Antihypertensive Drugs Metabolism: An Update to Pharmacokinetic Profiles and Computational Approaches

Aikaterini Zisaki1,2*, Ljubisa Miskovic1,2 and Vassily Hatzimanikatis1,2

1Laboratory of Computational Systems Biotechnology, Ecole Polytechnique Federale de Lausanne (EPFL), CH 1015 Lausanne, Switzerland; 2Swiss Institute of Bioinformatics, Quartier Sorge, Batiment Genopode, CH-1015, Lausanne, Switzerland

Abstract: Drug discovery and development is a high-risk enterprise that requires significant investments in capital, time and scientific expertise. The studies of xenobiotic metabolism remain as one of the main topics in the research and development of drugs, cosmetics and nutritional supplements.

Antihypertensive drugs are used for the treatment of high blood pressure, which is one of the most frequent symptoms of the patients that undergo cardiovascular diseases such as myocardial infarction and strokes. In current cardiovascular disease pharmacology, four drug clusters - Angiotensin Converting Enzyme Inhibitors, Beta-Blockers, Calcium Channel Blockers and Diuretics - cover the major therapeutic characteristics of the most antihypertensive drugs. The pharmacokinetic and specifically the metabolic profile of the antihypertensive agents are intensively studied because of the broad inter-individual variability on plasma concentrations and the diversity on the efficacy response especially due to the P450 dependent metabolic status they present.

Several computational methods have been developed with the aim to: (i) model and better understand the human drug metabolism; and (ii) enhance the experimental investigation of the metabolism of small xenobiotic molecules. The main predictive tools these methods employ are rule-based approaches, quantitative structure metabolism/activity relationships and docking approaches.

This review paper provides detailed metabolic profiles of the major clusters of antihypertensive agents, including their metabolites and their metabolizing enzymes, and it also provides specific information concerning the computational approaches that have been used to predict the metabolic profile of several antihypertensive drugs.

Keywords: Antihypertensive drugs, metabolism, computational approaches.

INTRODUCTION

Antihypertensive drugs are used for the treatment of high blood pressure, and more specifically, the primary and secondary hypertension. The complications of high blood pressure might trigger and/or cause several complex systematic cardiovascular disorders or syndromes such as stroke, unstable angina and myocardial infarction.

The most representative antihypertensive drugs are classified as: the Angiotensin Converting Enzyme inhibitors (ACE inhibitors), the beta-adrenergic blockers (β-blockers), the calcium (Ca2+) channel blockers and the diuretics. Additionally, the group of diuretics can be divided in four subgroups: potassium (K+) sparing diuretics, the loop diuretics, the thiazide and the thiazide-like diuretics (Fig. 1).

It has been comprehensively described that extensive variability of anti-hypertensive drugs plasma concentrations in patients, due to the P450-mediated drugs metabolism, has a great impact and influence on the clinical outcome as well as on the drugs' response [1]. Among the antihypertensive drugs, the ACE inhibitor captopril, the beta blockers alprenolol, carvedilol, metoprolol and propranolol, the calcium channel blockers diltiazem, felodipine, nimodipine nifedipine and verapamil and the potassium sparing diuretic spironolactone are known either for their response variability due to the participation of P450-dependent metabolism or they are known for low and variable oral bioavailability and their first pass drug interactions. Usually, Single Nucleotide Polymorphisms (SNPs) are responsible for the drug response variability and they are able to modify the enzymatic activity of the P450 isoforms [2]. For this reason there is a great interest in the scientific community for further investigation and improved knowledge of antihypertensive drugs metabolism and all their potential biotransformations either by experimental or in silico studies.

Predictive tools for the drugs metabolic status are useful since insufficient knowledge about drug metabolism is one of the most common causes for failure and incompletion during clinical trials. The metabolizing activity of the most known phase I enzymes [3] such as cytochrome P450 [4], monoamine oxidases [5], alcohol dehydrogenases [6], hydrolases [7] and more specifically carbonyldiases [8, 9] and phase II enzymes such as UDP-glucuronosyltransferases [10], sulforansferases [11], methyltransferases [12] and glutathione S-transferases [13] has been investigated in great detail by computational techniques and can be successfully predicted by several approaches.

In the history of drug development, several different computational approaches have been developed for the prediction of human drug metabolism. The majority of these approaches employ databases, rule-based approaches, quantitative structure metabolism relationships (QSMR’s), quantitative structure activity relationships (QSAR’s), pharmacophore, statistical QSAR, electronic or homology models and crystal structures with docking approaches, and combinatorial methodologies where data and/or rules are used to predict all the possibilities of a molecule metabolism. For the improvement of the prediction of drug metabolism combined methods have been also developed and applied respectively.

The interaction between a substrate and P450 in terms of inhibition and induction of P450 enzymes has been also extensively studied. Pharmacophore models and three-dimensional quantitative structure-activity relationships (QSARs) have been used either alone or in combination with protein homology models to provide metabolic information for cytochrome P450 [14, 15]. Unlike the
quantitative structure-activity relationships (QSARs), quantitative structure – property relationships methods have been developed in the drug discovery process [16]. These methods are used to generate by computational screening the ADME (Administration, Distribution, Metabolism, Excretion) profile components. Singh et al., have also developed a semi-quantitative model for evaluating the relative susceptibilities of different sites on drug molecules to metabolism by P450 [17].

Computational approaches have been employed for the scoring of compounds libraries in terms of their Km values to numerous P450 enzymes [18]. MetaDrug is a combinatorial method for predicting human drug metabolism. It has been used to predict some of the major metabolic pathways and to identify the role of P450 enzymes [19, 20]. Another computational method, called MetaSite, has been designed to identify the Site of Metabolism (SOM) for any human P450 acting on new substrates [21]. A statistical approach has also been applied to the prediction of aromatic hydroxylation sites for diverse sets of substrates [22]. Moreover, Jones et al., in 2002 suggested a combination of experimental data and semi-empirical molecular orbital calculations to predict activation energies for aromatic and aliphatic hydroxylation [23]. The development of model combined with analytical tools such as Mass Spectrometry (MS) or Liquid Chromatography LC/MS has facilitated the detection and the structural elucidation of chemical intermediates in drug metabolism [24, 25].

The META system is a commonly used drug metabolism prediction system. It is an expert system capable of predicting the potential for enzymatic attack sites and the nature of the chemical compounds formed by such metabolic transformations [26-28]. The model of PPS (Pathway Prediction System) also uses metabolic rules describing the transformation of chemical functional groups [29, 30]. On the other hand CATABOL is an approach that predicts chemical biodegradability in a quantitative manner [31]. It functions together with a probabilistic model that calculates probabilities of the individual transformation and matching substructure engine [31]. Furthermore, DEREK, SIAR and METEOR prescribe rules to describe the relationship between chemical structure and either toxicity (DEREK and SIAR) or metabolic fate (METEOR) [32].

In 2004, Hatzimanikatis et al. developed an alternative methodology called BNICE (Biochemical Network Integrated Computational Explorer) capable of generating every possible biochemical reaction based on enzyme reaction rules and starting or target compounds [33]. Recently, BNICE framework has been extended for the prediction of biodegradation pathways of xenobiotics, and has also been applied on xenobiotics compounds such as chlorinated...
aliphates, poly chlorinates biphenyls and polycyclic aromatic hydrocarbons [34]. BNICE was not only able to reproduce the proposed biodegradation routes but also to indicate new pathways consisting novel compounds and reactions.

