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ORIGINAL RESEARCH ARTICLE

Plasma Protein Binding Refinement of the Extended Clearance Classification System: Subclasses for Predicting Hepatic Uptake or Renal Clearance for Classes 1B and 3B

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Abstract

Background and Objectives The Extended Clearance Classification System (ECCS) was established to facilitate the timely anticipation of clearance rate determination according to the physicochemical characteristics of a given compound and in vitro passive membrane permeability. Unfortunately, distinguishing between renal and hepatic uptake clearance mechanisms using ECCS class 3B is not possible. We determined the effects of plasma protein binding (PPB) on major hepatic organic anion transporting polypeptide (OATP) and renal organic anion transporter (OAT) substrates. A modified ECCS could predict when renal or hepatic uptake mechanisms were the main clearance rate determinants (accounting for ≥ 70% of total clearance).

Methods A dataset of 66 human OATP and 41 OAT substrates was analyzed to determine the effect of PPB. A total of 63 acidic and zwitterionic, and high-molecular-weight (MW > 400 Da) compounds, including 50 drugs in ECCS classes 1B and 3B, were reanalyzed considering their PPB.

Results Statistical analyses revealed that hepatic uptake transporter (OATP1B1 and OATP1B3) substrates possess a high PPB rate of ≥ 90%, whereas OAT1 and/or OAT3 substrates possess low PPB rates of < 90%. By analyzing the 63 drugs on the basis of their PPB, the active hepatic uptakes of acids and zwitterions were determined to be the main clearance mechanisms, with PPB ≥ 90%, whereas renally eliminated drugs exhibited limited PPB (< 90%).

Conclusions Therefore, PPB is an effective parameter for defining clearance rate determination for acidic and zwitterionic drugs with high MWs. Using PPB as an additional parameter in ECCS, clearance mechanisms for class 1B and 3B compounds can be predicted, and OATP and OAT substrates may be readily distinguished.

1 Introduction

There are several routes by which drugs may be eliminated from the body. Most drugs are primarily eliminated via metabolism and biliary or renal excretion as unchanged drugs in bile and urine, respectively [1]. Early prediction of the clearance rate-determining step and extent of elimination from preclinical data can aid in choosing the appropriate preclinical strategy to predict human doses and plasma profiles following oral dosing. It can also enable the successful prediction of pharmacokinetic and potential drug–drug interactions in humans [1]. The amount of unchanged drugs excreted in urine and the extent of metabolic action are easily measurable both preclinically and in clinical trials. However, it is difficult to experimentally measure the biliary excretion of an unchanged drug in humans because of intermittent bile secretion into the gastrointestinal tract [2].

Various studies have shown that ionization state, molecular weight (MW), and polarity are common physicochemical determinants of biliary excreted compounds, and these characteristics predict the substrates of hepatic uptake transporters [3, 4]. Yang et al. [3] compiled a dataset from reports on the relationship between MW and biliary elimination and found a cutoff for anionic drugs with a MW of 400 Da in rats and 475 Da in humans. Furthermore, Varma et al. [4] found a significant overlap in the physicochemical space between the substrates of human hepatic organic anion transporting polypeptide (OATP) transporters and

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Key Points

PPB could be used to distinguish between renal and hepatic uptake in ECCS classes 1B and 3B, and among acidic and zwitterionic compounds with high MWs (> 400 Da), wherein a single clearance mechanism contributed ≥ 70% of the total clearance.

Hepatic uptake transporter (OATP1B1 and OATP1B3) substrates possess a high PPB rate of ≥ 90%, whereas OAT1 and/or OAT3 substrates possess low PPB rates of < 90%.

The MECCS was proposed as a refinement of the ECCS framework.

the compounds for which rat biliary excretion > 10%. Both human OATP substrates and biliary excreted drugs were found to have high MWs of > 400 Da, and approximately 60% of the compounds were acids.

In 2015, Varma et al. [5] proposed the Extended Clearance Classification System (ECCS) framework, which facilitates the identification of rate-determining step(s) in clearance mechanisms determined by the physicochemical features of a drug (i.e., ionization permeability and MW). They noted that OATP-mediated hepatic uptake is likely preferred by acids and zwitterions with MW > 400 Da. On the basis of ECCS, 50 drugs were identified as class 1B and 3B. Among the 14 class 1B drugs, 14% were cleared renally as unchanged drugs and the rest were mediated by active hepatic uptake. Of the 36 class 3B drugs, 64% were excreted renally, and hepatic uptake and metabolism accounted for 25% and 11% of the total clearance, respectively [5]. Unfortunately, distinguishing between renal and hepatic uptake clearance mechanisms using ECCS class 3B is not possible. Therefore, improving the predictivity of the clearance mechanisms for these two classes will be interesting.

Meanwhile, it has been demonstrated that plasma protein binding (PPB) plays a key role in drug therapies by affecting their pharmacokinetics and pharmacodynamics, and thus, it may influence their metabolic modification [6]. The extent of binding depends on the structure and physicochemical properties of the drug molecules. Lipophilicity (log *P*) and acid–base properties are significantly correlated with binding [7]. Studies have shown that highly lipophilic molecules and/or highly acidic groups result in greater PPB [8, 9]. For example, terbinafine, a highly lipophilic drug, has a PPB of 99% and log*P* = 5.55 [9].

