Exp Oncol 2007
29, 2, 132–136

Experimental ONCOLOGY

SERUM LEVELS OF sFAS AND sFasL DURING CHEMOTHERAPY OF LUNG CANCER

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The aim **of this study was to assess the clinical usefulness of determination of soluble Fas (sFas) and soluble Fas Ligand (sFasL) during chemotherapy of lung cancer.** ***Methods:*** **The study included 80 patients (69 males; 11 females; mean age 64 years; 48 with non-small cell lung cancer-NSCLC, 32 with small cell lung cancer-SCLC). The control group consisted of 15 healthy volunteers. The peripheral blood samples were taken before and after 4 cycles of chemotherapy. sFas and sFasL levels were assessed by Elisa method.** ***Results:*** **The serum sFas and sFasL levels observed at the end of the chemotherapy were higher in all patients with lung cancer compared to healthy volunteers. The levels of sFas and sFasL were higher after chemotherapy than before therapy. The levels of sFasL were significantly higher in SCLC patients than in NSCLC ones. There were no significant differences in serum sFasL levels in relation to clinical stage of lung cancer. After chemotherapy the levels of sFas were higher in patients with metastases. There were no significant differences in serum sFasL levels in relation to response to therapy. At the end of the therapy the serum levels of sFas were higher in Partial Response group than in Progressed patients. Before chemotherapy the levels of sFas were higher in Progressive Disease group than in No Change one. The levels of sFas observed after chemotherapy were higher in Partial Response group than in No Change one.** ***Conclusion:*** **Determination of serum sFas and sFasL levels can be useful in clinical practice, but their practical significance needs further studies.**
Key Words: **soluble Fas, soluble Fas ligand, small cell lung cancer, non-small cell lung cancer, chemotherapy.**

The Fas/FasL system is a major regulator of apoptosis [9]. Fas is a cell surface protein with a single transmembrane domain, belonging to the nerve growth factor receptor/TNF receptor family [15, 22]. FasL is a type II membrane protein that belongs to the TNF family [15, 22]. FasL is expressed in activated T cells and lung cancer cells [15, 19, 22]. Fas is expressed on the surface of cell membranes in a variety of normal tissue cells and malignant cells including lung cancer cells [19].

Fas-mediated apoptosis leads to the elimination of activated T-cells following an immune response. i. e., killing a tumor [1]. Deregulation of Fas-mediated apoptosis is thought to play a role in the cancer progression, lymph node involvement and metastasis [19].

It has been suggested that Fas/FasL systems induce apoptosis of activated immune cells and that the soluble isoforms of these proteins (sFas, sFasL) also inhibit their functions [3]. Elevated serum levels of sFas and sFasL have been observed in patients with many kinds of cancer [6, 7, 21]. The sFas function has not yet been fully elucidated, but there are several findings suggesting the role of sFas in cancer progression [21]. sFas has been reported to play an important role in the regulation of apoptosis as an inhibitor of Fas-mediated apoptosis [21]. It has been revealed that the Fas/FasL system is an important mechanism for tumor escape from the immune system: expression of FasL on tumor cell surfaces and emission of a soluble form of FasL [15, 22]. Soluble Fas and FasL levels are increased in peripheral blood of lung cancer patients [19]. However, the clinical significance of circulating sFas and sFasL has not been clarified, yet.

Received: January 7, 2007.
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Abbreviations used: NSCLC – non small cell lung cancer; sFasL – soluble Fas ligand; SCLC – small cell lung cancer.

MATERIALS AND METHODS

Patients. The study included 80 patients with carcinoma of the lung. They consisted of 69 males and 11 females (mean age of 64 years; ranged 29–78). The tumors were histologically classified as adenocarcinoma in 8 cases, squamous cell carcinoma in 40 cases and small cell carcinoma in 32 cases. None of the patients suffered from infectious, allergic, autoimmune, or other systemic diseases such as diabetes mellitus. The patients had not been previously treated with chemotherapy. The control group for serum sFas and sFasL concentrations comprised 15 healthy volunteers (12 males) with mean age of 61 years. There were no significant differences in age and sex between patients and controls. All patients had a history of smoking. Informed written consent was obtained from all healthy volunteers and all patients.

