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Chemical sympathectomy increases neutrophil-to-lymphocyte ratio in tumor-bearing rats but does not influence cancer progression

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ABSTRACT

The sympathetic nervous system regulates many immune functions and modulates the anti-tumor immune defense response, too. Therefore, we studied the effect of 6-hydroxydopamine induced sympathectomy on selected hematological parameters and inflammatory markers in rats with Yoshida AH130 ascites hepatoma. We found that chemically sympathectomized tumor-bearing rats had significantly increased neutrophil-to-lymphocyte ratio, leukocyte-to-lymphocyte ratio, and plasma levels of tumor necrosis factor alpha. Although our findings showed that sympathetic denervation in tumor-bearing rats led to increased neutrophil-to-lymphocyte ratio, that is an indicator of the disease progression, we found no significant changes in tumor growth and survival of sympathectomized tumor-bearing rats.

1. Introduction

The sympathetic nervous system (SNS) participates on neuronal regulation of immune functions (Leo and Bonneau, 2000; Mignini et al., 2003). In contrast to the parasympathetic and sensory nerves, a dense innervation by sympathetic nerves was found in all primary and secondary immune organs (Nance and Sanders, 2007). It was suggested that catecholamines and neuropeptides released from sympathetic nerve endings may influence three types of immune cells belonging to: 1) lymphoid stroma (e.g. epithelial cells and fibroblasts) and accessory cells (including antigen-processing and -presenting cells); 2) vasculature (including smooth muscle cells of arteriole and lymphatic vessels, and endothelial cells); and 3) immune "effector" cells, especially monocytes and lymphocytes (Downing and Miyan, 2000). Norepinephrine (NE), the main neurotransmitter of the SNS, regulates a variety of immune parameters via adrenergic receptors presented on above mentioned immune cells (Leo and Bonneau, 2000). NE is able to regulate the immune cells activity by initiating a change on the level of gene expression for cytokines and antibodies (Nance and Sanders, 2007). The SNS modulates also the anti-tumor immune defense response (Bhowmick et al., 2009; Peters et al., 2012; Wirth et al., 2014) by regulation of the activity of macrophages, cytotoxic T cells, NK cells, granulocytes, and by release of cytokines, mainly interleukin-2, interleukin-12, interferon-γ, tumor necrosis factor alpha (TNF-α), chemokines, and other humoral factors (Finn, 2012; Mantovani and Sica, 2010; Tugues et al., 2014).

For the study of the SNS role in the regulation of immune functions, interventions affecting the sympathetic neurotransmission are used. These approaches enable to investigate the role of NE in the modulation of immune responses during physiological conditions as well as in various disorders. A widely used method for depletion of tissue catecholamines is the chemical sympathectomy performed by administration of 6-hydroxydopamine (6-OHDA) (Picklo, 1997). Treatment with 6-OHDA has been shown to alter several immune functions including cytotoxic T lymphocyte generation and proliferation (Madden et al., 1989), antibody responses (Kasahara et al., 1977), cytokine production (Kruszewska et al., 1995), and lymphocyte trafficking (Madden et al., 1994a).

The activity and functions of immune cells documented by increase of inflammatory markers (e.g. C-reactive protein, TNF-α, interleukin-1, interleukin-6) reflect the severity of clinical condition during various diseases, including cancer (Zahorec, 2001). However, in addition to commonly used inflammatory markers, the ratio of neutrophil-to-lymphocyte (NLR) was proposed as an easily accessible parameter for systemic inflammatory response evaluation. NLR represents simple marker reflecting the intensity of stress and/or systemic inflammation in critically ill patients following shock, multiple trauma, major surgery or sepsis (Cho et al., 2009, 2014; Kim and Choi, 2012; Millrud et al., 2012; Perez et al., 2013; Rainer et al., 1999; Zahorec, 2001). Preliminary results have shown the correlation between the severity of clinical

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course and the severity of neutrophilia and lymphocytopenia (Zahorec, 2001). Moreover, NLR has been proposed as a reliable indicator and independent predictor of poor prognosis also in patients with cancer, while NLR elevation is associated with inefficient endogenous anticancer defense capability of the immune system (Seretis et al., 2013; Shimada et al., 2010).

Although many authors have proved that high NLR is associated with poor prognosis of oncological patients (Cho et al., 2009; Zahorec, 2001), there are no studies investigating the effect of sympathectomy on relationship between NLR and tumor progression. Therefore, the aim of present study was to elucidate the effect of 6-OHDA–induced sympathectomy in Yoshida AH130 ascites hepatoma-bearing rats on the count of blood neutrophils and lymphocytes as well as on values of other selected hematological parameters and plasma levels of proinflammatory cytokine TNF-α. Moreover, the effect of the chemical sympathectomy on tumor incidence and survival of tumor-bearing rats was investigated.