Recently, Kim et al. investigated the hepatic uptake clearance for a series of anionic drugs known as OATP substrates using suspended human hepatocytes. The results demonstrated that albumin facilitates the hepatic uptake to hepatocyte for anionic drugs with high protein binding [10]. Bowman et al. [11] proposed that transporters with high-affinity ligands may strip them away from their PPB sites; they called this a transporter-induced protein-binding shift (TIPBS). The notion of TIPBSs gained additional support when Miyauchi et al. [12] examined the active uptake of two organic anions, 1-anilino-8-naphthalene sulfonate (ANS) and pitavastatin, in rat hepatocytes with bovine serum albumin. They found that these two compounds were subjected to facilitated uptake, although pitavastatin showed higher unbound uptake compared with ANS, and pitavastatin had a greater affinity for OATP than did ANS. They stated that, with increased affinity for the transporter, albumin-mediated enhancement became more effective, which agreed with the TIPBS hypothesis [12]. In another study performed using HEK293 cells that overexpressed the OATP transporter, the uptake clearance of new chemical entities that were OATP1B3 substrates and had high PPB increased with increases in human serum albumin (HAS) concentrations, illustrating the role of albumin-mediated hepatic uptake for OATP1B3 substrates [13].

On the basis of a review of the literature, the potential utility of PPB must be assessed as a parameter to predict whether hepatic uptake is the rate-determining step for clearance of new chemical entities Hence, we investigated the effect of PPB on compounds that were OATP1B1 and/or OATP1B3 substrates, since the first step in drug elimination occurs via liver uptake into hepatocytes [14]. Additionally, we studied the effects of PPB on organic anion transporter (OAT1 and/or OAT3) substrates. Our objectives were to: (1) compile literature data for drugs known as substrates for human OATP1B1 and/or OATP1B3 and their PPB to investigate the effect of PPB on major hepatic OATPs; (2) compile literature data for drugs known as substrates for OAT1 and/or OAT3 and their PPB to investigate the effect of PPB on major renal OATs; (3) suggest a modified ECCS (MECCS) that could be used to distinguish between renal and hepatic uptake in classes 1B and 3B and predict overall routes of drug elimination.

2 Data and Methods

The data collection was carried out between 2018 and 2020, using the following keywords: ECCS, protein binding, hepatic uptake, OATP substrate, OAT substrate, permeability, renal clearance, and TIPBS. The dataset of hepatic uptake transporters (OATP1B1 and/or OATP1B3) substrates and renal uptake transporter (OAT1 and/or OAT3) substrates were compiled using the some of the widely accessible resources in the world including Google

Scholar, PubMed, and Science Direct. Only experimental human substrates were included, while any predicted and/or rat experimental data were excluded. Then their ionization state, MW and PB were collected and analyzed. The threshold of PPB to discriminate the high and low PPB was 90 as this value is a well-known value in the literature to distinguish the high PPB from the low PBB compounds [15, 16]. To modify the ECCS classes 1B and 3B, the same database that was developed to propose the ECCS by Varma et al. [5] was used. In the ECCS, 14 drugs were identified as class 1B and 36 drugs was identified as 3B class. Hence, in this research these drugs were used, and then 13 drugs with the same properties of either class 1B or 3B were added from the literature. Only compounds that have the same properties of these classes and have a known single human experimental clearance that contributed ≥ 70% of the total clearance have been added.

2.1 Hepatic Transporter Substrates

A dataset of 66 chemical substances, which are known to be human hepatic uptake transporters (OATP1B1 and/or OATP1B3), were collected from literature using Google Scholar, PubMed, and Science Direct to investigate the effect of PPB on drug clearance. The MWs and PPB values for these compounds were compiled from DrugBank [17] and from Google Scholar for compounds that were not listed in DrugBank [17] (Table S1).

2.2 Renal Transporter Substrates

A dataset of 41 drug compounds that are known to be human renal uptake transporter (OAT1 and/or OAT3) substrates were collected from the literature using Google Scholar, PubMed, and Science Direct to investigate the effect of PPB on these compounds. Their MWs and PPB values were compiled from DrugBank [17] and Google Scholar for those compounds that were absent from DrugBank [17] (Table S2).

2.3 MECCS

To modify the ECCS to successfully distinguish between hepatic uptake and renal excretion, we used the same database that was developed to propose the ECCS. Varma et al. [5] analyzed 307 drugs that had predominant clearance rate-determining steps (metabolism and renal or hepatic uptake) contributing ≥ 70% of the total clearance. According to this framework, 50 drugs were identified as classes 1B and 3B. Among the 14 class 1B drugs, 14% were cleared renally as unchanged drugs and the rest were mediated by active hepatic uptake, mainly by OATP transporters. Of the 28 Class 3B drugs, 64% were excreted renally, and hepatic uptake and metabolism accounted for 25% and 11% of the total clearance, respectively. However, montelukast and tesaglitazar were incorrectly predicted to be class 3B drugs. On the basis of their high passive permeability ($> 5 \times 10^{-6}$ cm/s), high MW (> 400 Da), and acidic natures, both drugs fit into class 1B [5, 18]. For the 50 drugs explored, their PPBs were further analyzed with respect to their mechanism of clearance in order to correctly predict when hepatic uptake was the predominant mechanism of excretion (Table S3). The PPB values for these 50 drugs, with clearance mechanism known from the study of Varma et al. [5], were collected from DrugBank [17] and Google Scholar for compounds that were absent from DrugBank [17] to correctly predict when hepatic uptake was the predominant mechanism of excretion (Table S3).