ANGIOTENSIN-CONVERTING ENZYME (ACE) INHIBITORS

The angiotensin-converting enzyme (ACE) inhibitors are widely used in the management of essential hypertension, among the other cardiovascular diseases such as stable chronic heart failure, myocardial infarction and diabetic nephropathy. They include three categories: (a) captopril, (b) the prodrugs such as ramipril and enalapril and (c) lisinopril, which is water-soluble and the only ACE inhibitor that it is not undergoing metabolism. Their mechanism of action involves the inhibition of angiotensin-converting enzyme, the decreased activity of renin-angiotensin aldosterone system that permits and assists in sodium excretion and the increased formation of bradykinin and vasodilatory prostaglandins.

All of these antihypertensive agents are characterized as having carboxyl functional groups requiring hepatic activation to form pharmacologically active metabolites [35]. The majority of ACE inhibitors are prodrugs converted by hepatic esterolysis to a major active diacid metabolite. Lisinopril is an exception because it does not follow a prodrug biodegradation [36]. Table 1 presents the ACE inhibitors, their metabolites, and the known enzymes causing their metabolic biodegradation.

More specifically, the prodrug of benazepril follows a hepatic carboxylation producing benazeprilat, which is the major active metabolite. Apart from the active metabolite only the glucuronides of benazepril and benazeprilat are found in the urine or in the feces [37].

Although the majority of ACE inhibitors belong to prodrugs and undergo hepatic carboxylation for releasing their active metabolites, the dominant urine metabolites of captopril are cysteine captopril and disulfide dimer captopril [38].

Cilazapril also belongs to the ACE inhibitors and it is rapidly converted to its active metabolite cilazaprilat, which is excreted unchanged through the kidneys [39].

Enalapril is extensively converted through hydrolysis to its active metabolite enalaprilot [35, 40]. Enalapril is also a prodrug, which is absorbed by the gastrointestinal tract and is extensively metabolized by hepatic esterases. In the elimination phase the enalaprilot is the only detectable metabolite [41].

Fosinopril, another ester prodrug, is metabolized in the liver by carboxylesterases to fosinoprilat. In addition to fosinoprilat, p-hydroxy fosinoprilat and the conjugated glucuronide fosinoprilat are the second generation detectable metabolites [42].

Lisinopril is the only ACE inhibitor that is not metabolized [36]. No information about the metabolism of resincamine, which belongs to the same drug group as lisinopril, is available.

Moexipril, an ACE inhibitor used for treatment of arterial hypertension, is also a prodrug, yielding moexiprilat, its active metabolite, by hydrolysis of an ethyl ester group [43].

Perindopril is hydrolysed in vivo to the active diacid metabolites perindoprilat and minor metabolic pathways lead to glucuronide-conjugates [44].

The prodrug of quinapril undergoes hepatic hydrolysis into its major active diacid metabolite quinaprilat [45].

Ramiprilam is another ACE inhibitor that acts as a prodrug. Its main metabolite, the diacid metabolite, is a potent angiotensin converting enzyme inhibitor. The minor metabolites ramipril glucuronide, diacid, diacid-glucuronide, diketopiperazine and diketopiperazine acid are associated with the metabolism of ramiprilam, and they are detectable in the urine and serum specimens [46].

Spirapril, is also a prodrug that has to be hydrolyzed by hepatic carboxylesterases to achieve its ACE inhibitory activity [35].

Similar to ramipril, trandolapril is metabolized to its main diacid metabolite. Trandolapril glucuronide, diacid, diacid-glucuronide, diketopiperazine and diketopiperazine acid are minor metabolites detectable in the urine and serum specimens from the metabolism of trandolapril [47].

BETA-BLOCKERS

The β-adrenergic blocking agents cause initially decrease in cardiac output, followed by reduction in peripheral vascular resistance and plasma renin activity decrease.

The majority of the beta-blockers are metabolized by P450 reactivity deriving dealkylated and hydroxylated metabolites and they appear to have similar metabolic characteristics. The elimination of the most of them occurs via hepatic metabolism and/or renal excretion of the unchanged drug [48]. Phase II glucuronidation reactions take place also in the most beta-blockers metabolic pathways. Atenolol and nadolol are the only beta-blockers that appear to be excreted in the unchanged form by the kidneys, while CYP1A2 and CYP2D6 seem to affect the propranolol biotransformation [49-68] (Table 2).

Acetubolol undergoes significant first pass metabolism. Its major metabolite, diacetolol, is formed during its first pass metabolism by CYP2D6 and contributes to beta blocking activity [49].

Both alprenolol and propranolol, and their hydroxyl glucuronides, undergo ring hydroxylated metabolites by P450 (CYP2D6 and CYP3A5/7) [50]. Propranolol is extensively metabolized with most metabolites appearing in the urine. It is metabolized through three primary routes: aromatic hydroxylation, N-dealkylation (CYP1A2), and direct glucuronidation [68].

The metabolism of atenolol is not extensive and the parent drug appears to be the only major radiolabelled component in blood [51]. Following similar metabolic profile, nadolol is a poorly lipid-soluble drug and is excreted unchanged in the urine [60].

According to betaxolol pharmacokinetics, approximately 15% of the administered dose is excreted as unchanged drug, the remainder being metabolites produced by the reactivity of CYP1A2 and CYP2D6 (α-hydroxy betaxolol and its acid) whose contribution to the clinical effect is negligible [52, 53].

Bisoprol is subject only to moderate hepatic metabolism. In the metabolism of bisoprolol only oxidative pathways have been detected, with no subsequent conjugation. It is metabolized primarily by CYP3A4 to inactive metabolites and it is also metabolized by CYP2D6, which does not seem to be clinically significant [54].

The product metabolites of carvedilol are excreted in the urine and only 0.3% is excreted as unchanged drug [55]. Carvedilol is metabolized by oxidation and glucuronidation in the aromatic ring. P450 is responsible for the carvedilol hydroxylations as well as for the demethylated metabolites to the aromatic ring [55].

Esmolol is rapidly metabolized by esterases mainly in red blood cells to a free acid metabolite and methanol [56].

Labetalol is identified in humans only by conjugated metabolites of the drug. Other detectable metabolites include the 3-amino-1-phenyl butane derivative as well as the D-hydroxy derivative of 3-amino-1-phenyl butane [57]. A family of catechol-like metabolites is formed by P450-dependent hydroxylation at the C3 position of the benzamide ring. These metabolites also undergo ring cyclization forming two novel indolic metabolites [58].

Approximately 70% to 80% of an oral metoprolol dose is metabolized by CYP2D6 in the liver. The α-hydroxylation reaction, which makes the formation of α-hydroxymetoprolol from metoprolol in the liver was mediated almost exclusively by CYP2D6 [59].

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Nebivolol human metabolism is complex and is subject to debridoquine type genetic polymorphism. N-dealkylation mainly in combination with hydroxylation, acyclic monoxidation and aromatic hydroxylation mediated by hepatic CYP2D6, followed by glucuronidation. The glucuronidation of the parent drug is also one of the major metabolic pathways [61].

Oxprenolol is extensively metabolized with direct O-glucuronidation being the major metabolic pathway. Glucuronide conjugates of unchanged and hydroxylated oxprenolol have been experimentally identified [62]. Moreover, the hydroxyl carboxylic acid and the monoallyl ether of catechol have been detected as oxprenolol metabolites from reactions mediated by hepatic CYP2D6 [63].

Penbutolol is extensively metabolized, and less than 4% of administered dose is excreted unchanged in urine [64]. The most important known metabolites of penbutolol are penbutolol glucuronides, 4-hydroxy penbutolol and 4-hydroxy penbutolol glucuronide [65]. The 4-hydroxy penbutolol has been described as a semi-active metabolite [66].

The liver extensively metabolizes pindolol, forming several groups of metabolites: first-generation hydroxylated metabolites (Phase I), second-generation sulphate metabolites, and conjugations with glucuronic acid with the parent compounds or with its hydroxylated derivatives (Phase II). 35 to 40% of the administered dose is excreted unchanged in the urine [67].

