Application of Artificial Neural Network for Modeling and Prediction of MTT Assay on Human Lung Epithelial Cancer Cell Lines

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Abstract

In this paper, a three-layer artificial neural network (ANN) was investigated to predict the inhibitory concentration (IC) values assessed via MTT cell viability assay on the four types of human lung epithelial cancer cell lines. In order to achieve this purpose, a multilayer perceptron (MLP) neural network trained with back-propagation algorithm was employed for developing the ANN model. To develop the model, the input parameters were concentrations and types of cell lines and the outputs were IC10, IC20, IC30, IC40, IC50, IC60, IC70 and IC80 values in the A549, H157, H460 and H1975 cell lines. The proposed ANN model has achieved good agreement with the experimental data and has a small error between the estimated and experimental values. The obtained results show that the proposed ANN model is a useful, reliable, fast and cheap tool to predict the IC values assessed via MTT cell viability assays.

Keywords: MTT assay; Artificial neural network; Multilayer perceptron; Modeling; Inhibitory concentration

Abbreviations: ANN: Artificial Neural Network; RBF: Radial Basis Function; ANOVA: Analysis of Variance; CF: Correlation Factor; CO2: Carbon Dioxide; DMSO: Dimethyl Sulfoxide; DOX: Doxorubicin; FBS: Fetal Bovine Serum; IC: Inhibitory Concentration; IC50: Inhibitory Concentration of 50% Of Enzyme Activity; MAF: Mean Absolute Error; MRE: Mean Relative Error; MTT: 3-(4,5-Dimethyl-2-Thiazol)-2:5-Diphenyl-2H-Tetrazolium Bromide; MLP: Multilayer Perceptron; RMSE: Root Mean Square Error; RPMI: Roswell Park Memorial Institute Medium

Introduction

Many biological assays require the measurement of surviving and/or proliferating mammalian cells. This can be achieved by several methods, e.g., counting cells that include/exclude a dye, measuring released 51Cr-labeled protein after cell lysis, and measuring several methods, e.g., counting cells that include/exclude a dye, and/or proliferating mammalian cells. This can be achieved by RMSE: Root Mean Square Error; RPMI: Roswell Park Memorial Institute Medium

Artificial neural network (ANN) is a highly simplified model of the biological network structure[16,17]. The basic advantage of ANN is that it does not need any mathematical model; an ANN learns from examples and recognizes patterns in a series of input and output data without any prior assumptions about their nature and interrelations [17]. Moreover, ANN is a good alternative to conventional empirical modeling based on polynomial and linear regressions [18]. Thus, ANN is a typical non-mechanistic model for modeling complex information and is known to have two intrinsic advantages. The first is its flexible capacity in apprehending the data used for training. Being intrinsically nonlinear, a trained ANN can grasp certain subtle patterns that tend to be overlooked by common statistical methods. The second advantage is its high predictive accuracy, i.e., the predictive capability for “new” data (untrained data) [19-23]. The high predictive accuracy is an assured outcome of the ability of ANN to apprehend the data [21,24,25]. On recognizing and application these advantages of ANN in MTT assays, in the current study, we report the design, training and validation of a feed-forward ANN to predict the inhibitory concentration (IC) data such that the designed ANN would (A) make sufficient use of the existing ICs data of a table available of experimental data about chrysin enhances doxorubicin-induced cytotoxicity in human lung epithelial cancer cell lines by Brechbuhl et al. [13], (B) predict the ICs evaluated with a MTT assay in human lung epithelial cancer cell lines treated with chrysin before exposure to DOX. The predicted ICs are expected to fill the data gap for untested IC values with less waste of time and resources.

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Materials and Methods

Compiling MTT assay data for the ANN model

Materials, cell culture and MTT assay conditions: For training the ANN model, we used experimental data evaluated by Brechbuhl [13]. The cytotoxic effects of combination drug therapy with chrysin and DOX were determined against cell lines using MTT [1]. The lung non-small cell epithelial cancer cell lines A549, H157, H460 and H1975 were cultured at 37°C with 5% CO₂ and grown in media and supplements purchased from Mediatech (Manassas, VA). All cells were grown in RPMI media containing L-glutamine and supplemented with 10% FBS (Gemini Bio-Products, West Sacramento, CA). Cells were maintained in T-150 flasks and split into 96-well plates at least 18 h prior to treatment. H157 and A549 cells were seeded for treatment at 12,000 cells per well. H460 and H1975 cells were seeded for treatment at 10,000 cells per well. At the time of treatment all wells were approximately 70-75% confluent and were treated with fresh media containing the indicated compounds. After exposure, 20 µl/well of MTT solution (5 mg/ml phosphate buffered saline) was added and incubated for 3-4 h. The medium was aspirated and replaced with 150 µl/well DMSO to dissolve the formazan salt. The color intensity of the formazan solution, which reflects the cell growth condition, was measured at 570 nm using a Spectra Max 340PC plate reader (Molecular Devices, Sunnyvale, CA).

