Artificial Neural Network Modeling for Drug Dialyzability Prediction

Kahina Daheb[1], Mark L. Lipman[2], Patrice Hildgen[3], Julie J. Roy[1]

[1]Department of Pharmacy and [2]Department of Nephrology, Jewish General Hospital; [3]Faculté de pharmacie, Université de Montréal, Montreal, Canada.

Received, June 12, 2013; Revised, July 15, 2013; Accepted, October 31, 2013; Published, November 6, 2013.

ABSTRACT – Purpose. The purpose of this study was to develop an artificial neural network (ANN) model to predict drug removal during dialysis based on drug properties and dialysis conditions. Nine antihypertensive drugs were chosen as model for this study. **Methods.** Drugs were dissolved in a physiologic buffer and dialysed *in vitro* in different dialysis conditions (UFRmin/UFRmax, with/without BSA). Samples were taken at regular intervals and frozen at -20ºC until analysis. Extraction methods were developed for drugs that were dialysed with BSA in the buffer. Drug concentrations were quantified by high performance liquid chromatography (HPLC) or mass spectrometry (LC/MS/MS). Dialysis clearances (CLDs) were calculated using the obtained drug concentrations. An ANOVA with Scheffe's pairwise adjustments was performed on the collected data in order to investigate the impact of drug plasma protein binding and ultrafiltration rate (UFR) on CLD. The software Neurosolutions® was used to build ANNs that would be able to predict drug CLD (output). The inputs consisted of dialysis UFR and the herein drug properties: molecular weight (MW), logD and plasma protein binding. **Results.** Observed CLDs were very high for the majority of the drugs studied. The addition of BSA in the physiologic buffer statistically significantly decreased CLD for carvedilol ($p= 0.002$) and labetalol ($p<0.001$), but made no significant difference for atenolol ($p= 0.100$). In contrast, varying UFR does not significantly affect CLD ($p>0.025$). Multiple ANNs were built and compared, the best model was a Jordan and Elman network which showed learning stability and good predictive results ($MSE_{testing} = 129$). **Conclusion.** In this study, we have developed an ANN-model which is able to predict drug removal during dialysis. Since experimental determination of all existing drug CLDs is not realistic, ANNs represent a promising tool for the prediction of drug CLD using drug properties and dialysis conditions.

INTRODUCTION

Hemodialysis techniques are in continuous evolution and new drugs are emerging on the market every year (1-6). However, dialyzer manufacturers are only providing few substance removal results in their membrane assessment (ex. urea, creatinine, etc.). The preponderance of existing data pertains to conventional dialysis techniques and may now be obsolete because of the wide use of high-permeability dialysis techniques (7). It is critical for optimal patient care to update our data on drug removal during dialysis. Unfortunately, the clinical investigations are costly and often impractical because of ethical concerns and *in vitro* experiments are also costly and time consuming. Therefore, a method validated to predict drug dialyzability would be a stepping stone in updating our data on drug removal during dialysis.

An artificial neural network (ANN) is a connectionist model composed of non-linear computational elements called 'neurons or axons' arranged in highly interconnected layers with a structure that simulates the transfer of information through the nervous system (8). By changing the transfer functions and the associated parameters, this constructed neural network adapts itself to the pattern of the input variables and eventually generates numbers that iteratively solves to values of the designated output variables (9). ANNs are used to detect complex patterns within data sets that may not be obvious with conventional statistical methods. Hence, ANNs have been increasingly

Corresponding Author: Julie J. Roy, Ph.D.; Department of Pharmacy; Jewish General Hospital; 3755, chemin de la Côte-Sainte-Catherine; Montreal (Quebec) Canada; Email: jroy@jgh.mcgill.ca

utilized as a helpful tool for clinical decision making in both diagnosis and treatment (10, 11).

Existing literature fails to define the precise impact of dialysis conditions and drug physicochemical properties on their removal during dialysis. The general principles are well recognized in the literature but the impact of each of the potential parameters (molecular weight, molecular size, charge, water/lipid solubility and protein binding) has not been addressed in detail. The challenge is to build mathematical prediction models, by understanding what are the most important factors affecting drug dialyzability and to what extent. The purpose of this study was to develop an ANN-based predictive model to predict drug removal during dialysis using ultrafiltration rate (UFR) and some drug properties (molecular weight, logD, plasma protein binding) as inputs for the model.

As a vast majority of end-stage renal disease (ESRD) patients suffer from cardiovascular diseases (CVD), two frequently prescribed family drugs for CVD, β-blockers and angiotensin converting enzyme inhibitors (ACE-Inhibitors), were chosen as models for this study (12-14).

METHODS

In Vitro dialysis

The chosen method for the evaluation of drug removal *in vitro* was adapted from the literature (15, 16). Drugs were dissolved in 6 or 9 litres of Krebs-Henseleit buffer containing 32 g/L of bovine serum albumin (BSA) (17). The dose used was 400 mg for all β-blockers (acebutolol, atenolol, labetalol, metoprolol, nadolol) except carvedilol for which the dose was 220 mg for cost reasons. For ACE-Inhibitors the doses were 100 mg, 5 mg, 5 mg, 3.8 mg and 4.8 mg for lisinopril, ramipril, ramiprilat, enalapril and enalaprilat respectively. Ramiprilat and enalaprilat are the active drugs that result from the de-esterification of the prodrugs ramipril and enalapril in the body. Drug analytical standards were purchased from Sigma-Aldrich (St. Louis, MO, USA); except ramipril, ramiprilat and enalaprilat dihydrate which were purchased from Toronto Research Chemicals (North York, ON, Canada). BSA was purchased from BioShop (Burlington, ON, Canada). The drug reservoir was equipped with a constant magnetic stirrer and maintained at 37ºC. Polyvinyl chloride tubing, identical to that used for patients, connected the reservoir to the dialyser and back. The former tubing served as the "arterial line", and the latter tubing as the "venous line" providing a closed loop system (Figure 1).

