

ADDITIVE ANTITUMOR EFFECT OF PLANT POLYPHENOLS AND SYNTHETIC INHIBITORS OF POLYAMINES BIOSYNTHESIS

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In the last years plant polyphenols attracted an attention in connection to their antitumor and anticarcinogenic properties [1, 2]. These properties are specific to polyphenols from many plant species and plant foods, for example, green and black tea (the most extensively studied — (–)-epigallocatechin-3-gallat), grape resveratrol, soybean genistein and daidzein and others.

Tea is among the most highly consumed beverages worldwide. Derived from the plant *Camellia sinensis*, tea is consumed in different parts of the world as green, black, or oolong tea. Green tea is favored in Japan and China, and it contains characteristic polyphenolic compounds including (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), (–)-epicatechin (EC), (+)-gallocatechin (GC), and (+)-catechin (C). To date, tea catechins have attracted considerable interest due to the potentially health-promoting properties of these substances, including strong antioxidant activity and cancer chemopreventive effects [3, 4]. In a lot of *in vitro* studies, plant polyphenols were found to suppress proliferation of human and animal malignant cells, especially lung, intestinal, prostate tumors, hepatoma, leukemia and others [5, 6].

Antitumor and antimetastatic activities of plant polyphenols were also shown in the *in vivo* experiments and were confirmed in extended epidemiological studies demonstrating the lowest levels of breast and prostate cancer morbidity in the countries where green tea and soybean foods consumption is widespread and regular. The exact mechanisms of action of green tea polyphenols remain obscure, but it is known that plant polyphenols possess antioxidative capacity derived from their ability to scavenge reactive oxygen species and trap hydroxyl and peroxy radicals and inhibits angiogenesis. The well-described antioxidant properties of tea catechins [7] may contribute to the antiproliferative and proapoptotic capacity of green tea by inhibiting NF-κB activity [8, 9]. Recent works demonstrated that polyphenols cause inhibition of polyamine (PA) synthesis in tumor cells.

PA — spermine, spermidine and putrescine are known as obligate agents for proliferation of any cells including malignant ones. It is necessary to point that PA *per se* and enzymes involved in their metabolism are regarded now as available targets for antitumor treatment. Inhibition of PA synthesis in tumor cells was shown to retard their proliferation and tumor growth. A lot of foreign publications as well as our own works show an essential growth retardation of different kinds of experimental tumors *via* inhibition of PA synthesis.

This effect was recently demonstrated in clinical trials, especially, the data on α-DFMO application to prevention and therapy of intestinal cancer [10]. Our previous studies showed that the influence of PA on the functioning of the NF-κB nuclear transcription factor may be an important way to control tumor growth. We have shown that PA, especially spermine, have specific affinity for the p50 subunit of NF-κB and contribute to the binding of p50 to specific sites of DNA (NRE-sequences) of NF-κB-dependent genes. Under intracellular PA depletion (after treatment with α-DFMO — a specific inhibitor of the key enzyme of PA biosynthesis — ornithine decarboxylase, ODC) we have observed growth retardation in MCF-7 human breast cancer cells and diminished activity of NF-κB classic form (p50/p65 heterodimer) in these cells. Introduction of putrescine into culture medium recovered PA level and NF-κB activity in these cells as well as culture growth rate [11].

These data suggest that one of the possible molecular pathways of PA influence on cell proliferation is the involvement of PA in the control of NF-κB activity and, through this, in transcription control of NF-κB-dependent oncogenes, for example, *c-myc*, *bcl-xl*, *cox-2*, *inos* etc. At the same time, molecular pathways of PA involvement in growth process are not clear in many respects. Especially, α-DFMO and some other synthetic inhibitors of PA metabolism (polyhexamethylenguanidine, PMG) are known to cause certain side effects such as ototoxic action, activation of cyclooxygenase (*cox-2*) and inducible NO-synthase (*inos*). Therefore, the search for natural compounds that will inhibit PA synthesis in tumor cells without side effects is very important for their further use in clinical oncology.

