Role of the Glutathione S-Conjugate Pump in Cisplatin Resistance

Recently, two reports (1,2) suggested that the adenosine triphosphate-depend-ent glutathione S-conjugate export pump (GS-X pump) is involved in cell lines with acquired cisplatin resistance in vitro. Fujii et al. (1) suggested that the GS-X pump plays a role in the efflux of platinum from their 25-fold cisplatin-resistant KCP-4 cells.

By analyzing cell lines that have acquired doxorubicin resistance in vitro and that overexpress the multidrug resistance-associated protein (MRP), as well as cell lines transfected with an MRP-expression vector, we and other investigators recently provided strong evidence that overexpression of MRP results in an increased activity of the GS-X pump (3-5).

From the small-cell lung carcinoma cell line GLC4, we derived in vitro a 150-fold doxorubicin-resistant cell line (GLC4/ADR) and an 8.6-fold cisplatin-resistant cell line (GLC4/CDDP) (3,6-8). There was an approximately 25-fold increase in MRP messenger RNA in the GLC4/ADR cell line, which parallels the amount of MRP in membrane subfractions as revealed by Western blotting (9). In contrast, no increased MRP level could be detected in plasma membranes of the GLC4/CDDP cell line. As compared with the parental GLC4 cell line, the GS-X pump activity, determined with the substrates leukotriene C4 and dinitrophenyl glutathione, was overexpressed eightfold and sevenfold, respectively, in the GLC4/ADR cell line, but the GLC4/CDDP cell line did not show any overexpression. Despite the extensive overexpression of the GS-X pump in the GLC4/ADR cell line, only a 2.1-fold cross-resistance for cisplatin was observed; however, in the cisplatin-resistant cell line, GLC4/CDDP, no overexpression of the GS-X pump was observed. In addition, the 10-fold cisplatin-resistant cell line of Ishikawa et al. (2) showed no cross-resistance for doxorubicin compared with the parent cell line, while the MRP-transfected cell line of Cole et al. (10) showed no cross-resistance to cisplatin compared with the original cell line.

These findings underscore the fact that overexpression of the GS-X pump does not necessarily have to result in cisplatin or doxorubicin resistance. The fact that overexpression does not necessarily result in cisplatin resistance suggests that the ultimate effect of the GS-X pump depends on other factors, such as the glutathione system (glutathione, glutathione S-transferase activity, or glutathione peroxidase activity). Ishikawa et al. (2) reported remarkably high glutathione levels in the cisplatin-resistant cell line. The report by Fujii et al. (1) provided only limited data concerning the glutathione system in their cells. The other reason for different results in different cell lines could be due to the fact that more than one GS-X pump does exist.

References

It is probable that GLC4/CDDP cells did not show any overexpression of the GS-X pump, since these cells do not seem to have an active efflux system for cisplatin (5).

We also noted that the glutathione level was seven times higher in KCP-4 cells than the level in KB cells, but the level of glutathione S-transferase was similar in both cells. The glutathione conjugation reaction may result in the bioactivation of these compounds, as opposed to their detoxification. In addition, the accumulation of the glutathione S-conjugates in cells may lead to a decrease in the detoxification activity of the conjugation system (6). Ishikawa and Ali-Osman (7) showed that cisplatin conjugated with glutathione, the glutathione-platinum complex, inhibited protein synthesis in vitro. The export pump for the glutathione-platinum complex, on the contrary, may play a protective role by decreasing the complex's intracellular concentration. Because the level of glutathione-platinum complex and its cytotoxic activity in the KCP-4 cells are not known, further study is needed to elucidate whether the GS-X pump is involved in the cisplatin resistance.

**References**

(1) Sumizawa T, Chuman Y, Sakamoto H, et al: Non-P-glycoprotein-mediated multidrug-resistant cell lines from human epidermoid carcinoma KB cells: C-A120, C-A500, and CA-1000 (7). Multidrug resistance-associated protein (MRP) was expressed in these multidrug-resistant cells, and the membrane vesicles from the cells transported [3H]leukotriene C4 (LTC4). We therefore examined the expression of MRP and MRP messenger RNA (mRNA) in our cisplatin-resistant KCP-4 cells by immunoblot and slot-blot analysis, respectively. MRP was not detected, and MRP mRNA was not overexpressed in KCP-4 cells. Cross-resistance to cisplatin of our non-P-glycoprotein-mediated multidrug-resistant cells was marginal (1), and cisplatin-resistant KCP-4 cells were not cross-resistant to doxorubicin, daunorubicin, and vincristine (2). Although the rat liver has a GS-X pump that transports LTC4 (3,4), and the GS-X pump is supposedly present in human liver, the expression of MRP mRNA in the human liver was found to be lower than that in KB cells.

Taken together, these findings suggest that the GS-X pump expressed in KCP-4 cells is different from MRP and appears to transport LTC4. It is probable that GLC4/CDDP cells did not show any overexpression of the GS-X pump, since these cells do not seem to have an active efflux system for cisplatin (5).

**Note**

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**Lactic Dehydrogenase Virus (LDHV) Contamination in Human Tumor Xenografts and Its Elimination**

Animal experiments using human or rodent transplantable tumors are useful in studies involving cancer-host interaction, such as those studies on the effects of biological response modifiers and metastasis and preclinical studies on gene therapy. Thus, these transplantable tumors are essential tools in the fields of experimental cancer.

Lactic dehydrogenase virus (LDHV), one of the unclassified Togaviridae, was first reported by Riley et al. (7) in 1960 as a factor in various transplantable tumors of the mouse that increases serum lactic dehydrogenase (LDH) activity in the host mice. LDHV infects subpopulations of mouse macrophages, thus altering humoral and cellular immune responses. Therefore, when implanted tumors used in experiments were contaminated with LDHV, the possibility that the results obtained were affected by LDHV-infected macrophages cannot be ruled out. In addition, because mice develop persistent viremia in the early stage of infection, the virus is transmitted by passaging of the tumor. Although LDHV contamination in mouse tumors has long been known, some human tumor xenografts may also be contaminated with LDHV because the stroma of the tumor tissue containing blood is derived from the host.