Chapter II

T-cell evaluation in patients with colon cancer: Di-Nitro-Chloro-Benzene skin testing versus plasma levels of sIL2r and sCD8

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Abstract

Background: Developing reliable methods to test the T-cell system can be important in the treatment of colon cancer patients with 5-Fluorouracil/Levamisole. In a pilot study we examined if DNCB (Di-Nitro-Chloro-Benzene) skin testing correlated with plasma levels of sIL-2r and sCD8 and secondly if the application of DNCB had any influence on the production of sIL-2r and sCD8.

Methods: In 10 patients with advanced colon cancer and in 10 healthy volunteers plasma levels of sIL-2r and sCD8 were measured before and 10 days after the application of 2mg DNCB on the inner side of the forearm.

Results: As expected colon cancer patients showed a depressed immune system compared to healthy volunteers (DNCB skin test: p=0.005, sIL2r (medians 700 vs. 295, p=0.002), sCD8 (medians 158 vs. 90: p=0.03, M-W test). The plasma levels for sIL-2r and sCD8 were significantly lower in the skin positive cases (p=0.01 and p=0.03, M-W test). However, a large overlap could be observed in plasma levels between the two skin categories. DNCB had no influence on the production of sIL-2r and sCD8; median change skin negative and skin positive -10 vs. +25, p=0.14 respectively 48 vs. 0, p=0.32 (M-W test).

Conclusions: DNCB skin testing and plasma levels of sIL-2r and sCD8 seem to be equally useful in evaluating the T-cell system and can be used simultaneously.

Introduction

Currently patients with a curative resected Dukes C colon cancer are treated with an adjuvant combination of 5Fluorouracil (5FU) and Levamisole. For many years levamisole has been used to improve T-cell function in patients with various oncological and immunological diseases.\(^1\) During the seventies many studies were published on the use of levamisole but without any remarkable clinical effect. After the publication of the Moertel-trial for colon cancer patients there was renewed interest in using levamisole.\(^2\) 5FU in combination with Levamisole showed a considerable improvement of survival and reduction of recurrences in patients with Dukes C colon cancer. Since treatment with levamisole is based on redressing the balance between the cancer and the host’s defense capacities, it seems to be relevant to evaluate the pretreatment immunological status of patients. Preexisting immune deficiency
may be a factor in defining patients likely to benefit from levamisole. T-cell function in cancer patients can be assessed using delayed hypersensitivity skin testing with DiNitroChloroBenzene (DNCB). Recent publications showed that serum soluble Interleukin-2 receptor (sIL-2r) and soluble CD8 (sCD8) levels in patients with cancer can be used also as parameters in the evaluation of the T-cell system. Especially in patients with lymphomas, disease activity can be monitored with sIL-2r. But also in patients with solid tumors (e.g. lung cancer, gastric cancer, breast carcinoma) it is possible to monitor disease activity with sIL-2r and sCD8. Being involved in a clinical trial of coloncancer patients treated with an adjuvant regime of 5FU and Levamisole, we became interested in different methods to evaluate the T-cell system. We examined in a pilot study the relationship between a semiquantitive method using DNCB skin testing and plasma levels of sIL-2r and sCD8 and secondly the influence of DNCB application on the production of sIL-2r and sCD8.

Methods

The study enclosed 10 patients with histologically or cytologically proven metastatic coloncancer (P-group) with an average age of 64 years (range: 46-84 years). Ten healthy, age matched volunteers with an average age of 59 (range 45-72 years) without symptoms suggesting chronic disease or cancer were used as control group (C-group). Patients or volunteers were excluded if they had former exposure to DNCB, a history of previous malignancies, recently (< 6 weeks) diagnosed viral or bacterial infections, treatment with immunosuppressive drugs or calcium channel blockers and recently performed surgery (< 6 weeks).

2mg DNCB in 0.1ml acetone was applied on the inner side of the forearm in a plastic ring with a diameter of 1.5 cm. After evaporation of the acetone, the DNCB was rubbed into the skin with a glass bar until a white color was seen.

Plasma samples were taken before and 10 days after the application of DNCB and were stored at -70°C. On day 10 skin reactions were considered positive when erythema with a diameter of >1.5 cm was noticed.

Sandwich enzyme immunoassays (T-cell science, Inc, Cambridge Massachusetts) for sIL-2r and sCD8 were used according to the manufacturers instructions and concentrations were expressed in Units/ml.
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Ethics

The protocol was approved by the local ethical committee. All patients and controls gave informed consent.