**CALCIUM CHANNEL BLOCKERS**

There are two types of calcium channel blockers: (i) the dihydropyridine group calcium channel blockers, such as nifedipine, amlodipine and felodipine and (ii) the non-dihydropyridine group
Table 2. Detailed metabolic profile of beta-blockers.

| Beta-blocker name | Metabolites                                      | Enzymes                      | References |
|-------------------|-------------------------------------------------|------------------------------|------------|
| Acebutolol        | Diacetolol                                      | CYP2D6                       | [49]       |
| Alpranolol        | 4-Hydroxy-alpranolol                            | CYP2D6                       | [50, 68]   |
| Atenolol          | Not metabolized                                 |                              | [51]       |
| Betaxolol         | α-Hydroxy betaxolol                             | CYP1A2, CYP2D6               | [52, 53]   |
| Bisoprolol        | Demethyl carboxylic acid                        | CYP3A4, CYP2D6               | [54]       |
|                   | Deethyl carboxylic acid                         |                              | [54]       |
|                   | Deethyl derivative                              |                              | [54]       |
| Carvedilol        | 8-Hydroxy carvedilol                            | CYP1A2, CYP3A4, CYP1A1       | [55]       |
|                   | 4'-Hydroxy carvedilol                           | CYP2D6, CYP2E1, CYP2C9       | [55]       |
|                   | 5'-Hydroxy carvedilol                           | CYP2D6, CYP2E1, CYP2C9       | [55]       |
|                   | O-Desmethyl carvedilol                          | CYP2C9, CYP2D6, CYP1A2, CYP2E1 | [55]   |
|                   | 1-Hydroxy carvedilol                            | Prostaglandin G/H synthase 1 | [55]       |
|                   | 4'-Hydroxyphenyl carvedilol                     |                              | [55]       |
| Esmolol           | Methanol                                        | Esterases                    | [56]       |
| Labetalol         | 7 Glucuronides metabolites                      | UDP-Glucuronosyltransferase  | [57, 58]   |
|                   | 3-Amino-1-phenyl butane                         | GI mucosa                    | [57, 58]   |
|                   | 3-Amino-1-(4-hydroxyphenyl) butane              | GI mucosa                    | [57, 58]   |
| Metoprolol        | α-Hydroxy metoprolol                            | CYP2D6                       | [59]       |
| Nadolol           | Not metabolized                                 |                              | [60]       |
| Nebivolol         | Hydroxy alicyclic metabolites                   | CYP2D6                       | [61]       |
|                   | Hydroxy aromatic metabolites                    | CYP2D6                       | [61]       |
|                   | 1st generation glucuronides                     | UDP-Glucuronosyltransferase  | [61]       |
|                   | N-dealkylation                                   | CYP2D6                       | [61]       |
|                   | 2nd generation glucuronides (after the hydroxylation) | UDP-Glucuronosyltransferase | [61]   |
| Oxprenolol        | Glucuronides of unchanged oxprenolol            | UDP-Glucuronosyltransferase  | [62, 63]   |
|                   | Hydroxylation to the aromatic ring of unchanged ox- | CYP2D6                       | [62, 63]   |
|                   | prenolol                                        |                               |            |
|                   | Glucuronides of hydroxylated oxprenolol         | UDP-Glucuronosyltransferase  | [62, 63]   |
|                   | N-dealkylation, producing the hydroxy carboxylic acid | CYP2D6                       | [62, 63]   |
| Penbutolol        | Glucuronide metabolite                          | UDP-Glucuronosyltransferase  | [64-66]    |
|                   | 4-Hydroxy metabolite                            |                              | [64-66]    |
| Pindolol          | Hydroxylated metabolites                        |                              | [67]       |
|                   | Glucuronides of hydroxylated metabolites        | UDP-Glucuronosyltransferase  | [67]       |
|                   | Sulfate metabolites                             | Sulphotransferases           | [67]       |
| Propanolol        | N-Desisopropyl propranolol                      | CYP1A2                       | [68]       |
|                   | 4'-Hydroxy propranolol                          | CYP2D6                       | [68]       |
Calcium channel blockers such as phenylalkylamine verapamil and benzothiazepine diltiazem. These drugs are able to decrease the concentration of free intracellular calcium ions leading to decreased contraction and vasodilation and they inhibit the aldosterone secretion. They also present diuretic activity through an increase in renal blood flow and glomerular filtration rate.

### Table 3. Detailed metabolic profile of calcium channel blockers

| Calcium channel blocker name | Metabolites                                | Enzymes          | References |
|------------------------------|--------------------------------------------|------------------|------------|
| Amlodipine                   | Pyridine derivative                        | CYP3A4           | [69]       |
|                              | De-amino amlodipine                        | CYP3A4           | [69]       |
|                              | Ester hydrolysis of the S-methoxycarbonyl group | Carboxylesterases | [69]       |
|                              | Amlodipine glucuronides                    | UDP-Glucuronosyltransferase | [69] |
| Diltiazem                    | N-desmethyl diltiazem                      | CYP3A4           | [70]       |
|                              | O-desacetyl-N-desmethyl diltiazem          | CYP3A4           | [70]       |
| Felodipine                   | Dehydro felodipine                         | CYP3A4           | [71]       |
| Isradipine                   | Pyridine of isradipine                     | CYP3A4           | [72]       |
|                              | Carboxylic acid of isradipine              | CYP3A4           | [72]       |
|                              | Carboxylic acid of isradipine pyridine     | CYP3A4           | [72]       |
| Nifedipine                   | Pyridine of nifedipine                     | CYP3A4           | [79]       |
|                              | Carboxylic acid of nifedipine              | CYP3A4           | [79]       |
|                              | Carboxylic acid of nifedine pyridine       | CYP3A4           | [79]       |
| Nimodipine                   | Dehydro nimodipine                         | CYP3A4           | [74, 98]   |
|                              | Carboxylic acid of nimodipine              | CYP3A4           | [74, 98]   |
|                              | Carboxylic acid of dehydronimodipine       | CYP3A4           | [74, 98]   |
| Nisoldipine                  | Hydroxylation of the isobutyl moiety       | CYP3A4           | [75]       |
|                              | Dehydrogenation of the 1, 4-dihydropyridine system | CYP3A4    | [75]       |
|                              | Oxidative ester cleavage                   | CYP3A4           | [75]       |
|                              | Hydroxylation of one of the methyl groups in 2-position and subsequent oxidation to the carboxylic acid | CYP3A4 | [75] |
|                              | Hydroxylation of one of the methyl groups in 6-position and subsequent oxidation to the carboxylic acid | CYP3A4 | [75] |
|                              | Oxidation of one of the methyl groups of the isobutyl moiety to the carboxyl group reduction of the aromatic nitro group | CYP3A4 | [75] |
|                              | Glucuronidation                             | UDP-Glucuronosyltransferase | [75] |
| Nitrendipine                 | Dehydro nitrendipine                       | CYP3A4           | [76]       |
|                              | Carboxylic acid of nitrendipine            | CYP3A4           | [76]       |
|                              | Carboxylic acid of dehydronitrendipine     | CYP3A4           | [76]       |
| Verapamil                    | O-Desmethyl verapamil                      | CYP2C8, CYP2C18, CYP2C9 | [77, 78] |
|                              | O-Desmethyl verapamil (D-702)              | CYP2C8, CYP2C9   | [77, 78]   |
|                              | O-Desmethyl verapamil (D-703)              | CYP2C8, CYP2C9   | [77, 78]   |
| Norverapamil                 |                                            | CYP2C8, CYP3A4, CYP3A5, CYP1A2 | [77, 78] |
| D-617                        |                                            | CYP2C8           | [77, 78]   |
Most calcium channel blockers have low and variable oral bioavailability because of extensive first pass metabolism. Just as the other groups of antihypertensive drugs, calcium channel blockers have similar ways of biodegradation. Most of them act as substrates of CYP3A4 causing aromatic hydroxylation and N-dealkylation (Table 3). Moreover, those belonging to dihydropyridine group undergo dehydrogenation of the same group [69-79].