To understand the relationship between PPB and the mechanism of clearance and to predict when hepatic uptake and renal excretion could be the main clearance mechanisms (i.e., ≥ 70% of the total clearance), a database of human clearance mechanisms was developed on the basis of published data. As shown in the ECCS framework, acidic and zwitterionic drugs with MW > 400 Da were expected to be uptaken hepatically or renally cleared. Therefore, we explored a set of 63 acidic and zwitterionic drugs with MW > 400 Da with a single clearance mechanism (renal or hepatic uptake) that contributed ≥ 70% of the total clearance, including 50 drugs from ECCS classes 1B and 3B that were cleared via hepatic uptake or were unchanged through renal excretion (Table S4).

2.4 Statistical Analyses

All data were coded, entered, and analyzed using the Statistical Package for Social Sciences SPSS v. 20 (IBM Corp., USA). Data were presented using descriptive analyses, including frequencies and percentages. For statistical testing, the chi-square test and the independent Student's *t*-test were used to evaluate associations among MW, PPB, and hepatic uptake or renal clearance. The results were considered statistically significant if $p < 0.05$.

3 Results

3.1 Effect of PPB on Hepatic Transporter Substrates

An analysis of 66 drugs with hepatic uptake transporter (OATP1B1 and OATP1B3) substrates (Table S1) revealed that nearly 86% exhibited high PPB (≥ 90%), whereas MW was > 400 Da for ~ 82% of them. These compounds were typically found as acids or zwitterions. We noted that drugs

could be OATP1 B1 and/or OATP1 B3 substrates for acids and zwitterions with MW > 400 Da and that the percentage of PPB ≥ 90%.

3.2 Effect of PPB on Renal Transporter Substrates

Analyzing the renal uptake transporter (OAT1 and OAT3) substrates (Table S2) of 41 drugs revealed that 81% exhibited limited PPB, which is less than the 90% cutoff value. Consequently, we predicted that OAT uptake was controlled by the concentration of the free substrate and that, as proposed by Bow et al. [19], OAT uptake was extremely limited by binding to human serum albumin.

3.3 ECCS Modification

By utilizing the simple physicochemical properties of drugs, including MW, permeability, and ionization, Varma et al. [5] proposed ECCS to anticipate the predominant clearance mechanisms of drugs (Fig. 1). On the basis of their ECCS, compounds that were identified as class 1B are mainly cleared via active hepatic uptake by OATP transporters. Additionally, these drugs have high metabolic activity (> 70%) and are excreted as phase-I and phase-II metabolites in bile and/or urine. In contrast, class 3B drugs are predominantly cleared by hepatic uptake or renal secretion. By analyzing these classes of drugs ($n = 50$) with respect to their PPB (Table S3), we found that for acidic and zwitterionic compounds with MW > 400 Da and PPB ≥ 90%, active hepatic uptake may be the main clearance mechanism. Statistical analyses further showed that ~ 87% of the compounds uptaken hepatically exhibited high PPB (≥ 90%), whereas ~ 96% of renally eliminated drugs displayed limited PPB (< 90%).

By examining the 15 drugs that were identified as class 1B with respect to their PPB values, we found that three compounds exhibited limited PPB (1Bx), with 67% (two of three drugs) being eliminated renally. In contrast, the other 12 compounds displayed high PPB values (1By), with ~ 92% (11 of 12 compounds) cleared by hepatic uptake. Meanwhile, 24 drugs from the 35 that were classified as 3B in the ECCS exhibited low PPB values (3Bx), with 92% (two of 24 compounds) eliminated renally; 11 compounds from this