Dialysis was undertaken with a Tina® or Aurora® dialyser (Baxter Inc., Mississauga, ON, Canada), and a polysulfone Optiflux® F160 NR membrane (Fresenius Medical Care North America, Lexington, MA, USA). They were set as follows: dialysis duration = 180 min, reservoir solution flow rate (Q_a) = 300 mL/min and dialyzate flow rate (Q_d) = 500 mL/min. UFR was set to 0,11 L/h or 1 L/h. Three-mL samples were collected from both arterial and venous line before and at 5, 10, 20, 30, 60, 90, 120, 150 and 180 minutes. Samples were frozen at -20ºC until analyzed.

Impact of drug plasma protein binding on CLD

Three *in vitro* dialysis sessions with albumin in the physiologic buffer have been performed with carvedilol, which is highly protein bound (98%), labetalol which has a medium protein binding (50%) and atenolol, which has a low protein binding (6 -16%) (18). In order to assess their CLD, a protein extraction method has been developed before HPLC analysis. For each of the three drugs an ANOVA with Scheffe's pairwise adjustments was used to compare their CLD in the two conditions UFmin with and without BSA.

Impact of ultrafiltration rate on CLD

The effect of UFR on drug CLD was investigated, by conducting three *in vitro* dialysis sessions with atenolol, carvedilol and labetalol, starting with a volume of 9 L. This volume represents the average blood volume of most patients before their dialysis. UFR was set at 1 L/h to get a volume of 6 L at the end of the 3-hour dialysis session.

Extraction procedure for atenolol

The liquid-liquid extraction method of Yilmaz *et al.* was adapted (19). One mL of each sample was introduced into a glass tube, followed by 10 µL of metoprolol tartrate as internal standard at a concentration of 10 µg/mL and 200 µL NaOH 1 M. Samples were vortex-mixed for 15 s. Seven mL of an ethyl acetate and diethyl ether 2:1 (v/v) mixture were added to all tubes, which were shaken horizontally for 30 min before 10-min centrifugation at 1750*g*.

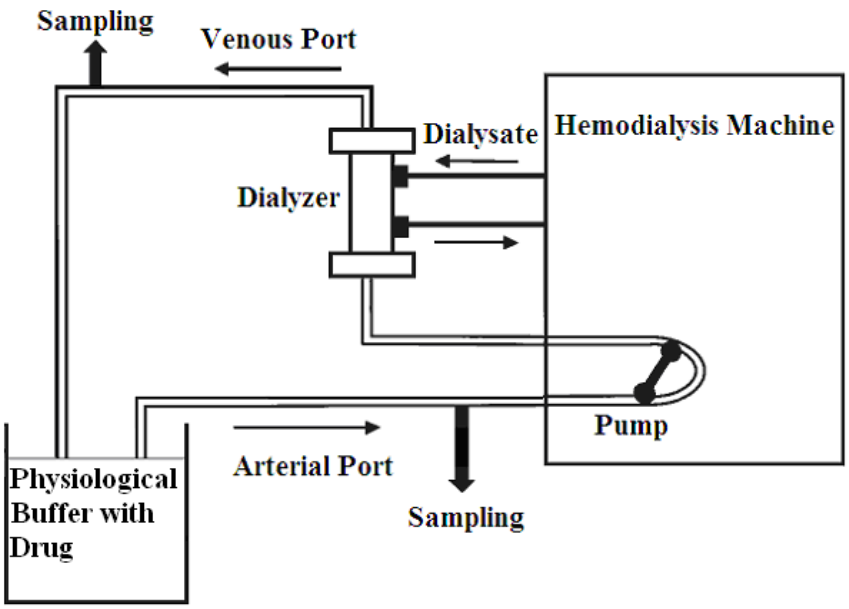

Figure 1. *In vitro* dialysis set up.

Six mL of the upper organic layer were transferred to another set of clean glass tubes and evaporated to dryness in a SpeedVac at medium temperature at around 40-45°C. Dry residues were dissolved in 1 mL of distilled water and vortex-mixed for 30 s. The reconstituted samples were subjected to a 0.45 µm nylon syringe filtration (Millex, Fisher, Mississauga, ON, Canada) and transferred to high-performance liquid chromatography (HPLC) vials for analysis.

Extraction procedure for labetalol

One mL of each sample was introduced into a glass tube, followed by 10 µL of metoprolol tartrate as internal standard at a concentration of 15 µg/mL and 200 µL of NaOH 1 M. Samples were vortex-mixed for 15 s. Seven mL of ethyl acetate were added to all tubes, which were shaken horizontally for 30 min before 10-min centrifugation at 1750*g*. Six mL of the upper organic layer were transferred to another set of clean glass tubes and evaporated to dryness in a SpeedVac at medium temperature at around 40-45°C. Dry residues were dissolved in 1 mL of mobile phase and vortex-mixed for 30 s. The reconstituted samples were subjected to 0.45 µm nylon syringe filtration (Millex, Fisher, Mississauga, ON, Canada) and transferred to high-performance liquid chromatography (HPLC) vials for analysis.

