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Oxidative stress response of tumor cells: microarray-based comparison between artemisinins and anthracyclines

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Abstract

The antimalarial artemisinins also reveal profound cytotoxic activity against tumor cells. Artemisinins harbor an endoperoxide bridge whose cleavage results in the generation of reactive oxygen species (ROS) and/or artemisinin carbon-centered free radicals. Established cancer drugs such as anthracyclines also form ROS and free radicals that are responsible for the cardiotoxicity of anthracyclines. In contrast, artemisinins do not reveal cardiotoxicity. In the present investigation, we compared the cytotoxic activities of different artemisinins (artemisinin, artesunate, arteether, artemether, artemisitene, dihydroartemisinylester stereoisomers) in 60 cell lines of the National Cancer Institute (N.C.I.), USA, with those of anthracyclines (doxorubicin, daunorubicin, 4′-epirubicin, idarubicin, deoxydoxorubicin, trifluoroacetyl-doxorubicin-14-valerate). The inhibition concentration 50% (IC_{50}) values of artemisinins and anthracyclines were correlated with the mRNA expression of 170 genes involved in oxygen stress response and metabolism as recently determined by microarray analysis and deposited in the N.C.I.'s database (http://dtp.nci.nih.gov). The genes whose expression was significantly linked to cellular drug response in Kendall's τ tests were subjected to hierarchical cluster analysis and cluster image mapping. Mathematical correction for false-positive correlations was done by a false discovery rate algorithm. One cluster contained predominately genes with a relationship to artemisinins and another one genes with a relationship to anthracyclines. In a third cluster, genes correlating to both drug classes were assembled. This indicates that different sets of genes involved in oxidative stress response and metabolism may contribute to the cytotoxic and differing toxic side effects of these drug classes.

Keywords: Artemisinin; Anthracyclines; Cardiotoxicity; Cluster analysis; Microarrays; Neurotoxicity; Oxidative stress

1. Introduction

Anticancer drug development remains a task of paramount importance of tumor pharmacology, as long as the efficiency of the available standard cytostatic drugs is hampered by the emergence of drug resistance and severe dose-limiting side effects.

The natural compound artemisinin (ARS) and its semisynthetic derivatives have gained considerable interest during the past years as novel treatment options for *Plasmodium falciparum* or *P. vivax* infections [1]. Different derivatives have been developed from the mother drug ARS that show improved features concerning activity, solubility, and pharmakokinetic behavior, e.g., arteether (ARE), artemether (ARM), artesunate (ART), artemisitene (ARTEMIS), and dihydroartemisinylester stereoisomers (ARTEST) [2,3]. In addition to their tremendous value for malaria treatment, artemisinins also reveal profound cytotoxic activity against tumor cells [3–7] and antiviral activity [8]. A salient feature of artemisinins is that they are active against multidrug-resistant *Plasmodium* strains [9]. As this has also be found in human cancer cell lines [6], artemisinins may be promising for the treatment of refractory tumors. Another propitious characteristic is the good tolerability and the lack of significant adverse side effects [10].

The active moiety of artemisinins is an endoperoxide bridge. Its cleavage results in the generation of reactive

Abbreviations: AD32, trifluoroacetyl-doxorubicin-14-valerate; ARE, arteether; ARM, artemether; ARS, artemisinin; ART, artesunate; ARTEMIS, artemisitene; ARTEST1/2, dihydroartemisinyl ester stereoisomers 1/2; DNR, daunorubicin; DODOX, deoxydoxorubicin; DOX, doxorubicin; EPI, 4′-epirubicin; FDR, false discovery rate; IC_{50}, inhibition concentration 50%; IDA, idarubicin; N.C.I., National Cancer Institute; ROS, reactive oxygen species

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oxygen species (ROS) and/or artemisinin carbon-centered free radicals [11–13]. The antimalarial as well as the cytotoxic activities of artemisinins are influenced by oxidative stress [4,14]. Established cancer drugs also form ROS and free radicals, i.e., anthracyclines. While the anticancer activity of anthracyclines is mediated by inhibition of DNA topoisomerase II and intercalation into DNA, ROS and free radicals are responsible for cardiotoxicity of anthracyclines [15,16]. In contrast to anthracyclines, artemisinins do not reveal cardiotoxicity or even any other considerable side effects if used in the clinic to treat malaria [10]. Supratherapeutic doses of artemisinins cause neurotoxicity in animals [17], an effect that is not known from treatment with anthracyclines. Although both drug classes form ROS and free radicals, the molecular basis for the different profiles of side effects is unknown.