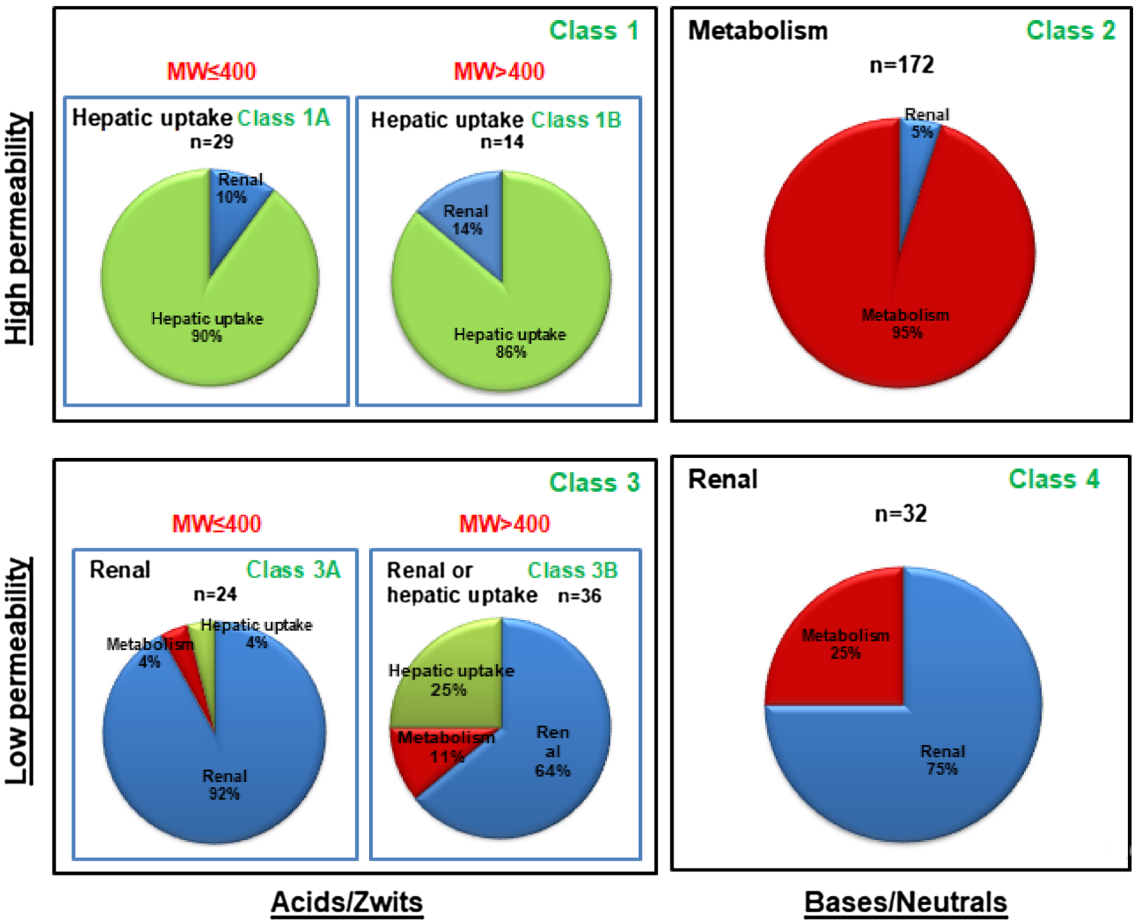

Fig. 1 The extended clearance classification system (ECCS) of Varma et al. [5]. *Zwits* zwitterions

class displayed high PPB values (3By), with ~82% (9 of 11 drugs) being cleared via hepatic uptake. We noted that, for acidic and zwitterionic drugs with MW > 400 Da and PPB ≥ 90%, hepatic uptake may be the predominant clearance mechanism.

To analyze MECCS, we constructed a dataset of 63 acidic and zwitterionic compounds with a single clearance pathway (hepatic or renal uptake) constituting > 70% of total clearance and MW > 400 Da, including 50 drugs of ECCS classes 1B and 3B plus, and 13 other compounds reported in the literature. The data were composed of ~79% acids and 21% zwitterions (Table S4), which were reclassified with respect to PPB. A total of 18 drugs were subsequently identified as class 1B and 45 as class 3B. For class 1B, five compounds exhibited low PPB values (1Bx), with 81% being eliminated renally. However, 13 compounds displayed high PPB (1By), with 92% of clearance occurring via hepatic uptake and a high extent of metabolic activity. For class 3B, 34 drugs displayed low PPB values (3Bx), 91% of which were eliminated renally. Meanwhile, 11 compounds exhibited high PPB (3By), with 82% being cleared by hepatic uptake (Figs. 2 and 3).

3.4 PPB among High-MW Acidic and Zwitterionic Compounds Exhibiting Hepatic Uptake or Renal Clearance

By studying the PPB distributions of the compounds that showed hepatic uptake or renal clearance (61 out of 63), we demonstrated that ~84% (21 out of 25) of the compounds with ≥ 70 hepatic uptakes were high-PPB (≥ 90%) compounds), with a mean PPB of 93.3%. Approximately 97% (35 out of 36) of the compounds with ≥ 70% renal excretion were found to exhibit less PPB < 90%, with a mean of 45.8%. Statistical analyses revealed that the PPB of acidic and zwitterionic drugs with high PPB values and MWs significantly affected the predominant clearance mechanism (hepatic uptake versus renal excretion), with $p = 0.000$, using both the chi-square and independent Student's *t*-tests. No distinct differences were noted in the distributions or

