The anti-malarial artesunate is also active against cancer

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Abstract. Artesunate (ART) is a semi-synthetic derivative of artemisinin, the active principle of the Chinese herb Artemisia annua. ART reveals remarkable activity against otherwise multiresistant Plasmodium falciparum and P. vivax malaria. ART has now been analyzed for its anti-cancer activity against 35 cell lines of the Developmental Therapeutics Program of the National Cancer Institute, USA. ART was most active against leukemia and colon cancer cell lines (mean G10 values: 1.1±0.56 µM and 2.1±0.74 µM, respectively). Non-small cell lung cancer cell lines showed the highest mean G10 value (25.6±21.4 µM) indicating the lowest sensitivity towards ART in this test panel. Intermediate G10 values were obtained for melanomas, breast, ovarian, prostate, CNS, and renal cancer cell lines. Importantly, a comparison of ART's cytotoxicity with that of other standard cytostatic drugs showed that ART was active in similar ranges comparable to those of established anti-tumor drugs. Furthermore, we tested CEM leukemia sub-lines resistant to either doxorubicin, vincristine, methotrexate, or hydroxyurea which do not belong to the N.C.I. screening panel. None of these drug-resistant cell lines showed cross resistance to ART. To gain insight into the molecular mechanisms of ART's cytotoxicity, we used a panel of isogenic Saccharomyces cerevisiae strains with defined genetic mutations in DNA repair, DNA checkpoint and cell proliferation genes. A yeast strain with a defective mitosis regulating BUB3 gene showed increased ART sensitivity and another strain with a defective proliferation-regulating CLN2 gene showed increased ART resistance over the wild-type strain, W303-1A. None of the other DNA repair or DNA check-point deficient isogenic strains were different from the wild-type. These results and the known low toxicity of ART are clues that ART may be a promising novel candidate for cancer chemotherapy.

Introduction

Chemical structures with biological activity in micro-organisms, plants, and animals have been developed during evolution of life and may be exquisite targets for drug research. Biogenic drugs are indispensable parts of the pharmacological repertoire to combat human diseases. Natural compounds can serve as lead structures and derivative compounds can be derived from large screenings with chemical drug libraries. Pter alkaloids (vinristine, vinblastine), epipodophyllotoxins (etoposide, teniposide), and taxanes (paclitaxel, taxotere) are important plant-derived drugs in cancer chemotherapy. Unfortunately, chemotherapy of tumors is frequently hampered by the emergence of drug resistance and the occurrence of severe adverse side effects. Hence, the development of less toxic drugs which retain activity against otherwise drug-resistant tumor cells is urgently warranted.

The Chinese herb Artemisia annua has been used in traditional medicine for more than 2,000 years to treat febrile symptoms associated with malaria. Since the isolation of artemisinin as the active principle of Artemisia annua in the early 1970s by Chinese scientists (1), the drug has attracted attention in the Western world. The inhibitory activity of artemisinins towards Plasmodium falciparum and P. vivax malaria has been demonstrated in a number of investigations (2,3). Semi-synthetic artemisinin derivatives with improved pharmacological features have been generated. Out of the different artemisinin derivatives, artesunate, arteether, and artesunate, the latter one is the most potent one in vitro (4).

The World Health Organization recommends the use of these compounds in geographical areas with multiplying resistant malaria. Proprietary features of artesunate (ART) are the activity against otherwise multi-drug-resistant Plasmodium strains (5), its good tolerability and the lack of significant adverse side effects (6).

Previously, we demonstrated that ART, apart from its anti-malarial activity, inhibits the growth of leukemia cells...
and induces apoptosis (7). In the present investigation, ART has been analyzed for its activity against 55 cell lines of different tumor types in collaboration with the Developmental Therapeutics Program of the National Cancer Institute of the USA. As drug resistance is a serious limitation of many established cytostatic agents, we then analyzed whether ART reveals cross-resistance in cell lines resistant to either doxorubicin, vincristine, methotrexate, or hydroxyurea. In an effort to gain insight into the molecular mechanisms of ART's cytotoxicity, we examined a recently described panel of isogenic S. cerevisiae strains with defined genetic defects (8) and identified two putative target genes, BUB3 and CLN2 (cyclin G1).

Materials and methods

Chemicals. Antitumor (ART) was obtained from Sankyo Co. Ltd. (Hanoi, Vietnam). The chemical structure of this sesquiterpene is depicted in Fig. 1.


