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Cheminformatics-based selection and synergism of herbal extracts with anticancer agents on drug resistance tumor cells—ACHN and A2780/CP cell lines

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ABSTRACT

The treatment of cancer usually involves lethal effect on normal body cells as side effects. Cheminformatics methodology can play a significant role in biomed/clinical scientific research. Similarity searching is a standard cheminformatics tool in drug discovery area and database design. In this study, five novel herbal extracts in combination with doxorubicin and cisplatin have been used to sensitize ACHN and A2780/cp cells. These herbal extracts have been selected on the basis of novel cheminformatics methodology and assayed for the first time. The findings confirmed predicted outcomes from the *in silico* research and the results introduced may bring to use the effects of these herbs in reversing of multidrug resistance (MDR) phenomenon.

1. Introduction

A major impediment in the anticancer chemotherapy process is the multidrug resistance (MDR) phenomenon. This phenomenon relates to the property of drug resistant tumors to exhibit simultaneous resistance to a number of structurally and functionally unrelated chemotherapeutic agents.

Three of the most important biological mechanisms involved in MDR phenomenon are [1]:

- Physiological and hematological properties of tumor cells
- Non-classical MDR mechanisms
- Transport-based classical MDR mechanisms

Increases in environmental interstitial fluid pressure, higher vascular permeability and acidic environment are some of the physiological and hematological properties of tumor cells that lead to reduction of anticancer agent effects on tumor cells. Also non-classical MDR mechanisms involved in abnormality mechanisms resulted from the change in the balance of proteins that control apoptosis such as p53, Bcl-2 and altered activity of specific enzyme systems such as S-transferase, GST, cyclooxygenase-2 and topoisomerase [1]. The transport-based classical MDR mechanisms lead to overexpression or activation of transmembrane proteins effluxing different chemical substances from tumor cells [1]. These transporter proteins are typically named ATP-binding cassette (ABC) family, which can reduce intracellular accumulation of drugs. Most typical efflux pumps overexpressed in the membrane of tumor cells are multidrug resistance associated protein (MRP) and P-Glycoprotein (P-GP) [1]. Today, wide ranges of scientific efforts are focused on blocking and reversal of ABC transporters involved in transport-based classical MDR mechanisms to overcome the MDR phenomenon.

Combinational drug therapy has a long history and its root can be found in traditional Chinese medicines. Today parallel to new advances in cancer chemotherapy, cancer combinational drug therapy has been developed extensively and wide ranges of scientific efforts were focused on and most are used in cancer therapy to (a) reverse effluxing of anticancer agents from tumor cells; (b) induce the efficacy of the therapeutic effect; (c) reduce the dosage but induce or maintain the efficacy of cytotoxic property; and (d) reduce or overcome drug resistance phenomena and achieve selective synergism against target or toxicity antagonism. Synergistic, additive and antagonistic effects are the three forms of combinational drug therapy effects on tumor cells [2].

Use of chemosensitizers in combination with anticancer drugs at the same time leads to sensitized MDR tumor cells, and also reverses and inhibits ABC transporters involved in multidrug

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resistance phenomenon [3,4]. Verapamil, cyclosporin A, rapamycin, and PSC-833, VX-710, LY335979, XR9051, XR9576 and flavonoid kaempferide are some of the chemosensitizers that were applied clinically in combinational drug therapy [5]. Doxorubicin and cisplatin are used widely in cancer chemotherapy but unfortunately they act as an exogenous substrate for ABC transporters (doxorubicin: MDR1, MRP2, MRP3, MRP5 and MRP6 transporters, cisplatin: MRP2 and MRP3 transporters) they are exposed by MDR pumps from tumor cells and cannot play efficient roles in chemotherapy [5].

Use of cheminformatics tools as a new born science that joins computational science to chemistry and biology has been applied in modern drug discovery to enhance the success rate from systematic knowledge use.

Similarity search is one of the most efficient methods in cheminformatics to detect specific biomolecular targets, which are expected to have an activity for a given disease such as cancer [6]. Molecular similarity analysis delves the way of molecules to cover a determined structural space and underlies many approaches for compound selection and design of combinatorial libraries. A proper diversity/similarity space puts molecules in a good position relative to others in a well parameterized way.

Three main parameters in similarity search are descriptors, the coefficients and the weighting scheme [6]. Each molecule in a similarity search process is identified with an ID code; one of the most common molecular structure codes is Simplified Molecular Input Line Entry Specification (SMILES) [6].

In the present study, candidate herbal extracts have been selected on the basis of novel logical algorithm using cheminformatics tools that were not explored before. Selected herbal extracts in combination with doxorubicin and cisplatin have been used to sensitize ACHN (human renal adenocarcinoma) and A2780/cp (human ovarian carcinoma, resistant to cisplatin) and to overcome the MDR phenomenon.