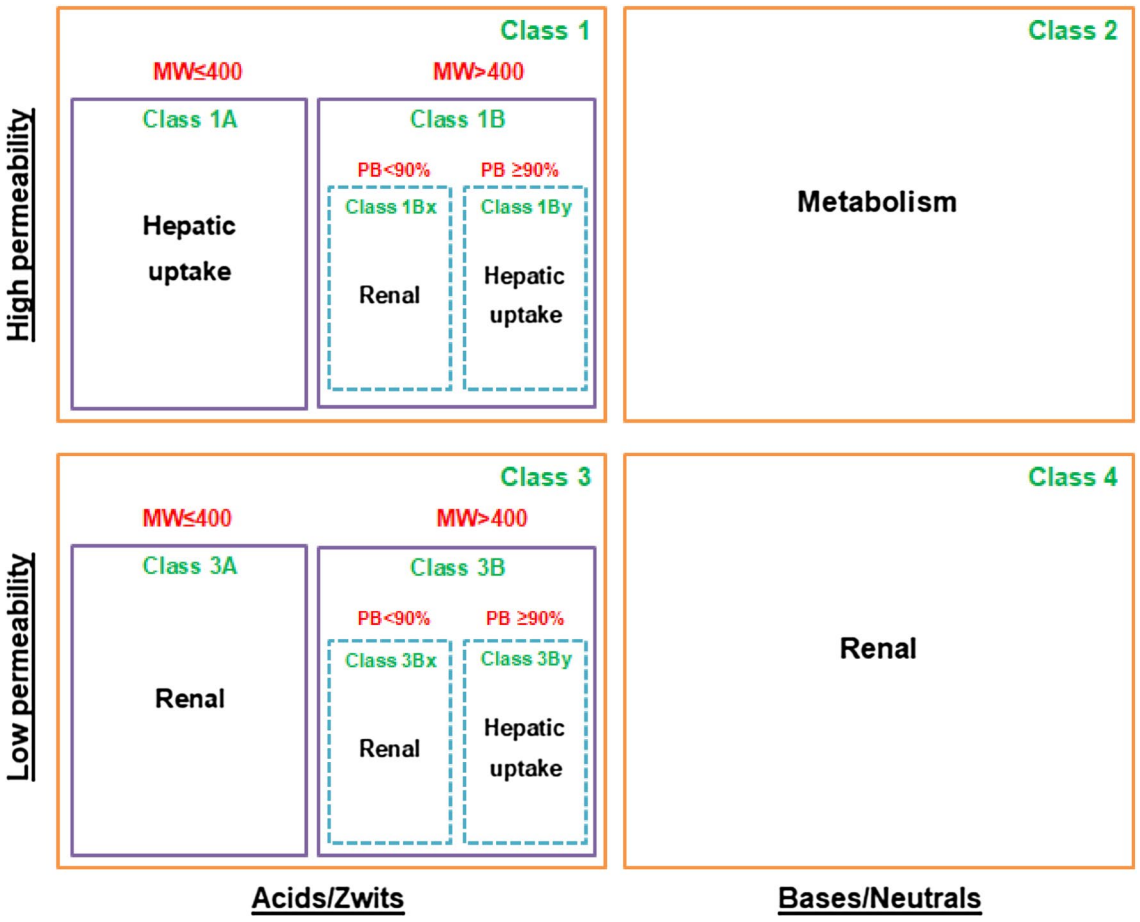

Fig. 2 The framework of the modified extended clearance classification system for identifying the predominant mechanism(s) determining the systemic clearance of drugs. *MW* molecular weight, *PB* protein binding, *Zwits* zwitterions

