Exp Oncol 2012
34, 1, 66–68

SHORT COMMUNICATION

Experimental ONCOLOGY

ACCELERATED REJECTION OF THE SECOND TRANSPLANTS OF IMMUNOGENIC TUMOR IN MICE UNDER INHIBITION OF INDOLEAMINE 2,3-DIOXYGENASE ACTIVITY BY ETHYL PYRUVATE

E.D. Vasilyeva[1], V. Kaledin[1], V.P. Nicolin[1], N.A. Popova[1], I.A. Kirilyuk[2], I.A. Grigor'ev[2]*

[1]Institute of Cytology and Genetics of Siberian Department of the Russian Academy of Sciences, Novosibirsk 630090, Russian Federation

[2]N.N. Vorozhtsov Institute of Organic Chemistry of Siberian Department of the Russian Academy of Sciences, Novosibirsk 630090, Russian Federation

***Aim*: A recently discovered enzyme, indoleamine 2,3-dioxygenase (IDO), is expressed in placenta, dendritic cells and also in many kinds of tumors and in tumor-infiltrating macrophages. By catabolizing tryptophan, IDO causes local depletion of this essential amino acid and excess of kinurenin, and suppresses *in situ* proliferation and functioning of T lymphocytes. Thus, immune resistance of tumors can be overcome by inhibiting IDO activity. *Materials and Methods*: C3HA mice immunized with non-syngeneic H-29 tumor were used to study the effect of the IDO inhibitor ethyl pyruvate, under systemic or local (at site of tumor cells localization) administration, on the occurrence and rate of rejection of the second transplants of this tumor. *Results*: Both systemic and local administration of ethyl pyruvate increases the incidence of and substantially accelerates tumor regression as compared with control. *Conclusion*: IDO inhibitors impairing immune resistance of tumors may appear useful in leveraging the efficacy of antitumor therapy.**

***Key Words*: indoleamine 2,3-dioxygenase, ethyl pyruvate, H-29 hepatocarcinoma, tumor regression.**

Since it was shown in 1998 that the capacity of an allogeneic embryo to escape rejection by the mother may somehow involve tryptophan catabolism [1], a possible mechanism of maternal immune tolerance has been widely investigated. Quite soon it was found that indoleamine 2,3-dioxygenase (IDO), an enzyme expressed in trophoblast and catabolizing tryptophan via the kynurenine pathway, is responsible for local depletion of the essential aminoacid tryptophan and accumulation of its toxic catabolites affecting T-cells [2, 3]. Suppression of IDO in the experiments with pregnant mice resulted in the rejection of allogeneic but not syngeneic embryos [1]. In oncology, these findings are critical for understanding how an intrinsically immunogenic tumor avoids or may avoid the immune attack in the host [4]. As a matter of fact, IDO was found to be expressed in various tumor cells and in tumor-infiltrating macrophages [5, 6]. In such cases high tumor IDO expression can predict unfavorable prognosis [4, 6, 7]. IDO inhibitors on their own slow down tumor progression and can enhance the therapeutic efficacy of a chemotherapy drug [8, 9]. A synthetic analog of tryptophan, D-1-methyl-tryptophan, is widely used as IDO inhibitor [9]. It is administered into animals chronically at daily doses up to 800 mg/kg b.w. [9]. However, since the IDO-induced immune tolerance to tumors can be overcome by suppressing the enzyme in the tumor cells and in tumor-infiltrating macrophages only, the dose of IDO inhibitor (and treatment costs) can be significantly reduced if the inhibitor is administered locally at tumor site. On the other hand, one of the recently discovered IDO-inhibiting compounds, ethyl pyruvate, a comparatively inexpensive and low-toxic anti-inflammatory agent, has drawn our attention [10, 11]. In the present study with the use of mice with an intrinsically immunogenic non-syngeneic tumor it was shown that chronic, systemic or local at tumor site, administration of ethyl pyruvate accelerates tumor rejection as compared with control.

Mice of C3HA strain and the transplantable tumor, Hepatocarcinoma-29 (H-29), were used [12]. All experimental procedures were perfomed in accordance with the normative rules of bioethics. The H-29 tumor originates from CBA mice but is transplantable to 100% of C3HA mice. In a considerable part of the grafted mice the tumor eventually stops to progress and regresses. At the preparatory stage, 5×10^5 H-29 cells were inoculated into the femur muscle of C3HA male mice. A month later the animals with progressing tumors were culled. A considerably higher dose of tumor cells (fivefold and tenfold) was then transplanted to immune animals which rejected the tumor. After that the animals were divided into two groups. The animals of one group were administered ethyl pyruvate for IDO inhibition, the other group was used as control. In the experiment with the systemic administration of ethyl pyruvate the inhibitor was dissolved in Ringer's solution (5 mg/ml) and administered intraperitoneally twice a day for 20 days. Control mice received no injections. In the experiment with local administration, 0.1 ml of 1% ethyl pyruvate solution was administered intramuscularly at tumor site once a day during 10 days after tumor cells inoculation. Control animals in the same manner were administered by 0.1 ml of saline. At regular intervals tumors were palpated and measured with a vernier caliper. The time of tumor resorption was registered. The experi-

Received: August 17, 2011.
*Correspondence: E-mail: nelly@bionet.nsc.ru
Fax: 7 383 333 12 78
Abbreviation used: IDO – indoleamine 2,3-dioxygenase.

ment was concluded 10 days after regression of the last tumor in the experimental group. The φ-criterion and Fischer's arcsin transformation were used to test the validity of differences in tumor regression values (percentage of mice with regressed tumors) between the experimental and control groups.

