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Feeding mice with *Aloe vera* gel diminishes L-1 sarcoma-induced early neovascular response and tumor growth

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Abstract

Aloe vera (Aloe arborescens, aloe barbadensis) is a medicinal plant belonging to the Liliaceae family. Aloe vera gel prepared from the inner part of Aloe leaves is increasingly consumed as a beverage dietary supplement. Some data suggest its tumor growth modulatory properties. The aim of the present study was to evaluate in Balb/c mice the in vivo influence of orally administered Aloe vera drinking gel on the syngeneic L-1 sarcoma tumor growth and its vascularization: early cutaneous neovascular response, tumor-induced angiogenesis (TIA test read after 3 days), and tumor hemoglobin content measured 14 days after L-1 sarcoma cell grafting.

Feeding mice for 3 days after tumor cell grafting with 150 μl daily dose of Aloe vera gel significantly diminished the number of newly-formed blood vessels in comparison to the controls. The difference between the groups of control and Aloe-fed mice (150 μl daily dose for 14 days) with respect to the 14 days' tumor volume was on the border of statistical significance. No difference was observed in tumor hemoglobin content.

Key words: *Aloe vera gel, mice, tumor growth, angiogenesis.*

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Introduction

Aloe vera gel is a colorless substance obtained from the parenchymatous cells in fresh leaves of *Aloe vera* (L) Burm. f. (*Aloe barbadensis* Mill) *Liliaceae*. Native to North Africa, Aloe has been introduced and is being cultivated in the warmer areas of the world. *Aloe vera* gel, rich in polysaccharides (pectins, hemicelluloses, glucomannan, acemannan, and other mannose derivatives) should not be confused with the drug "Aloe" – dried juice of *Aloe vera* leaves, bitter yellow exudate containing anthracene glycosides, mainly of the aloe-emodin anthrone 10-C-glucoside type [1, 2].

Aloe vera gel is commonly consumed as a beverage, dietary supplement. It is a traditional herbal remedy without unwanted side-effects. Consumed as a beverage it was not toxic *in vivo* for mice [3]. On the contrary, aloe latex and its hydroxyanthrone derivatives (aloin, aloe-emodin etc.) have strong laxative properties and their longer use requires medical supervision [2].

Traditionally, *Aloe* gel was widely used for the treatment of minor wounds, inflammatory skin disorders, and thermal and radiation burns. *In vitro*, *Aloe* gel suppressed bacteria-induced pro-inflammatory [tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β)] cytokines and matrix metalloproteinase 9 (MMP-9) production in human mononuclear leukocytes [4, 5].

In vivo, polysaccharides derived from *Aloe vera* gel, injected into mice, potently stimulated migration of macrophages to the peritoneal cavity [6].

In human, oral *Aloe vera* gel was used by patients with inflammatory bowel disease [7], osteoarthritis, and other inflammatory conditions. Oral and topical administration of *Aloe vera* gel diminished inflammation and eased joint immobility and pain [8-11].

In ophthalmology, Aloe vera extracts may be used in eye drops to treat inflammations and other cornea ailments [12].

Besides its anti-inflammatory activity, *Aloe vera* gel has antimicrobial properties and *in vivo* exerts a protective

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effect on polymicrobial sepsis in mice [13-17]. Anthraquinones, compounds present in the outer part of *Aloe* leaves and in their succus or extract, have been shown to have direct anti-cancer activity in different kinds of human cancer cell lines [18]. Moreover, aloe-emodin, a hydroxyanthraquinone from *Aloe vera*, can act as an anti-angiogenic agent [19].

Some data suggest that the inner part of *Aloe vera* leaves, *Aloe vera* gel and their polysaccharide components also have tumor growth modulatory properties, probably connected with their immunomodulatory activity [20, 21].

In our previous paper we reported the inhibitory effect of *Aloe barbadensis* fresh leaves aqueous extract (herbal drug Biostymina) on tumor-induced cutaneous angiogenesis in mice [22].

