

Communication

Lack of squamous cell lung carcinoma *in vitro* chemosensitivity to various drug regimens in the adenosine triphosphate cell viability chemosensitivity assay^{*⊙}

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A pilot study on squamous cell lung carcinoma (LC) chemosensitivity in adenosine triphosphate cell viability chemosensitivity assay (ATP-CVA) was performed. Besides the histological investigation, a modified ATP-CVA was used for the analysis of cancer cell chemosensitivity to four drug regimens, including topotecan, a promising agent for non-small-cell lung cancer (NSCLC) chemotherapy. Results of *in vitro* chemosensitivity testing showed chemoresistance or only weak response in the predominant amount of tumors.

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Abbreviations: AUC, area under chemoresponse curve; ATP-CVA, ATP cell viability assay; NSCLC, non-small-cell lung cancer; LC, lung cancer; TDC, test drug concentration; TGI, total growth inhibition.

About 40% of the non-small-cell lung cancer (NSCLC) patients are diagnosed in advanced stages and are candidates for systemic chemotherapy, despite the fact that at diagnosis most NSCLC are usually chemoresistant. Under clinical trials only a minor percentage of patients with NSCLC receive some benefit when the chemotherapy is used (Lilenbaum *et al.*, 1998). Due to the serious adverse effects of chemotherapy and its high costs, it is important to develop sufficient methods of prediction of the response to chemotherapy in patients before introduction of the treatment (Zabel *et al.*, 1992; Zabel *et al.*, 1997; Cree & Kurbacher, 1997). Adenosine triphosphate cell viability chemosensitivity assay (ATP-CVA) and its modifications are currently widely used in prediction of chemotherapy response or *in vitro* drug testing in a variety of cancers (Köchli *et al.*, 1994a; Köchli *et al.*, 1994b; Andreotti *et al.*, 1995; Cree *et al.*, 1996; Kurbacher *et al.*, 1998). The ATP-CVA has high evaluability rate (Köchli *et al.*, 1994a; Köchli *et al.*, 1994b; Andreotti *et al.*, 1995; Cree *et al.*, 1996; Kurbacher *et al.*, 1998) and the presence of non-cancerous cells in specimen evaluated does not affect its results (Köchli *et al.*, 1994c). ATP-CVA is individually able to detect, *in vitro*, a majority of patients with *in vivo* poor or good response to chemotherapy; its sensitivity seems to be more than 85% and its specificity not less than 80%, with the true positive predictive value of more than 92% and true negative predictive value of more than 70% (Köchli *et al.*, 1994a; Cree & Kurbacher, 1997; De Vita, V.T., Jr., 1997). To check the usefulness of ATP-CVA in the prediction of squamous cell lung carcinoma (LC) patients response to chemotherapy, we tested the four drug regimens in ATP-CVA: etoposide/carboplatin, cyclophosphamide (as its active metabolite, 4-hydroxycyclophosphamide)/etoposide/doxorubicin, paclitaxel/carboplatin, and topotecan.

PATIENTS, MATERIALS AND METHODS

The group of 15 squamous cell LC patients diagnosed and treated in the Thoracic and Cardiovascular Surgery Department of the St. Elisabeth Hospital (Ibbenbüren), was examined. All patients had primary and pathologically documented squamous cell lung carcinoma. The age of patients was 64.7 ± 7.0 years (mean \pm standard deviation, range 53–78 years). The tumors assessed were classified according to the TNM classification of lung cancer (Mountain, 1997). Tumors' staging was: pT1: 2 patients (13.3%), pT2: 10 patients (66.7%), pT3: 3 patients (20%). Tumors' grading was as follow: G2: 1 patient (6.7%), G3: 14 patients (93.3%). Nodal status classification was: pN0: 5 patients (33.3%), pN1: 3 patients (20.0%), pN2: 7 patients (46.7%). No patient has detectable metastasis (pM0: 15 patients, 100.0%).