In our previous study, under primary H-29 transplantation to non-syngeneic C3HA mice the inhibition of IDO by D-1-methyl-tryptophan resulted in enhancement rather than suppression of tumor outgrowth [13]. The effect observed may appear paradoxical at first sight. Actually, an arising tumor and primary tumor transplants develop in the so-called pathologic immune privilege conditions [14] with a complex immune response that may suppress tumor growth or enhance it. In this case, IDO inhibition may cause not only the proliferation of T-killer and T-helper cells but may also activate suppressor T-cells (Treg) contributing to the development of immune tolerance in the host, thereby enhancing the growth of the tumor. On the contrary, the clinicians have usually to deal with tumors which immune relationships with the host are explicit and which use IDO to suppress the effector phase of immune response. For this reason in the experiments on the tumor-suppressing potential of IDO inhibitors it is reasonable to use preimmunized animals. In the present study tumor recipients were tumor-grafted mice who rejected the tumors had been transplanted earlier.

At the preparatory stage for the first experiment, H-29 tumor cells were inoculated into the femur muscle of forty intact four-month old mice of C3HA strain at a dose of 5×10^5 cells. After a week tumors developed in all animals; subsequently tumor progression was observed in 13 animals and tumor inhibition and eventual regression – in 27 animals. The mice with tumor progression were culled. The mice rejecting the tumor transplant were re-inoculated with a significantly higher (fivefold, 1×10^6) dose of tumor cells at in the same femur muscle. Immediately after tumor cell inoculation the mice were divided into 2 groups. In the control group 13 animals were kept without any exposure. In the experimental group, 14 animals were exposed to injections of ethyl pyruvate twice a day as described in METHODS. The ethyl pyruvate injections at a single dose of 40 mg/kg were performed 40 times with a total course dose of about 1.6 g/kg b.w. As shown in Fig. 1, ten days after the transplantation tumors developed in 100% of the mice of both groups and then began to regress. During the first 4 days the rate of tumor regression was the same for both groups, and then somewhat faster in the experimental group as compared with control. Twenty six days after the transplantation the tumors regressed in all animals except two of the control group. Subsequently the tumor regressed in one animal and recurred and killed the other.

A month after tumor rejection the survived mice were used for H-29 cells inoculation at a dose higher than that used in the previous transplantation (5×10^6). After the transplantation the treated and untreated mice were divided equally between the experimental and control groups of the new experiment. The groups were enlarged by 4–5 mice with regressed tumors, which became available by that time, and then exposed to treatment (daily local administration of ethyl pyruvate or saline, see MATHERIAL AND METHODS).

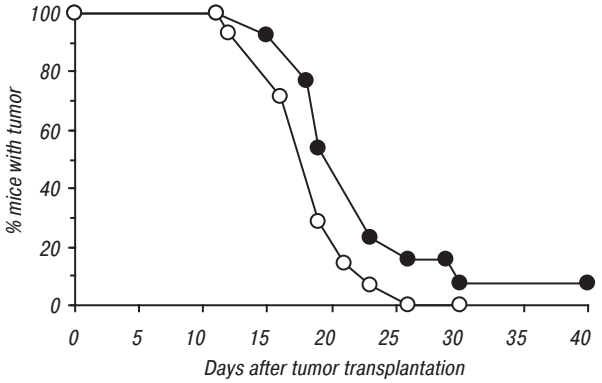

Fig. 1. Dynamics of H-29 transplant regression in C3HA mice under systemic administration of ethyl pyruvate, an inhibitor of indoleamine 2,3-dioxygenase (light circles), and in the control (dark circles). 40 doses of 40 mg/kg of the agent were administered intraperitoneally twice a day during 20 days after tumor cells inoculation. There are 13–14 animals in each group

Six days after tumor cells inoculation, tumors appeared in all animals of both groups. The average tumor volume was 0.30 ± 0.031 cm^3 for control and 0.20 ± 0.045 cm^3 for the experimental group. Two days later the average tumor volume was 0.23 ± 0.031 cm^3 and 0.14 ± 0.034 cm^3, respectively (insignificant differences). The tumors eventually diminished in both groups, but they became nonpalpable (fully regressed) at an earlier time and in more individuals treated with ethyl pyruvate as compared with control animals (Fig. 2). In both experiments ethyl pyruvate produced no notable toxic effect: both under systemic and local administration the mice's body weight decreased by less than 4% upon completion of the course.

