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Case Report

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Can the EGFR inhibitors increase the immunomodulatory effects of standardized plant extracts (mistletoe lectin and arabonoxylan) with clinical benefit? Case report of a patient with lung adenocarcinoma

Tibor Hajto*, Anna Horváth, Lilla Baranyai, Monika Kuzma and Pál Perjési

Institute of Pharmaceutical Chemistry, Medical University Pecs, Hungary

Abstract

Background: It is well documented that cancer cells are characterized by loss or down regulation of HLA-class-I molecules which are not reversible and reparable. It leads to a definitive escape of tumor cells from T cell lyse. Consequently, growing attention is focusing on the effector cells of innate immune system which are able to kill tumor cells in a non-MHC restricted manner. However, parallel with the tumor progression the tumor growth inhibiting type-1 innate immune cells are down regulated, in that among other reasons a tumor-associated dysregulation of EGF signaling can also play an important role.

Material and methods: Since a 74 year's old patient with inoperable lung adenocarcinoma showed a progression after four cycles Carboplatin and Paclitaxel, a second line treatment with 75 mg/day Erlotinib (Terceva) was started and given for seven months. This tyrosin-kinase inhibitor of Epidermal Growth Factor Receptor (EGFR) therapy was combined with standardized plant immunomodulators giving 0.75 ng/kg mistletoe lectin and 0.45 mg/kg arabinoxylan twice a week which were shown to be pathogenic associated molecular pattern (PAMP)-like molecules which can stimulate the type-1 innate immune cells.

Results: After the chemotherapy and prior to the start of second line treatment the patient was in terminal state of her disease requiring an intensive care. She had multiplex metastases in liver, in lymph nodes and in pleura. After the treatment with Erlotinib and immunomodulators for seven months a nearly complete remission (CR) was established in CT and her quality of life has been excellent.

Conclusion: This case report may support a hypothesis that EGFR inhibitors and type-1 immune cells activating immunomodulators together can synergistic inhibit the tumor growth. Further clinical investigations are necessary to clarify this question.

Introduction

Since a long time it was often observed that an enhanced natural cytotoxicity, measured in peripheral blood of tumor patients, may be one of the factors contributing to a lower cancer risk [1]. The ability of cancer cells to evade immunosurveillance mediated by highly specific T lymphocytes suggests a tumor (oncogene) – induced cellular dysregulation. Indeed, it was found that rectal tumors showing loss of HLA class-I expression which was related to a worse overall and progression-free survival [2]. In various other human tumors it was also documented that cancer cells are characterized by loss or down regulation of HLA class-I molecules [3]. Various epigenetic modifications (such as dysregulations in the expression of genome writers, erasers, or readers) can be responsible for this down regulation [4]. Despite of the fact that tumor infiltrating lymphocytes (TIL) are mainly cytotoxic T lymphocytes only a small fraction of malignant cells responds to CD8+ T cells since they can destroy only HLA class-I positive tumor cells expressing the specific tumor associated antigens. These genetic dysregulation-related quantitative and qualitative alterations of HLA class-I antigens are not reversible. Therefore, tumor cells will not able to recover HLA class-I antigen expression and as consequence they can definitively escape from the T cell lyse [4].

Missing self-recognition makes cancer cells insensitive to T effector cells but not to killer cells of innate immune system. Growing attention is focusing on the mechanisms of innate immune system which are able to kill tumor cells in a non-MHC-restricted manner and appear to exhibit more reversible escape mechanisms if it is compared with adoptive system. In the tumor-associated dysregulation of innate immune system a disturbed immune balance can play an important role. As known, the effector cells of innate immune system are committed in two directions. Type-1 macrophages (M1) and from the monocytes originating type-1 dentritic cells (DC1) generate proinflammatory cytokines, IL-12 and activate cytotoxic effector (such as NK and NKT) cells which are potent inhibitors of tumor growth. However, they are defective in tumor patients. Available information suggests that tumor-associated macrophages belong to the prototypic M2 population [5,6]. M2 macrophages and from the plasmocytoid precursors originating DC2 dentritic cells generate IL-4 and IL-10 which facilitate the generation of Th2 cells and inhibit Th1 cells and the type-1 natural system [6]. M2 macrophages and CD2 dentritic cells affect inflammation, promote cell proliferation by producing growth

Correspondence to: Tibor Hajto Meggyes u. 26, H-2045 Törökbálint, Hungary, Tel: +36 309 735 337, **E-mail:** drhajtot@t-online.hu

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factors, stimulate angiogenesis and tissue repair [5]. Tumor patients can have up to 40% more M2 peripheral monocytes than healthy individuals who have only 10% M2 monocytes [6].

