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Enhancement of phytohemagglutinin-induced lymphoproliferative response by indomethacin, Thymex L or their combination in lung cancer patients

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Several studies showed that thymic factors and prostaglandin synthesis inhibitors enhance *in vitro* lymphoproliferative response (LPR) to mitogens in cancer patients. In this study we investigated whether indomethacin and thymic extract (Thymex L), applied in combination, may in a synergistic pattern influence phytohemagglutinin-induced LPR in lung cancer patients. The results demonstrate that the use of the investigated agents enhances LPR to a similar level in hyporeactive patients before, as well as after, therapy. However, this drug combination exerts an additive effect on LPR, but only in patients who underwent cytoreductive radiation therapy, indicating the potential usefulness of this drug combination as an adjuvant treatment of these patients.

Key words: Indomethacin, lymphoproliferative response, lung cancer, Thymex L

Introduction

A number of studies demonstrated the presence of an alteration of various aspects of cell-mediated immunity in lung cancer patients.[1–3] Immunodepression in cancer patients is attributed, among various host- or tumor-related factors,[4,5] to abnormal monocyte immunoregulation.[6,7] The suppressive activity of monocytes is thought to be mediated by prostaglandin E (PGE), as it was shown that indomethacin and other PG synthesis inhibitors enhance the lymphocyte response *in vitro*.[8–10] Furthermore, the cell-mediated immune response in cancer patients could be affected by all conventional treatments, especially radio- and chemotherapy.[3,12,14] Immunosuppression caused by cytoreductive therapy might reflect a direct effect of the treatment used on lymphocyte responsiveness[15] and/or an indirect effect via a PGE-dependent suppression mechanism.[10,16] This disease- as well as therapy-related depression of immunity of cancer patients may be repaired by various thymic hormone preparations, as has been suggested by encouraging data obtained in clinical trials.[17–21] *In vitro* studies showed that thymic preparations may enhance various T cell-mediated immune functions,[19,23,24] including proliferative response to lectins or alloantigens.[15,19,22] The *in vitro* testing of the effectiveness of thymic preparations is thought to be useful as a good indicator of potential *in vivo* efficacy.[19]

In our previous reports[11,25] we found that indomethacin improved the *in vitro* lymphoproliferative response (LPR) to mitogens in lung cancer patients and that this effect was not affected by radio- or chemotherapy. As the indomethacin-induced recovery of LPR was only partially achieved, the aim of the present study was to investigate whether the enhancing effect of indomethacin might be improved by the addition of a thymic preparation (Thymex L) in untreated and treated lung cancer patients.

Materials and methods

Patients

The following three groups of lung cancer patients were included in the study:

(i) Twenty-four newly diagnosed, untreated patients (40–70 years old, $\bar{x} = 57$ years); 22 of them had unresectable (stage IIIa), histologically verified squamous cell carcinoma and two patients had small cell lung cancer (limited disease).

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(ii) Sixteen patients with squamous cell carcinoma (stage IIIa) (48–75 years old, $\bar{x} = 52$ years), who were tested within 24 h after completing radiotherapy (RT).

(iii) Thirty one patients (48–68 years old, $\bar{x} = 56$ years), who received two or four cycles of chemotherapy (ChT); 19 of these patients had metastatic squamous cell carcinoma (stage IV) and 12 patients had small cell carcinoma (limited disease). Blood samples of ChT-treated patients were taken on the 21st day after the last drug injection.

Controls

The control group included 37 healthy persons (laboratory personnel and blood donors), who were age and sex matched with patients whenever possible.

Radiation therapy

Radiotherapy was administered by a linear accelerator with external radiation photon energies of 10 MV at a daily dose of 2 Gy up to the total dose of 45–60 Gy, five times per week. The technique of two opposed parallel fields encompassing the primary tumor with 2 cm margin and a part of the mediastinum was used. Both fields were treated daily. Chest X-rays and computed tomography scans were used in treatment planning.

Chemotherapy

Drug treatment of squamous cell carcinoma included mitomycin C (8 mg/m^2) and vindesine (3 mg/m^2) on the first day, and cisplatin (120 mg/m^2) on the second day, or mitomycin C plus vindesine on the first day, and carboplatin (500 mg/m^2) on the second day of each treatment cycle. Patients with small cell lung cancer were treated with a combination of epirubicin (120 mg/m^2), cisplatin (60 mg/m^2) and vincristine (1.4 mg/m^2) on day 1 of each cycle. The free interval between the cycles of chemotherapy was 28 days.