In the present investigation, we compared the cytotoxic activities of different artemisinins (ARS, ARE, ARM, ART, ARTEMIS, ARTEST) in 60 cell lines of the National Cancer Institute (N.C.I.), USA, with those of anthracyclines (doxorubicin, daunorubicin, 4′-epirubicin, idarubicin, deoxydoxorubicin, trifluoroacetyl-doxorubicin-14-valerate). As a next step, we correlated the inhibition concentration 50% (IC_{50}) values of artemisinins and anthracyclines with the mRNA expression of 170 genes involved in oxygen stress response and metabolism determined by microarray analysis. The results were subjected to hierarchical cluster analysis and cluster image mapping. The mRNA expression as determined by microarray analyses has been reported [18] and deposited in the N.C.I.'s database (http://dtp.nci.nih.gov). The IC_{50} values for ART in 60 cell lines of the N.C.I. have been reported by us [6], while the IC_{50} values for the other artemisinins and for the anthracyclines have been deposited in the N.C.I.'s database.

2. Material and methods

2.1. Cell lines of the Developmental Therapeutics Program of the N.C.I., USA

The panel of 60 human tumor cell lines of the Developmental Therapeutics Program of the N.C.I., USA, consisted of leukemia (CCRF-CEM, HL-60, K-562, MOLT-4, RPMI-8226, SR), melanoma (LOX-IMVI, MALME-3M, M14, SK-MEL2, SK-MEL28, SK-MEL-5, UACC-257, UACC-62), non-small cell lung cancer (A549, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-460, NCI-H522), colon cancer (COLO205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620), renal cancer (786-0, A498, ACHN, CAKI-1, RXF-393, SN12C, TK-10, UO-31), ovarian cancer (IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, SK-OV-3), tumors of the central nervous system (SF-268, SF-295, SF-539, SNB-19, SNB-75, U251), prostate carcinoma (PC-2, DU-145), and breast cancer (MCF-7, NCI-ADRRes, MDA-MB-231, Hs578T, MDA-MB-435, MDA-N, BT-549, T-47D). Their origin and processing have been previously described [19].

2.2. Drug response

The sulforhodamine B assay for the determination of drug sensitivity in these cell lines has been reported [20]. The response of the 60 tumor cell lines to artesunate (ART) has been determined in a previous collaboration with the N.C.I. [6]. The inhibition concentration 50% (IC_{50}) values for ART as well as for other artemisinins (artemisinin (ARS), arteether (ARS), artemether (ARM), artemisitene (ARTEMIS), dihydroartemisinylester stereoisomers 1 and 2 (ARTEST 1 and 2)) and anthracyclines (doxorubicin (DOX), daunorubicin (DNR), 4′-epirubicin (EPI), idarubicin (IDA), deoxydoxorubicin (DODOX), and trifluoroacetyl-doxorubicin-14-valerate (AD32)) have been deposited in the database of the Developmental Therapeutics Program of the N.C.I. (http://dtp.nci.nih.gov).

2.3. Statistical analyses

The mRNA expression values of 60 cell lines of genes that are related to oxidative stress response, oxidative damage or oxidative metabolism were selected from the database of the National Cancer Institute (N.C.I.), Bethesda, MA, USA (http://dtp.nci.nih.gov). The mRNA expression has been determined by microarray analyses as reported [18]. This database has been searched for the following search strings: antioxidant, oxidase, oxygenase, peroxidase, oxygen, oxidoreductase, redox, hypoxia, damage-inducible, glutathione, cytochrome P450, quinone, and ubiquitin. In addition, a search using the PUBMED literature database (http://www.ncbi.nlm.nih.gov) was performed for genes that are related to oxidative stress and oxidative damage. The mRNA expression of genes by PUBMED searches were also taken from the N.C.I. microarray database. Thereby, the mRNA expression values of a total number of 170 genes were included into the present study.