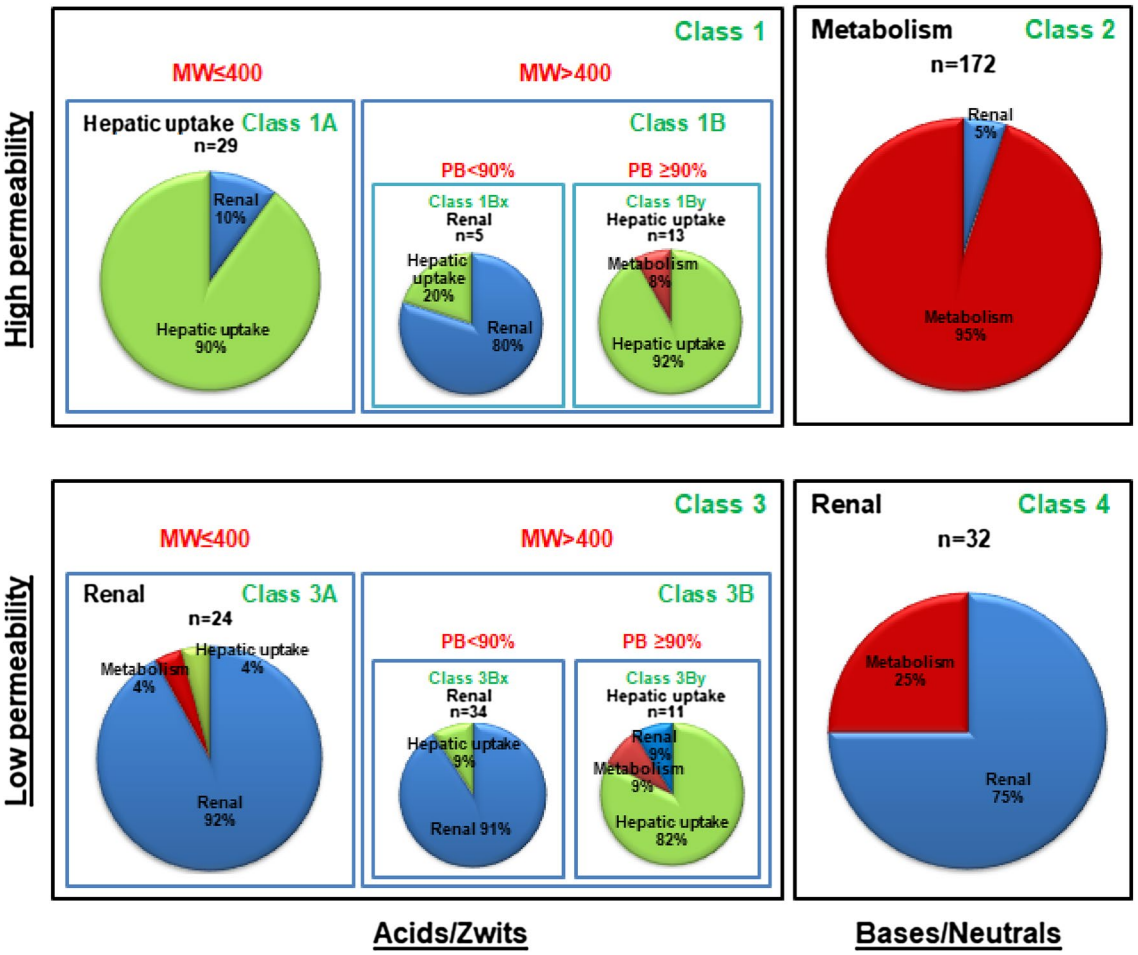

Fig. 3 Results of the modified extended clearance classification system obtained in this study. *MW* molecular weight, *PB* protein binding, *Zwits* zwitterions

mean MWs for renally versus biliary excreted compounds ($p = 0.825$, chi-square; $p = 0.751$, Student's *t*-test).

3.5 MECCS

On the basis of the assumption that drug excretion is affected by nonspecific and reversible drug binding to plasma proteins, the MECCS was proposed as a refinement of the ECCS framework of Varma et al. [5]. In the MECCS, PPB was used as an essential and independent variable, along with other physicochemical properties, including ionization state, MW, and membrane permeability, to predict the rate-determining process driving the systemic clearance of drugs. On the basis of this theory, the MECCS classified drugs into the following categories, as illustrated in Figs. 2 and 3:

1. Class 1A—low-MW acidic or zwitterionic compounds with high-permeability compounds mainly cleared by hepatic uptake mediated by OAT2 transporter.
2. Class 1Bx—low-PPB acidic and zwitterionic compounds, with high MWs and permeabilities, that are predominantly cleared via renal excretion;
3. Class 1By—high-PPB, high-MW and high-permeability acidic and zwitterionic compounds, for which hepatic uptake is the predominant clearance mechanism;
4. Class 2—high-permeability basic and neutral compounds, for which metabolism is the predominant clearance mechanism;
5. Class 3A—acidic and zwitterionic compounds that have low MWs and permeabilities and are predominantly cleared renally;
6. Class 3Bx—acidic and zwitterionic compounds with limited PPB and permeability values, and high MWs, for which renal clearance is the dominant mechanism;
7. Class 3By—acidic and zwitterionic compounds with high PPB and low permeability, for which hepatic uptake is the predominant clearance mechanism;
8. Class 4—low-permeability basic and neutral compounds that are mainly cleared renally.

4 Discussion

Binding to plasma proteins is an important factor that influences the volume of distribution and renal elimination. With higher free drug concentration, the displaced drug will distribute throughout the volume of distribution and the elimination rate will increase (if CL is unchanged)

PPB is an important factor that influences the volume of distribution and renal elimination [20]. With the higher unbound drug concentrations, the displaced drugs freely circulate throughout this volume, thereby increasing the rate of elimination if clearance remains unchanged [20]. Organic anion transporting polypeptides make up a family of uptake transporters that are expressed in various tissues and are important for drug disposition. Researchers have demonstrated that the rate-determining step in the hepatic clearance of substances in bile is hepatic sinusoidal uptake [14, 21–23]. This active transport is primarily mediated by OATP transporters. Of the 11 human OATP transporters, the primary hepatic ones—OATP1B1 and OATP1B3—are mainly localized on the basolateral side of the hepatocyte and mediate the influx of their substrates from the blood into hepatocytes. After crossing the basolateral membrane and entering hepatocytes, drugs are either excreted unchanged into the bile or metabolized [21, 24, 25]. Recently, Varma et al. [5] analyzed 219 diverse compounds using human OATP substrate data. All the OATP substrates used in this study exhibited similar physicochemical properties; they are acidic compounds with molecular weights greater than 400 Da and also possess high polar surface area, which is consistent with the features of known hepatic uptake system substrates [5]. As explained by free drug theory, only free drug concentrations have been thought to interact with target receptors and drive drug efficacy [26]. In 1966, Baker and Bradley [27] were the first to break from free drug theory and suggested that the hepatic uptake of drugs was high when most drugs were bound to plasma proteins, and therefore hepatic uptake may occur directly from the albumin–drug complex and not just from free drugs. Subsequent trials have demonstrated that the hepatic uptake of compounds and OATP substrates display albumin-mediated hepatic transport kinetics [10, 12, 28, 29].

The present study found that PPB could be used to distinguish between renal and hepatic uptake in ECCS classes 1B and 3B, and among acidic and zwitterionic compounds with high MWs (> 400 Da), wherein a single clearance mechanism contributed ≥ 70% of the total clearance.