The aim of the present study was to evaluate in Balb/c mice the *in vivo* influence of commercial *Aloe vera* gel product (*Aloe vera* drinking gel) on the syngeneic L-1 sarcoma tumor growth and its vascularization: a) early cutaneous neovascular response, tumor-induced angiogenesis (TIA test), and b) tumor hemoglobin content measured 14 days after L-1 sarcoma cell grafting.

Material and methods

Drug. Tru-Alo 99% *Aloe vera* Drinking Gel (*Aloe barbadensis* Miller folium succus), Aloin content < 40 ppm; produced by HI TECH ALOE VERA PTY LTD, Bundaberg, Australia.

Animals. The study was performed on 59 female inbred Balb/c mice 6-8 weeks old, weighing about 20 g, delivered from the Polish Academy of Sciences breeding colony. For all performed experiments animals were handled according to the Polish law on the protection of animals and NIH (National Institutes of Health) standards. All experiments were accepted by the local Ethical Committee.

Mice were housed 4-5 per cage and maintained under conventional conditions (room temperature 22.5-23.0°C, relative humidity 50-70%, 12 h day/night cycle) with free access to standard rodent diet and water.

Experiments were performed in anesthesia: ketamine 100 mg/kg (prep. Ketamina 10%, BIOWET, Pulawy, Poland); xylazine 10 mg/kg (prep. Sedazin, BIOWET, Pulawy, Poland); 3.6% chloral hydrate 0.1 ml per 10 g of body mass (Sigma Aldrich, USA); Morbital (BIOWET Pulawy, Poland).

Evaluation of sarcoma L-1 growth and angiogenic activity was performed as previously described [23, 24]. L-1 sarcoma cells were delivered from Warsaw Oncology Center collection, passaged twice *in vivo* and grafted subcutaneously (for evaluation of tumor growth and its hemoglobin (Hb) content) or intradermally (for evaluation of their angiogenic activity) to syngeneic Balb/c mice.

Preparation of tumor cells after *in vivo* passage. Briefly, sarcoma L-1 cells from *in vitro* stock were grafted (10^6/0.1 ml) subcutaneously into the subscapular region of Balb/c mice. After 14 days the tumors were excised, cut to smaller pieces, rubbed through the sieve and suspended in 5 ml of phosphate buffered saline (PBS). The suspension was left for 10 min at room temperature. After sedimentation, the supernatant was collected and centrifuged for 10 min at 1500 rpm. Obtained sarcoma cells were washed once with PBS for 10 min, then centrifuged at 1500 rpm, and resuspended in Parker medium in concentration of 4×10^6/ml or 10^7/ml.

Cutaneous angiogenesis assay (tumor-induced angiogenesis, TIA test)

Multiple 0.05 ml samples of 200 thousand sarcoma cells were injected intradermally into partly shaved, narcotized Balb/c mice (at least 2-4 mice per group). In order to facilitate the localization of cell injection sites, the suspension was colored with 0.1% trypan blue. Mice obtained *Aloe vera* gel (150 µl for one mouse daily) in drinking water for 3 days. After 72 hours mice were sacrificed with a lethal dose of Morbital. All newly formed blood vessels were identified and counted in the dissection microscope, on the inner skin surface, at magnification of 6×, in 1/3 of the central area of the microscopic field. Identification was based on the fact that new blood vessels are thin, directed to the point of cell injection, with ramifications, and some of them are tortuous (Fig. 1). Test calculations were performed by two independent observers and the results were averaged.

Subcutaneous tumor growth assay

0.1 ml samples of 1 million sarcoma cells were grafted subcutaneously into the sub-scapular region of Balb/c mice. On the day of cell grafting and on the following 13 days mice obtained 150 µl of *Aloe vera* gel in drinking water, or water as a control. After 14 days mice were sac-