Kendall's τ test was used to calculate significance values and rank correlation coefficients as a relative measure for the linear dependency of two variables. This test was implemented into the WinSTAT Program (Kalmia, Cambridge, MA, USA). Kendall's τ test determines the correlation of rank positions of values. Ordinal or metric scaling of data is suited for the test and are transformed into rank positions. There is no condition regarding normal distribution of the data set for the performance of Kendall's τ test.

In addition to the calculation of *P*- and *R*-values, the problem of multiple hypothesis testing was addressed. Significance tests might be prone to type I errors (false conclusions of significance) [21,22]. Therefore, a step-up re-sampling multi-comparison procedure was applied to control the false discovery rate (FDR) among the

significant correlations at a given significance level. This program was developed by Reiner et al. [23] and is available on http://www.math.tau.ac.il. The FDR is the expected proportion α of erroneous rejections among all rejections of the null-hypothesis. The χ^2 test was used as implement of the WinSTAT program (Kalmia) to prove bivariate frequency distributions for pairs of nominal scaled variables for dependencies.

Objects were classified by calculation of distances according to the closeness of between-individual distances by means of hierarchical cluster analysis. All objects were assembled into a cluster tree (dendrogram). The merging of objects with similar features leads to the formation of a cluster, where the length of the branch indicates the degree of relation. The distance of a subordinate cluster to a superior cluster represents a criterion for the closeness of clusters as well as for the affiliation of single objects to clusters. Thus, objects with tightly related features appear together, while the separation in the cluster tree increases with progressive dissimilarity. Recently, cluster models have been validated for gene expression profiling and for approaching molecular pharmacology of cancer [18,24,25]. Cluster analyses applying the complete-linkage method were done with the WinSTAT program (Kalmia). Missing values were automatically omitted by the program, and the closeness of two joined objects was calculated by the number of data points they contained. In order to calculate distances between all variables included in the analysis, the program automatically standardizes the variables by transforming the data with a mean = 0 and a variance = 1.

3. Results

Dose response curves of the cytotoxicity of all drugs tested have been determined over a dose range from 10^{-8} to 10^{-4} M in 60 cell lines of the National Cancer Institute (N.C.I.), USA and IC_{50} values have been calculated thereof. We used the mother drug ARS and its derivatives ARM, ARE, ART, ARTEMIS, ARTESTl, and ARTEST2. The anthracyclines DOX, DNR, EPI, IDA, DODOX, and AD32 were analyzed. The IC_{50} mean values of the cytotoxicity to the 60 cell lines for each drug are shown in Fig. 1. The $\log_{10}$ IC_{50} values of the artemisinins were in a range from -4.06 ± 0.002 M to -6.77 ± 0.012 M and the $\log_{10}$ IC_{50} values of the anthracyclines ranged between -7.42 ± 0.011 M and -6.11 ± 0.029 M. The most cytotoxic artemisinin was ARTESTl, while ARS and ARM were less active. ART, ARTEMIS, and ARTEST2 showed intermediate activity. Among the anthracyclines, IDA and DODOX were most cytotoxic, DOX, DNR, and EPI were intermediary, and AD32 was less inhibitory against the 60 cell lines. Altogether, artemisinins inhibited cancer cells at higher concentrations as compared to anthracyclines with the exception of ARTESTl (Fig. 1).

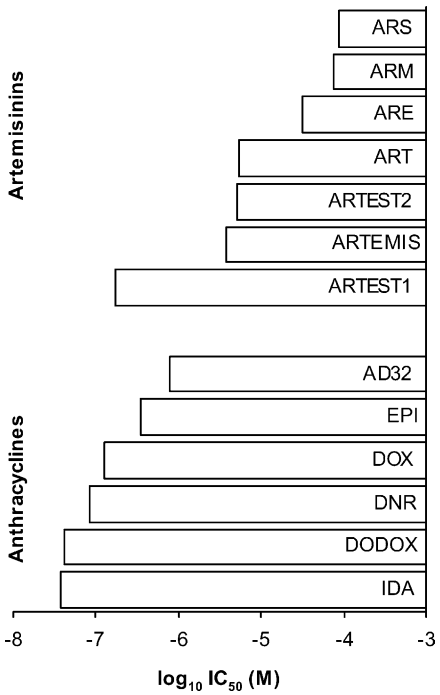

Fig. 1. IC_{50} values for artemisinins in comparison to those for anthracyclines determined in 60 cell lines of the N.C.I. tumor panel.

