The Use of 3-dimensional Cultures for Pharmacokinetic and Pharmacodynamic Study Of Anticancer Agents

Hyo-Jeong Kuh, Sang-Hak Lee, Jung-Won Lee, Jong-Kook Park

Clinical Research Coordinating Center, Catholic Research Institute of Medical Science, The Catholic Univ of Korea

Chemotherapeutic efficiency in human solid tumors is pretty low, possibly due to pre-existing metastasis and moreover, frequent drug resistance. Tumor resistance to anticancer agents, especially in human solid tumors, is a real obstacle for successful treatment and chemotherapy development. The dominant mechanism of anticancer drug resistance is often considered to be MDR mechanism. However, research effort on mdr1 and Pgp has not yielded improvement in cancer chemotherapeutic efficiency. A new mechanism, so-called **multicellular resistance (MCR)** has been proposed, which represents the tumor resistance induced when cancer cells establish contact with their 3-dimensional microenvironment. The interactions in the 3-D microenvironment in human tumors in vivo include cell-to-cell interaction and cell-to-extracellular matrix interaction, which may affect the signal transduction pathway, and consequently alter the pharmacodynamics of anticancer agents. Human solid tumors in vivo also show limited penetration of drugs due to 3-D structure, which may change tissue pharmacokinetics and then pharmacodynamics of anticancer agents.

Two-dimensional cellular assays have been commonly employed in the early stage of new drug development and also in pharmacodynamic studies. However, the lack of predictability and poor clinical relevancy of the data is attributed to the fact that these systems do not mimic the response of cells in the 3-D microenvironment present in human tumors in vivo. In order to evaluate the activity (i.e., pharmacodynamics) as well as pharmacokinetics of anticancer agents, an in vitro system is preferred in terms of speed, cost and ethical issue. There are several in vitro 3-D culture models of human cancer cells available, such as multicellular layers (MCL), multicellular spheroids (MCS), and histocultures. In this presentation, recent data from our laboratory on pharmacokinetics and pharmacodynamics of anticancer agents using these systems will be presented along with preliminary proteomics data to develop pharmacodynamic makers.

(1) Multicellualr layer (MCL)

For the efficient screening of anti-solid tumor activity of anticancer agents, an in vitro experimental system that mimics the characteristics of solid tumors in vivo, i.e., multicellualr resistance, is required. We developed a bioassay system using cytotoxicity assay in conjunction with multicellualr layer (MCL) of cancer cells for rapid determination of anticancer activity. DLD-1 human colorectal carcinoma cells were grown on Transwell insert membrane to form MCLs. The activity of several anticancer agents including 5-fluorouracil, irinotecan and its active metabolite SN-38, paclitaxel, cisplatin, oxaliplatin and heptaplatin were assessed after penetration through MCL with loading concentration close to clinical Cmax or in vitro IC_{50}'s.

Anti-proliferative activity of paclitaxel was reduced after the penetration through MCL in a concentration dependent manner. On the other hand, the activity of 5-fluorouracil was increased after MCL penetration. The anticancer activities of platinum agents including cisplatin and oxaliplatin, but not heptaplatin, were significantly reduced after MCL penetration, which is attributed to the instability of these agents in culture media. These data suggest that the cytotoxicity assay in combination with MCL penetration may be a novel experimental system for the determination of anti-solid tumor activity of new agents in vitro. This system also can be used for lead optimization in new drug development and in the search for better combination regimen against human solid tumors.

(2) Multicellular spheroid(MCS)

Multicellular spheroid(MCS) is an *in vitro* 3-D model of human solid tumors, which closely mimics *in vivo* tumor condition. We evaluated the anti-proliferative activity of 5-FU and its pharmacodynamic markers in MCS and monolayer culture of human tumor cells. MCS was established using a human colon cancer cell line, DLD-1. MCS of DLD-1 showed a steady growth over 11 days reaching 700 ㎛ in diameter with significantly increased G_0/G_1 phase cells. Anti-proliferative activity of a hypotoxin(tirapazamine) was similar in both culture systems, suggesting a reduced O_2 concentration in MCS. On the other hand, IC_{50} of 5-FU in MCS was 10 fold higher than that in monolayers. After 72hr exposure to 10 •M of 5-FU, S phase arrest was observed only in monolayers and apoptotic cell death(TUNEL-positive) was not observed in either culture system.

The basal expression of p53 was significantly higher in monolayers than in MCS and time-dependent increase in its expression was observed in both culture systems when exposed to 5-FU. The expression level of Akt and caspase-3 were similar in both culture systems. The expression of E-cadherin, PTEN and $p27^{kip1}$ were significantly higher in MCS than in monolayers. On the contrary, the expression of phospholipase D(PLD) was higher in monolayers compared to MCS. However, none of these proteins showed any changes in

their expression after 5-FU treatment. This study suggests that MCS may be not only a valid *in vitro* 3D model for screening of anti-cancer agents against human solid tumors, but also an exploitable system for searching biomarkers that may predict response to anti-cancer agents.

(3) Histocultures of human tumor xenografts

In this study, we evaluated the pharmacodynamics of paclitaxel (PTX) and tirapazamine (TPZ) in human NSCLC cells grown in monolayers and histocultures. In A549 human NSCLC cells, the relative importance of conc (C) and exposure time (T) for the anti-proliferative activity was evaluated using $C^n xT=k$ model, and cell cycle arrest effect and apoptosis were measured using flow cytometry.

The decreased activity of PTX and TPZ was observed in histocultures compared to monolayers. Hypoxic cytotoxicity ratio (HCR, ratio of normoxic IC_{50} to hypoxic IC_{50}) in monolayers increased with increasing exposure time, indicating the greater efficacy with longer drug exposure duration. The n values of $C^n xT$ model for PTX and TPZ in monolayers were less than 1, however, the n value for TPZ in histocultures was 1.3, suggesting that the exposure duration is more important in monolayers, but drug conc in histocultures. In both culture conditions, TPZ induced G_2/M arrest and apoptosis from G_1 phase. PTX induced significant G_2/M arrest in monolayers, but not in histocultures. Our data clearly showed the difference in pharmacodynamics between monolayers or histocultures. Overall, the present study indicates that the histocultures of human solid tumors, which best represents *in vivo* solid tumors, may be a feasible and better experimental in vitro system to obtain clinically relevant pharmacodynamic data.