Extraction procedure for carvedilol

The liquid-liquid extraction method of Borges *et al* was adapted (20). One mL of each sample was introduced into a glass tube, followed by 15 µL of metoprolol tartrate (Sigma-Aldrich) as internal standard at a concentration of 15 µg/mL of and 200 µL of $NaHCO_3$ 0.5 M. Samples were vortex-mixed for approximately 10 s. Ten mL of diethyl-ether were added to all tubes, which were shaken horizontally 30 min and then frozen for another 30 min at -80 ºC. The upper organic phase was transferred to another set of clean glass tubes and evaporated to dryness in a SpeedVac at medium temperature at around 40-45°C. Dry residues were dissolved in 1 mL of mobile phase, vortex-mixed for 10 s and sonicated for 10 min. The reconstituted samples were subjected to 0.45 µm nylon syringe filtration (Millex, Fisher, Mississauga, ON, Canada) and transferred to high-performance liquid chromatography (HPLC) vials for analysis.

Extraction procedure for enalapril, ramipril and their active metabolites

A liquid-liquid extraction was performed by adding 0.5 mL of formic acid 2% to each 0.05 mL sample in order to achieve maximal ionization of the molecules. Then, samples were spiked with 0.1 mL of internal standards at a concentration of 0.5 µg/mL, ramipril and ramiprilat were used as internal standards for enalapril-enalaprilat analysis

and vice versa. Seven mL of ethyl acetate were added to all tubes, which were mechanically shaked for 10 min before 10-min centrifugation at 1888*g*. Six mL of the upper organic layer were transferred to another set of clean glass tubes and evaporated to dryness under nitrogen stream for 20 min at 40°C. Dry residues were dissolved in 0.2 mL of a mixture of acetonitrile, methanol and 0.05% formic acid 1:1:8 (v/v/v). A 5-µL aliquot of the solution was injected onto the LC/MS/MS system for analysis.

Chromatographic analysis with HPLC-UV

All drug concentrations were quantified by HPLC with ultraviolet (UV) detection, except for enalapril, ramipril and their active metabolites which were analyzed with mass spectrometry (see below). The HPLC system used was a Shimadzu Prominence UFLC chromatographic system (Shimadzu Corporation, Tokyo, Japan) with a Phenomenex column (HyperClone 5 µm BDS C8 130A 150 x 4.60 mm 5 microns, Torrance, CA, USA). The different mobile phases were pumped at a flow rate of 1 mL/min and consisted of various proportions of phosphate buffer and methanol, as illustrated in Table 1.

The chromatographic analysis for atenolol and metoprolol (used as internal standard in the extraction procedure) was performed nonstereopecifically with an HPLC gradient elution method. The mobile phase consisted of A (methanol with 0.1% trifluoroacetic acid) and B (water with 0.1% trifluoroacetic acid). Gradient conditions were: initial 0-10 min linear change from A-B (15:85 v/v) to A-B (60:40 v/v), 10-11 min linear change from A-B (60:40 v/v) to A-B (15:85 v/v), and 11-13 min isocratic elution A-B (15:85 v/v). The mobile phase was pumped at a flow rate of 1 mL/min. Injection volume was 20 µL. Detector wave length was set at 223 nm and oven temperature at 30ºC.

Chromatographic and mass spectrometry (LC/MS/MS) analysis of enalapril, ramipril and their active metabolites

An API 2000™ triple quadrupole mass spectrometer (Applied Biosystems-SCIEX, Concord, ON, Canada) equipped with a Turbo IonSpray source and an 1100 Series HPLC system (Agilent, Mississauga, ON, Canada) were used for LC/MS/MS analyses. Chromatography was performed on a C8 column (5 µm, 50 x 2.1 mm, Intertsil, Torrance, CA, USA). The mobile phase consisted of an acetonitrile, methanol and 0.1% formic acid, 4:4:5 (v/v/v) mixture. The mobile phase was pumped at a flow rate of 0.21 mL/min through the column which was maintained at 55°C. The mass spectrometer was operated in the positive ion detection mode with nitrogen as the nebulising, turbo spray and curtain gas with the optimum value set at 60 psi. The turbo-gas temperature was set at 450ºC and electrospray ionisation (ESI) needle voltage was adjusted to 5400 V. Quantitation was performed using multiple reaction monitoring (MRM) of the transitions of *m/z* 377.20 → *m/z* 234.10 for enalapril, *m/z* 349.20 → *m/z* 206.10 for enalaprilat, *m/z* 417.20 → *m/z* 234.10 for ramipril and *m/z* 389.10 → *m/z* 206.00 for ramiprilat.

Table 1. Chromatographic conditions

Drugs	***Mobile phase composition***		***Oven T°***	***Detector wave length***
	Methanol	***KH_2PO_4 10 mM***		
Atenolol	20 %	80 %	30ºC	275 nm
Labetalol	50 %	50 %	30ºC	303 nm
Metoprolol	30 %	70 %	35ºC	275 nm
Carvedilol	50 %	50 %	35ºC	242 nm
Nadolol	30 %	70 % (buffer pH adjusted to 2,8 with phosphoric acid)	25ºC	270 nm
Acebutolol	35 %	65 %	30ºC	225 nm
Lisinopril	20 %	80 % (buffer pH adjusted to 2,5 with phosphoric acid)	40°C	212 nm

DATA ANALYSES

Drug concentrations were quantified by plotting the area obtained for each sample against a calibration curve. Correlations between peak areas and drug concentrations were linear for the full range of calibration curve concentrations. The correlation coefficients were superior to 0.98 for all calibration curves ($r^2 > 0.98$). Coefficients of variation for quality controls were less than 15 % for all drugs, except for carvedilol in one experiment where the coefficient of variation was inferior to 20% for the small quality controls (QCs).
Drug clearance during dialysis was calculated as follows:

$$CLD = [(Q_a \times C_a) - (Q_v \times C_v)]/ C_a \qquad (1)$$

where Q_a was the arterial blood flow rate, C_a was drug concentration in the arterial line, Q_v was the difference between Q_a and UFR, and C_v was drug concentration in the venous line (21). CL_D was calculated at each sampling time. A Trapeze area integration method was employed to compute average CL_D in the dialysis sessions.