The IC_{50} values of the 60 cell lines for all drugs were subjected to Kendall's τ test. As shown in Table 1, the relationships between the artemisinins were highly significant indicating that the cell lines responded similarly towards these artemisinins. The same applies to the anthracyclines tested. Out of the panel of the artemisinins, only ARTEMIS correlated with anthracyclines (DOX, DNR, IDA, and AD32). In addition to ARTEMIS, DNR correlated also with ARTESTl and ARTEST2. The other artemisinins did not correlate with anthracyclines indicating that cross-resistance between artemisinins and anthracyclines in these 60 cell lines was not a major issue.

Next, we correlated the IC_{50} values for artemisinins and anthracyclines with the baseline mRNA expression levels of 170 genes of the 60 N.C.I. cell lines by means of Kendall's τ test. These genes were selected, because they are known to be involved in cellular oxidative stress response or oxygen metabolism. Of these 170 genes, 78 correlated with a significance level of $P \leq 0.05$ and a correlation coefficient of $R \geq 0.2$ with the IC_{50} values of at least one drug. As a next step, this data set for 78 genes was further subjected by using false discovery rate (FDR) calculation. Adjusting the significance level to $P = 0.05$ revealed an α-value of 0.00598 which means that correlations with $P < 0.00598$ have a probability of $\geq$5% to correlate erroneously to cellular response to the drugs investigated. A subset of 54 genes was identified by this procedure. These genes are listed in Table 2.

Then, a hierarchical cluster analysis was performed and a cluster image map constructed using the *P*-values from

Table 1
Correlation of the IC_{50} values of cytotoxicity to the 60 N.C.I. cell lines for artemisinins and anthracyclines to each other by means of Kendall's τ test

	ARE	ARM	ARTEMIS	ARTEST1	ARTEST2	DOX	DNR	IDA	EPI	DODOX	AD32
ARS	0.001	9.22×10^{-6}	0.010	0.004	0.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
ART	5.01×10^{-7}	2.24×10^{-4}	4.68×10^{-4}	1.61×10^{-10}	9.15×10^{-9}	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
ARE		3.29×10^{-10}	0.011	5.23×10^{-9}	1.59×10^{-7}	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
ARM			0.004	9.38×10^{-6}	2.84×10^{-6}	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
ARTEMIS				1.89×10^{-5}	2.48×10^{-6}	0.011	0.010	0.037	n.s.	n.s.	0.003
ARTEST1					4.73×10^{-14}	n.s.	0.010	n.s.	n.s.	n.s.	n.s.
ARTEST2						n.s.	0.035	n.s.	n.s.	n.s.	n.s.
DOX							9.01×10^{-21}	1.18×10^{-9}	1.38×10^{-12}	3.36×10^{-9}	2.56×10^{-14}
DNR								1.01×10^{-10}	3.32×10^{-12}	1.01×10^{-10}	4.28×10^{-14}
IDA									1.43×10^{-5}	2.37×10^{-7}	2.39×10^{-9}
EPI										1.12×10^{-7}	6.90×10^{-9}
DODOX											1.15×10^{-7}

Abbreviations: ARS, artemisinin; ART, artesunate; ARE, arteether; ARM, artemether; ARTEMIS, artemisetene; ARTEST 1/2, dihydroartemisinylester stereoisomer 1/2; DOX, doxorubicin; DNR, daunorubicin; IDA, idarubicin; EPI, 4′-epirubicin; DODOX, deoxydoxorubicin; AD32, trifluoroacetyl-doxorubicin-14-valerate.

