Cisplatin Intralesional Chemotherapy of Recurrence

Schwannoma in Horse: A Case Report

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Abstract

A 7-year-old crossbred stallion had multiple solitary cutaneous masses at the right infraorbital area which were recurrent for two months. The biggest mass size was 5 x 7 x 1 cm\textsuperscript{3} and had superficial bleeding. This case was diagnosed as peripheral nerve sheath tumors based on cytological and histological examination which showed numerous spindle cells with cancerous characteristics, cell patterns of the tumor were interlacing bundle and a whirl. Most areas showed tense palisading nuclei of tumor cells (Verocay bodies) known as Antoni A area. The rest showed loose paucicellular tissue known as Antoni B area. Within histologic sections, the cells showed positive immunoreactivity for S100 and calretinin, whereas immunoreactivity was not detected for CD34. Rapidly recurrent tumor was found after excision combined with cryotherapy. Consequent treatment using cisplatin (1mg/cm\textsuperscript{3}) in autologous serum was intralesionally injected twice, 2 weeks apart. After the two injections, the horse responded with effective wound healing two months later. Therefore, intra-lesional injection of cisplatin should be considered for recurrent dermal Schwannoma or inadequate surgical removal of tumor.

Keywords: cisplatin, equine, Schwannoma

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Introduction

Equine cutaneous masses are classified as neoplastic masses (sarcoïd, squamous cell carcinoma, fibroma, and melanoma) and non-neoplastic masses (Carstanjen et al., 1997). Sarcoïd and squamous cell carcinoma occur more frequently in equine neoplasm but cutaneous benign peripheral nerve sheet tumors (PNSTs) in dogs, cats, horses, and cattle are rare (Goldschmidt and Hendrick, 2002; Valentine, 2006; Schöniger and Summers, 2009).

Schwannomas and neurofibromas are the most common benign peripheral nerve neoplasms. Schwannomas are relatively uncommon slow-growing tumors that originate from the Schwann cells of the nerve roots, while neurofibromas display a mixture of cell types, including Schwann cells, perineurial cells, and endoneurial fibroblasts. The etiology of Schwannoma is still unknown and is generally asymptomatic (Enoz et al., 2006). Gross characteristics of Schwannoma are firm to soft, well-circumscribed, unencapsulated masses in the dermis as single or multiple masses. Schwannomas in horses may occur at the age of 3 to 16 years, no breed or sex predisposition (Fernandez et al., 1996). In domestic animals, benign PNSTs occur more commonly on the eyelids (Pascoe and Summers, 1981; Schöniger et al., 2011) and, in humans, mostly occur in the head and neck region (Pandarakalam et al., 2005).

Histopathology of Schwannoma is a whirl of spindle-shaped to ovoid with elongated cytoplasmic extensions in a collagenous matrix form Antoni A and Antoni B areas, which could be differentiated from equine sarcoïd (Goldschmidt and Hendrick, 2002). In general, Schwannoma may be misdiagnosed as fibroma, fibrosarcoma or sarcoïd (Scott and Miller, 2003).

Histological characteristic features with immunohistochemistry for cellular markers are usually diagnosed in human Schwannoma and neurofibroma (Weiss and Goldblum, 2008; Skovronsky and Oberholtzer, 2004). Schwann cells were immunopositive for S100 protein and expression of laminin and glial fibrillary acidic protein (Schöniger et al., 2011).

Surgical resection is the principal treatment for skin tumors; however cutaneous tumors in horses have poorly defined margins, possibly local infiltrations and high recurrence rates. Various combining treatments include the use of a Nd:YAG or CO\textsubscript{2} laser (McCauley et al., 2002); intralesional chemotherapy with cisplatin (Hewes and Sullins, 2006) or 5-fluorouracil; cryotherapy; and brachytherapy (Saulze et al., 2009). Using Nd:YAG laser and brachytherapy gives highly successful results in periorbital neoplasms. However, the high cost of instruments, the need for professional technician and the hazard from radioisotope are the restrictions (Byam-Cook et al., 2006). Although cryotherapy is an easier and cheaper method compared to Nd:YAG laser and brachytherapy, the recurrence of tumor is in a higher ratio. Cisplatin oil emulsion was developed for equine skin tumor therapy (Theon et al., 1993), combined with electrical treatment it becomes highly potent (Tamzali et al., 2012). The purpose of this study was to report the success of injections of cisplatin intralesional chemotherapy without sesame seed oil emulsion.

