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Activity of Phagocytic Granulocytes in Patients with Prostatic Cancer

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Summary. Chemilumenescence (CL) occurs due to the phagocytosis of bacteria and of tumor cells by polymorphonuclear neutrophils (PMN). Levels of CL were measured in patients with prostatic cancer and from normal subjects. Patients with advanced disease (stage C, D) showed no elevated CL levels as compared to healthy individuals or patients with minimal disease (stage A, B). Following external radiation therapy in patients with stage A–C prostatic carcinoma high levels of CL were recorded. Estrogen medication also resulted in increased CL levels, while estramustine did not affect phagocytic activity. Intradermal BCG vaccination caused increased PMN activity. Progressive prostatic cancer in hormone treated patients was associated with increased CL as compared to patients with stable or regressive disease.

Key words: BCG vaccination, External radiotherapy, Chemilumenescence, Polymorphonuclear neutrophils, Prostatic carcinoma, Estrogen therapy, Estramustine therapy.

Polymorphonuclear neutrophils (PMN) phagocytose bacteria and tumor cells after opsonisation. This process is induced by preformed specific antibodies against antigen determinants on the particle surface, which bind the antibodies with their Fab parts causing activation of the complement system [24]. Alternatively the foreign particle itself activates the complement system pathway and induces phagocytosis of granulocytes. Following particle ingestion, the microbicidal mechanisms of PMN's are activated and several highly reactive intermediate products of oxygen reduction are generated [7]. Chemilumenescence is produced by oxygen reduction to "singlet oxygen" or by the carbonyl groups produced by O_2 mediated oxidation [10, 19, 20, 25]. "Natural fluorescence" can be augmented in vitro and measured photometrically [9, 22]. CL production directly correlates with the metabolic activation of PMN's during phagocytosis and can therefore be used to study the phagocytic activity of granulocytes against tumor cells [8, 13].

In the present study we used the chemilumenescence assay to assess the metabolic response of PMN's in patients with prostatic cancer (PC). Cells were obtained from patients with untreated PC, patients with estrogen or estramustine medication or BCG vaccination and patients following external beam radiation therapy.

Material and Methods

Isolation of Peripheral Blood Polymorphonuclear Cells (PMN)

10 ml whole blood (heparinized 10 U/ml) was mixed with 6% Dextran in physiological NaCl and was sedimented at 37 °C. After centrifugation of the supernatant (200 g/10 min), red cells were lysed with NH_4Cl solution (10 min, 4 °C, NH_4Cl 155 mM, $KHCO_3$ 10 mM, Titriplex II 0.1 mM) and the leucocytes were washed twice in 30 ml phosphate buffered saline (PBS).

Differential smear of the separated cells showed more than 90% granulocytes, about 1–2% monocytes and 5–7% lymphocytes.

Opsonisation of Target Cells

K562 cells were coated with autologous patients plasma for opsonisation. 1 ml K562 lymphoma cells (10^7 cells/ml) was incubated with 1 ml patient plasma (37 °C, 30 min). After centrifugation, the sediment of the opsonized target cells was diluted in 0.25 ml PBS for K562 cells ($4 \cdot 10^7$ cells/ml).

Quantitation of CL

CL was measured with LKB Biolumat 1251. 100 µl granulocytes and 100 µl Luminol (1:10 diluted, Lumac) were preincubated for 10 min to determine spontaneous activity. Thereafter 100 µl target cells were added and CL measured for 20 min. The results were recorded as counts for an interval of 20 min. Individual activity was determined according to the formula:

K562 total activity (20 min) – spontaneous activity (20 min).

CL assays were repeated twice.

CL levels were attributed to the activity of PMN's only, because the PMN's were enriched to 90–95% in the sediment. No activity

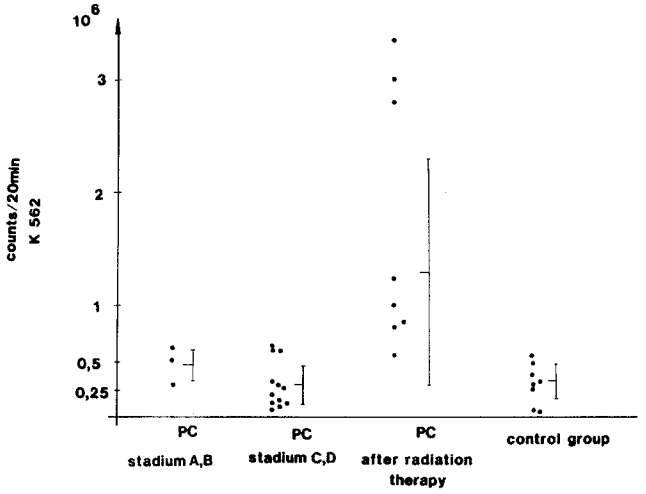

Fig. 1. Activity of phagocytic granulocytes in untreated patients with prostatic carcinoma – stage A, B (n = 3) and stage C, D (n = 12) – patients after external radiotherapy (stage A–C) (n = 8) and age-matched healthy men