Methods. Before treatment, patients underwent standard staging procedures consisting of physical examination, serum chemistry examination, bronchoscopy, chest CT scan and ultrasonography of the abdomen. Further imaging techniques were used when required clinically. The clinical stage of non small cell lung cancer (NSCLC) was assigned according to the International Union Against Cancer (TNM classification). The classifications of small cell lung cancer (SCLC) were made according to the Veterans Administration Lung Cancer Study Group (LD-limited disease; ED-extensive disease). After staging, the patients were placed on cisplatin or platin-derived chemotherapy, which was accompanied by radiotherapy in the locally advanced forms. Standard criteria for an objective response to therapy were used (WHO guidelines). To exclude the possible interference of chemotherapy, subsequent blood samples were

obtained at least 28 days after the last administration of cytotoxic drugs. To determine sFas and sFasL serum concentrations, venous blood samples were collected from each patient before and after IV cycles of chemotherapy (some of the patients underwent later radiotherapy). Serum samples were obtained by centrifugation and stored at –80 °C until assayed. Serum sFas and sFasL concentrations were measured by a single laboratory with an enzyme immunoassay (Human sFas Immunoasay — R & D systems; human sFas Ligand ELISA — Bender MedSystems) according to the manufacturer's instructions.

Statistical analysis. Data were presented as mean ± 1 SD or median (range), depending on their normal or skewed distribution provided by Shapiro-Wilk's W-test. Data for sFas and sFasL concentrations in the serum samples from healthy subjects and from patients with lung cancer were analyzed using Student's *t*-test for independent samples. Differences among groups of patients before and after chemotherapy were determined using Student's *t*-test for dependent samples. In the case of skewed distribution, the data were analyzed using Wilcoxon's-test and Mann-Whitney's U-test for unpaired data. The correlation between the parameters was calculated by the Spearman's and Pearson's rank tests.

All *p* values were two-tailed, and the values less than 0.05 were considered statistically significant. Computations were performed using Statistica 6.0 for Windows (StatSoft Inc., Tulsa, OK., USA).

RESULTS

Serum sFas and sFasL levels in healthy volunteers and patients with lung cancer. As shown in Table 1, the serum sFas and sFasL levels observed at the end of the chemotherapy, were significantly higher in 80 patients with lung cancer, compared to 15 healthy volunteers ($p = 0.017$; $p = 0.037$). The levels of sFas and sFasL were higher after chemotherapy than before chemotherapy ($p = 0.00001$; $p = 0.023$).

The levels of sFasL were higher in SCLC patients than in NSCLC ones. There were no significant differences in serum sFas with regard to a histologic type (Table 1).

There were no significant differences in serum sFasL levels in relation to clinical stage of NSCLC and SCLC (Table 1).

Serum sFas and sFasL levels in relation to response to therapy. There were no significant differences in serum sFasL levels in relation to response to therapy (Table 2). At the end of the therapy the serum levels of sFas were higher in Partial Response group than in Progressed patients ($p = 0.032$) (Fig. 1). Before chemotherapy the levels of sFas were higher in Progressive Disease group than in No Change group ($p = 0.02$) (Table 2). In NSCLC group, the levels of sFas observed after chemotherapy were higher in Partial Response group than in No Change group ($p = 0.03$) (Table 2). After chemotherapy the levels of sFas were higher in patients with metastases ($p = 0.02$) (Fig. 2).

Table 1. Serum sFas and sFasL levels in lung cancer patients and controls

Disease stage	Before chemotherapy (*p*-value vs controls)	After chemotherapy (*p*-value vs controls)	Controls (n = 15)
Lung carcinoma patients (n = 80)			
sFas	991.8 ± 299*	1229.9 ± 404** $p = 0.017$	227.3 ± 92
sFasL	357.2 (166–801)#	402.3 (144–3490)## $p = 0.037$	326.7 (151–870)
NSCLC (n = 48)			
sFas	1007.5 ± 296	1253.0 ± 389	
sFasL	349.6 (166–650)[1]	364.8 (144–418)[3]	
III B (n = 25)			
sFas	966.2 ± 288	1182.7 ± 358	
sFasL	364.8 (197–389)	372.5 (144–418)	
IV (n = 23)			
sFas	1075.7 ± 315	1351.8 ± 412	
sFasL	349.6 (166–650)	354.9 (166–403)	
SCLC (n = 32)			
sFas	968,0 ± 306	1194.9 ± 431	
sFasL	367.4 (263–801)[2]	382.3 (166–3490)[4]	
LD (n = 15)			
sFas	910.9 ± 305	1245.6 ± 442	
sFasL	363.7 (278–801)	382.3 (255–3490)	
ED (n = 17)			
sFas	1040.5 ± 307	1170.5 ± 428	
sFasL	367.4 (263–404)	374.9 (166–441)	

Notes: sFas – soluble Fas (pg/ml); sFasL – soluble Fas Ligand (pg/ml); * vs ** $p = 0.00001$; # vs ## $p = 0.023$; [1] vs [2] $p = 0.03$; [3] vs [4] $p = 0.01$.