It has been demonstrated that plasma proteins play a vital role in the hepatic uptake. Recently, Bowman et al. evaluate the uptake of four known OATP substrates, with different protein binding values, in buffer and human plasma incubations. They found that the $K_{m,u}$ and V_{max} values decreased in the plasma as protein binding increased [30]. This led to higher CLint ($V_{max}/K_{m,u}$) values in the human plasma incubations for the highly bound compounds. These results support the recent hypothesis of a transporter-induced protein-binding shift for compounds with high protein binding and high affinity for transporters. Therefore, it will be interesting to compare K_m values for OATP substrates with different PPB and evaluating their hepatic uptake. This will be our ongoing study [30]. In 2021, Francis et al. studied the plasma protein-mediated uptake and its impact on clearance prediction to improve in vitro–in vivo extrapolation (IVIVE) [31]. They found that including the plasma protein-mediated uptake improved the IVIVE of hepatic clearance irrespective of compounds ionization, ECCS groups, assay conditions, and species [31].

4.1 Comparison of Classification Systems

In 2005, Wu and Benet [32] proposed the Biopharmaceutics Drug Disposition Classification System (BDDCS), which categorizes drugs into four classes according to their solubility and metabolic extent based on the association between the permeability of the compound and its metabolism. The BDDCS predicts drug disposition, including the route of elimination, drug–drug interactions, and the effects of transporters. However, it should be taken into consideration that the BDDCS projects only the extent of elimination and has limited utility in predicting the rate-determining step [33]. Using this scheme, the highly permeable classes 1 and 2 were found to be mainly eliminated via metabolism, whereas a large proportion of the low-permeability BDDCS classes (classes 3 and 4) were found to be eliminated unchanged via renal or biliary excretion (Fig. 4a) [32]. Subsequently, Varma et al. [5] proposed an alternative classification of compounds into six categories based upon their permeability, ionization state, and MW (Fig. 4b): class 1A—acidic or zwitterionic, low-MW and high-permeability compounds for which their rate-determining step is hepatic uptake mediated by OAT2 transporters followed by metabolism [34]; class 1B—acidic or zwitterionic, high-MW, and low-permeability compounds that are mainly excreted by active hepatic uptake; class 2—basic and neutral drugs that are highly permeable and dominantly cleared by metabolism; class 3A—acidic or zwitterionic, low-MW, and low-permeability compounds for which clearance is mostly determined by renal elimination; class 3B—acidic or zwitterionic, high-MW, and low-permeability compounds that are predominantly cleared by renal or hepatic uptake; Class 4—basic and neutral, low-permeability compounds that are mainly eliminated renally (Fig. 4b) [5].

A major difference between BDDCS and ECCS is providing predictability based on solubility. However, solubility has no importance from the point of view of the clearance mechanism and hence was not considered in ECCS.