the correlations of the mRNA expression of these 54 genes and the IC_{50} values for artemisinins and anthracyclines. The dendrogram on the right side of Fig. 2 shows that three major branches of the cluster tree can be separated from each other. Then, we performed a second cluster analysis using the artemisinins and anthracyclines and found a dendrogram which can be separated into two clusters (Fig. 2, top). Interestingly, artemisinins and anthracyclines were clearly separated from each other in these two clusters. The cluster image map that can be constructed from these two dendrograms (Fig. 2) shows that cluster 1 of the dendrogram on the right side of Fig. 2 contained mainly genes that correlated with artemisinins, whereas cluster 3 contained predominately genes with a relationship to anthracyclines. In cluster 2, genes correlating to both drug classes were assembled. As shown in Table 3, this pattern of distribution was statistically significant ($P = 2.32 \times 10^{-15}$, χ^2 test).

4. Discussion

In the present investigation, we compared the cytotoxicity of different artemisinins and anthracyclines. Among all artemisinins, ARTEST1 was the most active drug with lowest mean IC_{50} value in a panel of 60 cell lines. Among the artemisinins clinically used for the treatment of malaria (ARS, ARE, ARM, ART), ART revealed the greatest cytotoxicity in this cell line panel. Except for ARTEST1, artemisinins showed generally higher mean IC_{50} values than the anthracyclines analyzed in our study. Although this speaks for a lower antitumor activity of artemisinins as compared to anthracyclines, the clinical utility of drugs generally depends also on additional factors, i.e., the involvement in drug resistance phenomena and the therapeutic index, that is the ratio between tumor cell eradication and toxicity towards normal tissues. As artemisinins reveal minor or no side effects in malaria treatment, it is reasonable to speculate that their therapeutic indices may be sufficient to treat tumors. This point of view is further stressed by the peak plasma concentrations of artemisinins measured in clinical anti-malarial studies. Concentrations of 2640 ± 1800 μg/ml (6.88 ± 4.69 mM) have been achieved upon intravenous application of 2 mg/kg ART [26]. Hence, peak plasma concentrations were two to three orders of magnitude higher than the IC_{50} values for ART in the present study.

As of yet, artemisinin-resistant malaria infections have not been observed in the clinic and artemisinins are active against otherwise resistant *Plasmodium* strains [27]. In the present investigation, the N.C.I. cell line panel showed cross-resistance either between artemisinins or between anthracyclines. Cross-resistance of the cell lines to both artemisinins and anthracyclines was, however, rarely seen. This indicates that artemisinins may be useful to treat anthracycline-resistant tumors.

Table 2
Genes shown in the cluster analysis (Fig. 2) whose mRNA expression correlated with IC_{50} values of the cytotoxicity of artemisinins and/or anthracyclines to 60 N.C.I. cell lines