The aim of the present work was to clarify whether plant polyphenols are able to strengthen antitumor activity of the inhibitors of PA metabolism and to study their combined effect on NF-κB activation and on expression of the protein products of certain NF-κB-dependent oncogenes.

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Abbreviations: *cox-2* — cyclooxygenase; CP — cisplatin; α-DFMO — α-difluoromethylornithine; IκB-α — NF-κB inhibitory protein; GTE — green tea extract; GTEW — biocomposite; MDA — malondialdehyde; *inos* — inducible NO-synthase; ODC — ornithine decarboxylase; PA — polyamines; PAO — polyamine oxidase; PMG — polyhexamethylenguanidine; W-256 — Walker carcinosarcoma.

As we have found, consumption of green tea extract (GTE) significantly inhibits the growth of the Walker carcinosarcoma and to a lesser extent inhibits the growth of Guerin's carcinoma (Fig. 1, a). The introduction of the PMG with GTE increased the antitumor effect (Fig. 1, b). Also we have found that expression of ODC was decreased in tumor cells of the animals treated with GTE or GTE in combination with the inhibitors of PA (Fig. 2, a). We have shown also that growth suppression was accompanied by diminished levels of PA content in tumor cells and diminished polyamine oxidase activity (PAO) in tumor cells (Fig. 2, b, c). So, GTE were found to inhibit both ODC expression and PAO activity and, that's why, they diminish PA level in the tumor cells.

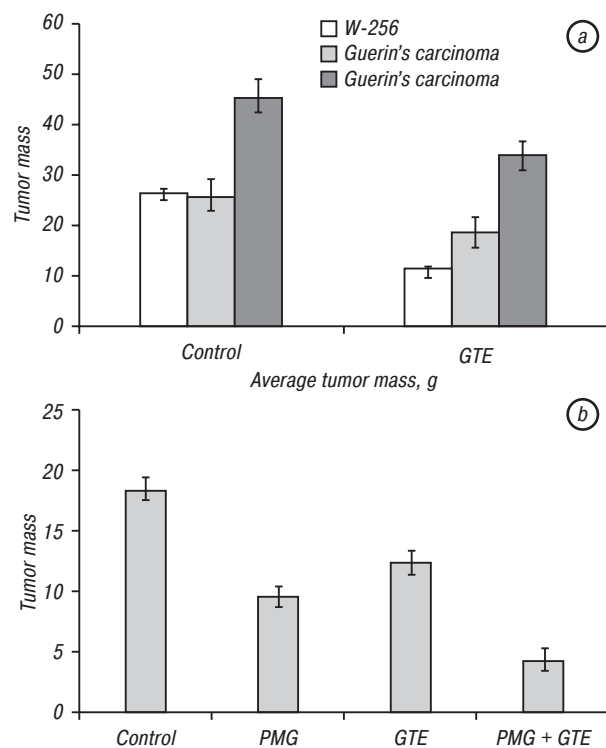


Fig. 1. Effect of GTE on the growth of grafted tumors (Walker W-256 carcinosarcoma and Guerin's carcinoma) (a); additive effect of GTE and PMG on Walker carcinosarcoma growth (b).

As we have found, Walker carcinosarcoma growth retardation caused by GTE or PMG or their combination was accompanied by diminished nuclear expression of subunits of the classic NF- κ B transcription factor — p50 and p65 proteins, enhanced nuclear expression of I κ B- α — NF- κ B inhibitory protein (Fig. 3). Retardation of Walker carcinosarcoma growth caused by GTE or PMG or their combination was accompanied by diminished expression of c-myc, bcl-xl, inos proteins and increased levels of p53 protein (see Fig. 3).