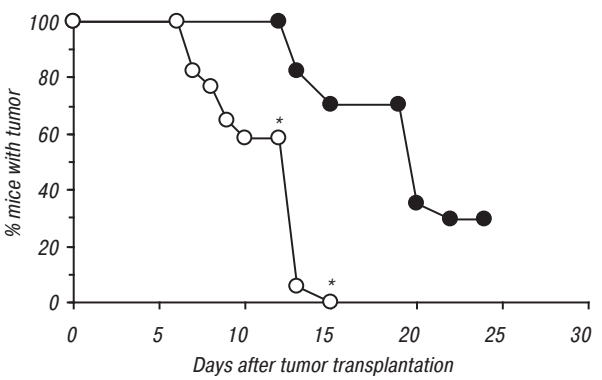

Fig. 2. Dynamics of H-29 transplant regression in C3HA mice under local administration of ethyl pyruvate, an inhibitor of indoleamine 2,3-dioxygenase (light circles), or saline (dark circles). 0.1 ml of 1% ethyl pyruvate solution (or saline in control) was administered intramuscularly at tumor site once a day during 10 days after tumor cells inoculation. There were 17 animals in each group. Asterisk marks a significant difference from the control ($p < 001$)

Thus, the results indicate that chronic, systemic or local, administration of ethyl pyruvate at a non-toxic dose to preimmunized mice results in an accelerated rejection of the repeated tumor transplants. In the present study (in particular in the first experiment)

we used a cumbersome regimen of ethyl pyruvate administration recommended by the authors who first suggested it as IDO inhibitor [11]. At the same time, from the literature ethyl pyruvate is known to be a food additive [10, 11], implicating it should retain at least some of its activity under *per os* administration. Therefore, it can not be excluded that ethyl pyruvate may inhibit IDO not only under parenteral administration. This question requires further investigation. Thus, future research is to focus on the applicability and clinical prospects of ethyl pyruvate (and/or other IDO inhibitors) for the treatment of tumors in combination with common chemotherapy drugs.

REFERENCES

1. Munn DH, Zhou M, Attwood JT, ***et al.*** Prevention of allogenic fetal rejection by triptophan catabolism**.** Science 1998; **281**: 1191–3.

2. Fallarino F, Grohmann U, Vacca C, ***et al***. T cell apoptosis by triptophan catabolism. Cell Death Differ 2002; **9**: 1069–77.

3. Munn DH, Shafizadeh E, Attwood JT, ***et al***. Inhibition of T cell proliferation by macrophage tryptophan catabolism. J Exp Med 1999; **189**: 1363–72.

4. Liu X, Newton RC, Friedman SM, Scherle PA. Indoleamine 2<3-dioxygenase, an emerging target for anti-cancer therapy. Current Cancer Drug Targets 2009; **9**: 938–52.

5. Munn DH, Sharma MD, ***et al.*** Expression of indoleamine 2,3-dioxygenase by plasmacytoid dendritic cells in tumor-draining lymph nodes. J Clin Invest 2004; **114**: 280–90.

6. Yoshida N, Ino K, Ishida Y, ***et al***. Overexpression of indoleamine 2,3-dioxygenase in human endometrial carcinoma cells induces rapid tumor growth in a mouse xenograft model. Cloin Cancer Res 2008; **14:** 7251–9.

7. Uyttenhove C, Pilotte L, Theate I, ***et al***. Evidence for a tumoral immune resistance mechanism based on triptophan degradation by indoleamine 2,3-dioxygenase. Nat Med 2003; **9**: 1269–74.

8. Frieberg M, Jennings R, Alsarraj M, ***et al***. Indoleamine 2,3-dioxygenase contributes to tumor cell evasion of T cell-mediated rejection. Int J Cancer 2002; **101**: 151–5.

9. Muller AJ, DuHadaway JB, Donover PS, ***et al***. Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy. Nat Med 2005; **11**: 312–9.

10. Fink MP. Ethyl pyruvate: a novel anti-inflammatory agent. J Intern Med 2007; **261**: 349–62.

11. Muller AJ, DuHadaway JB, Jaller D, ***et al***. Immunotherapeutic suppression of indoleamine 2,3-dioxygenase and tumor growth with ethyl pyruvate. Cancer Res 2010; **70**: 1845–53.

12. Kaledin VI, Zhukova NA, Nikolin VP, ***et al***. Hepatocarcinoma-29 — a metastasizing transplantable tumor provoking cachexia in host mice. Bul Exper Biol 2009; 148: **12**: 664–9.

13. Vasilieva ED, Nikolin VP, Popova NA, ***et al***. Inhiditor of activity of indoleamine 2,3-dioxygenase 1-methyl-D-triptophan may stimulate the growth of immunogenic tumors. Bul Exper Biol 2010; **149**: 559–61 (In Russian).

14. Kharchenko EP. Immunological privilege: pathological aspect. Immunology 2009; **4**: 249–55 (In Russian).

Подп. в печать 15.03.2012. Формат 60×84 1/8. Печать офс. Бумага офс. Усл. печ. л. 7,9. Уч.-изд. л. 8,0. Тираж 170 экз. Зак. №
Отпечатано в типографии ООО «Спринт-Украина», 03067, Киев, ул. Машиностроительная, 42