Gene symbol	GenBank	Name	Function
Cluster 1			
BAP29	N57499	B-cell receptor-associated protein 29	Caspase-8-mediated apoptosis and protein transport
CYP2C8	AA033966	Cytochrome P450, subfamily IIC, polypeptide 8	Coronary endothelium-derived hyperpolarization
FMO1	AA047666	Flavin-containing monooxigenase 1	Oxidative metabolism of xenobiotics
GPX4	AA046316	Glutathione peroxidase 4	Protection from toxicity of lipid hydroperoxides
HAAO	H78063	3-Hydroxyanthranilate 3,4-dioxygenase	Synthesis of the excitotoxin quinolinic acid
HSD3B7	W92245	3 β-Hydroxy-δ 5-C27-steroid oxidoreductase	Active against 7-α-hydroxylated sterols; bile acid synthesis
MUTYH	N24004	MutY homologue (*Escherichia coli*)	A/G mismatch-nicking endonuclease in DNA mismatch repair
YWHAG	N62323	Tyrosine 3-monooxygenase/tryptophan, 5-Monooxygenase activation protein, γ polypeptide	Multifunctional regulator of cell signaling processes mediated by protein kinases
Cluster 2			
COX6A1	AA054707	Cytochrome *c* oxidase subunit VIa polypeptide 1	Polypeptide of the multimeric cytochrome *c* oxidase complex of the mitochondrial respiratory chain
COX7C	AA044596	Cytochrome *c* oxidase subunit VIIC	See COX6A1
COX15	R59852, N35161	COX 15 homologue, cytochrome *c* oxigenase, assembly protein (yeast)	See COX6A1
CP	H86554	Ceruloplasmin (ferroxidase)	Iron and copper homeostasis, ferroxidase, amine oxidase, and superoxide dismutase activities
CPO	AA033872	Coproporphyrinogen oxidase	Heme biosynthesis
CYP19A1	AA034003	Cytochrome P450, family 19, subfamily A, polypeptide 1	Formation of aromatic C18 estrogens from C19 androgens
CYP2B7	T72862	Cytochrome P450, family 2, subfamily B, polypeptide 7	Metabolic activation of xenobiotics
DIA1	W46212	Diaphorase (NADH) (cytochrome *b*-5 reductase)	Drug metabolism; microsomal electron transport system
EPHX2	AA029947	Epoxide hydrolase 2, cytoplasmic	Xenobiotic metabolism by degrading toxic epoxides
ESD	AA047429	Esterase D/formylglutathione hydrolase	Hydrolysis of *S*-formylglutathione to reduced glutathione and formate
FDX1	N89718	Ferredoxin 1	Electron transport intermediate for mitochondrial cytochromes P450
FMO5	N54316	Flavin-containing monooxygenase 5	Oxidation of nucleophilic nitrogen, sulfur, and phosphorus atoms in many compounds
GLRX2	AA056193	Glutaredoxin 2	Glutathione-dependent hydrogen donor in cellular redox reactions
GSTA2	AA025243	Glutathione *S*-transferase A2	Conjugation of reduced glutathione to exogenous and endogenous hydrophobic electrophiles
GSTM5	AA056232	Glutathione *S*-transferase M5	See GSTA2
GSTT2	AA009800	Glutathione *S*-transferase T2	See GSTA2
GSTZ1	W79918	Glutathione *S*-transferase Z2	See GSTA2
HIF1A	W47003	Hypoxia-inducible factor 1, α subunit	Induction of oxygen-regulated genes
LOX	AA037733	Lysyl oxidase	Crosslinking of collagens and elastin by oxidative deamination reactions; role in tumor suppression
MAT2A	AA039310	Methionine adenosyltransferase II, α	Formation of *S*-adenosylmethionine from methionine and ATP
MGST1	N99964	Microsomal glutathione *S*-transferase 1	See GSTA2
NOS2A	H67843	Nitric oxide synthase 2A (inducible, hepatocytes)	Production of the messenger molecule nitric oxide (NO)
ODC1	AA055467	Ornithine decarboxylase 1	Polyamine synthesis, transcriptional target of MYC and modifier of APC-dependent tumorigenesis
P4HA2	W93222	Procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), α polypeptide II	Posttranslational formation of 4-hydroxyproline in collagens
PLOD	W94131	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase, Ehlers-Danlos syndrome type VI)	Formation of hydroxylysine in collagens and stabilization of intermolecular collagen crosslinks
PLOD3	AA058886	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3	See PLOD
STIP1	T47637	Stress-induced phosphoprotein 1 (HSP70/HSP90-organizing protein)	Stress-inducible mediator of the heat shock response
YWHAZ	W85726	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, ζ polypeptide	See YWHAG
Cluster 3			
AOX1	AA035245	Aldehyde oxidase 1	Production of hydrogen peroxide and formation of superoxide
CAT	W89002	Catalase	Protection from toxic effects of hydrogen peroxide
COX7B	AA004674	Cytochrome *c* oxidase subunit VIIb	See COX6A1
MGST3	AA054332	Microsomal glutathione *S*-transferase 3	See GSTA2
NDUFA5	AA031646	NADH dehydrogenase (ubiquinone) 1 α subcomplex, 5 (13 kD, B13)	Transfer of electrons from NADH to the respiratory chain

Table 2 (*Continued*)