Also we have performed the experiments devoted to combined effect of cisplatin and a biocomposite made of green tea and red grape wine polyphenols (GTEW). This biocomposite was shown to strengthen the antitumor effect of cisplatin (Fig. 4, a). In addition, this biocomposite sharply diminished the basic side effect of cisplatin — its nephrotoxicity (by the indices of urea and creatinine in blood serum) (Fig. 4, b).

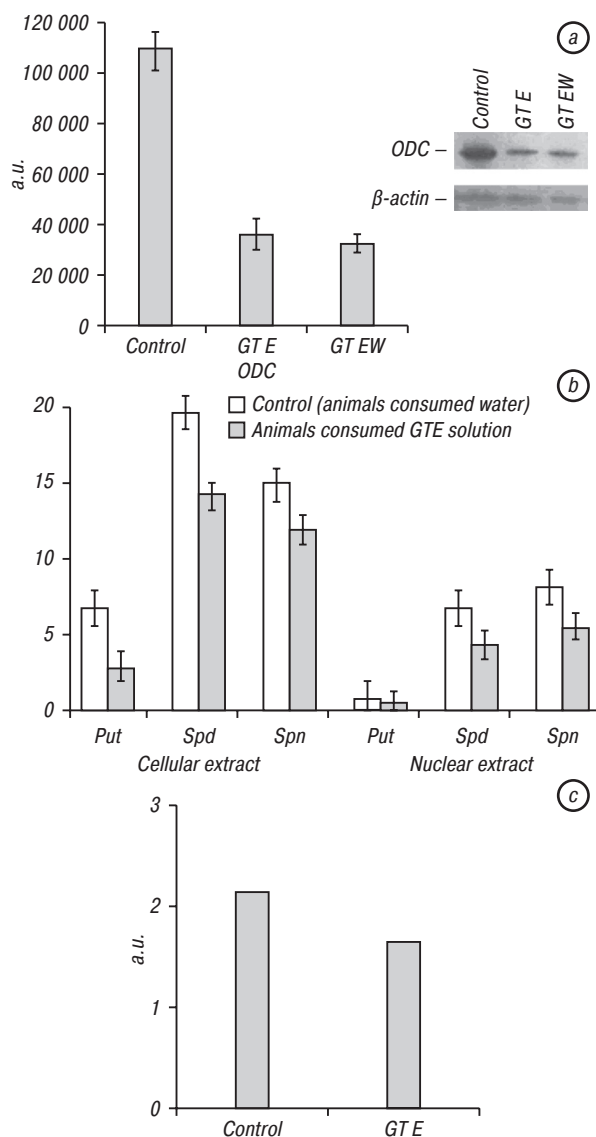


Fig. 2. Effect of GTE on the level of ODC protein (a), the PA content (putrescine — put; spermidine — spd; spermine — spn) (b) in the nuclear and cellular extracts of Walker carcinosarcoma (nmol/mg of protein) and activity of PAO (c).

It is known that a significant increase in lipid peroxidation in different tissues of the organism occurs during cancer development. Lipid peroxidation significantly increased during chemotherapy in patients with malignant tumors. Therefore it was important to examine the ability of the studied plant polyphenols to prevent this process. One of the important criteria of lipid peroxidation intensity is known to be the content of MDA. We found that GTE and GTEW, which we have used, have antioxidant properties. Consumption of GTE and GTEW essentially decreased malondialdehyde level (MDA) in the liver of the tumor-bearing animals. MDA level sometimes was even lower than in the liver of the intact animals (Fig. 4, c).

It was shown also, that GTE in combination with cisplatin magnified not only tumor growth retardation but also increase the animal lifetime (Table).

