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Oleanolic acid derivative methyl 3-hydroxyimino-11-oxoolean-12-en-28-oate inhibits ABCC1/MRP1 protein function and reduces its level in acute promyelocytic leukemia cells

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Background: The aim of our research work was to find modulator of ABCC1/MRP1 protein which is related to the multidrug resistance of cancer cells. Thus, concerning the commonly known biological activity of oleanolic acid, we decided to test a group of its semisynthetic derivatives.
Materials and Methods: We tested four oleanolic acid derivatives which have been chemically modified comparing to the parental compound base structure at C-3, C-11 and C-28 positions: methyl 3,11-dioxoolean-12-en-28-oate, methyl 3-hydroxyimino-11-oxoolean-12-en-28-oate (Fig. 1), 12α-bromo-3-hydroxyiminoolean-28->13-olide and methyl 3,12-dioxo-12a-aza-C-homoolean-28-oate. As an experimental model two cell lines were used: HL-60 (acute promyelocytic leukemia cells) and its multidrug resistant subline HL-60/AR overexpressing *ABCC1* gene. MTT test was performed to assess the potency of compounds in reduction of leukemic cells viability. The influence of the compounds on the MRP1 function was checked using fluorescent MRP1 substrate − calcein accumulation and retention assay with cytometric detection method and changes in MRP1 protein level were measured using SDS-PAGE electrophoresis and Western Blot technique.
Results: The studied oleanolic acid derivatives showed high activity revealing significant reduction of leukemic cells viability (MTT assay). All of tested compounds were more effective than parental compound. Methyl 3-hydroxyimino-11-oxoolean-12-en-28-oate (HIMOXOL) possessing = NOH group in C-3 position showed even higher activity against multidrug resistant cells than against wild type after 72 h of treatment (IC_{50}: 3.17±0.48 μM, 4.68±0.28 μM, respectively). Test assessing the ABCC1 function modulating activity (calcein retention assay) gave the information that HIMOXOL used in 10 μM almost completely blocked calcein efflux from HL-60/AR cells within three hours after the loading of the cells with calcein-AM. Calcein accumulation test confirmed effectiveness of the compound. Moreover 24 h treatment of HL-60/AR cells with 5, 10 and 20 μM HIMOXOL yielded dose dependent reduction of MRP1 protein level.
Conclusion: Obtained results prove that oleanolic acid derivative − methyl 3-hydroxyimino-11-oxoolean-12-en-28-oate is efficient ABCC1/MRP1 modulator able to decrease level of MRP1 protein and inhibit transport of its substrate out of the cell.

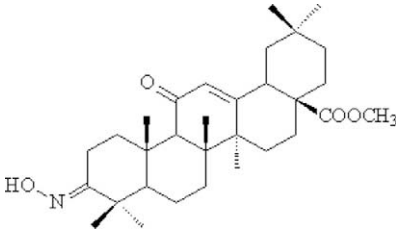

Figure 1. Structure of 3-hydroxyimino-11-oxoolean-12-en-28-oate.

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Iron chelators that overcome drug resistance