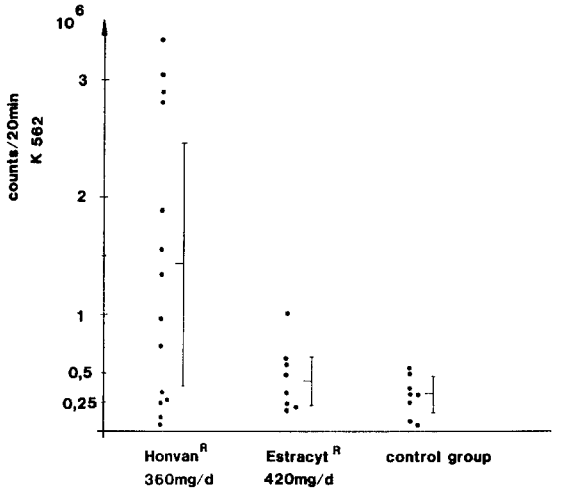

Fig. 2. Activity of phagocytic granulocytes in patients with prostatic carcinoma stage D after orchiectomy treated with estrogens (Honvan 360 mg) (n = 14) or estramustine (Estracyte 420 mg) (n = 8)

could be measured with the other cells (monocytes, NK cell triggered monocytes [17].

Patients Studied

15 patients with previously untreated prostatic carcinoma and 8 patients previously treated by external radiation therapy (Betatron) were included in the study. 14 patients were treated with estrogens (Honvan 360 mg/d) and 8 patients with estramustine (Estracyte 420 mg/d). All patients taking hormone treatment had been previously orchiectomized because of advanced PC stage D. Patients included in the study had been treated with hormones for a minimum of four weeks following orchiectomy.

Five patients (stage D) received adjuvant BCG immunotherapy plus hormone treatment with intradermal injection of BCG (Connaught $8 \cdot 10^7$ viable organisms). Patients with prostatic cancer were investigated by physical examination, IVP, bone scans and usual serum assays. Data from patients with diseases other than prostatic carcinoma were not included in the study.

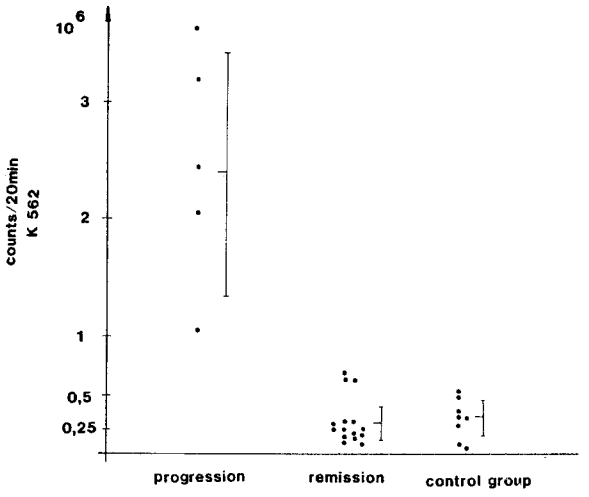

Fig. 3. Activity of phagocytic granulocytes in patients with prostatic cancer stage D according to their response to therapy (EORTC scheme)

Patients were classified according to the criteria of the EORTC group in terms of disease stage and response to therapy (remission, stable disease, progression). The control group consisted of 8 healthy men, ages over 60 years (mean 67,6 yrs.)

Statistical analysis: Wilcoxon rank sum test.

Results

When CL for a group of healthy individuals was compared with CL levels from patients with advanced prostatic cancer (stage D), no significant difference was found ($0.239 \pm 0.175 \cdot 10^6$ vs $0.322 \pm 0.170 \cdot 10^6$).

Patients with localized disease (stage A, B) without distant metastases exhibited slightly – but not significantly – elevated levels ($0.48 \pm 0.21 \cdot 10^6$) (Fig. 1).

Patients treated with external radiation therapy (Betatron) for stage A–C prostatic carcinoma exhibited higher levels of PMN activity compared to healthy individuals ($1.31 \pm 0.84 \cdot 10^6$) (Fig. 1). Three patients, who had undergone radiotherapy a few weeks previously (4, 5 and 9 weeks) showed significantly higher CL levels than the rest of the patients (average time after radiotherapy 2.3 years).

Patients in stage D treated with DES (Honvan 360 mg) after previous orchiectomy had highly elevated levels of PMN activity ($1.41 \pm 1.05 \cdot 10^6$). The group of patients taking estramustine therapy showed no significantly different PMN activity compared to healthy age-matched men ($0.43 \pm 0.22 \cdot 10^6$ vs $0.22 \pm 0.177 \cdot 10^6$) (Fig. 2).