2. Material and methods

2.1. Cheminformatics-based selection of candidate herbs

Selection of candidate herbs was carried out by a logical algorithm based on cheminformatics tools

- The herbs reported to have synergistic activities with anticancer compound were collected by searching in herbal medicine databases and previous findings, and then these findings were categorized as synergistic plants (SPs).
- Bioactive compounds from pointed herbs were selected and categorized in synergistic compounds collection (SCC).
- Chemical structures of herbal synergistic compounds were drawn by Chem. Draw Ultra-[0.8, 2004] program to obtain Simplified Molecular Input Line Entry Specification (SMILES) as a data query for molecules to carry out the similarity search [6].
- Two cheminformatics servers http://pubchem.ncbi.nlm.nih.gov/search/search.cgi and http://cactus.nci.nih.gov/ncidb2 were used to do the similarity search "Tanimoto index 98% and compounds, score $>=95\%$" and find similar compounds with the first set of synergistic compounds SCC and these were classified in similar synergistic compounds (SSCs).
- SSC derived from herbal source was gathered in similar herbal synergistic compounds (SHSCs).
- Herbs containing SHSC were searched in sources like www.google.com, www.scirus.com, www.sciencedirect.com and www.ncbi.nlm.nih.gov for the novelty item in the field of anticancer study.
- On the other hand, the herbs were checked by www.gbif.net, "A dictionary of Iran's vegetation plants" [7] and "Flora of Iran" [8], to study their existence and accessibility profile in Iran. The results were classified in novel candidate herbs (NCHs) to bioassay and determine their synergistic property with anticancer drugs *in vitro*.

2.2. Collection of candidate herbs

Vegetation regions of candidate herbs were guessed by searching "A dictionary of Iran's vegetation plants" [7] and "Flora of Iran" [8], and then candidate herbs were collected during three collection trips from North, North-West, South-West and East of Iran. Voucher herbarium samples of collected herbs are prepared and made available.

2.3. Preparation of herbal extracts and drugs

Plant materials were dried at room temperature for 4–7 days. Dried herbal powder was extracted by ethanol (Merck, Germany) (80%) according to the percolation method; the samples were steeped in solvent in the percolator for 24 h before each extraction and the process was repeated three times. The extracts were then collected and pooled (Table 2) [9,10]. After evaporating, each extract (10 mg) was dissolved in 1 ml ethanol (50%) as stock. Doxorubicin (50 mg/25 μl) and cisplatin (50 mg/100 μl) were obtained from the EBEWE Pharma Ges.m.b. Nfg. KG. A-4866 Unterach, Austria and were diluted with RPMI (10% FBS).

2.4. Cell lines

Anticancer drug resistance cancerous cell lines: A2780/cp (human ovarian carcinoma, resistant to cisplatin) NCBI C454, ACHN (human renal adenocarcinoma) NCBI C206 and a normal cell line HF2 (human fetal fibroblast) NCBI C336 as a cell of negative control in comparison with ACHN were obtained from the National Cell Bank of Iran, Pasture Institute of Iran (Tehran). These cells were maintained in RPMI-1640 (Gibco, England) medium supplemented with 10% FCS (Gibco, England) in a humidified incubator (37 °C and 5% CO_2).

2.5. Cytotoxicity experiments

Cells were cultured in RPMI-1640 medium supplemented with 10% heat inactivated FCS, 2 mM glutamine (Sigma, USA), penicillin (Sigma, USA) (100 IU/μl) and streptomycin (Sigma, USA) (100 μg/ml) at 37 °C in an incubator containing 5% CO_2.

Harvested cells with trypsin (Sigma, USA) (0.25%) were counted by the Neubauer slide and then were seeded into 96-well plates (104 cell/well). At the first step, for determination of cytotoxicity property of the samples, the cells were incubated with 100 μl of different concentrations of ethanol herbal extracts (100, 50, 25 and 12.5 μg/ml) diluted by RPMI (10% FBS), containing doxorubicin (8, 4, 2, 1 and 0.5 μM), or cisplatin (41, 20.5, 10.250 and 5.125 μM) and ethanol (50%) from 1% to 0.12% as solvent control for 24 h. Twelve cultured wells without any treatments were used as a negative control. At the same time, for studying the synergistic property, 50 μl of each ethanol herbal extract plus 50 μl of either of the anticancer drugs in the maintained concentration were added to the wells (serial dilution was performed by RPMI, 10% FBS). Each single dose and combination was in triplicate in each assay. For the MTT assay, the content of each well was taken out and 100 μl of MTT tetrazolium dye (Sigma, USA) (5 mg/ml in PBS) was added to each well and incubated at 37 °C for 3 h. The insoluble formazan produced was dissolved in a solution containing 100 μl DMSO (Fluka, USA) and the OD was read against a blank reagent with multiwell