Cell lines selected for drug resistance. Human CEM (CCR)-leukemia cells were maintained in RPMI medium (Gibco, Eggenstein, Germany) supplemented with 10% fetal calf serum in a 5% CO2 atmosphere at 37°C. Cells were passaged twice weekly. All experiments were performed with cells in the logarithmic growth phase. The development of drug-resistant subclones has been described (10,12). Drug-resistant cell lines were maintained in 5000 ng/ml doxorubicin (CEM-ADR5000), 100 ng/ml vincristine (CEM-VCR100), 2.0 µM MTX plus 10 nM leukovorin (CEM-MTX10000), 10 nM hydroxyurea (CEM-HUR500), respectively.

Sulfonated B testing procedure. Cell lines of the Developmental Therapeutics Program of the National Cancer Institute of the USA were added in aliquots of 100 µl per well into 96-well microtiter plates in a density of 5000 to 40,000 cells according to the growth characteristics of the particular cell type. Incubations were allowed to mature for 24 h at 37°C prior to the addition of ART (104 to 106 M). After a further 48-h incubation period, cells were assayed by means of a sulfonated B assay (13). The optical densities read by a plate reader were processed by a microcomputer. The drug concentration required to inhibit cell growth by 50% (GI50 value) was calculated according to the formula: 100 x (1 - T / T0) x 100 = GI50, where T is the optical density of the test well after a 48-h period of exposure to drug, T0 the optical density at time zero, and C the decimal optical density (14).

Growth inhibition curve. The minimum response to cytotoxic drugs was evaluated by means of a growth inhibition assay. Aliquots of 5 × 103 cells ml-1 were seeded in culture and drugs were added at different concentrations. Cells were counted 10 days after application. Cell numbers were determined each in triplicate independent determinations.

S. cerevisiae strains were used. A panel of drug-sensitive S. cerevisiae strains defective in 17 genes has been described in detail (8). Yeast strains S1 (MATa, ura3, ade2) or with genetic defects in cell proliferative growth arrest or damage repair genes (ubr, Cln3, ncl1, irh3, rds5, pcl1, rfa1, rfa2, snf9) were used for the present investigation. The toxicity of ART towards these strains was determined according to a test assay described previously. Two independent experiments were performed. First, their 3-fold dilutions with a high concentration of 100 µM ART. This is the standard range for toxicity assay. ART was tested over the 10 to 100 µM range. In second experiment, we tried to resolve minor differences using 2-fold dilutions. Aliquots of 1.5 ml yeast cells (1.4 × 106 cells/ml) were placed in 96-well microtiter plates. Fifteen µl of each ART concentration was added in triplicates to the yeast-containing wells. Plates were incubated for 15 min at 30°C and the absorbance (460nm) was read in a micro plate reader. The percentage of surviving cells was calculated relative to unirradiated control plates.

Results

Dose response curves of antitumor (ART) have been determined over a dose range from 10-8 to 10-4 M in 15 lines of different cancer types and GI50 values have been calculated therefrom. Among the tumor cell lines tested, leukemia and colon cancer cell lines revealed the lowest mean GI50 values (1.11 ± 0.56 µM and 2.12 ± 0.74 µM respectively). Fig. 2, shows that the cell lines were sensitive to both ART. The highest mean GI50 value (2.5 ± 0.75 µM) was observed for the colon cancer cell line.
In vitro, interestingly, the drug was also active in several non-CNS tumors (10) and some of these tumors showed a differential sensitivity. In addition, a panel of ART-resistant cell lines was investigated for their sensitivity to ART and showed a differential sensitivity. However, in vitro, ART-resistant cell lines showed a differential sensitivity. In addition, a panel of ART-resistant cell lines was investigated for their sensitivity to ART and showed a differential sensitivity. However, in vitro, ART-resistant cell lines showed a differential sensitivity. In addition, a panel of ART-resistant cell lines was investigated for their sensitivity to ART and showed a differential sensitivity. However, in vitro, ART-resistant cell lines showed a differential sensitivity. In addition, a panel of ART-resistant cell lines was investigated for their sensitivity to ART and showed a differential sensitivity. However, in vitro, ART-resistant cell lines showed a differential sensitivity. 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Figure 1: $C_{50}$ values of arsenic in comparison to $C_{50}$ values of common cytotoxic drugs determined in 35 cell lines of the NCI tumor panel. AraC, cytosine-arabinoside; ART, artemisinin; BCNU, carmustine; CA, chlorambucil; CCNU, lomustine; CP, cyclophosphamide; CTX, cyclophosphamide; DAC, dacarbazine; DDP, cisplatin; DOX, doxorubicin; DTX, docetaxel; EPI, epirubicin; ETO, etoposide; 6-MP, 6 mercaptopurine; MTX, methotrexate; PROC, procarbazine; PRED, prednisone; TAX, paclitaxel; 6-TG, 6-thioguanine; VBL, vincristine; VCR, vincristine; VP-16, etoposide; VAE-26, laposamide.

