Abstract. Background: Failure of cancer chemotherapy is largely caused by multidrug resistance in tumor cells, mediated by ABC transporters that pump many cytostatics out from the cells. Thus, inhibition of the activity of P-glycoprotein efflux pumps can improve the therapeutic results. Disiloxanes are synthetic resistance modifiers that suppressed not only the multidrug resistance gene but also MRP in various cancer cell lines. Among these compounds, SILA-409 [1,3-dimethyl-1,3-bis(4-fluorophenyl)-1,3-bis(3-morpholino-propyl)-disiloxane-dihydro chloride] showed a remarkable antiproliferative effect and markedly inhibited the P-glycoprotein-mediated efflux mechanism in vitro. The efficacy of this organosilicon drug was investigated in vivo, in a xenograft system. Materials and Methods: Human pancreatic cancer xenografts (PZX-40/19G) were treated s.c. with 10 mg/kg b.w. SILA-409 every second day for 34 days. Tumor volume changes were recorded every week. At the end of the experiment, a complete autopsy was performed and all the vital organs were evaluated histologically. The apoptotic and mitotic rates were counted and evaluated by morphometric methods, and the immunohistochemical expression of P-glycoprotein was determined using a monoclonal anti-p170 antibody. Results: This large dose of the organosilicon compound did not result in histologically observable toxic effects, and some tumor growth delay was noted. SILA-409 did not affect the mitotic activity, but the number of apoptotic cells per mm² was significantly increased. In the untreated tumors, 60% of the cells displayed p170-positivity, while in the treated group, P-glycoprotein was expressed in just 26% of the carcinoma cells. Conclusion: The multidrug reversal effect of SILA-409 was demonstrated in vivo without any apparent toxicity. In addition, it increased the apoptotic activity, exhibited some tumor growth delay, but did not affect the mitotic rate. This new organosilicon compound deserves further attention with a combination of multidrug-resistant substrate chemotherapeutic agents, especially in multidrug-resistant tumors.

Effect of SILA-409, a New Organosilicon Multidrug Resistance Modifier, on Human Pancreatic Cancer Xenografts

ATTILA ZALATNAI¹ and JÓZSEF MOLNÁR²

¹Semmelweis University, Faculty of Medicine, First Department of Pathology and Experimental Cancer Research, Budapest; ²University of Szeged, Department of Medical Microbiology and Immunobiology, Szeged, Hungary

Correspondence to: Dr. med. habil. Attila Zalatnai, Semmelweis University, Faculty of Medicine, First Department of Pathology and Experimental Cancer Research, H-1085 Budapest, Ulloï út 26, Hungary. Tel: +(36-1) 266-1638; 215-6920, Fax: +(36-1) 317-1074, e-mail: zalatnai@korb1.sote.hu

Key Words: Organosilicon compound, multidrug resistance modifier, P-glycoprotein, pancreatic cancer, xenograft.
by Japanese authors, who claimed that MDR1-gene expression was more significantly elevated in pancreatic carcinoma patients than in healthy people, and that over 70% of ductal adenocarcinomas were highly positive for P-gp (8). Reversal of multidrug resistance might offer a potential benefit in this tumor.

SILA-409 [1,3-dimethyl-1,3-bis(4-fluorophenyl)-1,3-bis(3-morpholino-propyl)-disiloxane-dihydro chloride] is a new water-soluble organosilicon compound that was patented in Germany (PCT/DE00/04110, 2000) and has previously been investigated in vitro (9). It acts specifically on P-gp without affecting MDR1 gene expression. It exerted a strong inhibitory effect on the efflux pump activity in MDR1 mouse lymphoma and colo-320 cell lines: at a concentration of 300-800 ng/ml, 100% inhibition was achieved. Moreover, it enhanced the antiproliferative action of daunorubicin in some tumor cells. A synergistic interaction between SILA-409 and epirubicin was found in human MDR1 gene-transfected mouse lymphoma cells and also in human colon cancer cells, measured by the checkerboard method. The compound itself did not induce apoptosis at a concentration of 0.1 to 2.0 µg/ml.

This work is the continuation and extension of the in vitro studies; the results of chronic administration of SILA-409 on human pancreatic cancer xenografts are presented.

**Materials and Methods**

Inbred, artificially immunosuppressed CBA mice (± 20 g), bearing subcutaneously-growing PZX-40/19G human pancreatic cancer xenografts, were treated with 10 mg/kg b.w. SILA-409 every second day for a month. The compound was dissolved in 0.9% saline and administered i.p. (0.1 ml per animal). Control mice were given saline only. The tumor diameters were measured weekly by microcaliper and the tumor volumes were calculated using the formula: \( V = \text{width}^2 \times \text{length}/2. \) At the end of the experiment, a complete autopsy was performed: in addition to the tumors, the lungs, liver, spleen, kidneys, pancreas, testicles, intestine and adrenals from each animal displayed a normal morphology without any sign of degenerative, toxic alterations.