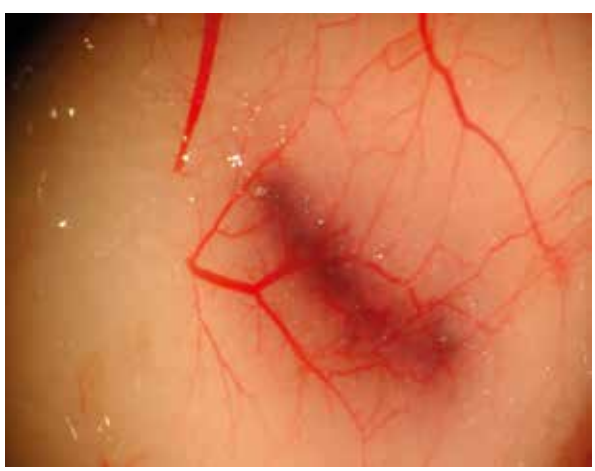

Fig. 1. Neovascular reaction 3 days after the intradermal injection of tumor cells

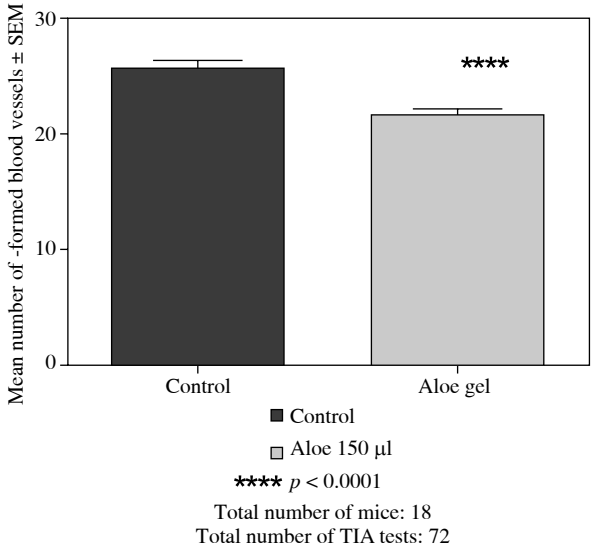

Fig. 2. Mean number of newly-formed blood vessels counted 3 days after the intradermal injection of sarcoma L-1 cells

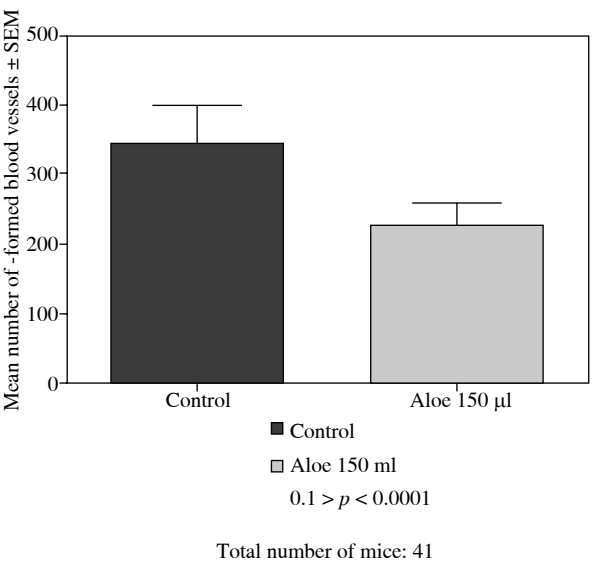

Fig. 3. The effect of feeding mice with *Aloe vera* drinking gel for 14 days after L-1 sarcoma subcutaneous grafting on the tumor volume (difference on the border of statistical significance)

rificed with Morbital. Tumors were removed, weighed and measured with an electronic caliper (The Fowler Ultra-Cal Mark III caliper).

Estimation of Hb concentration in tumors was done using the method described [25]. Briefly, tumors were homogenized in PBS using an ultrasonic sonificator (Virsonic, USA), then centrifuged for 20 min at 4000 × g. 20 µl of the supernatant was added to 5 ml of Drabkin reagent. The absorbance was read in a spectrophotometric reader Elx800 (Biotek Instruments, USA) at 570 nm. The reader for the Hb measurement was calibrated with hemoglobin standard solutions (Sigma). The results were shown as µg Hb in 1 mg of tumor mass.

Statistical analysis

Statistical evaluation of the results was performed by unpaired *t* test (GraphPadPrism).