demonstrates the anti-cancer activity of ART on cell lines of different tumor types, e.g. leukemia, melanoma and cancers of the colon, lung, prostate, breast, ovarian, and CNS. The anti-leukemic activity has previously been reported by us for ART (7) and by Woon-Seng et al for artesunate (14).

ART affects multidrug-resistant Phenoxazinium strains as shown by Loperaresówan et al (6) as well as multidrug-resistant cancer cells as shown in the present investigation. Our results indicate that ART may not be a substrate of the multidrug resistance gene product P-glycoprotein (CEM-ADR5005) and CEM-VCR100 cells express P-glycoprotein (20) and reveal the classical multidrug resistance phenotype with cross-resistance between Vinca alkaloids, taxanes, epipodophyllotoxins, and anthracyclines. The chemical structure of ART differs from other cytotoxic compounds involved in multidrug resistance. For example, essential
Growth inhibition assays of drug-sensitive and drug-resistant CEM leukemia cell lines (a), sensitive and doxorubicin-resistant cells tested with vincristine (e), sensitive and micrometoxate-resistant cells tested with methotrexate (f) and hydroxyurea-resistant cells tested with hydroxyurea (p). ART was tested in sensitive and (j), doxorubicin-resistant, dacarbazine-resistant, and (k), hydroxyurea-resistant. Each cell line was tested at 0.05, 0.1, 0.25, and 0.5 mM concentrations. Control (100%) for each cell type represents cell growth without drug addition (EM of each three independent determinations).

A peptide of ART renders theophylline derivatives resistant. Dose response curves of two cell lines (a 445, 497500) and varying with chelating agents (see inset in the figure) are shown. The log concentration represents growth without drug addition, and different bands of (g) indicate determinations of one. A repeated experiment revealed similar results.

The structural features required for a drug to bind to P-glycoprotein are the presence of a tertiary amine (2122). ART lacks this feature. Likewise, dihydropytrate reductase which is responsible for resistance to methotrexate was not able to protect from the detrimental effects of ART. CEM-MR1500V cells which amplify the DHFR gene (11) had similar responsiveness to ART as the sensitive parental CEM cells. At hydroxyurea-resistant cells were cross-resistant to ART in our experiments, ribonucleotide reductase, which is over-expressed in these cells (12), must also be excluded as target molecule for ART. Since cancer chemotherapy is frequently hampered by the emergence of drug resistance, the treatment of refractory and otherwise drug-resistant tumors with ART represents an attractive prospect. As P-glycoprotein is part of the blood-brain barrier (23), ART may be valuable for the treatment of CNS tumors.

As these mechanisms of drug resistance seem to be irrelevant for ART's cytotoxic action, the question for the molecular targets of ART arises. The mode of action of ART...
may be of a general anti-proliferative type. The iron-catalyzed
oxidation of a free myoglobin radical from the bridged endo-
peroxide group (Fig. 1) appears to be crucial for the anti-
mitotic activity of artesunate derivatives (28). Although
unproven as of yet, it may be assumed that ART's anti-cancer
activity is caused by a similar mechanism. ART's anti-
mitotic action is linked to protein damage (29). This raises
the question as to whether ART may target specific proteins in
cancer cells. To prove this possibility, we tested ART in a
panel of human leukaemia cell lines resistant to doxorubicin.
The use of yeast strains with genetic defects in cell prolifer-
tion, growth arrest, and damage repair genes is suitable to in-
vestigate the molecular target genes of anti-tumor drugs (30). We
found that a BUB3-defective S. cerevisiae strain was more sensitive
and a CLN2-defective strain was more resistant than the
wild-type strain. The human homologue of yeast BUB3, hBUB3, is
a mitotic spindle assembly checkpoint gene. It interacts with hBUB1 and hBUB3. These three genes may be part of a protein complex which localizes to kinetochores before chromosome alignment. As hBUB3, hBUB1, and hBUB1 dissociate from kinetochores in metaphase, they seem to modulate timing of anaphase initiation (31). The
human homologue of yeast CLN2 is cyclin G1. Its up-
regulation in G1 phase and constitutive expression throughout
the cell cycle implicates a role in growth regulation (27). Since
cyclin G1 is a transcriptional target of the tumor suppressor
p53, it may be part of a pathway leading to growth arrest and/or
apoptosis (28). Although a possible role of hBUB3 and
CLN2 genes for the action of ART needs to be corroborated in
further experiments with human tumor cells, the identification
of two growth-related genes by this means substantiates earlier observations of the anti-proliferative action of ART in
pro- and eukaryotes (27).