BCG vaccination and PMN activity was monitored in five patients before 4, 8 and 12 months after BCG vaccina-

tion. In four patients PMN activity was boosted, while in one patient activity remained constant.

Patients treated with hormone therapy (estramustine) were subgrouped according to their response to therapy: patients with clinically progressive disease revealed higher PMN levels than patients in stable disease or remission ($p < 0.001$) ($2.38 \pm 0.99 \cdot 10^6$ vs $0.28 \pm 0.163 \cdot 10^6$) (Fig. 3).

Discussion

The phenomen of PMN generating light or CL has been linked to the increased glucose oxidation via the hexose monophosphate shunt of granulocytes undergoing phagocytosis. CL reflects the direct oxidation of the particle being phagocytized, which results in the formation of electronically excited molecules of the substrate. On relaxation to the ground state, light is emitted [3, 25]. CL is directly related to the respiratory process and to the release of oxygen intermediates. Reduction of molecular oxygen leads to the generation of superoxide anion, which dissociates to hydrogen peroxide and O_2 [2, 12]. Singlet molecular oxygen may be released which seems to be essential for the phagocytic killing of foreign cells. CL reflects the direct metabolic response of PMN and the opsonic activity of serum. Beside phagocytic stimuli, membrane stimuli (e.g. phorbol myristate), antigen-antibody complexes, complement and lymphokines are able to evoke chemilumenescence [21].

In our study patients with advanced PC (stage C, D) revealed no elevated levels of PMN activity, whereas patients with stage A and B carcinoma showed only slightly activated CL. This finding indicates, that probably no activation of the granulocytes results from the tumor itself, either because the PMN's cannot reach the tumor or the tumor surface is not recognized as antigenetic.

Following radiotherapy for carcinoma, patients develop an elevated baseline CL, probably attributable to the long term influence of the altered antigenetic tumor cell surface. The high CL levels of recently irradiated patients could be caused by a localized, sterile inflammatory reaction due to the radiation of the prostatic tissue.

Patients with stable disease, receiving estrogen medication showed greater levels of CL than did healthy age-matched men, whereas patients with estramustine treatment showed no altered response. However, patients treated with estramustine had more advanced disease than did the estrogen treated patients. Moreover estramustine was mostly prescribed to patients, who had developed estrogen resistant disease. In individual patients estramustine treatment did not alter PMN CL levels during long term medication, while estrogen medication in most patients resulted in stimulation of PMN activity. In fact, DES is known to be a stimulator of the reticuloendothelial system [14]. The activity in DES treated patients therefore reflects the influence of estrogens because all studied patients had clinically stable disease. Patients with advanced disease (stage C, D) also exhibited highly elevated CL levels, so that in these cases tumor associated effects on CL might prevail.

Estramustine apparently had no influence on the phagocytic activity of granulocytes against tumor cells.

In our study patients were vaccinated intradermally with $8 \cdot 10^7$ viable organisms BCG, which caused a rise of PMN levels after each vaccination. Previously patients had been inoculated three times at a four month intervals with BCG. Only one patient failed to respond to BCG treatment. BCG has been widely used for cancer immunotherapy, although the exact impact on the immune system is still unknown. However, macrophages, NK cells and T-lymphocytes are principally involved [1, 23].

In hormone treated patients, irrespective of disease state, a significant difference in CL levels was demonstrated. Estramustine medicated PC patients were subgrouped. Five patients showed relapse, while 15 patients were in regression or stable disease. A highly significant difference between these two groups was observed: Patients with progressive disease had increased levels of PMN activity, possibly reflecting elevated antitumoral phagocytosis of PMN ($p < 0.001$).

Elevated PMN activity in patients with progressive disease might be explained by increasing phagocytic stimuli, represented by increased tumor effects. The nature of the reactions can not be defined, but ongoing immunological reactions to the tumor itself or stimulatory influences of tumor-derived products might be included [6]. Changes in the baseline CL levels may also have been produced by a serum factor. Meltzer and Stevenson observed, that increased opsonophagocytic activity of macrophages – monocytes produce approximately one third of the amount of CL of neutrophils [15] – can be transferred to normal mice by serum. Monocytes however seem to have a slower rate and degree of particle ingestion than PMN [16].

Our results are supported by the finding that PMN's of patients with active bacterial infections are in an activated state both functionally and metabolically [4, 5, 11].

A relationship between the respiratory burst, chemilumenescence and microbicidal and tumoridical activity in neutrophils was established. As a consequence, chemilumenescence of PMN has been used as an index of phagocytic activity in patients with prostatic cancer. The application of this assay in PC patients provides data about PMN function in patients taking various therapies and could help define criteria for assessment of the patient's individual response to hormone therapy. Also early signs of progression of the disease could be monitored with the PMN activity and could lead to changes in anti-tumor therapy.

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