Case History

A 7-year-old crossbred stallion was presented to the Kasetsart University Veterinary Teaching Hospital, Kamphaeng Saen Campus, with multiple nodular dermal masses that became larger within 2 months after an initial excision by the owner. The stallion was bright, alert and in good condition at the time of presentation. Normal cardiovascular and respiratory parameters were shown. The visual reflex, appearance and signs were always normal. There were externally visible, multiple fleshy nodules at the right infraorbital. Palpation of the right infraorbital revealed a subcutaneous swelling. The biggest expansive nodular mass was 5 x 7 x 1 cm\textsuperscript{3} with multifocal small hemorrhage (Fig 1A). Squamous cell carcinoma, fibrosarcoma, and sarcoïd were diagnosed based on the clinical signs and gross lesion.

Figure 1  Gross lesion finding demonstrated nodules at the right infraorbital. A, on the day presented. B-C, at day 2 after excision.
Diagnostic Method

Fresh surgical specimens of an infraorbital mass were fixed in 10% w/v buffered formalin, embedded in paraffin and stained with Hematoxylin and Eosin (HE). Histological section was prepared and stained for histological feature at Kasetsart University.

Fine needle aspiration, stained with Modified Wright’s staining, revealed a great number of spindle-shaped cells characterized by dense chromatin, 1-3 prominent nucleoli, anisokaryosis, anisocytosis, and basophilic cytoplasm. In addition, many eosinophils and other inflammatory cells were observed (Fig 2). As a result, cytological investigation suggested mesenchymal cell tumor. The histopathological findings demonstrated hemorrhagic ulcerative dermatitis due to rupture of epidermis, hemorrhage, and infiltration of inflammatory cells which were mainly eosinophils and neutrophils. The mass was non-encapsulated, poorly demarcated, and was composed of slightly pleomorphic fusiform cells with oval-shaped nuclei containing intracytoplasmic vacuoles in some cells. The cells were arranged in interlacing bundle and whirling pattern. Most areas showed abundant palisading nuclei of tumor cells known as Antoni A area. The rest showed loose paucicellular tissues known as Antoni B area (Fig 3). Histopathological diagnosis was benign peripheral nerve sheath tumor.

Figure 2  Cytology revealed spindle-shaped cells characterized by dense chromatin, 1-3 prominent nucleoli, anisokaryosis, anisocytosis, and basophilic cytoplasm (black arrow).

Figure 3  Histopathological findings demonstrated that the cells were arranged in interlacing bundle and whirling pattern (black arrow). Most areas showed abundant palisading nuclei of tumor cells known as Antoni A area (black arrow head). The rest showed loose paucicellular tissues known as Antoni B area (white arrow head).

Figure 4  Immunohistochemistry of the mass. A, S100 protein was positive as intense cytoplasmic staining. B, calretinin staining was positive as brown spot. C, CD34 marker staining was negative and D, negative control.
**Immunohistochemistry**

The diagnosis of Schwannoma was confirmed using antibodies against S100 protein (anti-S100 mouse monoclonal antibody, Cell Marque, USA), calretinin (anti-calretinin rabbit monoclonal antibody, Cell Marque, USA), and CD34 (anti-C34 mouse monoclonal antibody, Neo MARKER, USA) from Institute of Pathology, Thailand. For S100 and CD34, indirect immunohistochemistry method was performed with 1:100 dilution of primary antibodies and detected by peroxidase enzyme system, while for calretinin labeled streptavidin-biotin (LASB) method was used with 1:100 dilution of primary antibody and detected by peroxidase enzyme system. A characteristic histologic feature with immunohistochemistry for S100 was strongly positive, calretinin was positive and CD34 was negative (Figs 4A-4D). The positive results for S100 protein and calretinin suggested Schwannoma.