Amlodipine is extensively metabolized in the liver via cytochrome P4503A4 isoyzime. Firstly, it undergoes oxidation to the pyridine derivative, and then oxidative deamination or ester hydrolysis [69].

CYP3A4 metabolizes also diltiazem via deacetylation, N-demethylation, O-demethylation, N-oxidation and oxidative deamination. However, diltiazem acts simultaneously as substrate and inhibitor of CYP3A4 [70].

Following oral administration, felodipine is almost completely absorbed and undergoes extensive first pass metabolism. Different metabolites have been detected in plasma from felodipine metabolism including pyridine analogue, carboxylic monoacids, ester lactones, hydroxyl acid forms and a lactonic compound after its P450-dependent metabolism [71].

Isradipine is completely metabolized prior to excretion, and no unchanged drug can be detected in the urine. The major metabolites of isradipine are its pyridine, which is the product of the ring oxidation of the dihydropyridine moiety, and the carboxylic acids followed the ester cleavage [72].

Nifedipine is eliminated from the human body by oxidative hepatic metabolism catalyzied mostly by CYP3A4 to two major corresponding carboxylic acids and the pyridine of the parent compound [79].

Nimodipine follows hepatic metabolism mediated by CYP3A4. Pharmacokinetic studies on both healthy volunteers and patients reveal large variability in drug disposition that may be implicated in clinical effects diversification [73]. It is well known that nimodipine undergoes oxidative ester cleavage, oxidative demethylation, subsequent oxidation resulting in the generation of primary alcohols and a carboxylic acid, methyl hydroxylations and isopropyl hydroxylation. Reduction of the aromatic nitro group as well as in phase II reactions by glucuronidation have also been described [74].

Nisoldipine is an extensively metabolized calcium channel blocker whereby the unchanged drug cannot be detected either in the urine or in the bile. As in the case of nimodipine, it also follows hydroxylation of isobutyl moiety, dehydrogenation of the dihydropyridine group, and hydroxylation of the methyl group, subsequent aminogroup and finally conjugation with glucuronate regarding the phase II reactions [75]. Cytochrome P450 enzymes are believed to play a major role in the metabolism of nisoldipine and especially the CYP3A4 isozyme.

Nitrendipine is another dihydropyridine calcium channel blocker that follows a pattern of metabolism similar to nimodipine and nisoldipine. They all follow the oxidation of the dihydropyridine structure, hydroxylation to the methyl group and derivation of the corresponding carboxylic acids by the cleavage of the ester bonds [76].

Verapamil is extensively metabolized primarily by cytochrome P450 enzymes with less than 5% of orally administered dose being excreted unchanged [77]. Verapamil metabolism follows O-demethylation, N-demethylation or N-dealkylation and the derivation of norverapamil [78].

### DIURETICS

In general, all diuretics result initially in the reduction of plasma volume and cardiac output. In long-term usage, they decrease the total peripheral vascular resistance. More particularly, thiazide related diuretics lead to retention of water in urine. Loop diuretics result into the excretion of water in the urine by the inhibition of the sodium reabsorption at the ascending loop in the nephron. On the other hand, potassium-sparing diuretics do not promote

| K⁺ sparing diuretic name | Metabolites | Enzymes | References |
|-------------------------|-------------|---------|------------|
| Amiloride               | Not metabolized |         | [80]       |
| Eplerenone              | 21-Hydroxy eplererone | CYP3A4  | [81]       |
|                        | 6b-Hydroxy eplererone | CYP3A4  | [81]       |
|                        | 6b, 21-Dihydroxy eplererone | CYP3A4  | [81]       |
|                        | 3a, 6b-Dihydroxy eplererone | CYP3A4  | [81]       |
|                        | 6b, 15a-Dihydroxy eplererone | CYP3A4  | [81]       |
|                        | 3a, 6b, 21-Trihydroxy eplererone | CYP3A4  | [81]       |
|                        | 2a, 3b, 6b-Trihydroxy eplererone | CYP3A4  | [81]       |
| Spironolactone          | Spironolactone-thiole |         | [82]       |
|                        | Spironolactone-dethioacetate |         | [82]       |
|                        | Canrenone      | CYP3A4  | [82]       |
|                        | Thio-methyl-spiroloactone |         | [82]       |
|                        | Hydroxyl-thiomethyl-spiroloactone |         | [82]       |
| Triamterene             | 4-Hydroxy triamterene | CYP1A2  | [83]       |
|                        | 4-Hydroxy-sulfate-triamterene | Sulfo transferases | [83]       |
the secretion of potassium into the urine and they block the sodium channels.

**K⁺ Sparing Diuretics**

P450 complex of enzymes also participate in the potassium sparing diuretics metabolism causing mostly hydroxylations. Amlodipine is not metabolized by the liver but it is excreted unchanged by the kidneys [80]. On the other hand, triamterene is extensively metabolized to a major hydroxytriamterene sulfate metabolite and spironolactone is converted to several metabolites (Table 4).

Eplerenone is cleared predominantly by CYP3A4 metabolism. The major metabolic products excreted in the urine and the feces include all the hydroxylation and ketoreduction derivatives; however, no active metabolites have been identified in human plasma [81].

It is known that spironolactone is extensively metabolized and that its metabolites mediate its therapeutic activity. There are two metabolic pathways for spironolactone degradation. In the first one the drug undertakes dethioacetylation and in the second one the compound keeps its sulfur moiety [82].

The diuretic triamterene is an extensively metabolized potassium sparing diuretic. P450 enzymes predominantly catalyze its hydroxylation, and sulfotransferases catalyze Phase II conjugation reactions [83].

**Loop Diuretics**

Loop diuretics undergo hepatic metabolism in several different ways. Butamenide and torasemide are metabolized by cytochrome P450, whereas furosemide is only glucuronidated (Table 5). Only 20% of furosemide is metabolized in the liver. In contrast, almost 75% of torasemide is metabolized [84].

Pharmacokinetic studies of bumetanide indicate that more than 2/3 of urinary metabolites are unchanged bumetanide. The identified metabolites indicate that metabolism occurs on the butyl side chain, with the primary alcohol being the major metabolite. Conjugates of these metabolites were also found in the urine [85].

Pharmacokinetic studies have shown that approximately 60% to 70% of ethacrynic acid is excreted to the bile as an unchanged compound, while the remainder is biotransformation products. The glutathione conjugate as well as the mercapturate derivative are the two major final metabolites of ethacrynic acid [86].

On average, 70% of the oral furosemide dose is absorbed and only about 10% of the drug is eliminated from the body during hemodialysis. The liver appears to play a minor role in the metabolism of furosemide and an indirect evidence suggests that the kidneys could metabolize this compound [87]. The only known metabolite is the 4-chloro-5-sulfamoylantranilic acid, which has also a potential diuretic activity [88].

Torasemide is rapidly absorbed following oral administration and metabolized by hepatic cytochrome P450 enzymes. In the case of intravenous administration 25% of a dose is traced in the urine as unchanged drug. This diuretic undergoes different hydroxylations, oxidations and reductions mediated by CYP2C8 and CYP2C9 in order to produce its semi active metabolites [89].