Gene symbol	GenBank	Name	Function
NDUFA8	W94366	NADH dehydrogenase (ubiquinone) 1 α subcomplex, 8 (19 kD, PGIV)	See NDUFA5
NDUFA10	H68542	NADH dehydrogenase (ubiquinone) 1 α subcomplex, 10 (42 kD)	See NDUFA5
NDUFS3	AA043188	NADH dehydrogenase (ubiquinone) Fe-S protein 3 (30 kD) (NADH-coenzyme Q reductase)	See NDUFA5
NFIC	W87473	Nuclear factor I/C (CCAAT-binding transcription factor)	Activation of transcription and replication
OSR1	AA039663	Oxidative stress responsive 1	Activation by oxidative and osmotic stress
P4HA1	W72635	Procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), α polypeptide I	See P4HA2
PLOD2	N98463	Procollagen-proline, 2-oxoglutarate 5-dioxygenase (lysine hydrolase) 2	See PLOD
PRDX1	AA037489	Peroxiredoxin 1	Reduction of peroxide through thioredoxin system
RAC1	AA057070	Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Racl)	GTP-binding signal transducer; phagocytosis of apoptotic cells, etc.
SCO2	AA001905	SCO cytochrome oxidase deficient homologue 2 (yeast)	Copper transport cytochrome *c* oxidase subunit II (COX2)
TXNRD1	AA055408	Thioredoxin reductase 1	Regulation of the intracellular redox environment

Gene information was taken from the OMIM database, National Cancer Institute, USA (http://www.ncbi.nlm.nih.gov/Omim/) and from the GeneCard database of the Weizman Institute of Science, Rehovot, Israel (http://bioinfo.weizmann.ac.il/cards/index.html).

The clinical application of artemisinins as antimalarials demonstrated that these drugs are safe without severe side effects [10]. In contrast, the tremendous side effects such as myelosuppression, alopezia, mucositis, emesis, nausea, and cardiotoxicity are well-known for anthracyclines. Proliferation-associated toxicities (myelosuppression, alopezia, mucositis) represent general cytostatic and cytotoxic effects of many cytostatic drugs including anthracyclines