STATISTICAL ANALYSIS

Data are expressed as mean ± SD. The significance of differences among groups was tested using analysis of variance (ANOVA). Scheffe method was used for pairwise multiple comparisons. The level of significance was set at 5%. All the statistical analyses were done on Stata statistical software (Intercooled version 8.2, StataCorp, College Station, TX, USA).

Artificial neural networks

All ANNs were built using Neurosolutions® 6. The networks discussed in this study are described in Neurosolutions® as follows:

Multilayer Perception (MLP): the most widely used neural network either for classification or regression problems.

Generalized Feedforward MLP: MLP plus additional layer-to-layer forward connections. It shows additional computing power over standard MLP.

Jordan and Elman: MLP with non-adaptable recurrent feedback. It adds fixed memory to the MLP for simple temporal problems with fixed temporal dependencies.

The verification and validation process that was undertaken to choose the best model was the following: we applied our data (drug properties and experimental dialysis clearance results) to different models already built in the software. Among all models tested, we chose to report in our article what we found to be the best 3 models. The best models are those that can successfully predict the dialysis clearance of drugs included in the testing set. These latter drugs were not used to train the model. Finally, the model that shows the highest correlation factor (r^2) and the lowest mean square error is chosen as the best model among all the models tested.

Data used to build ANNs were divided as inputs and outputs. In this study, the outputs consisted of CLDs of nine antihypertensive drugs obtained with *in vitro* experiments in different conditions (UFR*min*/UFR*max* and *with*/*without* BSA). The inputs consisted of dialysis UFR and some drug properties: molecular weight (MW), logD and plasma protein binding. Data were divided into three sets: training, cross-validation (CV) and testing set (Table 4). The training set must be representative of the data and large enough to allow building a good network that will be able to generalize on new data. As described in Neurosolutions®: periodically, during training on training data set, the network is tested for performance on the CV set. If the network is starting to over train on the training data (i.e. memorize the data), the CV performance will begin to degrade. Thus, the CV data set is used to determine when the network has been trained as well as possible without "overtraining" (i.e. maximum generalization). When varying the network parameters, the best network weights are automatically saved during training and will be loaded into the network before the testing process is run. Finally, the testing set consists of a set of data excluded from the training process in order to test the generalizing ability of the network on new data.

Neurosolutions® allows for choosing and varying several parameters in the networks. The main parameters that were varied when building the networks were: number of hidden layers, number of processing elements (axons) in each layer, type of transfer axon (Tanhaxon, bias axon, etc.) and

mathematical functions (momentum/Levenberg Marquat, etc.). The training iterations were always set at 10 runs with 10000 epochs for each run, enabling the termination option when the CV performance do not improve after multiple epochs.

The three best ANN models were selected for comparison: Multilayer Perception (MLP), Generalized Feedforward MLP (GFF) and Jordan-Elman. The selection of these models was based on their description on Neurosolutions® and the resulting Mean Square Error (MSE) after their training. The MSE is defined as:

$$MSE = \frac{\left(V_{act} - V_{pred}\right)}{N} \qquad (2)$$

Errors are squared to penalize the larger errors and to cancel the effect of the positive and negative values of the differences (22). r^2 was useful in assessing the performance of the training sets but not for the CV and testing sets because they contained only two exemplars each.

In order to evaluate the learning stability of the network models selected, new networks were built thrice using exactly the same parameters.

RESULTS

An ANOVA with Scheffe's pairwise adjustments was performed on the collected data in order to investigate the impact of drug plasma protein binding and ultrafiltration rate (UFR) on dialysis clearance (CLD). The results are showed in Figure 2 and in Tables 2 and 3.

It was found that the addition of BSA in the physiologic buffer statistically significantly decreased CLD for carvedilol ($p= 0.002$) and labetalol ($p<0.001$), but made no significant difference for atenolol ($p= 0.100$) (Table 2). A two-group t-test method was also used for the above mentioned comparison and the results were concordant with the Scheffe' adjusted method (t-test results not shown).

An ANOVA with Scheffe's pairwise adjustments was used to compare drug CLD between the two conditions UFRmin = 0.11 L/h and UFR= 1L/h. It showed that UFR does not significantly affect CLD (Table 3). A two-group t-test method was also used for the above mentioned comparison and the results were concordant with the Scheffe' adjusted method (t-test results not shown). Data used to build the ANNs were obtained *in vitro* and are shown in Table 4.

