PRECLINICAL ANTITUMOR ACTIVITY OF THE DIINDOLYLMETHANE FORMULATION IN XENOGRAFT MOUSE MODEL OF PROSTATE CANCER

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Aim: Preclinical study of the specific anticancer pharmacological activity of the formulation containing active substance 3,3'-diindolylmethane (DIM), cod liver oil, polysorbate 80 and α-tocopherol acetate (vitamin E), in vivo in a xenograft animal model of LNCaP. Materials and Methods: The DIM, cod liver oil, polysorbate 80 and α-tocopherol acetate (vitamin E) formulation was intragastrically administered to BALB/c-nude (nu/nu) mice during 33 days post inoculation at the dose of 133 mg/kg/day. Antitumor activity of the test drug was estimated by the rate of tumor growth inhibition (T/C% — treated versus control), dividing the tumor volumes from treatment groups with the control groups. Results: Statistically significant tumor xenograft regressions have been shown in group which received the DIM, cod liver oil, polysorbate 80 and α-tocopherol acetate (vitamin E) on the 37th day of observation post inoculation. The highest antitumor activity was achieved on the 39th day (T/C = 16,8%). Therapeutic effect lasts for 6 days after the end of therapy period. Conclusion: Our findings demonstrate inhibitory effect of the formulation on tumor development in the xenograft animal model due to the tumor growth rate reduction.

Key Words: 3,3'-diindolylmethane, bioavailability, anticancer activity, xenograft model, LNCaP cell line, preclinical studies.

3,3'-Diindolylmethane (DIM) — one of the most prospective compound with antitumor and immunomodulatory properties. A great number of studies have revealed that DIM is able to block the multiple molecular mechanisms which cause cancer in different organs and tissues [1, 2]. In addition to the suppression of proliferation of transformed (tumor) cells and stimulation of their apoptosis, DIM inhibits pathologic angiogenesis and reduces the metastatic potential of cancer cells by affecting targets which mediate processes of cell migration and invasion [3, 4]. It has been recently found that DIM is a selective inhibitor of certain tumorigenic minor population of non-differentiated cancer cells — the so-called “cancer stem cells” [5], which are the main source of recurrence and metastasis according to modern ideas. DIM may provide some protection against hormone-dependent cancers by altering hormone levels, particularly due to down-regulation of androgen receptors [2].

The only significant problem in creating DIM-based anticancer drug is its low bioavailability and, as a result, the inability to achieve the therapeutic concentrations of active substance in target tissues. Generally, DIM exhibits low solubility in physiological fluids and has limited ability to permeate through membrane barriers [6, 7]. Based on this, and taking into account the uniqueness of the candidate substance DIM, the drug DIM, cod liver oil, polysorbate 80 and α-tocopherol acetate (vitamin E) was developed. Drug formulation is enclosed in a capsule as solution containing the active substance — DIM (150 mg), as well as organic auxiliary components — cod liver oil, polysorbate 80 and α-tocopherol acetate (vitamin E) providing high bioavailability and storage stability of the drug [8]. It is known that cod liver oil in combination with polysorbate significantly improves absorption in the gastrointestinal tract and distribution in the body, and vitamin E increases storage stability [8].

The new drug formulation, created by us on the basis of modern technological solution, contains DIM in a dissolved state, whereby DIM quickly enters the blood and the target organs, and reaches concentrations which manyfold exceed the concentrations of crystalline forms [9]. It was revealed that 5-fold higher concentration of DIM was observed in blood plasma of rats who received the 2,000-fold lower dose of liquid DIM formulation (the DIM, cod liver oil, polysorbate 80 and α-tocopherol acetate (vitamin E)) compared to crystalline form and non-formulated crystalline DIM. We decided to carry out preclinical in vivo study of pharmacological activity of the formulation as a therapeutic antitumor agent.

Prostate cancer (PC) is one of the most common cancers in the world affecting men. PC in Europe is 2nd/3rd cause of cancer deaths, in the USA — 1st. PC leads to 29% of fatal causes among patients with malignant tumors [10]. In the absence of organized mass screening programs of early detection of PC disease in many cases becomes metastatic and incurable.

