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Chronic adriamycin treatment impairs CGRP-mediated functions of meningeal sensory nerves

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ABSTRACT

Adriamycin is a potent anthracycline-type antitumor agent, but it also exerts potentially serious side effects due to its cardiotoxic and neurotoxic propensity. Multiple impairments in sensory nerve functions have been recently reported in various rat models. The present experiments were initiated in an attempt to reveal adriamycin-induced changes in sensory effector functions of chemosensitive meningeal afferents.

Meningeal blood flow was measured with laser Doppler flowmetry in the parietal dura mater of adult male Wistar rats. The dura mater was repeatedly stimulated by topical applications of capsaicin, a transient receptor potential vanilloid 1 (TRPV1) receptor agonist, or acrolein, a transient receptor potential ankyrin 1 (TRPA1) receptor agonist, which induce the release of calcitonin gene-related peptide (CGRP) from meningeal afferents. The blood flow increasing effects of CGRP, histamine, acetylcholine and forskolin were also measured. Capsaicin- and acrolein-induced CGRP release was measured with enzyme-linked immunoassay in an *ex vivo* dura mater preparation. TRPV1 content of trigeminal ganglia and TRPV1-, CGRP- and CGRP receptor component-immunoreactive structures were examined in dura mater samples obtained from control and adriamycin-treated rats.

The vasodilator effects of capsaicin, acrolein and CGRP were significantly reduced in adriamycin-treated animals while histamine-, acetylcholine- and forskolin-induced vasodilatation were unaffected. Measurements of CGRP release in an *ex vivo* dura mater preparation revealed an altered dynamic upon repeated stimulations of TRPV1 and TRPA1 receptors. In whole-mount dura mater preparations immunohistochemistry revealed altered CGRP receptor component protein (RCP)-immunoreactivity in adriamycin-treated animals, while CGRP receptor activity modifying protein (RAMP1)-, TRPV1- and CGRP-immunostaining were left apparently unaltered. Adriamycin-treatment slightly reduced TRPV1 protein content of trigeminal ganglia.

The present findings demonstrate that adriamycin-treatment alters the function of the trigeminovascular system leading to reduced meningeal sensory neurogenic vasodilatation that may affect the local regulatory and protective mechanisms of chemosensitive afferents leading to alterations in tissue integrity.

1. Introduction

Anthracyclines comprise an important class of chemotherapeutic agents still indispensable in the treatment of various malignancies (Carvalho et al., 2009; Kalyanaraman et al., 2002). Adriamycin, despite its potentially serious side effects, is one of the most commonly used antracycline derivative for its favorable antineoplastic activity. Besides its deleterious effects on cardiac muscle resulting in congestive cardiomyopathy, adriamycin exerts neurotoxic effects on primary sensory neurons in experimental animals and also in man (Bigotte and Olsson, 1982; Katona et al., 2004; Kondo et al., 1987; Minow and Gottlieb, 1975). Recent studies demonstrated marked structural, neurochemical and functional impairments of primary sensory neurons in animal models of adriamycin toxicity (El-Agamy et al., 2017; Kosoko et al., 2017). Neurotoxic propensity of adriamycin manifests as deleterious actions on chemosensitive sensory neurons which express transient receptor potential nociceptive ion channels (Boros et al., 2016). The non-selective cation channels transient receptor potential vanilloid 1 (TRPV1) and transient receptor potential ankyrin 1 (TRPA1) are multimodal sensor proteins that sense temperature (cold or hot), the presence of exogenous or endogenous noxious chemicals and acidic pH (Nilius et al., 2012; Numazaki and Tominaga, 2004; Szallasi, 1994).

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Chemosensitive afferent neurons innervate most somatic and visceral organs and tissues (Jancsó et al., 1987) and express the TRPV1 (Nilius et al., 2012) and the TRPA1 (Bodkin and Brain, 2011; Zygmunt and Högestätt, 2014) receptors, which play crucial roles in nociceptive and neurogenic vascular and inflammatory reactions (Dux et al., 2003; Earley and Brayden, 2015; Eberhardt et al., 2014; Geppetti et al., 2008; Gouin et al., 2017). Activated chemosensitive afferent nerves transmit nociceptive signals towards the central nervous system and concomitantly release neuropeptides, such as calcitonin gene-related peptide (CGRP) and substance P (SP) from their peripheral terminals leading to arterial vasodilatation, increase in vascular permeability and degranulation of mast cells (Brain, 1997; Holzer, 1998; Jansen-Olesen et al., 2014; Maggi, 1995). Since significant proportions of TRPV1 and TRPA1 expressing chemosensitive sensory neurons are peptidergic (Aubdool and Brain, 2011), the demonstration of peptide mediated vascular reactions and the quantitative measurement of the release of CGRP are reliable functional indicators of the activation of this particular class of nociceptors.

Local regulatory functions induced by neuropeptides released from chemosensitive afferents contribute also to protective mechanisms in peripheral tissues (Kashem et al., 2015; Russell et al., 2014). Preclinical experiments indicate that CGRP released from cardiac chemosensitive afferents is protective against adriamycin-induced cardiomyopathy (Ferdinandy et al., 1997; Katona et al., 2004; Shi et al., 2011). Recent observations from our laboratory have revealed profound structural and functional changes in cutaneous chemosensitive nociceptors following systemic adriamycin treatment. Adriamycin impaired both the sensory afferent function of chemosensitive nociceptors and the local efferent functions mediated by the release of neuropeptides (Boros et al., 2016; Katona et al., 2004). Testing peripheral neurogenic vascular reactions seems to be a useful tool to predict possible risks of adriamycin-induced impairments of organ function. Since adriamycin-induced changes in sensory functions develop prior to the manifestation of its cardiotoxic effect, they may also signalize upcoming cardiac injury (Boros et al., 2016).