As shown in the present investigation, the molecular ranges of
GloP values of ART and other approved cytoplac性和 agents are
comparable in the cell lines of the NCI-60 screening panel. It
remains to be proven, whether dose ranges of ART necessary
to affect cancer cells can be reached in vivo. A comparison of
pharmacokinetic data with the in vitro data of the present
investigation do indeed speak for this possibility. Concentrations of ART applied for the treatment of malaria (e.g. 2 mg/kg intravenously) reach peak plasma drug
dosages of 260±100 μg/l (6.8±4.6 mg/l) (29). The overall mean GloP value of all 55 cell lines tested in this
study is three orders of magnitude lower (4.68 μg/l). As shown in clinical studies with more than 1,000 malaria
patients, ART and other artemisinin-like derivatives are well tolerated with few and insignificant adverse side effects (30-32).

Effectiveness which may be disease- rather than treatment-related are transient fever, dizziness, itching, and vomiting (33-35). Neurotoxicity has been found in animal studies using supra-therapeutic concentrations (36). It can be speculated that ART may also be well tolerated in cancer treatment.

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References

1. Klayman D: Qinghao (artemisin) in anti-malarial drug
2. Menschik HM and Olsavsky P: Artemisinin derivatives for
the treatment of severe malaria. Curr Drug Discov Technol
3. Menschik HM and Olsavsky P: Artemisinin derivatives for
treating multidrug-resistant malaria. Curr Drug Discov
4. Banaszek MC, Vahid P and Dieter B: In vitro tests on Philippine
isolates of Plasmodium falciparum against four standard
antimalarials and four artemisinin derivatives, Bull World
5. Sven R, von Vegg TR, Nossen R, Leuermann C, Brockman A,
Plodner L, Gruenpergbuch R, and Welle N: Artemisinin
durability versus artesunate for the treatment of multidrug-
resistant Plasmodium falciparum. Am J Trop Med Hygiene 98:
6. Larsson A and Sigvardsson S: Overview of clinical studies on
7. Efferen T, Lotgering F, Efremov D, Mavro D, Oberich A,
Fahr J and Ostrach R: Detection of artemisinin in whole
human cells treated with artemisinin. Arzneimittel Forsch
8. Simon JA, Sinden R, Niyonkuru D, Lushd C, Bruns BM,
Roberts J, Jennie EL, Fairwell LI and Parid SH: Differential
tolerance of myeloid leukemia cells against DNA repair and
checkpoint systems of noncytochrome C leukemia. Cancer Res
9. Alvey MC, Sinden R, Marks A, Hasley ML, Czerwinski MJ,
et al. (1997): Abber BL, Mabie JC, Siderman RH and Boyd MJ:
Fertility of drug resistance with models of human tumor cells
using a multidrug-resistant human leukemia cell line, Cancer
10. Myerson A, Takemoto Y, Kawashita Y and Asano Y:
Cytotoxicity of artemisinin against multidrug-resistant human
T-cell leukemia cell lines developed in rodent or human cells.
Increased excretion of hydroxyacids resistant leukemia cells
12. Lohmeyer J, Sviderski RM, Paul K, Simon J, Totila S,
Sinden R, Sinden DA, Marks A and Boyd MJ: Comparison of
in vivo and in vitro screening data generated with an
aristoloma-lease versus a microparticle assay against a diverse
panel of human tumor cell lines. J Natl Cancer Inst 82: 215-218,
13. Marks A, Sinden R, Sinden D, Stomrlik R, Paul K,
a high-throughput screen for a diverse panel of cultured human
14. Efferen T, Fodor G and Czakalek R: Anti-SH(2) polyocular
antibody, CL-11 detects glutathione S-transferases and
multiple transferrin receptor human leukemic cell lines. Blood
15. DeVecchi FA, Hultman S and Rosenberg SA: (eds): Cancer
16. Prater JL, Davis W, Hayn and Schneider A: Antimitotic
agents at the Third Congress 1966 Oncological Association