For ATP-CVA, a slightly modified method of Andreotti *et al.* (1994; 1995) was used. Fresh lung cancer tissues were mechanically minced into 0.5–2.0 mm³ fragments under sterile conditions. Then the fragments were dissociated into a cell suspension of single cells or small aggregates by incubation with 10 ml sterile Tumour Dissociation Enzyme Reagent (BATLE LE, Ft. Lauderdale, Florida, U.S.A.) for 12 h at 37°C. After the incubation, a Ficoll-Hypaque (Pharmacia, Germany) gradient was used to reduce erythrocyte contamination. Then cells were resuspended for assay (1.5×10^5 cells/ml) in Complete Assay Medium (DCS Innovative Diagnostik-Systeme, Germany). Therapeutic drug preparations from commercial sources were stored and used before expiration dates according to the manufacturers. Drug combinations (etoposide/carboplatin, 4-hydroxycyclophosphamide/etoposide/doxorubicin, paclitaxel/carboplatin, and topotecan) were tested in six di-

lutions corresponding to 200%, 100%, 50%, 25%, 12.5% and 6.25% of each drug standard test drug concentration (TDC, 100% TDC used were as follow: etoposide 43.0 $\mu\text{g/ml}$, carboplatin 15.8 $\mu\text{g/ml}$, 4-hydroxycyclophosphamide 3.0 $\mu\text{g/ml}$, doxorubicin 0.5 $\mu\text{g/ml}$, paclitaxel 13.6 $\mu\text{g/ml}$, topotecan 4.0 $\mu\text{g/ml}$) (Andreotti *et al.*, 1994; 1995). We have used fresh 4-hydroxycyclophosphamide (Asta Medica, Germany) as an active metabolite instead of cyclophosphamide (Hunter *et al.*, 1994). Cultures of about 15000–20000 cells/well were tested in 96 cell microplates (Costar 3790) which contain both 12 maximum inhibition control cells (DCS Innovative Diagnostik-Systeme, Germany) and 12 no inhibition control cells. Cultures were incubated for 6 days at 37°C in a > 98% humidified, 95% air and 5% CO₂ atmosphere, then cellular ATP was extracted and stabilized by mixing of Cell Lysing Reagent (Sigma, U.S.A.) into each well. ATP was measured in a bmg LUMIstar (bmg, Germany) using 50 μl cell lysate and 50 μl Luciferin-Luciferase counting reagent (Sigma, U.S.A.). Five second-count integration with a 1-second delay was used. Each measurement was performed in duplicate. Percentages of total growth inhibition (TGI) were calculated according to the formula:

$$1 - (\text{MR} - \text{MI}) / (\text{MO} - \text{MI}) \times 100 = \% \text{TGI};$$

where: MR: mean counts for replicate test drug cultures; MI: mean counts for maximum inhibition control cultures; MO: mean counts for no inhibition control cultures. Areas under response curves (AUC) were calculated. In addition, the *in vitro* chemoresponses were categorized as follow: the high chemosensitivity was defined as TGI percentage of more than 70% for 6.25–200% TDC, the intermediate chemosensitivity as TGI percentage of more than 70% for 50–200% TDC and 50–70%

for 6.25–25% TDC, the medial chemosensitivity as TGI percentage of more than 70% for 50–200% TDC and < 50% for 6.25–25% TDC, the partial chemosensitivity as TGI percentage 50–70% for 6.25–200% TDC, and the chemoresistance as TGI percentage < 50% for 6.25–200% TDC.

RESULTS AND DISCUSSION

To the best of our knowledge, this is a pilot study on modified ATP-CVA usefulness with the focus on squamous cell lung cancer. Among the 15 tumors tested, all gave the *in vitro* cultures (100%). One out of 15 cancer cell cultures was tested only with topotecan, and one with only three regimens (4-hydroxycyclophosphamide/etoposide/doxorubicin, paclitaxel/carboplatin, etoposide/carboplatin). Fourteen out of 15 cultures tested showed chemoresistance or only medial chemosensitivity to drug combinations used, as was defined above. Only in one culture the intermediate chemosensitivity to paclitaxel/carboplatin was found. Treatment of cell cultures *in vitro* with topotecan, a promising agent for NSCLC chemotherapy, gave also no positive results. The results of chemosensitivity testing are in agreement with the poor clinical response to chemotherapy in the majority of squamous cell lung cancer cases. Due to the only one positive *in vitro* chemoresponse we are not able to calculate any correlation between tumor staging and results of ATP-CVA. None statistically significant correlation between the patients' or tumors' parameters and chemoresponse to treatment (measured as AUC) were found. There was good conformity between results of *in vitro* chemosensitivity testing and lack of early positive clinical response to chemotherapy used in patients assessed (data not shown). The correlation between *in vitro* and late *in vivo* effects are now under investigation, and

if the clinical significance of the testing will be confirmed, this can be a rationale for choosing an adjuvant therapy of squamous cell lung cancer.

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