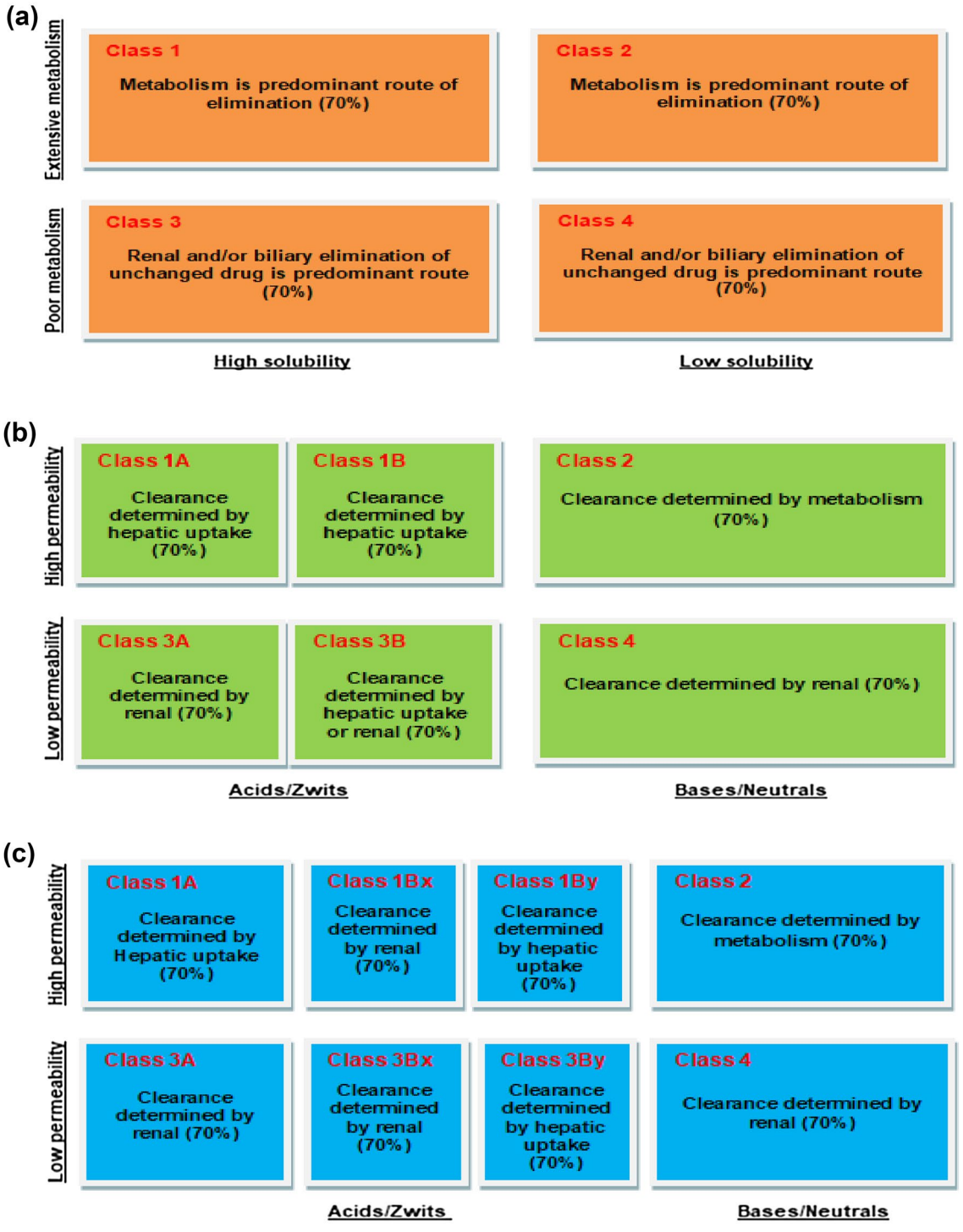

Fig. 4 Comparison among the **a** Biopharmaceutics Drug Disposition Classification System [27], **b** the Extended Clearance Classification System [5], and **c** the Modified Extended Clearance Classification System (this study). *Zwits* zwitterions

Moreover, ECCS correctly predicted the rate-determining clearance mechanism, while BDDCS projects only the extent of elimination and has limited utility in predicting the rate-determining step [5].

On the basis of the hypothesis that PPB is relevant for predicting clearance mechanisms or elimination routes for some classes, we proposed MECCS, an extension of ECCS [5]. The MECCS framework contains PPB as a new independent

physicochemical property, in addition to preexisting categories, such as MW, ionization state, and compound permeability, to increase predictive capabilities regarding drug elimination routes. Consequently, the MECCS classifies compounds into eight groups, as illustrated in Fig. 4c (also see Sect. 3.5).

Recently, the BDDCS and ECCS have played key roles in the early prediction of clearance mechanisms during drug discovery. However, neither scheme can differentiate between renal and hepatic uptake mechanisms in some classes. For BDDCS, both class 3 and 4 drugs are generally cleared by renal or hepatic uptake [35], whereas the predominant clearance mechanism for ECCS class 1B and 3B drugs is either hepatic uptake or renal excretion [5]. Here, we propose PPB as a basic principle in the MECCS framework, which solves the limitation of ECCS in predicting renal versus hepatic uptake for class 1B and 3B drugs. However, unlike BDDCS, both ECCS and MECCS do not include the solubility and formulation features of the oral absorption; hence, they cannot be utilized for biowaiver purposes. Finally, we did not study if PPB played a role in the other MECCS classes; therefore, future work should be focused on exploring this issue and on quantifying the MECCS scheme to optimize the predictability of major clearance mechanisms. This study should be considered as an observation, and future studies can validate these findings with large datasets of these known transporters. One of our future aims is to study whether the protein binding affects the site of drug excretion in OATP2B1 and OAT 2 transporters as these transporters are expressed in many organs [33, 36–38].

5 Conclusions

Overall, the results of this study showed that acidic and zwitterionic compounds with MW >400 Da and PPB $\geq 90\%$ could be OATP1B1 and/or OATP1B3 substrates. Meanwhile, OAT1 and/or OAT3 substrates typically exhibited limited PPB ($\leq 90\%$). We also demonstrated that hepatic uptake may be the predominant clearance mechanism for acidic and zwitterionic drugs with MW >400 Da and PPB $\geq 90\%$, whereas a renal clearance mechanism is predicted to be the predominant pathway for compounds with MW is >400 Da and PPB is $<90\%$.

It is clear that PPB can be used to predict whether a new compound is a substrate for OATP1B1 and/or OATP1B3 or whether hepatic uptake is the rate-determining step for its clearance. Moreover, it is worth considering PPB as one of the factors that could play a role in predicting the disposition of transporter-mediated compounds, which may include predicting the substrate and/or inhibitor affinity of the transporter. Therefore, PPB can be used in the early optimization of new compound properties, such as drug–drug interactions, pharmacokinetics, and tissue distribution. Our work further confirms previously reported experimental observations and theoretical models that suggested the phenomenon of albumin-mediated hepatic uptake via organic anion transporters and the concept of TIPBS. Future work is needed to quantify MECCS to optimize predictions of major drug clearance mechanisms.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13318-022-00806-4.

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Declarations

Authors Contributions Y.K. and F.H. made substantial contributions to the conception of the work as well as analysis and interpretation of data, drafted the work, and revised it critically for important intellectual content. H.H. supervised the work, approved the version to be published, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Availability of Data and Materials All data generated or analyzed during this study are included in this published article and its supplementary information files.

Code Availability Not applicable.

Conflict of interest Yahya Khawaja, Fatma Haddad, and Hussein Hallak declare that they have no conflicts of interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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