**Thiazide and Thiazide-Like Diuretics**

Despite the differences in effectiveness between the thiazide diuretics, they have several common pharmacokinetic properties. Most of these compounds appear to be well absorbed from oral dosage and the kidneys or the feces actively secrete them as unchanged substances. The degree of metabolism of the thiazide diuretics is not variable. In the case of chlorothiazide, hydrochlorothiazide and hydroflumethiazide it is essentially zero and almost zero for the rest of them [90].

The major portion of chlorthalidone is excreted unchanged by the kidneys [90]. In a similar pattern, metalazone is excreted unchanged in urine via glomerular filtration and active tubular secretion, and the possibility of undergoing enterohepatic recycling exists [91].

Although most of thiazide-like diuretics are excreted unchanged, indapamide is extensively hepatic metabolized. The metabolic studies of indapamide indicate the significance of the cytochrome P450 enzymes. More particularly, CYP3A4 is responsible for the dehydrogenation of indapamide, its hydroxylation and carboxylation (Table 6). Glucuronides of the hydroxylated metabolites have also been identified [92].

### COMPUTATIONAL APPROACHES FOR THE PREDICTION OF THE METABOLISM OF ANTIHYPERTENSIVE DRUGS

METEOR is a computational system for the prediction of drug metabolism that contains a knowledge base of reactions and rules relating structure and biotransformation [32]. In 2005, Testa et al. provided an evaluation of this system. The ACE inhibitor omaprilat

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**Table 5. Detailed metabolic profile of loop diuretics.**

| Loop diuretic name | Metabolites                                | Enzymes                      | References |
|--------------------|--------------------------------------------|------------------------------|------------|
| Bumetanide         | Debutylbumetanide                          |                              | [85]       |
|                    | Alpha-hydroxybumetanide                    |                              | [85]       |
|                    | Bumetanide carboxylic acid                 |                              | [85]       |
|                    | Bumetanide glucuronides                    | UDP-Glucuronosyltransferase  | [85]       |
| Ethacrynic acid    | Glutathione conjugation                    | Glutathione-S-transferases   | [86]       |
|                    | Mercapturic Acid                           |                              | [86]       |
| Furosemide         | 4-Chloro-5-sulfamoylantranilic acid        | CYP2C8, CYP2C9               | [87, 88]   |
| Torasemide         | Hydroxylation of the methyl group of the benzyl ring | CYP2C8, CYP2C9               | [89]       |
|                    | M1 carboxylic acid                         | CYP2C8, CYP2C9               | [89]       |
|                    | 4-Hydroxy torasemide                       | CYP2C8, CYP2C9               | [89]       |
was one of the drugs used in this evaluation [93]. According to the results, two of omaprilat’s reactions, amide hydrolysis and S-methylation, were in full qualitative agreement with the in vivo experimental results in humans. The system predicted five more oxidative reactions, which were false positives. METEOR was also able to produce conjugations such as S-glucuronidation and taurine conjugation that appear to be realistic predictions. Among the predictions for the metabolism of omaprilat there was only one false negative prediction, due to a gap in the knowledge base of the program.

In the case of the beta-blocker betaxolol, a combined protein and pharmacophore model for cytochrome P450 2D6 (CYP2D6) has been developed with a second pharmacophore in order to explain the CYP2D6 catalyzed metabolic reactions [94]. This computational approach indicated that the most favorable metabolic pathways would result in the hydroxylation at the aliphatic carbon atoms. The experimentally observed metabolism in humans involved the formation of acid metabolite and α-hydroxy betaxolol [53]. This computational approach was able to confirm only the formation of α-hydroxy betaxolol.

CYP2C9 is a predominant member of the 2C family with a major contribution to human drug metabolism. More particularly, a lot of anti-inflammatory compounds, antithrombotic agents and a major contribution to human drug metabolism. More particularly, a CYP2C9 is a predominant member of the 2C family with a CYP2C9 is a predominant member of the 2C family with a predominant member of the 2C family. An approach to improve the intracellular Ca²⁺ regulation, docking studies were used to predict the sites of metabolism (SOM) within the active site of CYP2C9, molecular dynamics (MD) simulations of docked complexes were performed to validate their stability and QM calculations were done to study the energy profiles for the oxidations [95]. This study demonstrated the ability of used computational approaches to provide reliable qualitative predictions of the novel thermoactive agent metabolites produced by the action of the P450 enzyme CYP2C9.

QM/MM calculations were used to model the phase I oxidation reactions of CYP2C9 by Lonsdale and coworkers [96]. Although this study does not include the metabolism of antihypertensive drugs, many antihypertensive drugs belong to CYP2C9 substrates. In this study, the authors provide mechanistic features of metabolizing reactions occurred by the catalytic activity of CYP2C9. In addition, they show that the active site is important for rationalizing the experimentally observed selectivity that is a likely significant feature for making predictions of novel pharmaceutical compounds metabolism.

Liu et al., based on a 2D substructure structure-based site of metabolism (SOM) prediction method called SMARTCyp [97] were able to predict the SOM of major drug metabolism enzymes belonging to the superfamily of P450 such as CYP1A2, CYP2C9 and CYP2C19 [98]. SMARTCyp is an alternative method that is able to estimate the reactivity of molecular fragments by high level quantum mechanical calculations on representative molecules and assign reactivity to different sites of a substrate by matching structural patterns [97]. In this study, the authors have also used some of the antihypertensive drugs catalyzed by CYP1A2 such as carvedilol, by CYP2C9, torasemide and carvedilol, and by CYP2C19 such as the beta-blocker of propranolol.

The CYP2C9 is not the only “prevalent” catalyzing enzyme of antihypertensive drugs. Several antihypertensive drugs are substrates of CYP2D6, which catalyzes some of the beta-blockers such as propranolol. Pharmacophore studies have been also developed to include the hydroxylation O- and N-dealkylation caused by CYP2D6 [94, 99-102].

The group of phase I enzymatic reactions does not include only the P450 reactivity but it can be expanded to other catalytic activities. Carboxylesterases is a major and important group of enzymes that catalyzes the metabolism of drugs and produgs including a great range of antihypertensive drugs such as ACE inhibitors. Most ACE inhibitors are produgs, depending on the action of carboxylesterases to express their pharmaceutical activity. In silico studies have been performed to predict the metabolic activity of carboxylesterase-1 and carboxylesterase-2 [8, 9]. These studies involve docking approaches as well as molecular dynamics simulations of known substrates including antihypertensive drugs to predict the catalytic activity of these two carboxylesterases.

Another study developed a structure-based hepatic metabolic clearance prediction model based on molecular descriptors [103]. In this study, the researchers used a set of 50 drugs to cover a range of molecular properties. This set also included a significant number of antihypertensive drugs such as beta-blockers (carvedilol, metoprolol, pindolol, propranolol), calcium channel blockers (diltiazem, nifedipine, verapamil) and loop diuretics (furosemide). The objectives of this study were the development of quantitative relationships between the molecular description and observed

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**Table 6. Detailed metabolic profile of thiazide diuretics**

| Thiazide diuretic name | Metabolites                              | Enzymes                | References |
|------------------------|------------------------------------------|------------------------|------------|
| Chlorthalidone         | Excreted unchanged in the urine          |                        | [90]       |
| Indapamidine           | Hydroxyl-indapamide                      | CYP3A4                 | [92]       |
|                        | Dehydro-indapamide                       | CYP3A4                 | [92]       |
|                        | Hydroxyl-dehydro-indapamide              | CYP3A4                 | [92]       |
|                        | Indapamid-epoxide                        | Microsomal epoxide hydrolase | [92]     |
|                        | Dihydroxy-indapamide                     | CYP3A4                 | [92]       |
|                        | Indapamide-glutathione conjugation       | Glutathione (non enzymatic) | [92]   |
| Metolazone             | Excreted unchanged in the urine          |                        | [91]       |

