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A liver schwannoma observed in a female Sprague-Dawley rat treated with MNU

Catarina Isabel Teixeira-Guedes[a,1], Ana Isabel Faustino-Rocha[a,1], Daniela Talhada[a], José Alberto Duarte[b], Rita Ferreira[c], Fernanda Seixas[a,d], Paula Alexandra Oliveira[a,e,*]

[a] Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[b] Research Center in Physical Activity, Health and Leisure (CIAFEL), Faculty of Sport Sciences, University of Porto, Porto, Portugal
[c] Organic Chemistry of Natural Products and Agrifood, Mass Spectrometry Center, Department of Chemistry, University of Aveiro, Aveiro, Portugal
[d] Animal and Veterinary Research Center (CECAV), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[e] Center for the Study of Animal Sciences (CECA), University of Porto, Porto, Portugal

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ABSTRACT

Background: Schwannoma is a tumor of the nervous system composed by Schwann cells. It can occur naturally in several tissues of the body in both humans and animals. Diaphragmatic hernia can be congenital or acquired and is defined as a protrusion of abdominal viscera into the thoracic cavity.
Materials and methods: The animal was a female rat from an experiment of mammary tumor chemically induced. It was injected with *N*-methyl-*N*-nitrosourea (MNU) and died spontaneously at 22 weeks of age.
Results: The animal had a diaphragmatic hernia and a hemorrhagic and multicystic mass in the liver herniated lobule. Microscopically the liver displayed a well circumscribed mass that was a tumor with hemorrhagic areas, necrosis and Antoni A and Antoni B patterns. It also displayed occasional positivity to vimentin and diffuse positivity to S-100 and NSE.
Conclusion: The tumor was a schwannoma with the origin in the Glisson's capsule.

1. Introduction

Schwannoma also named neurilemmoma is a nerve sheath tumor composed by Schwann cells derived from the neurilemma of a nerve fiber (Enzinger and Weiss, 2008; Frosch, 2007; Kazianis et al., 2001). This tumor type can occur spontaneously in various tissues (Enzinger and Weiss, 2008; Ikeda et al., 2003; Yoshizawa et al., 1996). In humans, the most common sites include the head, neck and flexor surfaces of extremities (Enzinger and Weiss, 2008) and it rarely develops in the gastrointestinal tract, retroperitoneal cavity and other parts of the body, whereas development of a primary benign schwannoma in the liver is extremely infrequent (Brennan et al., 1997; Ota et al., 2012). In rats, although schwannoma is unusual, when present it is frequently malignant and occurs mostly in maxillary region and paravertebral region of the mediastinum and retroperitoneum (Cardesa et al., 1983; Rice and Ward, 1989).

Histologically, schwannomas are characterized by Antoni type A and B growth patterns associated with multiple cysts (Laber-Laird et al., 1988; Rice and Ward, 1989). Antoni A pattern is characterized by elongated cells arranged in fascicles in areas of moderate to high cellularity with little stromal matrix. In the Antoni B pattern the tumor has less cellularity with a loose meshwork of elongated cells with microcysts and myxoid changes. In both growth patterns, the cytology of the individual cells is similar, with elongated cytoplasm and regular oval nuclei. These tumors have a uniform immunoreactivity to vimentin, S-100 protein and NSE (neuron-specific enolase), which are very important to confirm diagnosis (Baderca et al., 2008; Frosch, 2007).

Diaphragmatic hernia is a protrusion of abdominal viscera into the thoracic cavity through an abnormal opening or defect of diaphragm muscle, being sometimes covered by a membranous sac (Skari et al., 2000; Yoshitomi and Boorman, 1991). It can occur due to defects in the diaphragm muscle present at birth date (congenital hernia) or as a consequence of trauma (acquired hernia) (Tenbrinck et al., 1990).

In the present paper we report a case of a diaphragmatic hernia and hepatic schwannoma in a female Sprague-Dawley rat, which was treated with MNU as part of a mammary tumor study.

2. Materials and methods

2.1. Animal

All animal procedures were done in accordance with the European Directive 2010/63/EU on the protection of animals used for

* Corresponding author at: Department of Veterinary Sciences, CECA, University of Trás-os-Montes and Alto Douro, 5001-911 Vila Real, Portugal.
Tel.: +351 259350651; fax: +351 259350480.
E-mail address: pamo@utad.pt (P.A. Oliveira).
[1] These authors contributed equally to this work.

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scientific purposes. The Portuguese Ethics Committee for Animal Experimentation approved all experiments and procedures carried out on the animals (Direcção Geral de Veterinária, Approval no. 008961).

Forty-two female Sprague-Dawley rats (*Rattus norvegicus*) were acquired from Harlan Interfauna Inc. (Barcelona, Spain) at four-weeks and integrated in a study of chemically induced mammary cancer. During the experimental protocol the animals were maintained under controlled conditions of temperature ($23 \pm 2\,^{\circ}C$), humidity ($50 \pm 10\%$) and light-dark cycle (12–12 h). A standard diet (4RF21®, Mucedola, Italy) and tap water were supplied ad libitum. At seven-weeks of age the animals were divided into three groups: a MNU exercised group, a MNU sedentary group and a control group (without any treatment). Animals from MNU groups received an intraperitoneal injection of *N*-methyl-*N*-nitrosourea (MNU) (ISOPAC®, lot 100M1436V, Sigma Chemical Co., Madrid, Spain) at a dose of 50 mg/kg. In the exercised group, after MNU administration, animals were trained on a Treadmill Control LE8710 (Harvard Apparatus, USA) at a speed of 20 m/min, 60 min/day, 5 days/week, to evaluate the effect of physical exercise on mammary tumors development. Animals from sedentary group were not trained.