In conclusion, GTE and GTEW can essentially retard experimental tumor growth; the molecular pathway of GTE and GTEW action includes inhibi-

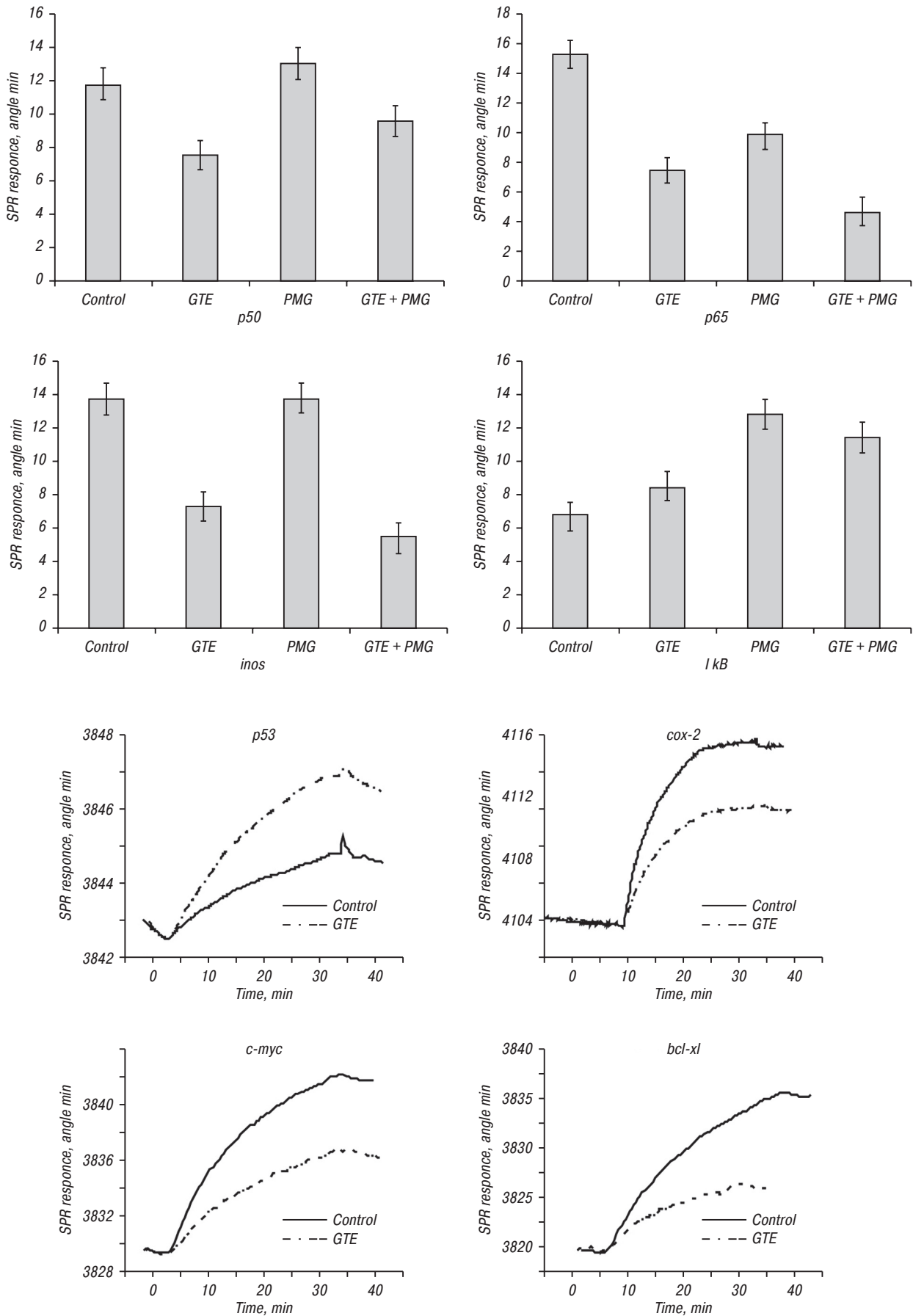


Fig. 3. Walker carcinosarcoma growth retardation caused by GTE or PMG or their combination was accompanied by diminished nuclear expression of subunits of the classic NF- κ B transcription factor (p50 and p65 proteins), enhanced nuclear expression of I κ B- α (NF- κ B inhibitory protein), decreased expression of inos, cox-2, c-myc, bcl-xl proteins and increased p53 expression.

