CORRECTION OF BIOCHEMICAL AND IMMUNOLOGIC PARAMETERS IN LARGE BOWEL CANCER BY OPTIMAL DOSES OF RETINYL ACETATE AND ASCORBIC ACID

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Carcinoma of the colon (CC) is nowadays one of the commonest forms of human malignant neoplasm. In tumors in this situation a state of immunodepression is frequently observed [5], and this, as we know, is a bad prognostic sign for patients with cancer [2]. One of the factors contributing to the development of immunodepression may be a low concentration of vitamin A and ascorbic acid (AA) in the blood plasma of patients with CC [2]. If these vitamins are deficient, reactions of humoral and cellular immunity are depressed and this is accompanied by increased sensitivity of the patient to infection [3, 4]. In the early postoperative period patients with CC often develop suppurative inflammatory complications despite the extensive use of antimicrobial therapy.

It was accordingly decided to investigate the possibility of correcting biochemical and immunologic parameters in the pre- and postoperative periods in patients with CC with moderately increased (optimal) doses of retinyl acetate (RA) and AA.

Observations were made on 144 patients with CC in stage II and III of the disease and aged between 30 and 65 years. Of this total number 36 patients formed the control group. Patients of group I (22 individuals) received RA alone in a daily dose of 50,000 I.U. in the form of an oily solution or capsules (Uman' Vitamin Factory) for 8-12 days in the preoperative period and for 15-20 days after the operation. Patients of group 2 (20 individuals) received AA alone in a dose of 1 g twice a day for the 8-12 days before the operation. Patients of group 3 (46 individuals) received RA and AA simultaneously by the scheme described above in the preoperative and postoperative periods. All patients under observation, including the control group, recieved the polyvitamin preparation "Undivit" in a dose of 1 dragée twice a day.

Retinol (ROL) in the blood plasma was determined by high-pressure liquid chromatography on a Spectra-Physics (West Germany) SP-8000 chromatograph with "Partisil ODS" analytical column. C_{19}-retinoid, obtained from L. A. Vakulova from the Laboratory of Chemistry of Polyene Compounds, "Vitaminy" Scientific Production Combine, was used as the internal standard. The quantity of AA in the plasma and lymphocytes was measured by a spectrophotometric method with phenylhydrazine. Parahydroxyphenyllactic and homogentesic acids (PHPLA and HGA respectively) in the 24-hourly sample of urine were determined on an MAT-311A chromatomass-spectrometer (from "Varian," West Germany), connected by capillary inferface to a Varian 3700 gas chromatograph, equipped with WCOT capillary glass column with SE-30 on Gas Chrome Ω (80-100 mesh) measuring 25 m × 0.5 mm.. [$^2H_4$]Parahydroxyacetic acid ([$^2H_4$]-PHPAA) was used as the internal standard. The mass spectra of the fragments were recorded along lines with m/e of 179, 183, and 326 for PHPLA, [$^2H_4$]-PHPAA, and HGA respectively.

Functional activity of the lymphocytes was judged from the rate of proliferation of the cells in mixed lymphocyte culture (MLC) and in the blast transformation reaction in response to mitogen concanavalin A (conA, from Sigma, USA) and pokeweed mitogen (PWM, from Gibco, USA). Lymphocytes of healthy blood donors and patients with CC were used as reacting cells. A pool of lymphocytes from not less than three healthy donors, irradiated in a dose of 2000 R, was used as the stimulating cells. The lymphocytes were incubated in a volume of 0.2 ml in 96-well flat-bottomed 3040 plates in an incubator containing 5% CO_2 for 2 h (for conA),

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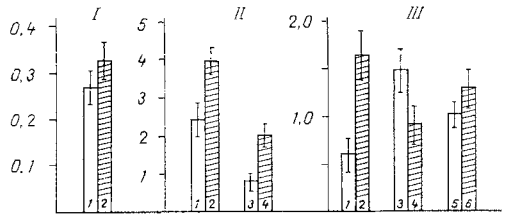

Fig. 1

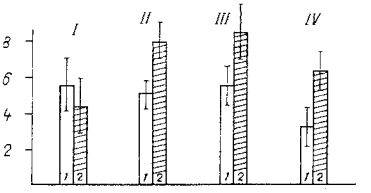

Fig. 2

Fig. 1. Effect of RA and AA on biochemical parameters in patients with CC. I) Plasma ROL concentration (in μg/ml): 1) initial level; 2) 8-12 days after adminstration of RA (n = 22, P > 0.05); II) AA concentration: 1, 2) in plasma (in μg/ml); 3, 4) in lymphocytes (in $\mu g/10^7$ cells); 1, 3) initial level; 2, 4) 8-12 days after vitamin C administration (n = 17, P < 0.05); III) concentration (in mg/day) of PHPLA (1, 3, 5) and HGA (2, 4, 6) in urine of healthy blood donors (1, 2) and patients: 3, 4) initial level; 5, 6) after taking AA (n = 24).