LPR

The proliferative response of peripheral blood lymphocytes (LPR) to phytohemagglutinin (PHA) was estimated by the whole blood method as described earlier.[11] In short: 20 drops of heparinized blood were added to sterile bottles with 2 ml of RPMI 1640 medium supplemented with 20% heat-inactivated fetal bovine serum (Gibco, Grand Island, NY), 100 U/ml penicillin and 100 μg/ml streptomycin. In addition to the duplicate samples prepared for the determination of the control PHA response, the additional samples were prepared in the same way with either indomethacin or Thymex L, or both agents simultaneously. PHA (reagent grade, Wellcome, UK) was used in optimal concentration of 80 μg/ml. Indomethacin (Sigma, St Louis, MO) was added at a concentration of 1 μg/ml. Thymex L (Thymoorgan GmbH Pharmazie and Co, Vienenburg, Germany), commercially available preparations of calf thymus containing about 30 proteins of different molecular weight (1 mg of dried substance contains 0.6 mg of protein) was added at a concentration of 5 μg/ml. Indomethacin and/or Thymex L were added to the cultures at the time when the PHA assays were initiated and incubation continued for the entire culture period. After 4 days of incubation at 37°C, colchicine (Sigma) was added to arrest mitoses, and smears were made after hypotonic treatment and fixation. The Giemsa stained cells were counted. The number of mitoses was counted per 1000 cells. The results were expressed as the percentage of the PHA response found in control cultures with peripheral blood cells from healthy persons. The culture conditions used have consistently elicited an optimal response in healthy subjects and cancer patients in our previous experiments.[11,25]

Data analysis

Wilcoxon's paired test was used for statistical analysis.

Results

In 37.5% of untreated lung cancer patients the LPR *in vitro* to PHA was below the lower limit of values found for control subjects. In the RT-treated group, the percentage of hyporeactive patients was much greater (87.5%), while after chemotherapy this percentage was only 25.8% (Table 1). The addition of indomethacin to cell cultures significantly increased ($p < 0.005$) the LPR to PHA in hyporeactive patients regardless of the previous therapy used. Similarly, significant enhancement of

Table 1. The mean PHA response in hyporeactive[a] and normally reactive lung cancer patients

Patients	*n*	%	Mean PHA response
Before therapy	24		
hyporeactive	9	(37.5)	37.3 ± 13.9
normally reactive	15	(62.5)	91.8 ± 14.0
After RT	16		
hyporeactive	14	(87.5)	29.8 ± 12.0
normally reactive	2	(12.5)	85.9
After ChT	31		
hyporeactive	8	(25.8)	49.6 ± 22.3
normally reactive	23	(74.2)	92.6 ± 16.8
Controls	37		96.7 ± 15.4

[a] Patients whose LPR to PHA were below the lower limit of normal response ($\bar{x} \pm$ SD of healthy subjects).

LPR to PHA was obtained by Thymex L in all patient groups (Figure 1). The addition of indomethacin and Thymex L simultaneously to peripheral blood cell cultures of untreated patients increased the PHA response to the same level as did each of the agents added alone. However, in RT-treated patients the increase of LPR obtained when both agents were present in the culture was significantly higher ($p < 0.025$) than that obtained with either single agent. The mean value of LPR to PHA in the presence of both agents, obtained in patients treated with chemotherapy, was not different from the value found in the presence of indomethacin alone, but it was higher ($p < 0.025$)

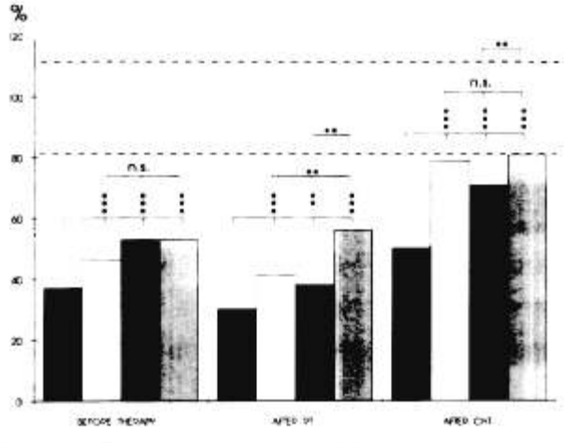

Figure 1. The enhancement of LPR to PHA *in vitro* by indomethacin (INDO), Thymex L (TxL) or their combination in hyporeactive lung cancer patients. $^{**}p < 0.025$. $^{***}p < 0.005$. Dotted lines represent the normal range ($x \pm$ SD of healthy individuals). (■) PHA, (□) PHA + INDO, (■) PHA + TxL, (▨) PHA + INDO + TxL.