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Introduction: Previously, our laboratory demonstrated that the di-2-pyridylketone thiosemicarbazone (DpT) and 2-benzoylpyridine thiosemicarbazone (BpT) series of iron chelators possess highly potent and selective anti-cancer activity *in vitro* and *in vivo*. Interestingly, one of our leading compounds, Dp44mT, was more cytotoxic to drug resistant KBV1 cells which over-express P-glycoprotein (P-gp), a classical drug export pump, than the parent cells, KB31 which do not possess P-gp (Whitnall *et al.* 2006).
We investigated if this observation was indeed dependent on P-gp function and assessed the cytotoxicity using specific inhibitor of P-gp, PSC833. We also determined whether increased toxicity was due to reduced efflux of the chelator, Dp44mT.
Materials and Methods: KB31 and KBV1 (P-gp over-expressing) cells have been derived from the HeLa cell line. To maintain drug resistance, KBV1 was cultured in the presence of vinblastine (1 μg/mL). The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) assay was used to assess the effect chelators have on cellular proliferation. ^{14}C-labeled Dp44mT was used to study the uptake and efflux of drug in the cells.
Results: We have demonstrated using the MTT cell proliferation assay that both Bp4eT and Dp44mT were more cytotoxic in P-gp over-expressing KBV1 cells compared to the parent KB31 cells which do not express P-gp. This increased cytotoxicity in P-gp expressing cells was reversed in presence of the P-gp inhibitor, PSC833 (1 μM), making the resistant cells far less sensitive to our chelators (IC_{50} increased 13.5-fold for Bp4eT and 3.4-fold for Dp44mT relative to control). In contrast, PSC833 had no significant effect on the sensitivity of non resistant cells to Dp44mT or Bp4eT (see Table).
We have also examined whether this increased cytotoxicity in P-gp cells was due to reduced efflux of the chelator. We used ^{14}C-labeled Dp44mT to compare the efflux of drug over various time points at 37ºC. It was shown over a 2 h re-incubation period that ^{14}C-Dp44mT was consistently effluxed more in KBV1 compared to KB31 cells. However, this was unlikely to be due to P-gp as PSC833 had no effect on the efflux of Dp44mT in KBV1 cells.
Conclusion: Further studies are underway to elucidate the mechanism of increased cytotoxicity of Dp44mT and Bp4eT in P-gp over-expressing cells. This study has shown that these iron chelators are more effective against P-gp over-expressing cells than those without P-gp. Further understanding of the mechanism of how these chelators overcome drug resistance will be crucial in developing chemotherapeutic drugs that are able to combat multi-drug resistance in cancer patients.

	IC_{50} (μM) ±SD			
Cell line	Bp4eT	Bp4eT + PSC833	Dp44mT	Dp44mT + PSC833
KB31	0.022±0.002	0.018±0.001	0.017±0.001	0.013±0.001
KBV1	0.013±0.005	0.176±0.024	0.007±0.001	0.024±0.006

References

Whitnall M *et al* (2006) Proc Natl Acad Sci USA, 103, 14901−6.

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Targeting the MAPK pathway to increase sensitivity of ovarian carcinoma cells to platinum compounds

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Aberrant activation of the MAPK pathway may be relevant in reduced sensitivity of cancer cells to conventional and target specific agents. We previously showed that ovarian carcinoma cells selected from the IGROV-1 cell line for resistance to oxaliplatin (IGROV-1/OHP) exhibited decreased sensitivity to gefitinib, due to increased phospho-Extracellular signal Regulated Kinase (ERK)1/2 levels, suggesting that the signaling occurring through EGFR-mediated activation of downstream events may contribute to resistance of ovarian carcinoma cells. The present study was designed to investigate whether targeting the MAPK cascade may be a promising approach to improve cell sensitivity to platinum compounds and to explore the molecular mechanisms leading to activation of survival pathways in these cell systems. Since MEK1/2 regulates ERK activation, we examined whether the pharmacological inhibition of the MEK1/2 kinase may increase sensitivity to platinum compounds in the platinum-sensitive and -resistant cells. When IGROV-1 cells were exposed to cisplatin/oxaliplatin in combination with the MEK1/2 inhibitor CI1040, a synergistic effect was observed, as evaluated by the combination index method. In the IGROV-1/OHP subline, a supra-additive interaction was found with the cisplatin/CI1040 combination. In IGROV-1 cells, the drug combinations resulted in increased caspase 3 cleavage as compared to single drug treatment, thereby supporting an increased apoptotic response. Because ERKs are known to be dephosphorylated by Dual Specificity Phosphatases (DUSPs), we used a genome-wide approach to examine the expression levels of DUSPs in the studied cell lines. We found that the mRNA level of different members of the DUSP family (e.g., DUSP5, DUSP6, DUSP23) were significantly reduced in IGROV-1/OHP and in other Pt drug-resistant ovarian carcinoma cell lines as compared to parental cells. In silico analysis of transcription factor binding sites indicated that p53 may bind DUSP5 but not DUSP6 or DUSP23 promoters, thus suggesting that p53 mutation of IGROV-1/OHP cells may lead to DUSP5 down-regulation. Overall, our results support that targeting the MAPK pathway may increase sensitivity of ovarian cancer cells to platinum compounds. However, the efficacy of

this approach appears related to the molecular alterations of tumour cells including the down-regulation of specific DUSPs and may be reduced in cell systems with acquired drug resistance.