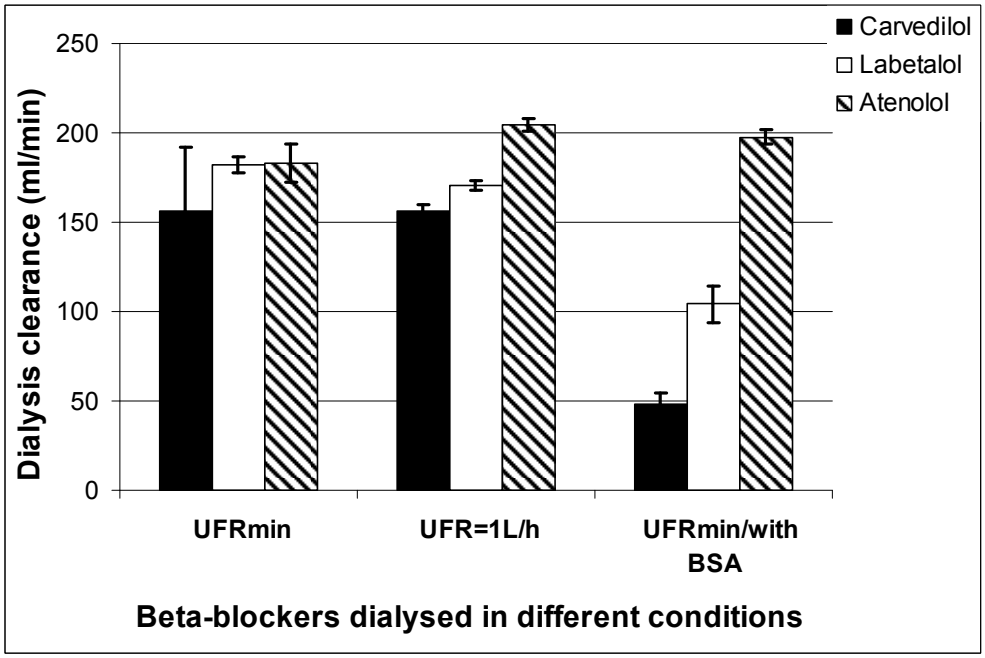

Figure 2. *In Vitro* dialysis clearances (Mean ± SD, n=3) of carvedilol (99% protein bound), labetalol (50% bound) and atenolol (6-16% bound) in different conditions:

1. UFmin: minimal ultrafiltration rate (0.10 – 0.11 L/h) *without* bovine serum albumin (BSA)
2. UF = 1 L/h without BSA
3. UFmin and *with* BSA in the physiologic buffer

Table 2. Comparison of atenolol, labetalol and carvedilol dialysis clearances (Mean ± SD, n=3) in two conditions: minimal ultrafiltration rate (UFmin = 0.11 L/h) *with* and *without* bovine serum albumin (BSA) in the buffer.

	Atenolol (10% prot. bound)	*Labetalol (50% prot. bound)*	*Carvedilol (98% prot. Bound)*
		CLD (mL/min)	
UFmin *without* BSA	183 ± 11	182 ± 5	156 ± 36
UFmin *with* BSA	198 ± 4	104 ± 10	48 ± 7
P-Value*	0,100	<0,001	0,002

*Scheffe' adjusted method; significant if p-value <0,025

Table 3. Comparison of atenolol, labetalol and carvedilol dialysis clearance (Mean ± SD, n=3) in the two conditions: minimal ultrafiltration rate (UFmin) and Ultrafiltration rate = 1 L/h.

	Atenolol	*Labetalol*	*Carvedilol*
			CLD (mL/min)
UFmin = 0.11 L/h	183 ± 11	182 ± 5	156 ± 36
UF = 1L/h	203 ± 3	170 ± 3	156 ± 4
P-Value*	0,033	0,195	1,000

* Scheffe' adjusted method; significant p-value if <0,025

Table 4. Drug properties and dialysis conditions used as inputs to build the artificial neural networks, and experimental drug dialysis clearances considered as outputs.

Drugs	***MW (g/mol)*** (23)	***logD**** (24)	***Protein Binding (%)***	***Ultrafiltration rate (L/h)***	***CLD (mL/min)***	***Sets***
Enalapril/BSA**	376.45	-1.21 (25)	60 (26)	0.11	145	
Enalaprilat/BSA	348.4	-4.85	60 (26)	0.11	174	
Ramipril/BSA	416.51	-0.33 (25)	73 (27)	0.11	45	
Ramiprilat/BSA	388.46 (28)	-2.22 (25)	56 (27)	0.11	138	
Lisinopril	405.49	1.01 (25)	0 (26)	1	197	
Atenolol	266.34	-1.57 (23)	0	0.11	183	
Atenolol/BSA	266.34	-1.57 (23)	3 (26)	0.11	198	**Training**
Atenolol	266.34	-1.57 (23)	0	1	203	
Labetalol	328.41	-0.17	0	0.11	182	
Labetalol/BSA	328.41	-0.17	50 (26)	0.11	104	
Labetalol	328.41	-0.17	0	1	170	
Carvedilol	406.47	2.89	0	0.11	156	
Carvedilol/BSA	406.47	2.89	95 (26)	0.11	48	
Carvedilol	406.47	2.89	0	1	156	
Acebutolol	336.43	-0.88	0	0.11	192	**Cross-**
Metoprolol	267.36	0.61	0	0.11	191	**Validation**
Nadolol	309.40	-0.63	0	0.11	208	**Testing**
Lisinopril	405.49	1.01	0 (26)	0.11	190	

* $\log D = \log P - \log (1 + 10^{pKa - pH})$

** BSA: with Bovine Serum Albumin in the buffer

Three ANN models were selected for comparison: Multilayer Perception (MLP), Generalized Feed forward MLP (GFF) and Jordan and Elman. The results for each model are shown in Figure 3 and Table 5. Since the Jordan and Elman network showed the highest r^2 and the lowest MSE, coupled with a learning stability, it was considered as the best network model. Although showing a learning stability, the relative importance of inputs was different each time this model was re-built. Figure 4 shows the best relative importance of inputs, see discussion for details.