As it was mentioned, the dominance of M2 and CD2 cells in tumor patients can lead to an enhanced production of growth factors which are also contribute to the down regulation of the type-1 natural immune cells. Indeed, it was shown that EGF can inhibit the NK cytotoxicity against cancer cells by down regulating the expression of NKG2D ligands: UL-16 binding proteins (ULBP1 and ULBP2) or MICA and MICB on the tumor cell membrane [7,8]. In this paper a case report is presented which suggests a synergistic effect on the stimulation of type-1 natural immune cells using a combination EGFR inhibitor (Tarceva) and standardized plant immunomodulators (arabinoxylan given in BioBran and mistletoe lectin given in mistletoe extract). The complete remission of adenocarcinoma in lung after this combinative treatment may open new perspectives in tumor therapy.

Material and methods

Mistletoe extract standardized by the determination of its mistletoe lectin level

Iscador^R is a fermented aqueous mistletoe plant extract manufactured and supplied by Iscador AG (CH-4144 Arlesheim, Switzerland). The active (sugar-binding) lectin content of commercially available mistletoe extracts (*Iscador*^{R} M spec 5 mg) was measured in the research laboratory of Pharmceutical Chemistry Department of Medical University Pécs. The determination of sugar binding mistletoe lectin (ML) level in extracts was carried out by an optimized ELLA technique as published previously [9]. Standardized mistletoe extracts (ME) exhibited a bell-shaped dose-response relationship and 0.5-1.0 ng/kg lectin doses were found to be most effective as it was always assessed previously using healthy volunteers. Since two and three therapy-free days were found to be necessary for an immunologically optimal effect, the subcutaneous ME injections were regularly given twice a week.

Standardized rice bran extract (*BioBran/MGN-3)*

The second immunomodulatory drug used in the combinative treatment of the presented patients is *BioBran/MGN-3* which is manufactured and supplied by Daiwa Pharmaceutical Co, Ltd, Tokyo, Japan. BioBran/MGN-3 is composed of denaturated hemicellulose, which is obtained by rice bran hemicellulose reacting with multiple carbohydrate-hydrolyzing enzymes from shiitake mushrooms. BioBran/MGN-3 is standardized for its main chemical component: arabinoxylan with a xylose (in its main chain) and with an arabinose polymer (in its side chain). To the presented patient BioBran/MGN-3 was given orally in doses of 45 mg/kg twice a week parallel to the optimized, lectin-oriented mistletoe extract therapy.

Epidermal growth factor receptor (EGFR) inhibitor (Erlotinib)

Erlotinib (Tarceva) is a tyrosin-kinase inhibitor of EGFR. Tarceva film tablets are usually applied in a daily dose of 150 mg. The majority of clinical experiences originate from patients with advanced NSCLC. Because several side effects the presented patient was treated with 75 mg doses five times a week.

Ethics committee

Ethics committee proposed to observe and publish case reports of own patients treated by ME standardized in terms of lectin activity. The patient has given an informed consent to process and publish her dates. This case report may stimulate an interest for other research groups according to the opinion of the ethics committee.