**Treatment**

The initial treatment was surgical removal and suture. The wound was dehiscence and hypergranulation tissue was present. Excision combined with topical cryotherapy, metal probe cooled with liquid nitrogen directly touching the skin over a period of 2 min, was performed two weeks later. This treatment was ineffective again; there were recurrent multiple nodules and continuous growth within 48 h after excision. The size was 2 x 5 x 1 cm$^3$ (Figs 1B-C). The horse was restrained and premedicated with acepromazine (0.04 mg/kg, iv.) and xylazine hydrochloride (0.5 mg/kg, iv.) and auriculopalpebral and supraorbital nerves were blocked with 2% lidocaine. Intralesional administration of cisplatin 1 mg/cm$^3$ in autologous serum (2:1 ratio) was injected into the lesion twice at two-week intervals. The injection was spaced 6 mm apart in 2 planes and in a 1 cm wide outwardly healthy margin.

**Results and Discussion**

Only surgical removal or excision combined with cryotherapy was unsuccessful; the mass recurred within 48 h. Intralesional cisplatin was chosen for additional therapy. The intralesional cisplatin injection twice at 2-week intervals was performed. There were no oedematous reaction located on thin skin regions, healing delay and recurrence in this stallion after two months. However, the mass should be followed up at a minimum of 6 months.

Histopathology findings was a whirl of spindle to ovoid shape with elongated cytoplasmic extensions in a collagenous matrix form Antoni A and Antoni B areas which are the characteristics of benign peripheral nerve sheath tumors (PNSTs). In veterinary literature, PNSTs, Schwannoma and neurofibroma were not clearly classified (Koestner et al., 1999; Scott and Miller, 2003). All of these terms were used interchangeably. However, distinction of the tumors is very important for prognosis and treatment.

Immunohistochemistry for S100 protein markers have been studied for separating neural tumors from nonneural origin tumors and also for differentiating Schwannomas from neurofibromas (Valentine, 2006; Weiss and Goldblum, 2008; Skovronsky and Oberholtzer, 2004). Calretinin is a 29-kd, calcium-binding protein expressed primarily in reliable types of neurons in the central and peripheral nervous systems. Samson et al. (2004) demonstrated that calretinin staining was positive in 24 of 25 (96%) Schwannomas and, therefore, this staining was useful for differentiating Schwannomas from neurofibromas.

CD34 is a transmembrane glycoprotein normally expressed by lymphoid and myeloid lineage (Civin et al., 1984). Anti-CD34 antibodies also react with endothelial cell, periadnexal spindle cell and dendritic cells (Nickoloff, 1991). In human, anti-CD34 antibody was used in the immunohistochemistry method to exclude tumor from Schwann cell origin from other PNSTs because it can react with dendritic cell within endoneurium of peripheral nerve but not react with conventional Schwannoma (Weiss and Nickoloff, 1993).

To our knowledge, the CD34 markers are usually used for differentiating human Schwannomas and there are no reports of this marker in horse. The positivity for both S100 protein and calretinin as well as the negative immunolabeling for CD34 marker in this case could suggest that the tumor was from Schwann cell origin.