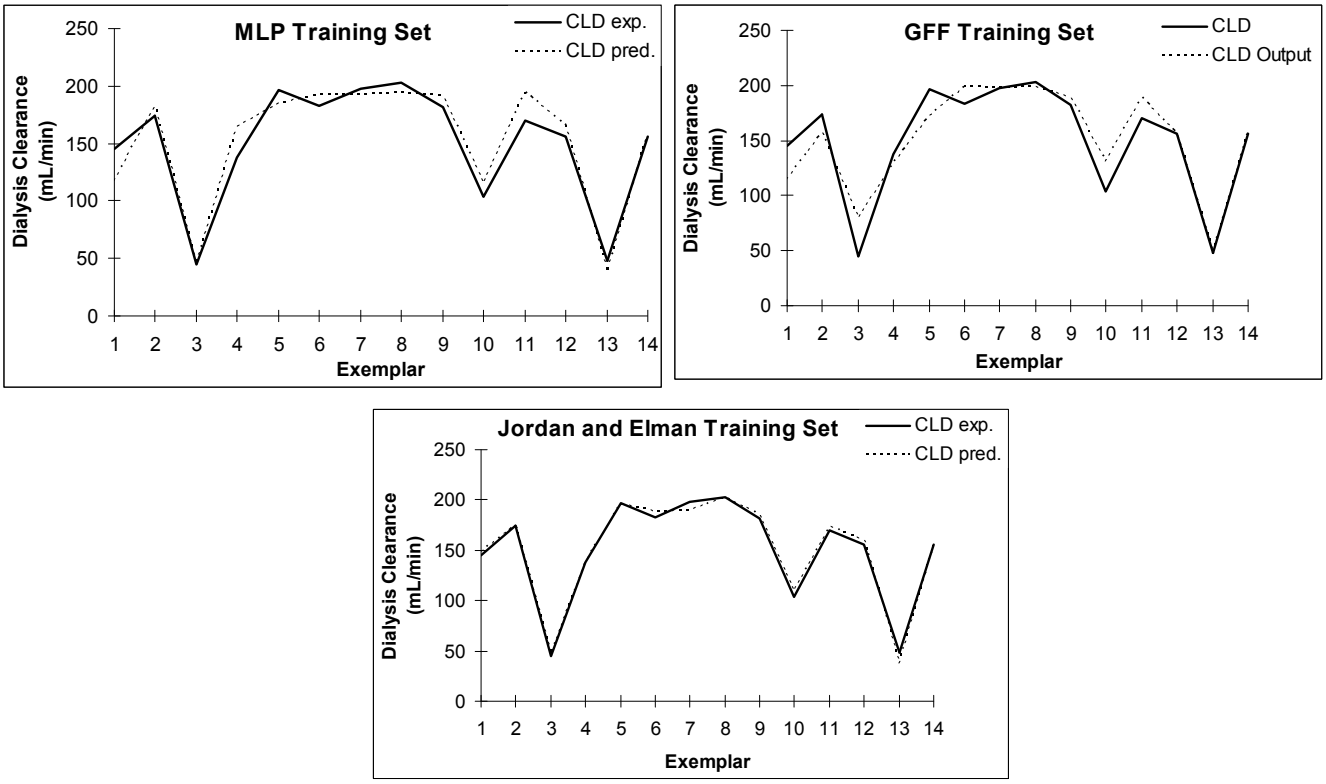

Figure 3. Experimental and predicted drug dialysis clearances (CLD) for the 14 exemplars (drugs) of the training sets with three artificial neural network models: Multilayer perception (MLP), Generalized Feedforward (GFF) and Jordan-Elman.

Table 5. Artificial neural network models performance on drug dialysis clearance (CLD) prediction and comparison with the experimental CLD obtained *in vitro*.

	Training Set		*Cross-validation Set*	*Testing Set*		
	MSE	r^2	*MSE*	*MSE*	*CLD exp. (mL/min)*	*CLD pred. (mL/min)*
MLP	204	0.92	0.14	148	208	191
					190	185
GFF	323	0.86	0.20	282	208	193
					190	172
Jordan-Elman	21	0.99	0.33	129	208	192
					190	193

DISCUSSION

First and foremost, it should be noted that the *in vitro* model used in this study was validated with *in vivo* results obtained in a previous study (manuscript accepted herein). Observed CLDs were very high for the majority of the drugs studied (Table 4). These drugs were almost entirely eliminated from the buffer before the end of the 3-hour dialysis session. Prior to build the ANNs, a good understanding of the data is required. Therefore, the impact of drug plasma protein binding and UFR on CLD was investigated.

Impact of drug plasma protein binding on CLD

The fact that adding BSA to the buffer made no difference for atenolol CLD could be explained by its very low protein binding. Indeed, Figure 2 shows clearly the impact of drug protein binding on their CLD: in the experiments without BSA, the three drugs CLD are very close, while in the experiments with BSA, CLD decrease markedly as a measure to