Statistical analysis of the MTT assay data: Experimental data evaluated by Brechbuhl [13] were expressed as the mean ± standard error of the mean (S.E.M.). All experiments included at least triplicate treatment groups and each experiment was repeated at least two times. ANOVA comparison, Tukey comparison, t-tests, linear regression curves and cytotoxities (IC₅₀) were calculated using Prism version 5 (GraphPad, San Diego, CA).

Modeling Approach

Artificial neural network

Artificial neural networks (ANN) is a good technique used to handle problems of modeling, prediction, control and classification [22]. An ANN is based on the operation of biological neural networks. The fundamental processing element of ANN is an artificial neuron (or simply a neuron). A biological neuron receives inputs from other sources, combines them, performs generally a nonlinear operation on the result, and then outputs the final result [20,23,25]. ANNs have been used in many different applications such as finance, medicine, engineering, geology and physics [19,22,23]. ANN eliminates the limitations of the classical approaches by extracting the desired information using the input data. Applying ANN to a system needs sufficient input and output data instead of a mathematical equation (or simply a neuron). A biological neuron receives inputs from other sources, combines them, performs generally a nonlinear operation on the result, and then outputs the final result [20,23,25]. ANNs have been used in many different applications such as finance, medicine, engineering, geology and physics [19,22,23]. ANN eliminates the limitations of the classical approaches by extracting the desired information using the input data. Applying ANN to a system needs sufficient input and output data instead of a mathematical equation (or simply a neuron). A biological neuron receives inputs from other sources, combines them, performs generally a nonlinear operation on the result, and then outputs the final result [20,23,25]. ANNs have been used in many different applications such as finance, medicine, engineering, geology and physics [19,22,23]. ANN eliminates the limitations of the classical approaches by extracting the desired information using the input data. Applying ANN to a system needs sufficient input and output data instead of a mathematical equation (or simply a neuron).

The output from qth neuron of the first hidden layer is given by [20]:

\[ y_q = f \left( \sum_{k=1}^{p} (x_k W_{1q}) + b_1 \right) \quad q = 1,2,\ldots,Q \quad (1) \]

Where \( x \) is the inputs, \( Q \) is the number of neurons in the first hidden layer, \( p \) is the number of neurons in the input layer, \( b \) is the bias term, \( W \) is the weighting factor and \( f \) is the activation function of the first hidden layer. The output of the mth neuron in the output layer is given by:

\[ y_m = \sum_{n=1}^{M} (\theta_n W_{mn}) + b_m \quad m = 1,2,\ldots,M \quad (2) \]

Where \( b \) is the bias term, \( W \) is weighting factor, \( s \) is the number of neurons in the second hidden layer, \( M \) is the number of neurons in the output layer.

Developing the model

The simplified overview of the proposed MLP model is shown in Figure 2. The inputs are concentrations and types of cell lines and the outputs are IC₁₀, IC₂₀, IC₃₀, IC₄₀, IC₅₀, IC₆₀, IC₇₀ and IC₈₀ values in the A549, H157, H460 and H1975 cell lines. The data set required for training the network is obtained using the experimental values [13]. For developing the ANN model, the experimental data are divided into two sets. The number of samples for training and testing are 21 (about 75%) and 7 (about 25%).

In this study, different ANN structures were tested and optimized to obtain the best ANN configuration. We tested many different structures with one, two, three and four hidden layers with different number of neurons in each layer also we tested Radial basis function (RBF) for prediction output. Table 1 show the comparison between these structures, where the mean relative error percentage (MRE %) is given by:

\[ MRE\% = 100 \times \frac{\sum_{i=1}^{N} |X_i(Exp) - X_i(Pr ed)|}{X_i(Exp)} \]

Where \( X_i(Exp) \) is the experimental value, \( X_i(Pr ed) \) is the predicted value and \( N \) is the number of samples.
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Where N is the number of data and 'X(Exp)' and 'X(Pred)' stand for experimental and predicted (ANN) values respectively.