The mechanisms of adriamycin-induced pathophysiological changes affecting sensory and vascular functions are not fully understood. The susceptibility of the different compartments of the primary sensory neuron to adriamycin appears to be diverse, too; nociceptor endings seem to be more affected than the rest of the neuron. In the skin, adriamycin treatment decreases the density of epidermal nerve fibers while leaving the density and distribution of subepidermal nerve fibers apparently normal (Boros et al., 2016). Adriamycin seems to impair also the contractility of vascular smooth muscle, probably by oxidative stress modifying protein functions (Murata et al., 2001).

The present experiments were initiated in an attempt to reveal adriamycin-induced changes in vascular and sensory effector functions of dural chemosensitive afferent nerves in a well-established animal model of meningeal nociception. Morphological and functional traits of trigeminal afferents innervating meningeal tissues in rats have already been extensively studied in our laboratory (Dux et al., 2009, 2016a; Marics et al., 2016). These studies have revealed that a substantial proportion of dural afferent nerves are capsaicin-sensitive, contain CGRP and express the nociceptive ion channels TRPV1 and TRPA1 (Dux et al., 2017; Dux et al., 2016b). Therefore, in the present study changes in meningeal blood flow and CGRP release were measured in control and adriamycin-treated rats upon dural applications of specific agonists of TRPV1 and TRPA1 in an open cranial window model. Changes in TRPV1 protein content of trigeminal ganglia, expression and distribution of TRPV1 receptor immunoreactivity, CGRP immunoreactivity and immunoreactivity of vascular CGRP receptor components in the dura mater were also determined.

2. Methods

2.1. Animals

The experiments were approved by the Ethical Committee for Animal Care of the University of Szeged. Study procedures were carried out in accordance with the Directive 2010/63/EU of the European Parliament. All efforts were made to minimize the number of animals used and their suffering.

Adult male Wistar rats weighing 270–330 g were used. One group of animals received a cumulative dose of 15 mg/kg of adriamycin (Doxorubicin, Pharmacia Italia, Italy) by intravenous injection of 2.5 mg/kg of the drug three times a week for 2 weeks (Tong et al., 1991). Control rats received equivalent amounts of the vehicle (saline). All experiments were performed 2–7 days after cessation of the treatment.

2.2. In vivo measurement of meningeal blood flow

Control and adriamycin-treated animals were anesthetized with thiopental sodium (100 mg/kg, i.p. Insera Arzneimittel GmbH Freiburg, Germany). The animals were tracheotomized and breathed spontaneously. Systemic blood pressure was recorded with a pressure transducer connected to a cannula inserted into the femoral artery. The body temperature was monitored with a thermoprobe inserted into the rectum and was held at 37–37.5 °C by a heating pad. For the measurement of meningeal blood flow, a cranial window was prepared according to Kurosawa et al. (1995). The head of the animal was stabilized in a stereotaxic frame, the scalp was incised in the midline and the parietal bone was exposed on one side. A cranial window was drilled into the parietal bone to expose the underlying dura mater. Blood flow was recorded with a needle-type probe of a laser Doppler flowmeter (Perimed, Sweden) positioned over a branch of the middle meningeal artery. Data on meningeal blood flow and systemic blood pressure were processed with the Perisoft program (Perimed, Sweden). Stimulation of the dura mater was performed by repeated topical applications of capsaicin (100 nM), acrolein (300 μM) and CGRP (10 μM) at a volume of 40 μl for 3 min followed by repeated washouts with synthetic interstitial fluid (SIF, containing in mM: 135 NaCl, 5 KCl, 1 $MgCl_2$, 5 $CaCl_2$, 10 glucose and 10 Hepes, pH 7.4). During washout periods the basal blood flow was restored. The effects of single histamine (10 μM), acetylcholine (100 μM) and forskolin (100 μM) applications were also tested. All drugs were purchased from Sigma-Aldrich Chemie Gmbh, Hungary. A stock solution of capsaicin (32 mM) was prepared with the aid of 6% ethanol and 8% Tween 80 in saline and was further diluted with SIF. All the other drugs were dissolved in SIF immediately before use. Basal blood flow was determined as the mean flow during a 3 min period prior to drug application. Percentage changes in meningeal blood flow in response to capsaicin, acrolein, CGRP, histamine, acetylcholine and forskolin were determined as mean flow values within the 3 min application period relative to the basal flow. At the end of the experiments, the animals were sacrificed by an overdose of thiopental sodium (200 mg/kg, i.p.).

2.3. Ex vivo measurement of CGRP release

Measurement of the release of CGRP from dural afferents was performed by the method of Ebersberger et al. (1999). Control and adriamycin-treated animals were deeply anesthetized with thiopental sodium (200 mg/kg, i.p.) and decapitated. After removal of the skin and muscles, the skull was divided into halves along the midline and the cerebral hemispheres were removed. The skull preparations were washed with carbogen-gassed SIF at room temperature for 30 min and then mounted in a humid chamber at 37 °C. The cranial fossae were filled with 300 μl of carbogen-gassed SIF solution. Samples of the superfusate were collected at periods of 5 min by carefully removing the