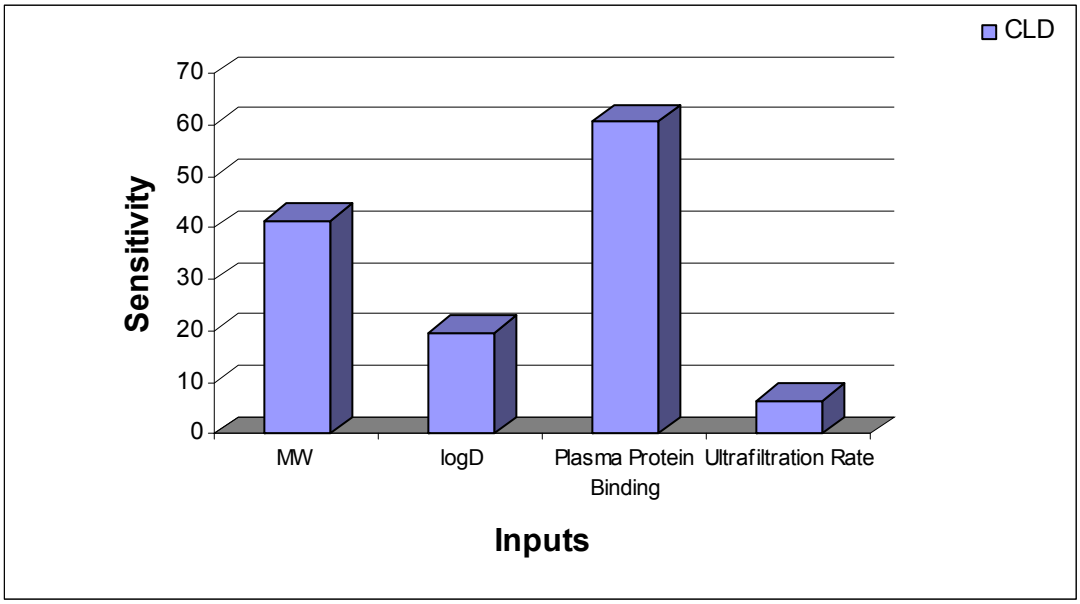

Figure 4. Sensitivity about the mean (with the best network "Jordan-Elman"). As described in Neurosolutions®: this testing process provides a measure of the relative importance among the inputs of the neural model and illustrates how the model output varies in response to variation of an input. By default the first input is varied between its mean +/- a user-defined number of standard deviations while all other inputs are fixed at their respective means. The network output is computed for a user-defined number of steps above and below the mean. This process is repeated for each input.

the increase of drug protein binding percentage. Furthermore, carvedilol, labetalol and atenolol CLD were significantly different, when compared in the same condition "UFmin with BSA", using an ANOVA with Scheffe's pairwise adjustments (all 3 pairwise Scheffe's adjusted p-values were < 0.001).

ANN Modeling

Among all drug physicochemical properties, MW and logD were chosen as inputs for ANN modeling because of their effect on drug dialyzability. In fact, it is well known that the heavier the molecule, the slower it diffuses in water (Fick's first law). Hence, the importance of MW in dialysis is directly related to the diffusion coefficient in water. logD is a good indicator of the drug behaviour in dialysis process, since it reflects the medium pH and the drug logP and pKa:

$$logD = logP - log(1 + 10^{pKa - pH}) \qquad (3)$$

pKa and pH together reflect the drug degree of ionization. This has an impact on the interaction between the drug and the dialyzer filter membrane. logP is strongly related to plasma protein binding. Indeed, it is well shown in Table 6 that the higher the logP the stronger is the drug plasma protein binding, which means that the hydrophobe drugs have a stronger plasma protein binding. Water solubility was not considered as input because the experiments were carried out at very low drug concentrations (very far from saturation).

Table 6. Drug logP and plasma protein binding.

	logP (18)	***Plasma Protein Binding (%)***
Atenolol	0.5	6-16
Labetalol	2.7	50
Carvedilol	3.8	98

According to Figure 4 plasma protein binding is the most important input. However, logD is strongly linked to plasma protein binding, as explained previously with logP. Furthermore, some drugs were dialyzed *in vitro with* and *without* BSA in the buffer, which emphasizes the effet of plasma protein binding on CLD. Therefore, the importance of logD might be underestimated with this experimental protocol.

Relative importance of inputs

Each of the four Jordan and Elman networks built with the default parameters have put different weights on the inputs. It was assessed previously with the Scheffe's method that UFR has not a statistically significant impact on CLD. Therefore, the network that has put the least weight on UFR during its training was considered as the best network. Its performance is shown in Figure 3 and Table 5 and its relative input weights are shown in Figure 4.

In the future, it would be valuable to include low dialysability drugs in the training set and in the testing set in order to verify if the model can be generalized to drugs that are even more heterogeneous.

In this study, we have developed an artificial neural network model which is able to predict drug removal during dialysis. It is a "Jordan and Elman" network which was built using the default parameters in the software and has put the least weight on the UFR input. Since experimental determination of all existing drug CLDs is not realistic, artificial neural networks represent a promising tool for the prediction of drug CLD using drug properties and dialysis conditions.

ACKNOWLEDGEMENTS

The authors are grateful for mass spectrometry analysis and study sponsorship provided by Pharmascience Inc. The authors would like also to thank the hemodialysis staff at the Jewish General Hospital for technical assistance, Prof. Grégoire Leclair at Université de Montréal for his support in drug analysis, and pharmacists for carrying out drug dialysis and HPLC analysis during their master: Assia Tlili, Jordine Felix, Helen Mantzanis, Maryam Alrifaee, Rosa Boudjemai and Elpiniki Kampouris.