Table 2. The number of hyporeactive patients whose LPR was augmented (>20% of baseline) by indomethacin (INDO), Thymex L (TxL) or their combination

Patients	INDO	TxL	INDO + TxL
Before therapy	7/9 (77.7%)	7/9 (77.7%)	8/9 (92.8%)
After RT	8/14 (57%)	9/14 (64%)	10/14 (71%)
After ChT	6/8 (75%)	5/8 (62.5%)	6/8 (75%)

than the value obtained in the presence of Thymex L alone (Figure 1). The percentage of hyporeactive patients in all three groups, whose PHA response was augmented by either single agent used or their combination, is shown in Table 2.

In patients with normal lymphocyte reactivity, regardless of the therapy, the addition of indomethacin or Thymex L, either alone or in combination, did not cause any significant changes of baseline values (data not shown).

Discussion

Many reports showed that the decreased LPR in cancer patients may be improved *in vitro* by PG synthesis inhibitors.[8,9] Similarly, thymus hormones are shown to enhance various T cell functions, including LPR to mitogens.[19,22] Indeed, some clinical trials with thymosins[18,19,21] suggested that they might be useful as adjuncts for patients who had been treated by conventional therapy. In addition, there is recent evidence that cancer patients, including lung cancer patients, have lower thymic hormone values than healthy age-matched individuals.[26]

Our previous studies of the effect of indomethacin on lymphocyte response to mitogens in untreated[11] and treated[25] lung cancer patients showed that indomethacin-induced improvement of this lymphocyte function was not completely achieved. In the current study we investigated whether indomethacin and a thymic extract (Thymex L), applied in combination, may be synergistic in their action on LPR to PHA *in vitro* in these patients.

We found that the lymphocyte response to PHA, which was decreased before as well as after cytoreductive therapy in lung cancer patients, was augmented to a similar level by each of the agents used in a high percentage of patients, but neither

of them normalized this lymphocyte function. This is consistent with other reports concerning the *in vitro* effect of either indomethacin or thymic hormone preparations in various cancer patients including lung cancer.[9,15,27] Our results indirectly suggest that diminished lymphocyte response is, at least partially, due to PGE-mediated suppressor activity, but also may be related to the thymic hormone-sensitive, intrinsic impairment of the T cell function exerted in the course of the disease itself. Alternatively, it is induced by the applied therapy. In this sense, several studies utilizing monocyte-depleted or T cell-enriched populations indicated that there may be a primary T cell defect playing a role in the decreased PHA response in cancer patients.[13,15]

In our study, the combination of indomethacin and Thymex L induced a significant improvement of PHA response only in previously treated patients; in irradiated patients this drug combination induced higher augmentation than did each single agent alone, while in chemotherapy-treated patients this improvement was evident only when compared to that with Thymex L. An additive effect of PG synthesis inhibitor-aspirin and thymosin fraction 5 (TF5) on PHA-induced IL-2 production was also demonstrated by Zatz *et al.*[28] Richner *et al.*[27] found that a combination of indomethacin and a thymomimetic drug (isoprinosine) had no additive effect on PHA-induced lymphocyte proliferation in cancer patients, including lung cancer patients. The discrepancy of the results obtained by Richner *et al.* and the data presented here may be explained by the use of different drugs.

Another question that arises from our study concerns the absence of any additive effect of indomethacin and Thymex L on the lymphocyte response in untreated patients. The possible explanation may be the fact that cytoreductive therapy can enhance,[10,16] but also diminish[4,13–15] some suppressor activities operating in cancer patients, which probably allows a better effect with the drug combination. Thus, in a study by Hersh *et al.*[29], irradiation diminished suppressor cell activity of peripheral blood lymphocytes of cancer patients for mitogen response in 75% of cases.

We observed the enhancing effect of the drugs only in those patients who were able to respond, their lymphocytes not being much affected by previous treatment. Indeed, in a few patients whose baseline lymphocyte response to PHA was very low, no improvement of LRP was seen in the presence of either agent or their combination. The significance of the initial PHA-induced LPR for the modulating action of the drugs was also evident in the chemotherapy-treated group. This patient population showed better initial LPR to PHA than the irradiated patients, and the addition of either agent or both simultaneously to the culture increased the LPR almost to the lower limit of the normal response of healthy individuals. This is in agreement with our previous results.[11,25]

In conclusion, our *in vitro* studies suggest that such a combination of two different approaches (the monocyte-based antisuppressor and the T cell restorative one) in the modulation of the lymphocyte function may provide a better effect than a single one in immunodepressed, irradiated lung cancer patients. As this patient population is considered a uniformly immunosuppressed group of subjects for evaluation of both *in vitro* and *in vivo* immunorestorative properties of various biological response modifiers,[15] our *in vitro* results may indicate a rationale for the possible use of thymic hormone preparations in conjunction with PG synthesis inhibitors as adjuvant treatment of these patients.

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