There are several treatments for benign cutaneous tumor (Carstanjen et al., 1997). Surgical resection is a primary treatment, however cutaneous tumors in horses tend to have poorly defined margins, may be locally infiltrative and have a high recurrence rate. Fifty per cent of periocular Schwannomas recurred following surgical removal within a 6-month period (Saulez et al., 2009), similar to this case. Additional therapies including laser, photodynamic therapy, radiation therapy, brachytherapy, and chemotherapy should produce more satisfied results. Saulez et al. (2009) reported the success of interstitial brachytherapy using Ir192 for malignant buccal Schwannomas. Cisplatin intralesional injection is a successful treatment for human Schwanna (Zhang et al., 2013). However, cisplatin was 83% (40/48) successful in treating cutaneous neoplasia in horses, which did not recur in 2 years (Hewes and Sullins, 2006) and 99.5% (193/194) in treating sarcom tumor, which did not recur in 4 years (Tamzali et al., 2012). Cisplatin solution should be prepared immediately prior to use with mixture of cisplatin, medical grade of sesame oil and autologous serum (Theon et al., 1993). Sequential therapeutic of cisplatin oil emulsion on large tumor was reported by Hewes and Sullins (2006) and Theon et al. (2007). This study showed that intralesional cisplatin without sesame oil after 2 treatments provided satisfied results. The suspension without sesame oil decreasing the frequency of treatment might be due to the increase in intracellular concentrations.

**References**


บทคัดย่อ

การรักษาเนื้องอกชวานโนมาแบบเกิดซ้้าในม้าโดยใช้เคมีบ้าบัดด้วยยาซิสปาติน: รายงานสัตว์ป่วย

รุ่งเรือง ยอดชีวัน

พ่อม้าพันธุ์ผสมอายุ 7 ปีมีก้อนเนื้อใต้ผิวหนังเกิดซ้้าที่บริเวณใต้เบ้าตาขวามาเป็นระยะเวลา 2 เดือน ก้อนเนื้อมีขนาด 5x7x1 ลูกบาศก์เซนติเมตรและผิวหน้ามีเลือดออก สามแพทย์ทำการตัดก้อนขึ้นเนื้อเพื่อศึกษาลักษณะทางเซลล์และจุลพยาธิวิทยา ผลการตรวจทำให้สังเกตว่าเป็นเนื้องอกของกลุ่มเซลล์ในระบบประสาทเนื่องจากพบเซลล์มะเร็งมีลักษณะของเซลล์มะเร็ง โดยเซลล์มะเร็งจัดเรียงตัวเป็นแถวต่อเนื่องกันเป็นมัดๆ และบางบริเวณมีกลุ่มเป็นก้อนหนวย มีสองบริเวณที่พบได้ชัดเจนคือ บริเวณที่มีเซลล์มากนั้นเซลล์เรียงตัวกันเป็นแถวหรือโคนยงบิล ศีรษะก้อนที่บริเวณนี้แน่นเอียงไปข้างหลัง และบริเวณที่มีเซลล์น้อยเรียงแน่นไปข้างหน้า จึงทำการตรวจเพิ่มเติมโดยใช้แอนติบอดีและพบว่าเซลล์เนื้องอกให้ผลบวกต่อโปรตีนและคาอีทิตามิค แต่ให้ผลลบต่อกับแอนติบอดีต่อซีดีสามสิบสี่ จากการวินิจฉัยจึงสรุปได้ว่าเนื้องอกนี้มีชวานโนมา การรักษาในขั้นต้นได้ทำการตัดก้อนเนื้อออกตามโปรแกรมการรักษา พบว่าเนื้องอกไม่เจริญอย่างคาดหวัง แต่ทำการฉีดซิสปาตินเข้าต่อเนื่อง 2 เดือนที่หนึ่งพบว่าเนื้องอกลดลงในขนาดและจำนวนเซลล์ และหลังการฉีดซิสปาติน 1 เดือนมีอาการเจริญซ้้าที่บริเวณเดิม ดังนั้นการฉีดซิสปาตินเข้าต่อเนื่องทำให้เนื้องอกชวานโนมาที่มีอุบัติการเกิดซ้้าไม่สามารถรักษาได้ด้วยวิธีการตัดก้อนเพียงอย่างเดียว

คำสำคัญ: ซิสปาติน หมา เนื้องอกชวานโนมา

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