REFERENCES

1. Clark WR, Hamburger RJ, Lysaght MJ. Effect of membrane composition and structure on solute removal and biocompatibility in hemodialysis. Kidney Int, 1999; 56(6):2005-2015.
2. Depner TA. "Artificial" hemodialysis versus "natural" hemofiltration. Am J Kidney Dis, 2008; 52(3):403-406.
3. Locatelli F, Martin-Malo A, Hannedouche T, Loureiro A, Papadimitriou M, Wizemann V, Jacobson SH, Czekalski S, Ronco C, Vanholder R, Grp MPOS. Effect of Membrane Permeability on Survival of Hemodialysis Patients. J Am Soc Nephrol, 2009; 20(3):645-654.
4. Parker TF. Technical advances in hemodialysis therapy. Seminars in Dialysis, 2000; 13(6):372-377.
5. Vanholder R, Glorieux G, Van Biesen W. Advantages of New Hemodialysis Membranes and Equipment. Nephron, 2010; 114(3):C165-C172.
6. Meert N, Eloot S, Schepers E, Lemke HD, Dhondt A, Glorieux G, Van Landschoot M, Waterloos MA, Vanholder R. Comparison of removal capacity of two consecutive generations of high-flux dialysers during different treatment modalities. Nephrol Dial Transplant, 2011; 26(8):2624-U1803.
7. Bailie GR, Mason NA, Ghannoum M. Dialysis of Drugs. CKD Insights, LLC, Verona, WI, USA, 2011.
8. Chiu JS, Chong CF, Lin YF, Wu CC, Wang YF, Li YC. Applying an artificial neural network to predict total body water in hemodialysis patients. Am J Nephrol, 2005; 25(5):507-513.
9. Chang YJ, Yeh ML, Li YC, Hsu CY, Lin CC, Hsu MS, Chiu WT. Predicting Hospital-Acquired Infections by Scoring System with Simple Parameters. PLoS One, 2011; 6(8).
10. Wang YF, Hu TM, Wu CC, Yu FC, Fu CM, Lin SH, Huang WH, Chiu JS. Prediction of target range of intact parathyroid hormone in hemodialysis patients with artificial neural network. Computer Methods and Programs in Biomedecine, 2006; 83(2):111-119.
11. Ramesh AN, Kambhampati C, Monson JRT, Drew PJ. Artificial intelligence in medicine. Ann R Coll Surg Engl, 2004; 86(5):334-338.
12. Agarwal R, Nissenson AR, Batlle D, Coyne DW, Trout JR, Warnock DG. Prevalence, treatment, and control of hypertension in chronic hemodialysis patients in the United States. Am J Med, 2003; 115(4):291-297.
13. Santos SFF, Peixoto AJ. Hypertension in dialysis. Curr Opin Nephrol Hypertens, 2005; 14(2):111-118.
14. Furgeson SB, Chonchol M. beta-blockade in chronic dialysis patients. Seminars in Dialysis, 2008; 21(1):43-48.
15. Hudson JQ, Comstock TJ, Feldman GM. Evaluation of an in vitro dialysis system to predict drug removal. Nephrol Dial Transplant, 2004; 19(2):400-405.
16. Maynor LM, Carl DE, Matzke GR, Gehr TWB, Farthing C, Farthing D, Brophy DF. An in vivo-in vitro study of cefepime and cefazolin dialytic clearance during high-flux hemodialysis. Pharmacotherapy, 2008; 28(8):977-983.
17. Krebs-Henseleit (KH) Buffer, Cold Spring Harbor Protocols http://cshprotocols.cshlp.org/cgi/content/full/2006/4/pdb.rec581?text_only=true Accessed May 4, 2010.
18. Drug Bank : Open Data Drug & Drug Target Database. http://www.drugbank.ca/drugs/DB00335;

http://www.drugbank.ca/drugs/DB01136; http://www.drugbank.ca/drugs/DB005981st. Accessed March 1, 2012

19. Yilmaz B, Asci A, Arslan S. Determination of metoprolol in human plasma and urine by high-performance liquid chromatography with fluorescence detection. J Sep Sci, 2010; 33(13):1904-1908.
20. Borges NCD, Mendes GD, Silva DD, Rezende VM, Barrientos-Astigarraga RE, De Nucci G. Quantification of carvedilol in human plasma by high-performance liquid chromatography coupled to electrospray tandem mass spectrometry - Application to bioequivalence study. Journal of Chromatography B-Annalytical Technologies in the Biomedical and Life Sciences, 2005; 822(1-2):253-262.
21. Rowland M, Tozer TN. Clinical Pharmacokinetics: concepts and applications. Lippincott Williams &Wilkins, Philadelphia, PA, USA, 1995.
22. Rizkalla N, Hildgen P. Artificial neural networks: Comparison of two programs for modeling a process of nanoparticle preparation. Drug Dev Ind Pharm, 2005; 31(10):1019-1033
23. The Merck Index 14th Edition. Merck Research Laboratories, Whitehouse Station, NJ, USA, 2006.
24. Sparc Performs Automated Reasoning in Chemistry V4.6 - SPARC on-line calculator. Accessed April 9, 2013.
25. Brion JD, Buxeraud J, Castel J, Couquelet J, Cussac M, Debaert M, Fournier JP, Fulcrand P, Huet J. Médicaments du Système Cardio-Vasculaire. AFECT, Paris, FR, 1992.
26. Aronoff GR, Bennett WM, Berns JS, Brier ME, Kasbekar N, Mueller BA, Pasko DA, Smoyer WE. Drug Prescribing in Renal Failure-Dosing Guidelines for Adult and Children. American College of Physicians, Philadelphia, PA, USA, 2007.
27. Compendium of Pharmaceuticals and Specialities (CPS). Canadian Pharmacists Association, Ottawa, ON, Canada, 2009.
28. ChemBlink : Online Database of Chemicals from Around the World. http://www.chemblink.com/products/87269-97-4.htm. Accessed April 9, 2013.