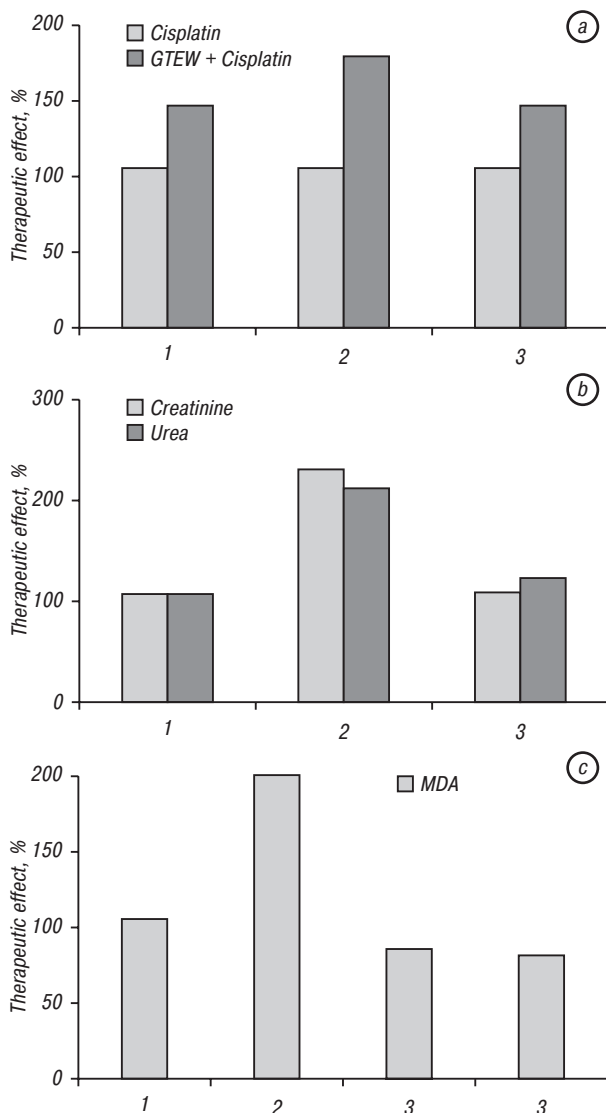


Fig. 4. Biocomposite in combination with cisplatin enhanced antitumor effect of cisplatin (a). Biocomposite sharply diminished the basic side effect of cisplatin — its nephrotoxicity (by the indices of urea and creatinine in blood serum) (b). GTE and GTEW- biocomposite have anti-inflammatory and antioxidant properties (c); (a — increasing antitumor effect of cisplatin by GTEW; 1, 2 — Guerin's carcinoma; 3 — Walker carcinosarcoma; b — creatinine and urea content (%) in blood serum (1 — non-treated tumor-bearing animals; 2 — tumor-bearing animals obtained cisplatin alone; 3 — tumor-bearing animals obtained cisplatin and GTEW; c — GTE and GTEW normalize MDA content in rat liver (1 — intact animals; 2 — non-treated animals with Walker carcinosarcoma; 3 — tumor-bearing animals obtained GTE; 4 — tumor-bearing animals obtained GTEW).

tion of PA synthesis and interconversion and also inhibition of NF-κB activation and expression of NF-κB-dependent oncogenes; green tea polyphenols increase the antitumor effect of CP and PMG and have antioxidant and antitoxic properties; green tea polyphenols are perspective for cancer prevention in the cancer risk groups and for treatment of cancer patients, especially in combination with CP-like drugs.

Table. Effect of GTE and GTE in combination with cisplatin on the life span of animals with Walker carcinosarcoma

Group of animals	Average lifetime (days)
Control	22.9 ± 2.2
Cisplatin	Animals lived 29 days, two tumors completely degraded
Cisplatin + GTE	All treated animals were alive more than 2 months

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