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| Thiazide diuretic name | Metabolites                              | Enzymes                | References |
|------------------------|------------------------------------------|------------------------|------------|
| Chlorthalidone         | Excreted unchanged in the urine          |                        | [90]       |
| Indapamidine           | Hydroxyl-indapamide                      | CYP3A4                 | [92]       |
|                        | Dehydro-indapamide                       | CYP3A4                 | [92]       |
|                        | Hydroxyl-dehydro-indapamide              | CYP3A4                 | [92]       |
|                        | Indapamid-epoxide                        | Microsomal epoxide hydrolase | [92]     |
|                        | Dihydroxy-indapamide                     | CYP3A4                 | [92]       |
|                        | Indapamide-glutathione conjugation       | Glutathione (non enzymatic) | [92]   |
| Metolazone             | Excreted unchanged in the urine          |                        | [91]       |
Table 7. Detailed description of antihypertensive drugs metabolism according to the 3rd level E.C. classification of BNICE framework generalized reaction rules. The + indicates sequential reactions given by generalized reaction rules and the | indicates alternative generalized reaction rules given the same metabolite.

| Category        | Drug name | Metabolites                          | Enzymes                        | 3rd level E.C. number of BNICE generalized reaction rules |
|-----------------|-----------|--------------------------------------|--------------------------------|----------------------------------------------------------|
| ACE Inhibitors  | Benazepril| Benazeprilat                          | Carboxylesterases              | 3.1.1.-                                                  |
|                 |           | Benazeprilat glucuronic acid          | UDP-Glucuronosyltransferase    | 2.4.1.-                                                  |
|                 | Captopril | Cysteine-captopril disulfide          |                                | 1.8.1.-|1.11.1.-                                                |
|                 |           | Capropril dimer disulfide             |                                | 1.8.1.-|1.11.1.-                                                |
|                 | Cilazapril| Cilazaprilat                          | Carboxylesterases              | 3.1.1.-                                                  |
|                 | Enalapril | Enalaprilat                           | Carboxylesterases              | 3.1.1.-                                                  |
|                 | Fosinopril| Fosinoprilat                          | Carboxylesterases              | 3.1.1.-                                                  |
|                 |           | p-Hydroxy fosinopril metabolite       |                                | 1.14.13.-                                                |
|                 | Lisinopril| Not metabolized                       |                                |                                                          |
|                 | Moexipril | Moexiprilat                           | Carboxylesterases              | 3.1.1.-                                                  |
|                 | Perindopril| Perindoprilat                         | Carboxylesterases              | 3.1.1.-                                                  |
|                 |           | Perindopril glucuronides              | UDP-Glucuronosyltransferase    | 2.4.1.-                                                  |
|                 | Quinapril | Quinaprilat                           | Carboxylesterases              | 3.1.1.-                                                  |
|                 | Ramipril  | Ramiprilat                            | Carboxylesterases              | 3.1.1.-                                                  |
|                 |           | Diketopiperazine ester                |                                | 3.5.1.-                                                  |
|                 |           | Diketopiperazine acid                 |                                | 3.5.1.-|3.1.1.-                                                  |
|                 |           | Glucuronides of ramipril              | UDP-Glucuronosyltransferase    | 2.4.1.-                                                  |
|                 |           | Glucuronides of ramiprilat            | UDP-Glucuronosyltransferase    | 2.4.1.-                                                  |
|                 | Rescinnamine| No information available              |                                |                                                          |
|                 | Spirapril | Spiraprilat                           | Carboxylesterases              | 3.1.1.-                                                  |
|                 | Trandolapril| Trandolaprilat                        | Carboxylesterases              | 3.1.1.-                                                  |
|                 |           | Diketopiperazine ester                |                                | 3.5.1.-                                                  |
|                 |           | Diketopiperazine acid                 |                                | 3.5.1.-|3.1.1.-                                                  |
|                 |           | Trandolapril glucuronides             | UDP-Glucuronosyltransferase    | 2.4.1.-                                                  |
|                 |           | Trandolaprilat glucuronides           | UDP-Glucuronosyltransferase    | 2.4.1.-                                                  |
| Beta-blockers   | Acebutolol| Diacetolol                            | CYP2D6                         | 2.1.1.-|2.1.1.-                                                  |
|                 | Alprenolol| 4-Hydroxy-alprenolol                  | CYP2D6                         | 1.14.13.-                                                |
|                 | Atenolol  | Not metabolized                       |                                |                                                          |
|                 | Betaxolol| α-Hydroxy betaxolol                   | CYP1A2, CYP2D6                 | 1.14.13.-                                                |
|                 | Bisoprolol| Demethyl carboxylic acid              | CYP3A4, CYP2D6                 | 2.7.8.-|1.1.1.-|1.2.1.-                                                  |
|                 |           | Deethyl carboxylic acid               |                                | 2.7.8.-|1.1.1.-|1.2.1.-                                                  |
|                 |           | Deethyl derivative                    |                                | 2.7.8.-|1.1.1.-|1.2.1.-                                                  |
|                 | Carvedilol| 8-Hydroxy carvedilol                  | CYP1A2, CYP3A4, CYP1A1         | 1.14.13.-                                                |
(Table 7) Contd….