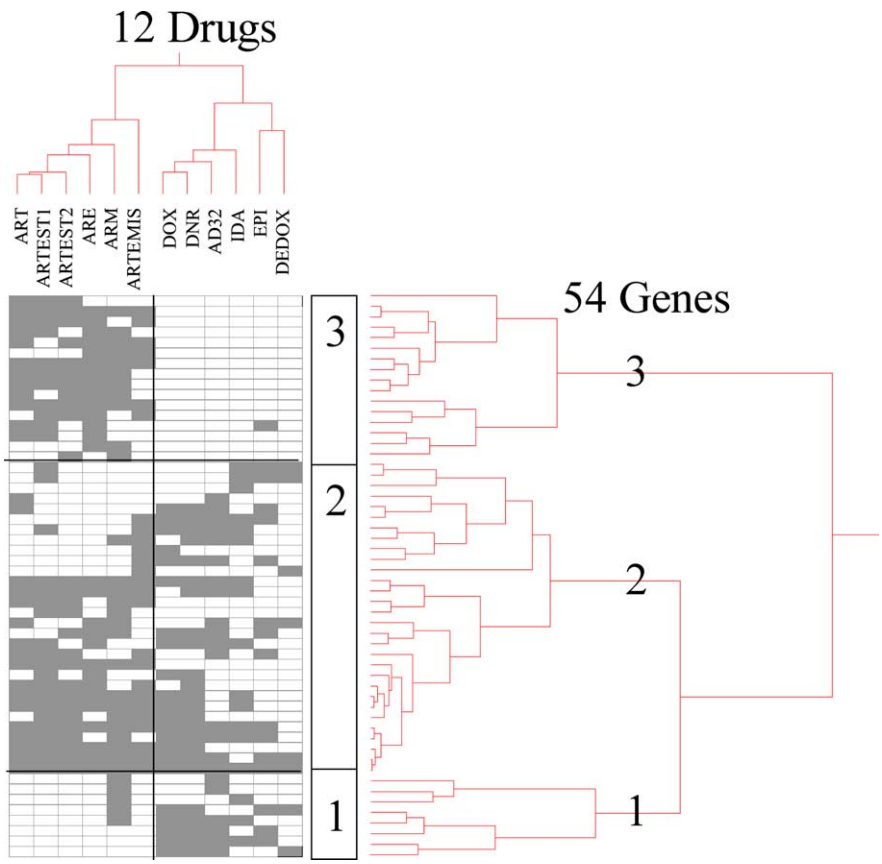

Fig. 2. Dendrograms and clustered image map obtained by hierarchical cluster analysis (complete linkage method) of 54 genes correlating with IC_{50} values for at least one artemisinin or anthracycline in 60 N.C.I. cell lines. The genes are listed in Table 2. The dendrogram on the right shows the clustering of genes and the dendrogram on the top shows the clustering of drugs. Light fields indicate correlations with $P < 0.00598$ according to FDR calculations and dark fields show correlations of $P \geq 0.00598$.

Table 3
Separation of clusters of 60 N.C.I. cell lines obtained by the hierarchical cluster analysis shown in Fig. 2 according to the correlation of gene expression with the IC_{50} values for the cytotoxicity to artemisinins or anthracyclines

	Artemisinins	Anthracyclines
Cluster 1	66	1
Cluster 2	102	90
Cluster 3	5	24

χ^2 test: $P = 2.32 \times 10^{-15}$.

towards growing cells including those of normal tissues. Emesis and nausea in cancer therapy represent a consequence of the excitation of the ligand-driven 5-HT_3 serotonin receptor by cytostatic drugs [28]. Cardiotoxicity is caused by the generation of reactive oxygen species (ROS) by anthracyclines that are insufficiently detoxified in cardiac tissue [15,16]. Interestingly, artemisinins are also thought to produce ROS [11–13], however, without exhibiting cardiotoxicity. The reasons are unknown and the question arises, as to whether different molecular mechanisms are operating after challenging cells with artemisinins or anthracyclines. To address this question, we have chosen a strategy of correlating the mRNA expression of genes of the N.C.I.'s cell line panel with response to artemisinins and anthracyclines.

By means of hierarchical cluster analysis we found a set of genes whose expression correlated predominately with IC_{50} values for cytotoxicity of artemisinins, while another set correlated with IC_{50} values for cytotoxicity of anthracyclines. The expression of a third group of genes showed overlapping IC_{50} values for both artemisinins and anthracyclines. The genes identified by this approach have different cellular functions (Table 3). One group represents oxidative stress response genes that protect cells from oxidative damage (CAT, GPX4, MGST3, OSR1, PRDX1, and TXNRD1), while another set of genes is involved in oxidative metabolism in cells (AOX1, COX7B, CYP2C8, FMO4, HAAO, HSD3B7, NDUFA5, NDUFA8, NDUDA10, and NDUFS3). The other genes play a role for DNA repair (MUTYH), signal transduction and cell proliferation (RAC1, YWHAG), protein transport and apoptosis (BAP29), collagen metabolism (PLOD2, P4HA1), or act as transcription factors (NFIC). It is worth speculating that the expression profiles may account not only for cytotoxic effects towards tumor cells but also for toxicity in normal tissues. Genes accumulated in cluster 3 of the cluster analysis were statistically correlated with the IC_{50} values for cytotoxicity of anthracyclines of the 60 cell lines more expressed in heart than in brain tissues. This indicates that these genes might be linked with cardiotoxicity, since the mRNA expression of these genes were also correlated with the IC_{50} values for anthracyclines. This is in accordance with recent reports showing the multifactorial nature of cardiotoxicity [29,30]. These data together with the results of the previous investigation speak for a complex network of genes that is responsible for the toxic effects of anthracyclines toward heart tissue. On the other hand, the genes of cluster 1 of the cluster analysis might point to potential neurotoxic effects of artemisinins. Neurotoxicity was only observed at extremely high concentrations in animal experiments that were beyond the therapeutic range in humans. A large meta-analysis of clinical studies showed that neurotoxicity is not an issue associated with treatment with artemisinins [10].

The present analysis represents a first step to deepen the insights into the functional relevance of genes predicting both antitumor and adverse side effects of antitumor drugs. Further extensive genomic and functional studies are warranted to link expression profiles to the cellular drug response for a comprehensive understanding of both antitumor action and toxicity.

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