Modern methods of treating PC — hormone-dependent cancer — are primarily directed to total androgen blockade (androgen deprivation therapy), and may involve surgery (radical prostatectomy,  

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Abbreviations used: BR-DIM — bioreesponse 3,3'-diindolylmethane; DIM — 3,3'-diindolylmethane; PC — prostate cancer.
The following microclimate parameters were maintained: light conditions: 12 h — light, 12 h — dark; air temperature 24–26 °C; relative humidity 30±70%; air exchange 8–10 room volumes per hour. The animals had free access to distilled water and food (PMI LabDiet® 5K67). Food and water were pre-sterilized (autoclaving).

All animals were inoculated with LNCaP cells subcutaneously along the spine (in left scapular region) after adaptation period. LNCaP cell line inoculation was carried out by injection of 0.2 ml of tumor suspension in sterile solution of PBS (Sigma, USA), containing 5 ml cells (25 · 10^6 cells/ml). All manipulations with animals were approved by the local Animal Care and Use Committee.

**Treatment protocols.** At 3 days after LNCaP cell inoculation, animals which met criteria for inclusion were randomized and individually labeled. Two groups of animals were formed: control and experimental (animals treated with the formulation) — 30 mice each. In experimental group animals were administered with the formulation twice daily (in the morning and evening) with an interval of 8–9 h intragastrically at a dose of 133 mg/kg/day (per DIM) by atraumatic gavage for 33 days. The control group treated with the same amount of solvent (cod liver oil + polysorbate) on the same schedule. Tumor growth was monitored for a further 6 days after the end treatment period. In the days of administration of drug/solvent animals were inspected at a specified time prior to and two hours after administration.

Euthanasia was carried out by CO₂-inhalation.

**Assessment of antitumor activity.** Tumor volume measurements began at the initiation of tumor growth and continued twice a week. Tumor volumes were measured with caliper and were calculated by the following formula (Vt):

\[ V_t = \frac{3}{2} LW^2 \]

\[ W = \text{the shortest tumor diameter.} \]

Antitumor activity of the DIM formulation was estimated by tumor growth inhibition ratio (T/C%), where T and C represent the means of the tumor volumes of the control (Vc) and treatment mice (Vt) in each experiment day (Vt/Vc · 100%). Another value of inhibition ratio (D%) was reckoned by the formula:

\[ D\% = \frac{V_c - V_t}{V_c} \times 100\% \]

Behavior and general condition of animals have been registered both in experimental and control groups. Animals were weighted 2–3 times a week.

**Statistical analysis.** Statistical differences in tumor growth inhibition ratio (T/C%) between treated and untreated groups were determined using Student’s t-test by means of GraphPad Prism 5 software. Differences were statistically significant at p < 0.05.

**RESULTS AND DISCUSSION**

**Study of the antitumor activity of the formulation in subcutaneous LNCaP xenografts.** The effect of the DIM formulation on tumor growth dynamics was evaluated by measuring changes in tumor size.
choline, has been successfully studied by different authors in xenograft model of PC [2, 13–15], and other cancers [3, 16–19]. However, bioavailability of DIM in BR-DIM formulation compared with crystalline DIM was enhanced only in 1.5- to 2-fold, which demonstrate that administration of BR-DIM formulation (per os) doesn’t ensure peak concentrations of the active substance (DIM) and, consequently, desired therapeutic effect. This could help to explain why no significant clinical effect was achieved with BR-DIM formulation in treatment of cervical dysplasia [20].

However, animal in vivo experiments revealed that crystalline DIM and BR-DIM formulation — by various routes of administration — demonstrate significant dose-dependent antitumor effect. They induced a reduction in tumor volume which was established by implantation of PC3 cells, and/or reduce the amount of newly formed metastases. According to the study in a SKOV-3 xenograft tumor model of ovarian cancer T/C ratio was 47.2%, in other study this value was 51.63% (PC3 xenograft tumor model of PC) (T/C values were calculated according to the original experimental data reported in these publications).

Our findings confirm inhibitory effect of the new formulation containing DIM, cod liver oil, polysorbate 80 and α-tocopherol acetate (vitamin E) on tumor development in the xenograft animal model and complement previously obtained experimental data.

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