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Mechanisms associated with Sunitinib-resistance in human breast carcinomas

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Background: Despite potent activity in several tumor types, sunitinib showed disappointingly no benefit when combined with cytotoxics in patients with breast cancer. Aiming understanding resistance in breast cancer, we examined molecular changes in carcinoma models protractedly exposed to sunitinib.
Materials and Methods: MCF-7 models were selected to investigate the effects of sunitinib in vitro (MTT and Matrigel assay) and in xenografts. RT-PCR and western blot assays were used to assess a panel of 75 genes and proteins possibly affected by exposure to sunitinib.
Results: The MCF-SUNI cell line was established from the parental MCF-7 cell line using a stepwise exposure to increasing sunitinib concentrations for more than 6 months. Exposure to 48-hour sunitinib led to IC_{50}s of 8.6 and 17.8 μM in MCF7 and MCF-SUNI cells, respectively. Protracted exposure to sunitinib led to a 3-fold increase mRNA expression of VEGFC, VEGFR1, VEGFR3, Neuropilin-1, CXCL12 (SDF-1), CXCR4, HIF1-alpha, PDGFRA, endothelin-1, RET in MCF-SUNI as compared to parental MCF-7 cells. We also observed a basal up regulation of MAPK and AKT survival signalling pathways as measured by p-ERK1/2 and p-AKT levels in MCF-SUNI cells. Interestingly, MCF-SUNI cells also displayed an increased invasive capacity in matrigel as compared to MCF-7 cells. Consistent with the potential role of SDF-1/CXCR4 cell signalling in spontaneous invasion, we observed that AMD3100, a CXCR4 inhibitor, was capable of inhibiting invasion in MCF-SUNI cells. In MCF-7 xenografts protractedly exposed to cytostatic doses of sunitinib, tumor resistance occurred around day 30 and was associated with increased expressions of SDF-1, CXCR4, and PDGFRA mRNAs.
Conclusions: Our data suggest that acquired resistance to sunitinib involves an increased expression of several survival molecules such as SDF-1/CXCR4 (chemokine/GPCR signalling also involved in resistance to cytotoxics) in MCF-7 breast carcinomas. Our data provide a rationale to further investigate inhibitors of SDF-1/CXCR4 to prevent and/or counteract resistance to sunitinib.

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Food does not affect the pharmacokinetics of CS-7017 in healthy subjects: results from an open label, phase I, two-treatment, three-period, crossover study

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Background: CS-7017 is a novel, highly selective peroxisome proliferator activated receptor gamma (PPARγ) agonist which has shown anti-cancer effects in preclinical studies. The aim of this phase I clinical study was to evaluate the effect of a high fat meal on the pharmacokinetics and safety of CS-7017 in healthy subjects.
Methods: This was a phase I, single-centre, open-label, randomized, two-treatment, three period, crossover study in healthy subjects. Subjects received single doses of 0.5 mg (2 × 0.25 mg) of CS-7017 under fasting conditions (A) or following a high fat meal (B) in an ABB or BAA sequence. Each treatment was separated by 6 days. PK samples for CS-7017 were collected on Days 1−4 (Period 1), Days 7−10 (Period 2) and Days 13−16 (Period 3). The primary endpoint was the *ln*-transformed PK parameters of CS-7017 (AUC_{last}, AUC_{0-inf} and C_{max}) when CS-7017 was administered with food (B), relative to when CS-7017 was administered without food (A). Furthermore, intra-subject variability of CS-7017 pharmacokinetics in the fed and fasted state was also determined. The secondary endpoint included a safety assessment.
Results: Twenty-one subjects were enrolled and randomized, two discontinued due to personal reasons. Based on the bioequivalence criteria (90% confidence interval to be within 80−125% of the control), the total exposure (AUC) of CS-7017 was equivalent and the peak exposure (Cmax) of CS-7017 was almost equivalent under fasting and fed conditions (Table). Based on ANOVA results, the intra-subject CVs were 1.6−2.7 folds lower when CS-7017 was given with a high fat meal (Treatment B). No deaths, serious adverse events (SAEs) or discontinuations due to AEs occurred in this study. Four subjects reported treatment-emergent AEs, all of which were mild and resolved by the end of the study without medication.
Conclusions: If CS-7017 is administered with food there may be a slight decrease in CS-7017 exposure. However, this reduction in exposure is not considered clinically significant and therefore, no dose modification is recommended. The administration of a single oral dose of 0.5 mg CS-7017 appeared to be well tolerated in this group of healthy subjects.