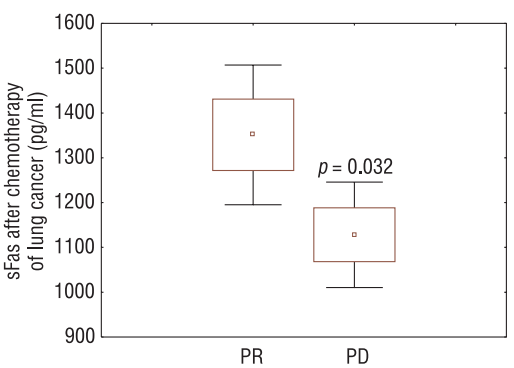

Fig. 1. The serum levels of sFas after chemotherapy of lung cancer in respect to response to therapy

Table 2. Values of sFas and sFasL before and after chemotherapy of lung cancer patients

	PR (n = 15)		NC (n = 17)		PD (n = 16)	
	before chemotherapy	after chemotherapy	before chemotherapy	after chemotherapy	before chemotherapy	after chemotherapy
NSCLC (n = 48)						
sFas	1000.3 ± 272	1519.0 ± 376*	901.2 ± 314*#	1115.8 ± 420**	1120.5 ± 275##	1140.3 ± 251#
sFasL	349.6 (248–389)	364.8 (285–397)	357.2 (166–650)	364.8 (144–418)	338.2 (197–382)	368.6 (237–395)
	PR (n = 17)		NC (n = 3)		PD (n = 12)	
	before chemotherapy	after chemotherapy	before chemotherapy	after chemotherapy	before chemotherapy	after chemotherapy
SCLC (n = 32)						
sFas	1032.6 ± 272	1204.4 ± 441	853.0; 1378.9; 1313.3	1977.4; 1189.7; 1280.2	809.8 ± 312	1110.6 ± 397
sFasL	374.9 (263–801)	382.3 (166–958)	392.2; 382.3; 382.4	389.8; 367.4; 352.5	348.8 (292–397)	382.3 (203–3490)

Notes: sFas – soluble Fas (pg/ml); sFasL – soluble Fas Ligand (pg/ml); PR – partial response; NC – no change; PD – progressive disease vs ** $p = 0.03$; * vs # $p = 0.008$; *# vs ## $p = 0.02$.

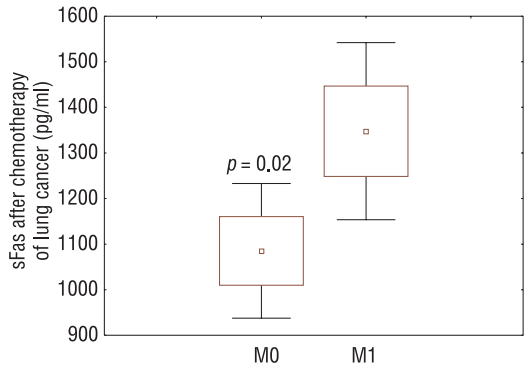

Fig. 2. The serum levels of sFas after chemotherapy of lung cancer in Mo and M1 patients

DISCUSSION

Many studies have revealed that the Fas/FasL system is an important mechanism for tumor escape from the immune system: expression of FasL on tumor cell surfaces and release of a soluble form of FasL [15, 22]. The high serum sFas concentration was proved in patients with cancers: hepatocellular carcinoma [6], renal cell carcinoma [7] and breast cancer [21], which is in accordance with the results of our study. We proved that sFas concentration observed after chemotherapy was higher in the serum of patients with lung cancer than in healthy individuals. Higher concentrations were also observed in lung cancer patients before chemotherapy in comparison with healthy individuals, though the differences were not statistically significant. Similarly, Yoshimura et al. [23] and Shimizu et al. [19] showed higher serum sFas concentrations in patients with lung cancer compared to healthy individuals.

The origin of sFas in the serum remains unclear, though there are three possible theories. sFas may be derived from the tumor itself [18], or from peripheral blood lymphocytes [8]. The third theory indicates that the surrounding stromal tissue may produce sFas in response to the tumor or immune activation [12]. sFas is formed due to cleavage of the external part of extracellular Fas and acts as a FasL inhibitor to bind Fas and prevent Fas- mediated apoptosis. Nonomura et al. [16] claimed that immune cells, essentially lymphocytes, NK and T cells were the most important sources of sFas in response to a developing tumor. However, the results of our study suggest that cancerous cells are the main source of sFas in the serum of patients with lung cancer.

The enhanced serum concentration if an apoptosis inhibitor (sFas) in patients with lung cancer reflects an intense inhibition of cancerous cells apoptosis, which promotes the development of the tumor. Micheau et. al. [11] proved that cytostatics caused the increase in serum sFas, whereas Shimizu et al. [19] showed that sFas increased in the serum together with clinical staging of cancer. Our study confirmed this finding — sFas concentration was higher after chemotherapy than before treatment, where as patients with distant NSCLC metastases had higher sFas concentration than patients without metastases.