Also we tested many ANN configurations with different structure, different training algorithm and different number of epochs. Table 2 shows the obtained MRE% for these different ANN configurations. The best obtained ANN structure in Table 1 is the latest ANN structure in Table 2.

As it is shown in Tables 1 and 2, the MLP model with 2-11-8-9-8 structure (i.e., 2 neurons in the input layer, 11 neurons in the first hidden layer, 8 neurons in the second hidden layer, 9 neurons in the third hidden layer and 8 neurons in the output layer) has the least MRE%. Therefore, we selected this structure in this paper.

**Results and Discussions**

Table 3 shows the specification of the proposed ANN model. The training and testing results for the proposed ANN model in comparison with experimental results [13] are shown in Tables 4 and 5 respectively.

Table 6 shows the obtained errors for the proposed ANN model, where the mean absolute error percentage (MAE %), the root mean square error (RMSE), and the correlation factor (CF) of the proposed ANN models are calculated by:

\[
MAE\% = 100 \times \frac{1}{N} \sum_{i=1}^{N} \frac{|X(Exp) - X(Pred)|}{X(Exp)} \quad (7)
\]

\[
RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (X(Exp) - X(Pred))^2} \quad (8)
\]

\[
CF = 1 - \frac{\sum_{i=1}^{N} (X(Exp) - X(Pred))^2}{\sum_{i=1}^{N} X(Exp)^2} \quad (9)
\]

Where N is the number of data and 'X(Exp)' and 'X(Pred)' stand for experimental and predicted (ANN) values respectively.

**Conclusion**

In this paper, the inhibitory concentration (IC) values assessed via MTT cell viability assay on the four types of human lung epithelial cancer cell lines is modeled and predicted by artificial neural network. The proposed ANN model has achieved good agreement with the experimental data with minimum error. According to the obtained results from the ANN model and comparing them with the experimental results, it can be shown that ANN can be used in modeling and output prediction of the IC values assessed via MTT cell viability assays. Seems that the biggest achievement of the Modeling and prediction of the inhibitory concentration values assessed via MTT cell viability assay on human lung epithelial cancer cell lines co-treated with chrysin and doxorubicin.

**Table 1:** Average of MRE% for all outputs for different ANN structures.

| ANN Structure     | Average of MRE% |
|-------------------|-----------------|
|                   | Train | Test  |
| 2-8-11-8          | 1.203 | 15.375|
| 2-12-10-8         | 4.635 | 22.25 |
| 2-7-8-8           | 6.505 | 21.625|
| 2-9-8             | 8.95  | 23.5  |
| 2-11-8-9-8        | 0.311 | 11.375|
| 2-10-8-11-8       | 2.78  | 16.05 |
| 2-7-2-12-8        | 3.437 | 16.5  |
| 2-7-10-12-8       | 2.020 | 14.5  |
| 2-9-7-11-8        | 7.046 | 14.625|
| RBF               | 1.977e-13 | 61.323 |

**Table 2:** Comparison of the ANN configurations with different training algorithm, number of hidden layers and number of epochs.
Table 3: Comparison of the ANN configurations with different training algorithms, number of hidden layers and number of epochs.

| Type of cell lines | Concentration | Experimental (Brechbuhl et al., 2012) | ANN |
|-------------------|---------------|--------------------------------------|-----|
|                   |               | IC10 | IC20 | IC30 | IC40 | IC50 | IC60 | IC70 | IC80 |
| A549              | 0             | 0.04 | 0.039 | 0.158 | 0.245 | 0.365 | 0.543 | 0.639 | 1.43  |
|                   | 5             | 0.041 | 0.085 | 0.137 | 0.203 | 0.291 | 0.418 | 0.62  | 1     |
|                   | 10            | 0.04  | 0.081 | 0.128 | 0.187 | 0.264 | 0.374 | 0.545 | 0.864 |
|                   | 20            | 0.029 | 0.061 | 0.099 | 0.147 | 0.212 | 0.306 | 0.456 | 0.742 |
|                   | 25            | 0.018 | 0.048 | 0.085 | 0.14  | 0.223 | 0.354 | 0.587 | 1.087 |
| A549              | 30            | 0.017 | 0.045 | 0.086 | 0.146 | 0.238 | 0.388 | 0.661 | 1.267 |
| H157              | 5             | 0.075 | 0.135 | 0.199 | 0.274 | 0.367 | 0.492 | 0.678 | 1     |
|                   | 10            | 0.095 | 0.153 | 0.212 | 0.275 | 0.35  | 0.446 | 0.581 | 0.801 |
|                   | 15            | 0.072 | 0.124 | 0.179 | 0.241 | 0.317 | 0.417 | 0.562 | 0.809 |
|                   | 20            | 0.062 | 0.114 | 0.171 | 0.239 | 0.325 | 0.442 | 0.671 | 0.928 |
|                   | 25            | 0.075 | 0.132 | 0.193 | 0.264 | 0.351 | 0.467 | 0.638 | 0.932 |
| H157              | 30            | 0.075 | 0.132 | 0.193 | 0.264 | 0.351 | 0.467 | 0.638 | 0.932 |