Table. Pharmacokinetic parameters of CS-7017 under fasting and fed conditions.

Parameter CS-7017	Geometric LSM Single oral 0.5 mg dose of CS-7017 under		Ratio B/A, % (95% CI)	Intra-subject CV (%) Single oral 0.5 mg dose of CS-7017 under	
	fasting conditions (A)	fed conditions (B)		fasting conditions (A)	fed conditions (B)
AUC_{last} (ng·h/mL)	368.4	314.8	85.4 (80.3, 90.9)	15.8	9.8
AUC_{0-inf} (ng·h/mL)	396.1	341.3	86.2 (81.3, 91.3)	16.2	6.0
C_{max} (ng/mL)	30.5	25.6	84.1 (79.3, 89.2)	15.3	8.9

AUC_{last}, area under the plasma concentration curve from the time of dosing to last measurable concentration; AUC_{0-inf}, AUC from the time of dosing extrapolated to infinity, calculated as: $AUC_{0-inf} = AUC_{last} + Clast/\lambda z\ C_{max}$, Maximum (peak) observed plasma concentration; LSM, least-squares-means.

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Co-administration of a CYP3A4 inhibitor (ketoconazole) increased the bioavailability of CS-7017 but did not affect tolerability: results from an open-label, phase I, two-way crossover study in healthy subjects

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Background: CS-7017 is a novel, highly selective, peroxisome proliferator activated receptor gamma agonist (PPARγ) agonist showing anticancer activity in preclinical models. CS-7017 is metabolized via the CYP3A4 enzyme. The aim of this phase I clinical study was to determine the effect of concomitant administration of a CYP3A4 inhibitor, ketoconazole, on the pharmacokinetics and safety of CS-7017 in healthy volunteers.
Methods: Healthy male subjects aged 20−40 years were eligible for enrolment in this phase I, open label, randomized, two-treatment period, two-way crossover study. Subjects were randomized to receive two treatment sequences either in the order AB or BA. Treatment A comprised of a single oral dose of 0.25 mg CS-7017 (1 × 0.25 mg tablet) on the morning of day 4. In treatment B, subjects received an oral dose of ketoconazole, 400 mg (2 × 200 mg tablets) in the morning of days 1 to 6 and a single oral dose of 0.25 mg CS-7017 (1 × 0.25 mg tablet) in the morning of day 4. There was a washout period of 14 days between treatments. The primary endpoint of this study was the geometric mean ratio of the PK parameters of CS-7017 in combination with ketoconazole compared with CS-7017 administered alone. The safety and tolerability of CS-7017 with and without concomitant ketoconazole administration were also evaluated.

Table 1. Pharmacokinetic parameters of CS-7017 with and without concomitant administration of ketoconazole

Parameter CS-7017	Geometric LSM		Ratio B/A (%)	90% Confidence interval (%)	95% Confidence interval (%)
	Treatment A (Reference)	Treatment B (Test)			
AUC_{last} (ng·h/mL)	193.4	330.4	170.81	(161.57, 180.58)	(159.69, 182.70)
AUC_{0-inf} (ng·h/mL)	214.3	367.5	171.48	(161.62, 181.94)	(159.62, 184.21)
C_{max} (ng/mL)	14.9	16.2	108.8	(102.46, 115.61)	(101.17, 117.09)
	Medians				
	Treatment A (Reference)	Treatment B (Test)	Hodges-Lehmann Estimator for B-A	90% Confidence interval (%)	95% Confidence interval (%)
t_{max} (hours)	2.000	2.000	0.4917	(-0.008, 0.983)	(-0.017, 0.992)
$t_{1/2}$ (hours)	10.50	15.31	6.0717	(5.213, 6.749)	(5.118, 6.931)

$AUC_{0-inf} = AUC_{last} + C_{last}/\lambda_z$.; LSM, least-squares-means.

Results: A total of 22 patients completed the study and were evaluable. The PK parameters of CS-7017 as monotherapy or in combination with ketoconazole are summarized in Table 1. Concomitant administration of CS-7017 with ketoconazole significantly increased total exposure to CS-7017 by approximately 71% and extended the half-life of CS-7017 by 46%