Statistics

The results were presented as a mean ± standard error of the mean. Patients and controls were compared for their DNCB response with the chi-square test. Comparison of patients with controls and of skin-positive with skin-negative on plasma levels were performed with the non-parametric Mann-Whitney test. A p-value below 0.05 was considered significant.

Results

Skin reactions

Local application of DNCB induced a distinct erythema in all healthy volunteers and in 3 of 10 patients with advanced colon carcinoma (p=0.005, chi-square test). This indicates a depressed T-cell function in the P-group, as expected. Severe skin reactions were noticed in 3 healthy volunteers after the period of 10 days. Most volunteers complained about itching. After a few weeks all skin reactions disappeared, leaving a slight brownish discolorisation in 3 healthy volunteers with severe skin reactions. Although slight erythema existed in three patients, severe reactions were not noticed.

sIL-2r and sCD8

Before application of DNCB, plasma levels of sIL-2r were significantly higher in the P-group compared to the C-group (medians 700 vs. 295, p=0.002, M-W test). Plasma levels of sCD8
were also significantly higher in the P-group compared to the C-group (medians 158 vs. 90, p=0.03, M-W test).

The first question about the correlation of DCNB skin testing and sIL-2r and sCD8 plasma levels is answered in figure 1. There is some correlation: the skin positive cases have significantly lower plasma levels for sIL-2r as well as for sCD8. However as the figure also indicates, there is a large overlap in plasma levels between the two skin categories. Concerning the second question, an influence of DNCB on the production of sIL-2r and sCD8, we correlated the skin testing to the plasma changes over 10 days. For sIL-2r we did not find a correlation: median change -10 versus +25 (p=0.14, M-W test). Similar results were observed for skin negative versus skin positive on the changes of sCD8: medians 48 versus 0, p=0.32 (M-W test).

**Discussion**

DNCB can be used in the evaluation of the T-cell system in cancer patients. After the application of DNCB uptake in Antigen Presenting Cells (Langerhans cells) takes place. T-cells interact with DNCB modified peptides presented in HLA class II that leads to the induction of cytokine release (e.g. IL2) and an up regulation of IL-2 receptor molecules on the membranes. The latter molecules can also be released into the circulation and can be taken as an indication of immune activation. Increased levels of sIL-2r may depress the effectiveness of target cell stimulated release of IL2. CD8 antigen is a surface membrane component of suppressor/cytotoxic T-cells and NK cells. A soluble form of CD8 antigen (sCD8) has been shown to be released by activated CD8+ lymphocytes. Measurement of sCD8 may serve as an index of suppressor/cytotoxic cell activity. High sCD8 levels have been shown to be indicative of tumor bulk in breast cancer patients, and may reflect enhanced suppressor T-cell activity, which can compromise the host antitumor response. Furthermore measurement of sCD8 might give more insight into the pathogenesis of malignancies such as Hairy Cell Leukemia, kidney tumors and breast cancer and may serve as parameter for monitoring different phases of the disease and response to therapy. In this study we confirm that patients with advanced colon cancer have increased plasma levels of sIL-2r and sCD8 as compared to healthy volunteers. The evaluation of the skin test took place after 10 days although some skin reactions of healthy volunteers intensified after this period. Evaluation of the T-cell function by DNCB skin testing and by measurement of sIL-2r and sCD8 plasma
levels correlated with each other. DNCB skin testing showed a decrease in local T-cell activity in colon cancer patients but had no systemic influence on sIL-2r and sCD8. Although in vitro, lymphocytes can be stimulated by PHA to produce sIL-2r we could not measure an effect in vivo with a strong working sensitizer as DNCB. Increased levels of sIL-2r and sCD8 are measured in a variety of tumors, especially leukemia’s. In solid tumors current evidence suggest that there is a slight but significant increase in sIL-2r and sCD8 plasma levels. The increase of sIL-2r in patients treated with levamisole and the release of sIL-2r and sCD8, suggests that the systemic activity in colon cancer patients may be an interesting factor to monitor in 5FU/levamisole treated patients. Therefore it is mandatory to examine whether the induction of sIL-2r correlates with the beneficial effect of levamisole in the adjuvant therapy of colon carcinoma.

In this study we found a correlation between DNCB skin testing and plasma levels of sIL-2r and sCD8. However, local application of DNCB has no influence on sIL-2r and sCD8. Therefore DNCB skin testing and plasma levels of sIL-2r and sCD8 can be used simultaneously in assessing the T-cell function. Further research is necessary to address the real value of DNCB skin testing, sIL-2r and sCD8 in the evaluation of T-cell function in colon cancer patients with regard to the adjuvant treatment with chemotherapy.

References