Table 4: Specification of the best proposed ANN model.

| Type of cell lines | Concentration | Experimental (Brechbuhl et al., 2012) | ANN |
|-------------------|---------------|--------------------------------------|-----|
|                   |               | IC10 | IC20 | IC30 | IC40 | IC50 | IC60 | IC70 | IC80 |
| A549              | 0             | 0.031 | 0.062 | 0.1   | 0.147 | 0.21  | 0.3   | 0.441 | 0.707 |
|                   | 5             | 0.065 | 0.147 | 0.253 | 0.393 | 0.589 | 0.884 | 1.38  | 2.36  |
|                   | 10            | 0.091 | 0.15  | 0.209 | 0.247 | 0.351 | 0.451 | 0.591 | 0.823 |
|                   | 20            | 0.05  | 0.016 | 0.021 | 0.026 | 0.033 | 0.035 | 0.052 | 0.07  |
|                   | 25            | 0.004 | 0.007 | 0.009 | 0.013 | 0.016 | 0.026 | 0.026 | 0.036 |
| H157              | 0             | 0.014 | 0.025 | 0.036 | 0.05   | 0.066 | 0.087 | 0.119 | 0.173 |
|                   | 5             | 0.018 | 0.024 | 0.03  | 0.036 | 0.042 | 0.049 | 0.058 | 0.072 |
|                   | 10            | 0.013 | 0.017 | 0.021 | 0.024 | 0.028 | 0.033 | 0.038 | 0.047 |
|                   | 15            | 0.013 | 0.019 | 0.024 | 0.029 | 0.034 | 0.041 | 0.056 | 0.065 |
|                   | 20            | 0.013 | 0.019 | 0.024 | 0.029 | 0.034 | 0.041 | 0.056 | 0.065 |
|                   | 25            | 0.015 | 0.025 | 0.033 | 0.043 | 0.041 | 0.049 | 0.057 | 0.065 |

Table 5: The Comparison between experimental and predicted ANN results for training data.
Testing data.

Table 6: The Comparison between experimental and predicted ANN results for testing data.

| IC   | Output | MAE | RMSE | CF  |
|------|--------|-----|------|-----|
|      | Train  | Test | Train | Test | Train  | Test  |
| IC10 | 1.52E-05 | 0.006638 | 2.62E-05 | 0.010782 | 0.999999 | 0.935947 |
| IC20 | 4.28E-05 | 0.007065 | 7.21E-05 | 0.011347 | 0.999999 | 0.98275  |
| IC30 | 7.02E-05 | 0.006433 | 1.01E-04 | 0.011026 | 0.999999 | 0.96767  |
| IC40 | 0.000134 | 0.009188 | 0.000218 | 0.016406 | 0.999998 | 0.994751 |
| IC50 | 0.000138 | 0.009188 | 0.000218 | 0.016406 | 0.999998 | 0.994751 |
| IC60 | 0.000121 | 0.035157 | 0.000216 | 0.077989 | 0.999999 | 0.9862   |
| IC70 | 0.000139 | 0.072462 | 0.000288 | 0.157204 | 0.999999 | 0.971827 |
| IC80 | 0.000139 | 0.072462 | 0.000288 | 0.157204 | 0.999999 | 0.971827 |
| IC90 | 1.52E-05 | 0.006638 | 2.62E-05 | 0.010782 | 0.999999 | 0.935947 |

Figure 3: Comparison with the experimental for IC\textsubscript{50} as a function of Chrysin on DOX-induced cytotoxicity in A549, H157, H460 and H1975 cell lines.

Figure 4: Comparison of the ANN and experimental results for IC\textsubscript{50} as a function of Chrysin on DOX-induced cytotoxicity in A549, H157, H460 and H1975 cell lines.

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