We found that patients with NSCLC that showed partial remission (PR) after chemotherapy, had higher sFas concentration than patients with No Change (NC) or Partial Response (PR). Cytostatics destroying cancerous cells may release sFas from these cells.

The results of our study are in agreement with Kondera-Anasz's et al. study [9] based on the group of women with cervical cancer, and with Midis's et al. [12] findings based on nonhematopoietic human malignancy. The high serum sFas concentration found in patients with metastases, may cause resistance to treatment by inhibiting Fas-mediated apoptosis in cancer cells [21]. Various observations of different cancers may indicate that the mechanism of sFas induction might differ depending on tumor type.

We did not prove that determination of sFas concentration might be useful in diagnostics of lung cancer Shimizu et al. [19] indicated such a possibility, however, it requires further studies performed in more numerous groups of patients.

In addition to Fas, FasL also exists in a soluble form released from cell surfaces after cleavage by metalloproteinases [20]. FasL is expressed in activated T cells and lung cancer cells [2]. An increase in serum sFasL concentration was proved in patients with various cancers [4, 17]. It has been proposed that cancer cells expressing FasL have an advantage to evade human immune surveillance by inducing apoptosis in infiltrating lymphocytes expressing Fas [1]. sFasL, cleft by metalloproteinases, protects FasL of tumor cells against their recognition by Fas of T lymphocytes (imitation of tumor cells). Shimuzu et al. [19] suggested that soluble FasL played an important role in tumor genesis and anticancer cytotoxic activity, similar to soluble Fas.

The behavior of serum sFasL in cancer patients is controversial. Enjoji et al. [3] showed that the levels of sFasL were not detectable in biliary carcinoma patients. In Murakami's et al. [14] study, the serum levels of sFasL were significantly lower in bile duct carcinoma patients than in healthy individuals. Conversely, Ichikura et al. [5] showed that the levels of sFasL were higher in gastric carcinoma patients than in healthy volunteers. Our results are in agreement with observations made by Ichikura et al. [5]. We showed that the levels of sFasL in lung cancer patients (observed after chemotherapy) were higher than in controls. The same observations were made by Mouawad et al. [13] in melanoma patients. In this study, the levels of sFas and sFasL in patients were higher in patients than in healthy donors. We did not observed significant differences in serum sFasL level in relation to clinical stage of the tumor. The same observations were made by Melzani et al. [10] in patients with melanoma.

When SCLC is diagnosed, cancer cells are already present in the whole organism, transported via blood vessels (independently of classification by imagining tests as ED or LD). Thus, it is justified that we found the higher sFasL concentration in patients with SCLC than NSCLC.

Summing up, sFas and sFasL play a significant role in patients with lung cancer. Determination of serum sFas and sFasL concentrations may be helpful to assess clinical staging and effects of chemotherapy. However, it requires further studies.

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УРОВЕНЬ sFAS И sFasL В СЫВОРОТКЕ КРОВИ БОЛЬНЫХ РАКОМ ЛЕГКОГО НА ФОНЕ ХИМИОТЕРАПИИ

Цель работы — **оценить клиническую целесообразность определения уровня растворимого Fas (sFas) и растворимого лиганда Fas (sFasL) в сыворотке крови больных раком легкого при химиотерапии.** *Методы:* обследовали 80 пациентов (69 мужчин и 11 женщин; средний возраст — 64 года; из них у 48 диагностирован немелкоклеточный рак легкого (НМКРЛ), у 32 — мелкоклеточный рак легкого (МКРЛ)). Контрольная группа состояла из 15 здоровых доноров. Образцы периферической крови брали до и после 4 курсов химиотерапии. Содержание sFas и sFasL анализировали иммуноферментным методом. *Результаты:* уровни sFas и sFasL в сыворотке крови всех больных раком легкого по окончании химиотерапии выше, чем таковые в контрольной группе и чем таковые до терапии. Уровень sFasL был значительно выше у больных МКРЛ, чем таковой у пациентов с НМКРЛ. Значительных различий в уровне sFasL в сыворотке крови в зависимости от клинической стадии заболевания не выявлено. По окончании химиотерапии уровень sFas выше у пациентов с метастазами, а также в группе с частичным ответом на терапию, чем у больных с прогрессирующим заболеванием. До начала терапии уровень sFas был выше у больных с прогрессирующим заболеванием, чем у пациентов со стабильным состоянием, а по окончании терапии – у больных с частичным ответом по сравнению с группой больных со стабильным состоянием. *Выводы:* определение уровня sFas и sFasL в сыворотке крови может быть применено в клинической практике, но значимость таких показателей необходимо определить в дальнейших исследованиях.

Ключевые слова: растворимый Fas, растворимый лиганд Fas, мелкоклеточный рак легкого, немелкоклеточный рак легкого, химиотерапия.