Results

The results of the TIA test were evaluated by unpaired *t* test. Two-tailed *P* value was lower than 0.0001 ($t = = 4.467$; df = 70). Hence, the mean number of newly formed blood vessels in the experimental, Aloe-fed group of mice was highly significantly lower than in the corresponding controls (Fig. 2).

The difference between the groups of control and Aloe-fed mice (150 µl daily dose for 14 days) with respect to the 14 days' tumor volume was on the border of statistical significance (Fig. 3). No difference was observed in hemoglobin content between control and experimental tumors (21.3 ±3.1 vs. 24.1 ±3.6 µg/mg, respectively).

Discussion

It was shown by other authors that some *Aloe vera* active components slow down the experimental tumor growth. Three anthraquinones (aloesin, aloe-emodin and barbaloin) extracted from *Aloe vera* leaves may exert their chemo-preventive effect through modulating antioxidant and detoxification enzyme activity levels [18]. Aloe-emodin induces cell death through S-phase arrest and apoptosis in the dose- and time-dependent manner [26]. Other researchers describe the anti-tumour effect of specific derivatives of the *Aloe vera* plant. Di(2-ethylhexyl)phthalate isolated from *Aloe vera* Linne may have anti-leukemic and anti-mutagenic properties [27].

The anti-tumor effect was also documented for the *Aloe vera* leaf pulp extract and the main lectin (Aloctin I) present in it, in the Ehrlich ascites tumor model [28]. Acemannan, the compound of the extract from the parenchyma of *Aloe vera/aloe barbadensis*, stimulates the synthesis of monokines and recruitment of immune cells and, by this mechanism, necrosis and regression of murine sarcoma [29]. The results of these studies suggest that this effect could be due to its immunomodulatory activity. Acemannan has been approved by the FDA-US as a potent immunomodulating and anti-viral agent. It was approved as an aid in the treatment of canine and feline fibrosarcoma [30].

However, the critical condition for the tumor to effectively metastasize is formation of the new vessels prompted by a group of cancer cells derived from the primary, transported by the blood circulation and grafted in "per-

missible" tissue environment. This "permissiveness" is conditioned by the agents released by the tumor cells that drive recipient tissue to facilitate new vessel growth in it. We have been able to show that *Aloe vera* drinking gel slows down an early phase of new vessel formation and their in-growth in hosting tissue. This may also explain *Aloe vera* gel anti-tumor activity. However, *Aloe vera* drinking gel has not caused the necrotical effect on the tumor volume and it has not influenced the vascularity of the mature tumor (as indicated by the lack of differences in hemoglobin content of tumors between groups). It may suggest that its effect is exerted only on the newly forming vessels during micrometastasis implantation.

Few cytotoxic and targeted drugs have been proven effective in adjuvant systemic therapies after most of the tumor was removed by surgery or radiotherapy. The presence of micrometastases at the time of primary therapy is emphasized as the cause of failure of loco regional therapies. The effective chemoprevention should be directed at the micrometastasis priming mechanism, that is among most important angiogenesis. *Aloe vera* drinking gel, having a low profile of side effects, may be a good candidate for supplemental therapy. However, *Aloe vera* is also known for its beneficial wound healing impact that might be partly attributed to its compound, β-sitosterol, pro-angiogenic properties. It was shown that in the presence of heparin, beta-sitosterol stimulated neovascularization in the mouse Matrigen plug assay, and the motility of human umbilical vein endothelial cells in an *in vitro* wound migration assay [31]. Therefore, further detailed studies on the specific compounds' contribution of the antiangiogenic effect and its mechanism are warranted.

Lissoni *et al.* performed a randomized study of chemotherapy versus chemotherapy plus *Aloe arborescens*, in 240 patients with lung, colorectal, gastric and pancreatic metastatic cancers [32]. *Aloe arborescens* was given orally at a dose of 10 ml thrice daily of a mixture consisting of 300 g of Aloe fresh leaves in 500 g of honey plus 40 ml of 40% alcohol, every day without interruption, either during or after chemotherapy, until the progression of disease, starting 6 days prior to the onset of chemotherapy. The results of this study suggest that *Aloe* may be successfully associated with chemotherapy to increase the tumor regression rate and survival time.

Authors declare no conflict of interest.

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