Fig. 2. Effect of RA and AA on proliferative and stimulating activity of cells in MLC of patients with CC. Ordinate, IP. I, II) Proliferative activity of lymphocytes in MLC of patients of control group (n = 30, P > 0.5) and of patients receiving RA + AA in preoperative period (n = 36, P < 0.01): 1) before administration, 2) 8-12 days after administration of preparations. III) Stimulating activity of lymphocytes of patients receiving RA + AA for 8-12 days (cells of healthy blood donors stimulated by irradiated lymphocytes from patients with CC): 1) before, 2) after administration of preparations. IV) Proliferative activity of lymphocytes from patients receiving RA + AA in postoperative period: 1) control group (without preparations, n = 36); 2) patients receiving RA + AA (n = 31, P < 0.01)

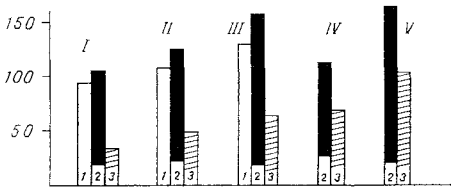

Fig. 3. Effect of optimal doses of RA and AA on proliferative response of lymphocytes stimulated by conA. Abscissa, concentration of conA: 1, 2, and 3) 1, 5, and 15 μg/ml respectively; ordinate, change in activity (in %): I, II, III) relative to initial parameters; IV, V) relative to parameters of control group. I, II, III) Patients receiving RA, AA, or RA + AA respectively in preoperative period; IV, V) patients receiving RA and RA + AA in postoperative period. In groups I, II, and IV) changes in activity with conA in a concentration of 15 μg/ml are not significant.

5 days for PWM), and 6 days (for MLC). At the end of incubation [^{3}H]thymidine was added to the culture and 18 h later the cells were harvested on 75-105-05 filters (from Flow Laboratories, England) by means of a multiharvester from the same firm. The ratio between the number of counts per minute in the experiment to the same parameter in the control (monoculture) was taken conventionally as the index of proliferation (IP).

EXPERIMENTAL RESULTS

In a preliminary study the immunopharmacology and pharmacokinetics of large doses of RA and AA in healthy blood donors and cancer patients was investigated. RA in a dose of more than 500,000 I.U. and AA in a dose of over 10 g were found to induce a state of temporary

immunodepression. The upper limit was accordingly set at doses of 50,000 I. U. for RA and 1 g for AA twice a day as optimal for immunocorrection.

Systematic administration of RA to the patients in optimal doses did not lead to any statistically significant rise in the level of ROL, the principal metabolically active form of vitamin A, in the blood plasma (Fig. 1). An increase in the ROL concentration was observed only in individual patients. By contrast, after administration of AA in a dose of 2 g daily, the vitamin C level rose in the patients' plasma and lymphocytes (Fig. 1). After cessation of AA administration the plasma vitamin level fell to its initial value after 2-4 days, and the lymphocyte level after 3-7 days. After operation on the patients the AA concentration in the plasma and lymphocytes fell considerably. There is evidence in the literature that operations, trauma, and infection lead to rapid exhaustion of the AA reserves in the body [4]. A change in the ratio between the PHPLA and HGA levels in the urine also was observed 10 days after the beginning of AA administration. In mammals conversion of PHPLA into HGA takes place in the presence of AA. Consequently, increased excretion of PHPLA with the urine may be evidence of the presence of endogenous vitamin C deficiency. The urinary PHPLA level in healthy donors and patients with CC, incidentally, fluctuates considerably depending on the season and character of the diet. However, values of the ratio of PHPLA to HGA in the urine, together with the plasma AA level give a more reliable indication of the patient's vitamin balance.

Simultaneous administration of RA and AA in optimal doses to patients in the preoperative and postoperative periods led to a statistically significant increase in proliferative activity of the lymphocytes in MLC in 70-75% of patients compared with its value in the control group (Fig. 2). A significant increase also was observed in the stimulating ability of the cells in MLC of patients receiving the vitamins (Fig. 2). Stimulating cells in MLC are known to be β-lymphocytes, and synthesis of immunoglobulins, on which the antigenic characteristics of the cells depend, is disturbed in avitaminosis A [3].

After administration of RA or AA alone in optimal doses to patients in the preoperative period some increase was observed in the proliferative activity of lymphocytes stimulated *in vitro* by conA (Fig. 3). Stimulation of the proliferative response under these circumstances was observed only when suboptimal quantities of the mitogen were used (Fig. 3). The simultaneous use of two vitamin for immunocorrection led to a statistically significant increase in the intensity of the blast transformation response to conA when optimal and suboptimal concentrations of mitogen were used. In the postoperative period simultaneous administration of RA and AA to the patients caused a significant increase in the proliferative response by 150-170% when suboptimal concentrations of conA were used (Fig. 3).

RA and AA, used separately for immunocorrection in the preoperative period, did not affect the blast transformation reaction of lymphocytes stimulated by the β-cell mitogen PWM. A very small increase in the intensity of proliferative activity of the cells in response to PWM was observed only when the lowest concentrations of the mitogen were used in patients receiving AA or a combination of AA and RA. Incidentally, a response of the patients' lymphocytes to PWM was affected by a much lesser degree than the response to alloantigens and to the T-cell mitogen conA. In the case of simultaneous administration of RA and AA a statistically significant increase was observed in the percentage of T cells in the peripheral blood, in the absence of changes affecting the total number of lymphocytes and immunoglobulin production.

The results show that correction of the parameters of T-cell immunity in patients with CC by optimal enhanced doses of RA and AA is possible in principle. Future investigations, to study the effect of these substances on the frequency of recurrences and metastases after radical surgery in the late postoperative period will be useful.

LITERATURE CITED

1. A. V. Sergeev, V. S. Anan'ev, et al., in: Proceedings of the 2nd Congress of Oncologists of the Belorussian SSR [in Russian], Minsk (1982), p. 96.
2. N. N. Trapeznikov, V. I. Kupin, and Z. G. Kadagidze, in: The Clinical Use of Zymosan and a Study of the Mechanism of Its Action [in Russian], Riga (1982), p. 26.
3. S. Sirisinha, M. D. Darip, and P. Moongkarndi, Clin. Exp. Immunol., 40, 125 (1980).
4. W. R. Thomas and P. G. Holt, Clin. Exp. Immunol., 32, 370 (1978).
5. H. J. Wanedo, R. Basker, F. Attiyeh, et al., Cancer (Philadelphia), 45, 1254 (1980).