Results

In the now 74 year old patient (female) the first diagnosis of an adenocarcinoma in the middle and lower lobes of the right lung took place in February 2015. In March 2015 a thoracotomy was carried out and an inoperable dissemination was found. At the same time multiple hepatic, lymph node and pleura metastases were established in CT and by histological examination (T4N2M1). From April 2015 until June 2015 the patient was treated with four cycles of Carboplatin (110 mg/m^2) and Paclitaxel (90 mg/m^2). In July 2015, a further progression was established in CT and the patient was in a terminal state of her disease requiring an intensive medical care. The molecular genetic investigation of her tumor cells showed in the 19. exon of EGFR gene a 47-S752 del mutation in a ratio of 23%. Therefore in July 2015 the chemotherapy was terminated and a second line treatment with erlotinib (Tarceva) was started. Because of major side effects (such as rash and diarrhea), Tarceva tablets were given in limited doses 75 mg/day five times a week.

In addition to the Erlotinib treatment an immunomodulatory therapy with standardized plant extracts were also administered. 0.75 ng/kg mistletoe lectin using standardized mistletoe extract and 45 mg/kg Arabinoxylan using standardized BioBran/MGN-3 preparation were given twice a week. At begin of this combinative therapy a surprising side effect is occured. Without Tarceva on the day of immunomodulatory treatments only a mild elevation in body temperature was usually observed. After the combination of these plant extracts with Erlotinib high temperature responses (up to 39.5°C) occurred suggesting a synergistic effect on the type-1 cells of innate immune system between them. Therefore on the day of immunomodulatory therapy (twice a week) the Erlotinib was not given.

In October 2015 after a treatment for seven months a nearly complete remission (CR) of the primary tumor and CR of all metastases was established in CT (Figures 1 and 2). The quality of life is now excellent and the patient is able to work 100%.

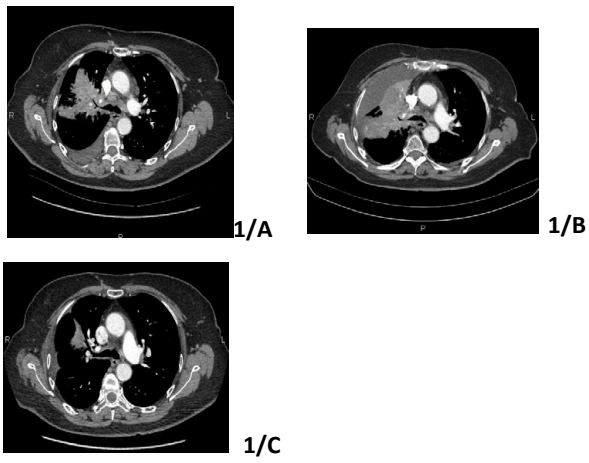

Figure 1. CT investigations of lungs in the patient with inoperable adenocarcinoma on the boundary of middle and lower right lobes which were accompanied with an extensive atelectasy: 1/A. prior the chemotherapy; 1/B. after four cycles Carboplatin (110 mg/m^2) and Paclitaxel (90 mg/m^2); 1/C. seven months after a combinative treatment with Erlotinib (75 mg five times a week) and standardized plant immunomodulators giving 0.75 ng/kg mistletoe lectin and 45 mg/kg arabinoxylan.

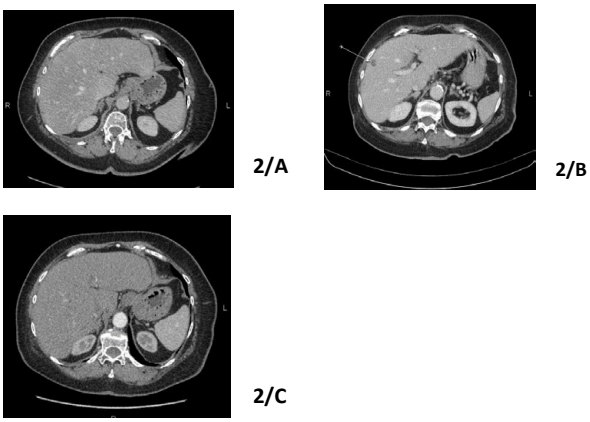

Figure 2. CT investigations of liver metastases in the patient with inoperable adenocarcinoma: 2/ A. prior the chemotherapy; 2/B. after four cycles Carboplatin (110 mg/m^2) and Paclitaxel (90 mg/m^2); 2/C. seven months after a combinative treatment with Erlotinib (75 mg five times a week) and standardized plant immunomodulators giving 0.75 ng/kg mistletoe lectin and 45 mg/kg arabinoxylan.