| Category     | Drug name       | Metabolites                  | Enzymes                      | 3rd level E.C. number of BNICE generalized reaction rules |
|--------------|-----------------|------------------------------|------------------------------|----------------------------------------------------------|
|              | 4'-Hydroxy carvedilol | CYP2D6, CYP2E1, CYP2C9       |                              | 1.14.13.-                                                  |
|              | 5'-Hydroxy carvedilol | CYP2D6, CYP2E1, CYP2C9       |                              | 1.14.13.-                                                  |
|              | O-Desmethyl carvedilol | CYP2C9, CYP2D6, CYP1A2, CYP2E1 |                              | 1.14.13.-                                                  |
|              | 1-Hydroxy carvedilol | Prostaglandin G/H synthase 1 |                              | 1.14.13.-                                                  |
|              | 4'-Hydroxyphenyl carvedilol |                        |                              | 1.14.13.-                                                  |
| Esmolol      | Methanol        | Esteras                      |                              | 1.14.13.-                                                  |
| Labetalol    | 7 Glucuronides metabolites | UDP-Glucuronosyltransferase |                              | 2.4.1B1                                                   |
|              | 3-Amino-1-phenyl butane | GI mucosa                  |                              | 1.4.1.-|1.4.3.-|1.5.99.-|4.3.1.-                                                  |
|              | 3-Amino-1-(4-hydroxyphenyl) butane | GI mucosa                  |                              | 1.4.1.-|1.4.3.-|1.5.99.-|4.3.1.-                                                  |
|              | Benzyl acetone  | GI mucosa                    |                              | 1.4.1.-|1.4.3.-|1.5.99.-|4.3.1.-                                                  |
| Metoprolol   | α-Hydroxy metoprolol | CYP2D6                     |                              | 1.14.13.-                                                  |
| Nadolol      | Not metabolized |                              |                              |                                                          |
| Nebivolol    | Hydroxy alicyclic metabolites | CYP2D6                    |                              | 1.14.13.-                                                  |
|              | Hydroxy aromatic metabolites | CYP2D6                    |                              | 1.14.13.-                                                  |
|              | 1st generation glucuronides | UDP-Glucuronosyltransferase |                              | 2.4.1B1                                                   |
|              | N-dealkylation  | CYP2D6                       |                              | 1.4.1.-|1.4.3.-|1.5.99.-|4.3.1.-                                                  |
|              | 2nd generation glucuronides (after the hydroxylation) | UDP-Glucuronosyltransferase |                              | 1.14.13.- +2.4.1.-                                         |
| Oxprenolol   | Glucuronides of unchanged oxprenolol | UDP-Glucuronosyltransferase |                              | 2.4.1.-                                                   |
|              | Hydroxylation to the aromatic ring of unchanged oxprenolol | CYP2D6                     |                              | 1.14.13.-                                                  |
|              | Glucuronides of hydroxylated oxprenolol | UDP-Glucuronosyltransferase |                              | 1.14.13.- +2.4.1.-                                         |
|              | N-dealkylation, producing the hydroxy carboxylic acid | CYP2D6                     |                              | 1.4.1.-|1.4.3.-|+1.1.1.-                                                  |
|              | Carbinol        | CYP2D6                       |                              | 1.4.1.-|1.4.3.-                                                  |
|              | Monoally ether of catechol | CYP2D6                    |                              | 3.3.1.-                                                   |
| Penbutolol   | Glucuronide metabolite | UDP-Glucuronosyltransferase |                              | 2.4.1.-                                                   |
|              | 4-Hydroxy metabolite |                              |                              | 1.14.13.-                                                  |
| Pindolol     | Hydroxylated metabolites |                              |                              | 1.14.13.-                                                  |
|              | Glucuronides of hydroxylated metabolites | UDP-Glucuronosyltransferase |                              | 1.14.13.- +2.4.1.-                                         |
|              | Sulfate metabolites | Sulfotransferases            |                              | 1.14.13.-                                                  |
| Propanolol   | N-Desisopropyl propanolol | CYP1A2                     |                              | 1.4.1.-|1.4.3.-                                                  |
|              | 4'-Hydroxy propanolol | CYP2D6                       |                              | 1.14.13.-                                                  |
| Category                     | Drug name   | Metabolites               | Enzymes                        | 3rd level E.C. number of BNICE generalized reaction rules |
|------------------------------|-------------|---------------------------|--------------------------------|---------------------------------------------------------|
| Calcium Channel Blockers     | Amlodipine  | Pyridine derivative       | CYP3A4                         | 5.3.3.-|1.3.1.-|1.5.3.-|1.5.1.- |
|                              |             | De-amino amlodipine       | CYP3A4                         | 1.4.3.-|1.5.99.- |
|                              |             | Ester hydrolysis of the S-methoxycarbonyl group | Carboxylesterases | 3.1.1.- |
|                              |             | Amlodipine glucuronides   | UDP-Glucuronosyltransferase    | 2.4.1.- |
|                              | Diltiazem   | N-desmethyl diltiazem     | CYP3A4                         | 1.4.3.-|1.5.99.- |
|                              |             | O-desacetyl-N-desmethyl diltiazem | CYP3A4 | 1.4.3.-|1.5.99.-|+1.14.13.-|3.1.1.- |
|                              | Felodipine  | Dehydro felodipine        | CYP3A4                         | 5.3.3.-|1.3.1.-|1.5.3.-|1.5.1.- |+3.1.1.-|1.14.13.- |
|                              | Isradipine  | Pyridine of isradipine    | CYP3A4                         | 5.3.3.-|1.5.1.-|1.5.3.- |
|                              |             | Carboxylic acid of isradipine | CYP3A4 | 3.1.1.- |
|                              | Nimodipine  | Pyridine of nifedipine    | CYP3A4                         | 5.3.3.-|1.5.1.-|1.5.3.- |
|                              |             | Carboxylic acid of nifedipine | CYP3A4 | 3.1.1.- |
|                              |             | Carboxylic acid of nifedine pyridine | CYP3A4 | 5.3.3.-|1.5.1.-|1.5.3.-|+3.1.1.- |
|                              | Nimodipine  | Dehydro nimodipine        | CYP3A4                         | 5.3.3.-|1.5.1.-|1.5.3.- |
|                              |             | Carboxylic acid of nimodipine | CYP3A4 | 3.1.1.- |
|                              |             | Carboxylic acid of dehydro nimodipine | CYP3A4 | 5.3.3.-|1.5.1.-|1.5.3.-|+3.1.1.- |
|                              | Nisoldipine | Hydroxylation of the isobutyl moiety | CYP3A4 | 1.14.13.- |
|                              |             | Dehydrogenation of the 1, 4-dihydropyridine system | CYP3A4 | 5.3.3.-|1.5.1.-|1.5.3.- |
|                              | Nitrendipine| Dehydro nitrendipine      | CYP3A4                         | 5.3.3.-|1.5.1.-|1.5.3.- |
|                              |             | Carboxylic acid of nitrendipine | CYP3A4 | 3.1.1.- |
|                              |             | Carboxylic acid of dehydro nitrendipine | CYP3A4 | 5.3.3.-|1.5.1.-|1.5.3.-|+3.1.1.- |
|                              | Verapamil   | O-Desmethyl verapamil     | CYP3A4                         | 1.14.13.- |
|                              |             | O-Desmethyl verapamil (D-702) | CYP2C8, CYP2C18, CYP2C9 | 1.14.13.- |
|                              |             | O-Desmethyl verapamil (D-703) | CYP2C8, CYP2C9 | 1.14.13.- |
(Table 7) Contd….