The growth of the xenografted pancreatic carcinomas was not significantly influenced by chronic administration of SILA-409; no size reduction was observed, although in some animals the volumes remained unchanged in the first 2 weeks of the experiment, which might suggest some growth delay effect (Figure 1) The parent tumor was a moderately-differentiated ductal carcinoma and the level of differentiation did not change during treatment. Similarly, there were no alterations in the amount and distribution of the picrosirius red-visualized connective tissue stroma, nor was mucin production influenced.

In the control tumors, the number of mitotic figures per mm² was 6.54±1.17 (mean±S.E.M.), with a similar value being found in the treated tumors (7.14±0.97). However, SILA-409 led to an increased apoptotic activity (13.03±1.07 apoptotic cells/mm² vs. 7.78±0.74/mm² in controls, \( p<0.01 \) by Student’s \( t \)-test) (Figure 2).

Immunohistochemically, p-170 positivity was detected in 59.7±4.1% of the tumor cells of the untreated controls. The positive reaction was linear in appearance and confined to the cell membrane. In the SILA-409-treated animals, the p-170 expression was significantly less (25.8±2.9, \( p<0.015 \)) in the tumor samples (Figures 3, 4).

![Figure 1. Volume changes of PZX-40/19G human pancreatic cancer xenografts during a continuous SILA-409 treatment suggest a growth delay. In the first 2 weeks the tumor volumes remained relatively constant. Individual lines represent different tumorous nodules.](image-url)
Discussion

The present work demonstrates, for the first time, that SILA-409, which was active as a multidrug resistance reversing agent \textit{in vitro} (9), also decreased the p170/P-gp expression in a human tumor xenograft. This organosilicon compound, administered continuously, was not toxic even at high doses in mice. In addition, it showed a modest, but demonstrable, antineoplastic effect.

One of the major problems in the treatment of malignant tumors is that many of them are initially resistant to chemotherapy or they acquire a drug-induced resistance during treatment. The pharmacological modulation of P-gp may reverse the multidrug resistance, potentially offering an enhanced antineoplastic effect when combined with chemotherapeutic agents.

Many chemical compounds capable of reversing multidrug resistance have long been recognized, but these reverters represent a wide range of structural and pharmacological characteristics (10, 11). The reversal activity on P-gp was found to be correlated with lipophilicity, molecular weight and the presence of at least one basic tertiary nitrogen atom (12). Unfortunately, the encouraging \textit{in vitro} results were difficult to reproduce in clinical practice. The early drugs (verapamil, quinidine, cyclosporine, amiodarone, tamoxifen, etc.) resulted in response rates of 60 – 80% in hematological malignancies, but rarely in patients with solid tumors. Moreover, significant cardiovascular side-effects were noted (13, 14). The second generation of drugs yielded unpredictable results. The new, third generation compounds have greater specificity, so much lower doses are needed to achieve a relevant effect, and some of them show promise in clinical trials (15).

In this work, the organosilicon compound SILA-409 was tested \textit{in vivo}. It was given at a dose of 10 mg/kg b.w., which was much higher than the dose necessary to achieve a multidrug resistance reversal \textit{in vitro}. We provided evidence that the drug was well tolerated without any local irritation and that it was devoid of toxic side-effects at this concentration, even after continuous (1-month) administration. No histological signs of toxicity were seen in the checked vital organs.
Although no direct antineoplastic effect was expected, some growth delay was noticed in the xenografted human pancreatic carcinomas. The tumor volumes remained constant in the first 2 weeks of the experiment, but after this they started to grow continuously. Despite this lack of volume reduction, the number of apoptotic cells was significantly elevated, while the mitotic activity was unchanged. These findings suggest a modest, but demonstrable, antineoplastic effect of this compound. No increased apoptotic rate was observed in the earlier in vitro studies (9), but with concentrations 1000- to 20,000-fold lower than the doses used in this experiment. The chronic treatment did not alter the pattern of differentiation, the extent of necrosis, the amount of stromal connective tissue or mucin production.

The multidrug reversal effect of SILA-409 on P-gp was established earlier in cell cultures (9), while this work demonstrated that the compound is also effective in vivo. In the untreated pancreatic cancer samples, almost 60% of the tumor cells expressed immunohistochemically detectable P-gp, but after treatment only about 26% P-gp-expressing cells were seen. These findings, together with the increased apoptotic activity, render this organosilicon compound suitable for further investigation.

Acknowledgements

The excellent technical assistance of Ms. Bernadette Baán is highly appreciated.

References