Discussion

EGFR is expressed in 40% to 80% of lung cancer which makes it an attractive target for molecular intervention in this disease. Erlotinib belongs to the first agents to target tyrosine kinase of the EGFR. Clinical trials of patients with advanced non-small-cell lung cancer (NSCLC) who had or had previously been treated with chemotherapy the response rate to EGFR tyrosine kinase inhibitor between 8% and 15% was found [10,11]. This rate was higher if selected patients with EGFR mutation or other benefits were investigated. In large randomized trials with advanced NSCLC patients the erlotinib therapy was associated with a significantly greater progression-free (2.2 *vs.* 1.8 months) and overall (6.7 *vs.* 4.7 months) survival duration [11,12]. Interestingly, in a very large study, in that the median survivals of 1466 patients were compared after chemotherapy (75 mg/m^2 Docetxel every 3 weeks) versus EGFR inhibitor (250 mg/daily Gefitinib), no significant differences were found [13]. Therefore, a beneficial combination of EGF inhibitors with standardized plant immunomodulators with Pathogenic Associated Molecular Pattern (PAMP) – like molecules may open new perspectives in the EGF research.

Standardized plant extracts administered in this case report have a great advantage; they do not cause any side effects, they are well tolerated and serious adverse events were not reported [14,15]. MGN-3/BioBran® is a modified arabinoxylan preparation obtained from rice bran. It was found to stimulate type-1 cells in innate immune system and these effects can with a great probability correspond to the PAMP –like properties of its arabinoxylan content [16-18]. Growing evidences support a tumor inhibitory effect of MGN3/BioBran [18-20]. Three year randomized clinical trial showed a significantly increased survival of patients with hepatocellular carcinoma after a combination of chemotherapy with arabinoxylan versus chemotherapy alone [18].

Until now standardized mitletoe lectin preparations were not investigated in prospective, randomized and controlled clinical trials. However, it is noteworthy referring to previous case reports [19,20] about patients with sarcoma or with liver metastases which showed astonishing remissions under an immunomodulatory treatment using plant extracts which were standardized to their mistletoe lectin (ML) content.

As reported previously [9], ML originating from plant leaves and stem and arabinoxylan isolated gently from rice bran may represent Pathogenic Associated Molecular Pattern (PAMP) like structures which can bond appropriate Pattern Recognition Receptor (PRR) (in the case of lectin gangliosides with terminal Neu5Ac alpha 2-6Gal beta 1-4GlcNAc residues or in the case of arabinoxylan lectin ligands) on membrane of the type-1 phagocytic cells (such as M1 macrophages or CD1 dentritic cells). As known, PAMP – PRR interactions on the membrane of type-1 phagocytic cell generate proinflammatory cytokines and IL-12 activating cytotoxic effector cells, such as Natural Killer (NK) and NK-T cells which are potent inhibitor of tumor growth [5,6,21-24]. Since NK cells are regularly present in peripheral blood, they are very suitable for monitoring the activation level of type-1 cellular cascades in innate immune system. With the monitoring of NK cells in peripheral blood it was shown that the effects of mistletoe lectin are synergistic increased by its combination with arabinoxylan [25].

EGF signaling is frequently dysregulated in various cancer. Oncogenic signaling reprograms cancer cell metabolism to augment the production of glycolytic metabolites in favor of tumor growth. It was shown that EGF signaling is associated with increased glycolysis leading to an accumulation of metabolic intermediates [26]. One of these intermediates, fructose 1.6 biphosphate directly binds to and enhances the activity of EGFR. This case report suggests that the improvement of dysregulation in EGFR signaling and parallel a bettering of the dysregulaion in innate immune system may open new perspectives in tumor research. Can the polarity of immune system and the polarity of the neuroendocrine system be stronger connected with each other than it was until now believed? We must learn to modulate the regulatory axis of these systems together.

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Disclosure statement

The authors declare that there is no competing or other conflicting interest in relation to this paper.

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