| Category | Drug name | Metabolites | Enzymes | 3rd level E.C. number of BNICE generalized reaction rules |
|----------|-----------|-------------|---------|----------------------------------------------------------|
|          | Norverapamil | CYP2C8, CYP3A4, CYP3A5, CYP1A2 | 1.4.3.- | 1.5.99.- |
|          | D-617     | CYP2C8     |         | 1.4.1.- | 1.4.3.- | 1.5.99.- |
| K⁺ sparing diuretics | Amiloride | Not metabolized |         |           |           |
|          | Eplerenone | 21-Hydroxy eplererone | CYP3A4 | 1.14.13.- |
|          |           | 6b-Hydroxy eplererone | CYP3A4 | 1.14.13.- |
|          |           | 6b, 21-Dihydroxy eplererone | CYP3A4 | 1.14.13.- | + 1.14.13.- |
|          |           | 3a, 6b-Dihydroxy eplererone | CYP3A4 | 1.1.1.- | + 1.14.13.- |
|          |           | 6b, 15a-Dihydroxy eplererone | CYP3A4 | 1.14.13.- | + 1.14.13.- |
|          |           | 3a, 6b, 21-Trihydroxy eplererone | CYP3A4 | 1.1.1.- | + 1.14.13.- | + 1.14.13.- |
|          |           | 2a, 3b, 6b-Trihydroxy eplererone | CYP3A4 | 1.1.1.- | + 1.14.13.- | + 1.14.13.- |
|          | Spironolactone | Spironolactone-thiole |         | 1.2.1.- | (2.3.1.- | 3.1.2.- |
|          |           | Spironolactone-dethioacetate |         | 1.2.1.- | (2.3.1.- | 3.1.2.- | + 3.3.1.- | + 4.2.1.- | + 1.3.1. |
|          |           | Canrenone |         | 1.2.1.- | (2.3.1.- | 3.1.2.- | + 3.3.1.- | + 4.2.1. |
|          |           | Thio-methyl-spiro nolactone |         | 1.2.1.- | (2.3.1.- | 3.1.2.- | + 2.1.1. |
|          |           | Hydroxyl-thiomethyl-spiro nolactone |         | 1.2.1.- | (2.3.1.- | 3.1.2.- | + 2.1.1.- | + 1.14.13A1 |
|          | Triamterene | 4-Hydroxy triamterene | CYP1A2 | 1.14.13.- |
|          |           | 4-Hydroxy-sulfate-triamterene | Sulfotransferases | 1.14.13.- | + 2.8.2. |
| Loop Diuretics | Bumetanide | Debutylbumetanide |         | 1.4.1.- | (1.4.3.- | 4.3.1. |
|          |           | Alpha-hydroxy bumetanide |         | 1.14.13.- | (1.14.18.- | 1.17.99. |
|          |           | Bumetanide carboxylic acid |         | 1.13.11.- |
|          |           | Bumetanide glucuronides | UDP-Glucuronosyltransferase | 2.4.1. |
|          | Ethacrynic acid | Glutathione conjugation | Glutathione-S-transferases | 2.5.1. |
|          | Mercapturic Acid | | | 2.5.1.- | + 3.5.1. |
|          | Furosemide | 4-Chloro-5-sulfamoyl antranilic acid | | 2.4.1.- |
|          | Torasemide | Hydroxylation of the methyl group of the benzyl ring | CYP2C8, CYP2C9 | 1.14.13.- | (1.17.99. |
|          |           | M1 carboxylic acid | CYP2C8, CYP2C9 | 1.13.11.- |
|          |           | 4-Hydroxy torasemide | CYP2C8, CYP2C9 | 1.14.13.- |
| Thiazide Diuretics | Bendroflumethiazide | No available information | | |
|           | Chlorothiazide | Chlorothiazide is not metabolized but is eliminated rapidly by the kidney | | |
|           | Cyclothiazide | No available information | | |
| Category                  | Drug name            | Metabolites                             | Enzymes                  | 3rd level E.C. number of BNICE generalized reaction rules |
|--------------------------|----------------------|-----------------------------------------|--------------------------|----------------------------------------------------------|
|                          | Hydrochlorothiazide  | Hydrochlorothiazide is not metabolized. |                          |                                                          |
|                          | Hydroflumethiazide   | Essentially unchanged                    |                          |                                                          |
|                          | Methyclothiazide     | No information available                 |                          |                                                          |
|                          | Polythiazide         | No information available                 |                          |                                                          |
|                          | Quinethazone         | No information available                 |                          |                                                          |
|                          | Trichlormethiazide   | No information available                 |                          |                                                          |
| Thiazide-like Diuretics  | Chlorthalidone       | Excreted unchanged in the urine          |                          |                                                          |
|                          | Indapamide           | Hydroxyl-indapamide                      | CYP3A4                   | 1.14.13.-                                                |
|                          |                      | Dehydro-indapamide                       | CYP3A4                   | 1.14.13.-|1.14.15.-|1.1.1.-                                            |
|                          |                      | Hydroxyl-dehydro-Indapamide             | CYP3A4                   | 1.14.13.-|1.14.15.-|1.1.1.- + 1.14.13.-                                 |
|                          |                      | Indapamide-epoxide                       | Microsomal epoxide hydrolase | 1.3.1.-|1.5.3.-|1.14.19.-                                           |
|                          |                      | Dihydroxy-indapamide                     | CYP3A4                   | 1.14.12.-                                                |
|                          |                      | Indapamide-glutathione conjugation       | Glutathione (non enzymatic) | 1.14.12.- + 2.5.1.-                                     |
|                          | Metolazone           | Excreted unchanged in the urine          |                          |                                                          |

The human hepatic clearance values, the estimation of predictability of this \textit{in silico} model by comparing it with the \textit{in vitro} hepatocytes model and the identification of important molecular descriptors influencing the hepatic metabolic clearance. In the prediction of hepatic metabolic clearance for the antihypertensive drugs the obtained \textit{in silico} values were in good agreement with those values obtained from human hepatocytes incubations. More specifically, only the “fold error” corresponding value of propranolol appeared to be unexpected with respect to the observed hepatic metabolic clearance.

Biopharmaceutics Drug Disposition Classification System (BDDCS) is an alternative study that develops computational classification models and predicts BDDCS classes for molecular properties. This study also elaborates antihypertensive drugs such as the calcium channel blocker amiodipine [104]. The Biopharmaceutics Classification System (BCS) has had a significant impact on the drug regulatory process and practice, and requires knowledge of solubility and permeability data [105, 106]. According to BCS, as defined by FDA, the drugs can be classified into four different classes according to their permeability and solubility. The BDDCS takes into account the BCS classification system as well as several different drug parameters such as elimination routes, pharmacodynamics properties, post absorption effects and intravenous dosing, drug-drug interactions, and it develops a four class classification system divided into extensive metabolic profile and poor metabolic profile [104, 107]. Among the drugs used for this study, several antihypertensive drugs have been classified into four categories as extensive metabolizing or poor metabolizing drugs with high and low solubility respectively as they have been also described in the literature. For instance, the antihypertensive drugs which are known not to undergo metabolism (amiloride, atenolol, hydrochlorothiazide, nadolol, chlorthalidone, chlorothiazide) have been classified among the two classes described as the poor metabolizing drugs. Following the same pattern, the antihypertensive drugs used in the study which are known to undergo extensive metabolism (diltiazem, enalapril, nifedipine, verapamil, propranolol, carvedilol, spironolactone) have been classified by the system as extensive metabolizing drugs, belonging to class 1 and 2 according to their solubility.

Recently, the computational framework of BNICE has been used in a yet unpublished study for the characterization of known drugs metabolism. The conceptual framework introduced in BNICE for the systematic formulation of generalized enzyme reaction rules is based on the E.C. (Enzyme Commission) classification system [108]. These rules are built through manual curation and analysis of all the known biochemical reactions and each rule is associated with a 3rd level EC class. The application of the formulated generalized reaction rules allows the users to produce all possible metabolites. The algorithm applies iteratively the generalized reaction rules to a set of starting compounds and a set of enzyme cofactors. Each starting compound is evaluated to determine if it contains the functional group that will be transformed by the reaction rules, generating all possible products. The rules are then applied to the products from the previous generation, and this process is repeated until no new compounds are created or the maximum specified number of iterations has been reached. In the next step, the BNICE algorithm continues with the pathway search, which tries to find out all the possible enzymatic routes from a starting compound to a target compound through the system. Among the used databases was also the DrugBank database, which combines drug chemical, pharmacological and pharmaceutical data with drug target information [109-111].

In this study 222 drugs, found in the pathway browser of the database, have been used. The pathway browser consists of drugs...
that are very well described according to their biochemical mechanism. 75 of these drugs belong to antihypertensive agents. For those that undergo metabolism, BNICE was able to reproduce all the drug metabolites reported in the literature indicating that the method is able to identify the known biodegradation routes, and it is also able to indicate new metabolic routes by producing new enzymatic reactions. The method also provides also the third level E.C. number of the enzymes producing metabolites that have never been confirmed by pharmacokinetics studies. Table 7 shows the E.C. numbers suggested by the BNICE methodology that are able to reproduce the metabolites found in experimental studies.

CONCLUSION

Several experimental pharmacokinetics studies and studies of metabolism have shown that the antihypertensive drugs are metabolized by the human body through different pathways and processes. Although ACE inhibitors are mostly metabolized by the liver carboxylesterases in order to express their antihypertensive activity, the most of the beta-blockers, the calcium channel blockers and the diuretics follow metabolic pathways that undergo the P450 reactivity. Only thiazide diuretics and the majority of thiazide-like diuretics are used essentially unchanged and they are known not to undergo metabolism. As was expected due to the structural similarity, the antihypertensive drugs belonging to the same drug category and presenting structural similarity are metabolized in the same pattern.

In recent years, only a few computational methods have been used for the prediction of antihypertensive drugs metabolism and none of them has been developed exclusively for the prediction of the metabolism of the antihypertensive drugs. The above-described methods use mostly rule-based approaches, molecular dynamics, quantum mechanical and molecular mechanical studies, docking approaches and statistical approaches. Even though all of them present reliable results regarding the metabolism of antihypertensive drugs, we believe that there is still a need for further development of in silico methods and for the evolution of the existing methods that will allow the better mechanistic understanding of the drug metabolism that would result in better efficacy of treatments or more efficient patient stratification into antihypertensive treatment.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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