2. Methods

2.1. Animals

One hundred and nine male Wistar rats weighing 175–200 g at the time of delivery, obtained from Charles River (Germany), were housed two or three per cage and maintained under controlled conditions (12 hr light–dark cycle, lights on at 6:00 am, ambient temperature 22 ± 2 °C and 55 ± 10% humidity). The animals had free access to tap water and standard pelleted rat chow. The rats were handled daily and protected from the external noises to minimize a possible stress. All the experimental procedures were approved by the Animal Care Committee of the Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia. The animals received care in compliance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health.

2.2. Experimental protocol

Prior to the start of the experiment, animals were acclimatized in the animal room for 7 days. Consecutively, chemical sympathectomy or vehicle treatment was carried out. One week later, a part of animals (nc86) was intraperitoneally injected with tumor cells (Fig. 1). Based on the type of above mentioned treatment, rats were randomly divided into four experimental groups: 1/control rats—animals without chemical sympathectomy and also without injection of tumor cells (control, n = 7); 2/sympathectomized rats without injection of tumor cells (6-OHDA, n = 8); 3/animals without chemical sympathectomy injected with tumor cells only (Yoshida, n = 38); and 4/sympathectomized rats injected with tumor cells (Yoshida + 6-OHDA, n = 48). All animals of control group and 6-OHDA group, 17 rats of Yoshida group, and 18 rats of Yoshida + 6-OHDA group were sacrificed on the 14th or 15th day after tumor cells injection, and blood, plasma, and spleen were collected for next processing. In addition, 21 rats of Yoshida group and 30 rats of Yoshida + 6-OHDA group were kept for an investigation of sympathetic denervation effect on incidence of tumors and survival of tumor-bearing rats (Fig. 1).

Moreover, eight animals were used to confirm the efficiency of chemical sympathectomy. Four of them were injected with 6-OHDA (6-OHDA, n = 4) and four with vehicle (control, n = 4). These animals were sacrificed seven days after 6-OHDA/vehicle treatment. During the experiment health status of animals was controlled daily. Moreover, in all rats injected with tumor cells, the presence of tumors was monitored (Fig. 1). All experimental procedures were performed between 8:00 a.m. and 13:00 p.m.

2.3. Chemical sympathectomy

Chemical sympathectomy was performed in conscious animals by intraperitoneal injection of 6-hydroxydopamine hydrobromide (100 mg/kg of body weight, Sigma-Aldrich, Germany) over two consecutive days. The 6-OHDA was dissolved in sterile saline containing 0.1% of the antioxidant ascorbic acid (Sigma-Aldrich, Germany). This dose has been shown to induce the destruction of sympathetic nerve endings after 3–5 days, and this effect lasted for at least 21 days in spleen (Kruszewska et al., 1995). An efficiency of the sympathectomy was confirmed immediately after application of 6-OHDA by presence of eyelids ptosis in the sympathectomized rats (Claude Bernard–Horner's syndrome) and blood in urine indicating the destruction of sympathetic nerve endings in the urinary tract. Moreover, seven days after 6-OHDA treatment we found decreased levels of NE in the spleen ($p < 0.0001$; Fig. 2). Control rats to 6-OHDA groups were intraperitoneally injected with vehicle (sterile saline containing 0.1% of the antioxidant ascorbic acid).

2.4. Injection of tumor cells, tumors incidence, and survival of animals

A Yoshida AH130 ascites hepatoma cells were used for induction of tumors in syngeneic male Wistar rats. One week after chemical sympathectomy, a Yoshida AH130 ascites hepatoma cell suspension was injected intraperitoneally as a single dose of 5×10^6 tumor cells in

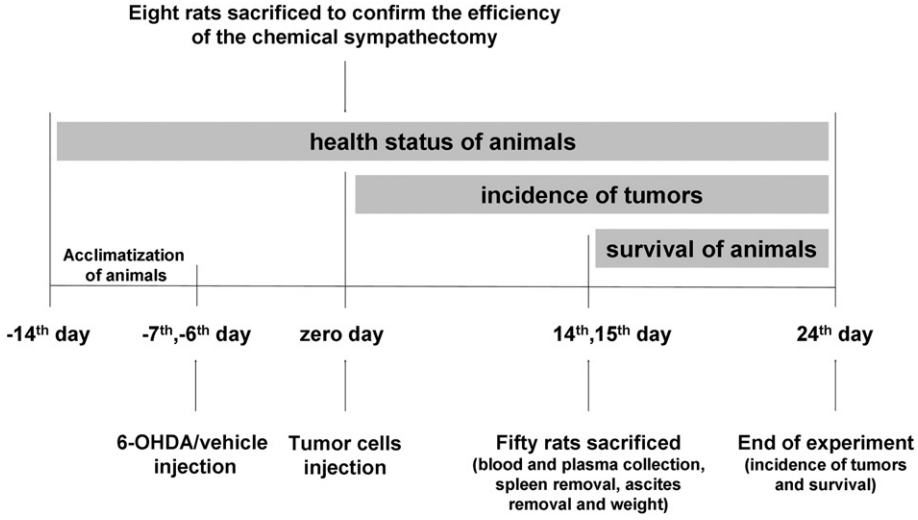

Fig. 1. Schematic diagram of experimental design. 6-OHDA–6-hydroxydopamine.