligande kan optree. Dit maak die sifting van groot hoeveelhede molekules in 'n beperkte tyd moontlik. Om te help met die identifisering van MAO inhibeerders, is farmakofoormodelle van die MAO-ensieme geskep met behulp van bekende kristallografiese strukture van MAO-A wat mede-gekristalliseer is met harmien en van MAO-B wat mede-gekristalliseer is met safienamied. Die Discovery Studio® sagtewarepakket van Accelrys is vir die doeleinde gebruik.

Vir die studie is virtuele biblioteke van die Verenigde State Voedsel en Geneesmiddel Administrasie se aanvaarde geneesmiddels en die Verenigde State Omgewingsbeskermingsagentskap se maksimum daaglikse dosis databasis gesif met die farmakofoormodelle van MAO-A en MAO-B. Van al die molekules wat deur die modelle geïdentifiseer is, is 26 geneesmiddels gekies op grond van beskikbaarheid en koste, en dié geneesmiddels is onderwerp aan *in vitro* biotoetse om hulle sterktes (IC_{50} waardes) as inhibeerders van rekombinante menslike MAO-A en MAO-B te bepaal. Van al die geneesmiddels wat getoets is, het 6 verbindings MAO-inhiberende aktiwiteit getoon. Van die 6 verbindings is pentamidien (IC_{50} = 0.61 µM vir MAO-A en IC_{50} = 0.22 µM vir MAO-B) en fenformien (IC_{50} = 41 µM vir MAO-A) gekies vir verdere analise.

'n Ondersoek na die herstel van die ensimatiese aktiwiteite, na die verdunning en dialise van die ensiem-inhibeerder-komplekse, toon dat beide pentamidien en fenformien omkeerbare interaksies met die MAO-ensieme ondergaan. 'n Kinetiese analise dui aan dat pentamidien as 'n kompeterende inhibeerder optree met geskatte K_i-waardes van 0.41 µM en 0.22 µM vir

die inhibisie van MAO-A en MAO-B onderskeidelik. ’n Analise van die beskikbare farmakokinetiese data en die tipiese terapeutiese doserings van pentamidien en fenformien dui daarop dat die MAO-inhiberende eienskappe (en omkeerbare binding) van fenformien waarskynlik nie van farmakologiese belang in mense is nie. Daar word egter verwag dat pentamidien interaksies met beide MAO-A en MAO-B sal hê teen normale terapeutiese dosisse. Omdat pentamidien MAO-A inhibeer, kan dit teoreties lei tot ’n tiramien-geassosieerde hipertensiewe krisis as dit gekombineer word met tiramien-bevattende voedsel. Dit is egter onwaarskynlik dat pentamidien MAO sentraal sal inhibeer omdat die mate waartoe pentamidien die senustelsel binnedring beperk is.

In ’n poging om verdere insig in die manier waarop binding aan MAO plaasvind te verkry, is pentamidien en fenformien vasgemeer in modelle van die aktiewe setels van MAO-A en/of MAO-B. ’n Analise van die interaksies tussen die ensiem-modelle en die ligande is uitgevoer en die resultate word in die verhandeling bespreek.

Die resultate van die studie toon dat die farmakofoormodel-benadering bruikbaar kan wees om bestaande geneesmiddels met potensiële MAO-inhiberende effekte te identifiseer. Die soektog na nuwe terapeutiese MAO-inhibeerders wat vir die behandeling van sekere neurologiese toestande, soos PD en depressie, gebruik kan word, kan versnel word deur ’n virtuele siftingsbenadering te volg. So ’n benadering mag ook meer koste-effektief wees as die *de novo* ontwerp van MAO-inhibeerders.

Sleutelwoorde:
Monoamienoksidase, heraanwending, Parkinson se siekte, virtuele sifting, toksikologie, ensiem-inhibisie

LIST OF ABBREVIATIONS

2-BFI	5-Isothiocyanato-2-benzofuranyl-imidazoline
3D	Three dimensional
4-HQ	4-Hydroxyquinoline
[ ]	Concentration of
A	
A	Actives
Aβ	β-Amyloid
Acc	Accuracy
AdoHcy	S-Adenosyl-homocysteine
AdoMet	S-Adenosyl-methionine
Ala	Alanine
APP	Amyloid precursor protein
Arg	Arginine
Asn	Asparagine
B	
Bcl2	B-Cell lymphoma 2
BDNF	Brain-derived neurotrophic factor
C	
COMT	Catechol-O-methyltransferase
Cys	Cysteine
D	
D	Dopamine receptor
DA	Dopamine
DMF	N,N-Dimethylformamide
Dopa	3,4-Dihydroxyphenylalanine
E	
E	Enzyme

EPA	United States Environmental Protection Agency
F	
FAD	Flavin adenine dinucleotide
FDA	United States Food and Drug Administration.
F-dopa	Fluorinated levodopa
G	
GABA	Gamma-aminobutyric acid
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GDNF	Glial cell line-derived neurotrophic factor
Gln	Glutamine
Gly	Glycine
GP	Globus pallidus
GPe	Globus pallidus externa
GPi	Globus pallidus interna
GSH	Glutathione
H	
HLA-DR	Human leukocyte antigen-DR
Hcy	Homocysteine
HRMS	High resolution mass spectrometry
Hsp	Heat shock protein
HTS	High-throughput screening
I	
I	Inhibitor
I_2	Imidazoline type 2 receptor
IC_{50}	Inhibitor concentration that produces 50% inhibition of an enzyme
IL-1	Interleukin-1
Ile	Isoleucine

K

K_i	Inhibitor constant
K_I	Equilibrium constant
K_M	Michaelis constant

L

I_{bal}	Balanced labelling performance
Leu	Leucine
LRRK2	Leucine-repeat rich kinase 2
Lys	Lysine

M

MAO	Monoamine oxidase
Met	Methionine
MPP^+	1-Methyl-4-phenylpyridinium
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mRNA	Messenger ribonucleic acid

N

n	Number of selected hits
N	Total number of hits
NGF	Neuronal growth factor
NMDA	N-Methyl-D-aspartate
NOS	Nitric oxide synthase
NTF	Neurotrophic factor

P

PD	Parkinson’s disease
PET	Positron emission tomography
Phe	Phenylalanine
PKC	Protein kinase C

R

ROS	Reactive oxygen species
ROC curve	Receiver operating characteristics curve
S	
S	Substrate
SD	Standard deviation
Se	Sensitivity
Ser	Serine
SN	Substantia nigra
SNpc	Substantia nigra pars compacta
SNpr	Substantia nigra pars reticularis
Sp	Specificity
T	
tHcy	Total homocysteine
Thr	Threonine
TNF-α	Tumor necrosis factor α
Tyr	Tyrosine
Trp	Tryptophan
U	
UCHL1	Ubiquitin carboxyhydrolase L1
V	
v_i	Initial velocity
V	Reaction velocity
V_{max}	Maximal velocity
Val	Valine
Y	
Ya	Yield of actives

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