3. Results

The animals were monitored twice a day for clinical signs of toxicity. Two rats died before the completion of the experiment, one at nine weeks and the other at 15 weeks after the beginning of the experimental protocol. The first animal did not show any signals of disease and belongs to the control group. The second animal belongs to MNU exercised group and showed reluctance to exercise since the start of the exercise protocol. Three days before the death, the rat exhibited lethargy, decreased activity, piloerection and weight loss. This animal died spontaneously at 22-weeks of age, 15-weeks after beginning the study.

3.1. Macroscopic examination

Immediately after the animal's death, a complete necropsy examination was performed. The animal was in good body condition. Macroscopically, we observed a severe herniation of bowel loops and the right lobule of the liver into the thoracic cavity compressing the lungs and heart (Fig. 1a). The diaphragm was pale and thin and there were two herniations of the cecum forming a dome into the thoracic cavity. The liver was congested and the herniated lobule was transformed in a well-delimited hemorrhagic and multicystic mass measuring 2 cm × 1 cm × 1 cm in its major dimensions (Fig. 1b). The cut surface revealed a whitish soft tumor with cystic and fibrotic areas replacing almost the entire right lobule. The remaining liver showed dystrophy.

3.2. Microscopic examination

After necropsy, organs were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 2 μm and stained with H&E for histopathological examination.

The liver showed a well circumscribed but not encapsulated tumor mass comprised of sheets or irregular bundles of loosely packed fusiform cells, separated by variable amounts of pale matrix, with cystic areas replete with fluid and blood (Fig. 2a). Nuclei ranged from round to fusiform, were hyperchromatic or vesicular, and showed up to two nucleoli. Mitoses were uncommon, one or two mitoses per high power magnification (HPM). Extensive liver capsular areas were replaced by the spindle cell proliferation and occasionally, we observed hypercellular areas with increased mitotic activity (4/HPM). The tumor showed hemorrhagic areas, necrosis and invaded the diaphragm and costal wall (Fig. 2b). The

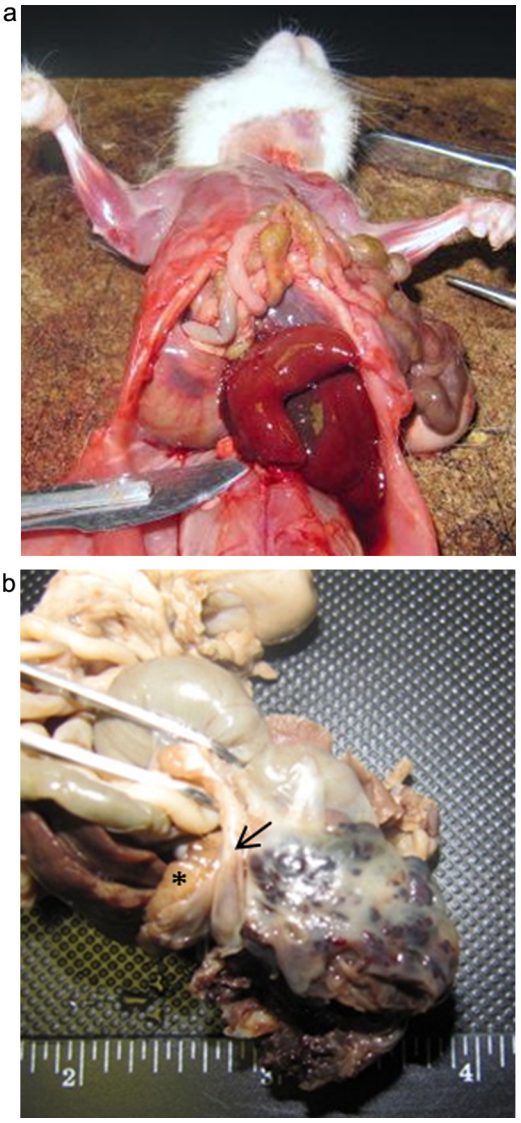

Fig. 1. Macroscopic examination. (a) Diaphragmatic hernia in situ; (b) diaphragmatic hernia after fixation. Non-herniated liver (asterisks) and diaphragm (arrow).

microscopic analysis showed Antoni A and Antoni B areas. Antoni A areas were very cellular, composed of spindle cells arranged in a fascicles and palisades (Fig. 2c) whereas in Antoni B areas cells were separated by abundant oedematous material forming cystic spaces (Fig. 2d). The tumor displayed rare macrophages, mast cells, polymorphonuclear cells infiltration and occasionally trapped hepatocytes. To confirm the nature of the tumor, additional sections were immunostained with a panel of antibodies using streptavidin-biotin-peroxidase method. The markers used were: Vimentin (1:100, NCL-Vim-Va, Dako), S100 protein (1:100, NCL-S100p, Novocastra) and NSE (1:400, CALP, Dako). The tumor showed occasional positivity to vimentin and diffuse positivity to S-100 protein (Fig. 2e) and NSE (Fig. 2f) confirming the diagnosis of schwannoma. The lung showed atelectasia and some arteries exhibited hyperplasia of the tunica media. The non-glandular region of the stomach showed epithelial hyperplasia, hyperkeratosis and superficial microbial contamination. The spleen presented haemosiderin deposits. In the kidney we observed acute tubular necrosis, cysts, hyaline casts and thickening of the glomerular basement membranes. The small intestine showed discrete infiltration of lymphocytes and plasma cells in the lamina propria. Haemolymphatic ganglia showed haemosiderin and mast cells. We