ANGIOGENESIS IN MALIGNANT ORAL-CAVITY TUMOURS IN DOGS

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Received for publication May 04, 2009

Abstract

The aim of this study was to determine the vascularisation level in malignant tumours of the oral cavity. The studied material comprised 10 samples of neoplastic tissue extracted from dogs with a suspicion of oral cavity carcinoma. The samples were collected from nine male and one female dogs of various breeds, aged between 6 and 16 years. The tumours were located within the areas of mandibular and maxillary gingiva. Tumour specimens were preserved in 10% buffered formalin for 24 h, embedded in paraffin, and then sections stained with haematoxylin and eosin were prepared. Tumour type was diagnosed according to the official WHO classification. In order to render the vascular endothelium visible, immunohistochemical staining was performed with the use of the polyclonal antibody against von Willebrand factor VIII and DAKO EnVision system. In the quantitative analysis of the blood vessels displaying the FVIII factor expansion, a system of computer-assisted microscopic analysis was utilised. In ten microscopic fields the vessels were counted and a mean value per 1 mm² was calculated. The largest numbers of blood vessels were observed in squamous cell carcinomas. In the remaining tumours, the vessel count fluctuated between 78-97/mm² (mean 87). The conducted research indicated that angiogenesis in malignant tumours of the oral cavity has potential diagnostic application in determining the malignancy levels of neoplasms.

Key words: dog, oral cavity, tumour, angiogenesis.

Angiogenesis is a process necessary for the correct development, growth, and maturation of an organism. It involves the creation of new blood vessels based on the already existing vascular network. Physiological angiogenesis is intensified in periods of embryogenesis, ovulation, pregnancy, and healing of wounds (5, 7, 9). Angiogenesis also plays a vital part in the formation of neoplasms as it conditions their growth and metastases (14). The significance of the process in tumour biology has been subject to extensive research in the last 30 years. Folkman (4) was a pioneer in the field, with his hypothesis proposed in 1971 suggesting that angiogenesis is in fact an indispensable condition in the growth of carcinoma outside the in situ stage (pre-invasive). In the early stages, a non-vascularised tumour is only 1-3 mm³ in volume and comprises approximately 10⁶ cells nourished via diffusion (4, 5, 14, 15). Most tumours can survive for months, even years in the pre-vascular stage (14). Invasive hyperplasia is related to the secretion of angiogenic agents by the tumour cells that stimulate endothelium cell proliferation and spatial reorganisation into new capillary tubes. Blood vessels facilitate the oxygenation of tumour cells and provide substances necessary for their proliferation (15). The number of capillary tubes per 1 mm² of the tumour can be 10 times the respective value in healthy tissue. The greatest vascular density can be observed on the rim of the neoplasm (5).

The aim of this study was to determine the vascularisation level in malignant tumours of the oral cavity of dogs.

Material and Methods

The studied material comprised 10 samples of neoplastic tissue extracted from dogs with a suspicion of oral cavity carcinoma. The samples were collected from nine male and one female dogs of various breeds aged between 6 and 16 years. The tumours were located within the areas of mandibular and maxillary gingiva (Figs 1, 2).

None of the dogs had undergone earlier treatment for the neoplastic disease. They had been fed commercially-available or home-prepared dog feeds. The clinical cancer stage was determined in each of the dogs according to the TNM system. Radiological examinations were performed of the splanchnocranium and thorax bones to rule out the presence of metastases. Tumour specimens were preserved in 10% buffered formalin for 24 h, embedded
in paraffin, and then haematoxylin and eosin stained sections were prepared. The tumour type was diagnosed according to the official WHO classification.

In order to render the vascular endothelium visible, immunohistochemical staining was performed with the use of polyclonal antibodies against von Willebrand factor F VIII and the DAKO EnVision system. Stained preparations were examined at the Department of Clinical Pathomorphology, Medical University in Lublin. In the quantitative analysis of the blood vessels displaying the FVIII factor expansion, a system of computer-assisted microscopic analysis was utilised. In ten microscopic fields the vessels were counted and a mean value per 1 mm² was calculated.

**Fig. 1.** Tumour in right mandible.

**Fig. 2.** Tumour in right maxilla.

**Fig. 3.** Squamous cell carcinoma – (HE, 200x).

**Fig. 4.** Fibrosarcoma – (HE, 200x).

**Fig. 5.** Squamous cell carcinoma immunohistochemical staining (200x).

**Fig. 6.** Fibrosarcoma – immunohistochemical staining (200x).

### Results

In the histopathological examination of the samples, the following tumour types were diagnosed: squamous cell carcinoma - three cases (Fig. 3), fibrosarcoma – three cases (Fig. 4), and amelanotic melanoma - four cases. Radiological examinations revealed the presence of thoracic metastases in two dogs.

Characteristics of the subjects and the results of the examination are concisely presented in Table 1.
The largest numbers of blood vessels were observed in squamous cell carcinomas (Fig. 5). In one of the tumours as many as 322 capillary tubes were noted; in the remaining two they numbered 218 and 286/mm², respectively. In one of the amelanotic melanoma cases, the vessel count per 1 mm² of tumour surface amounted to 125. In the remaining tumours - three fibrosarcomas (Fig. 6) and three amelanotic melanoma - the vessel count fluctuated between 78-97/mm² (mean 87).

The juxtaposition of the blood-vessel-density values in particular tumours is presented in Fig. 7.

![Graph showing blood-vessel density in different oral cavity tumours]

**Fig. 7.** Comparison of mean blood-vessel density in particular oral cavity tumours.

**Table 1**

General results breakdown

<table>
<thead>
<tr>
<th>No.</th>
<th>Dogs</th>
<th>Location of tumour</th>
<th>Histopathological diagnosis</th>
<th>Microvessel density number/mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mixed breed, ♂, age 8</td>
<td>Incisive and canine teeth right maxilla</td>
<td>Squamous cell carcinoma</td>
<td>286</td>
</tr>
<tr>
<td>2</td>
<td>German Shepherd, ♂, age 11</td>
<td>Molar teeth right mandible</td>
<td>Squamous cell carcinoma</td>
<td>322</td>
</tr>
<tr>
<td>3</td>
<td>Collie rought, ♂, age 10</td>
<td>Molar teeth left mandible, metastases in lung</td>
<td>Squamous cell carcinoma</td>
<td>218</td>
</tr>
<tr>
<td>4</td>
<td>Dog, ♂, age 8</td>
<td>Premolar and molar teeth right mandible, metastases in lung</td>
<td>Fibrosarcoma</td>
<td>78</td>
</tr>
<tr>
<td>5</td>
<td>Rottweiler, ♂, age 8</td>
<td>Premolar teeth left mandible</td>
<td>Fibrosarcoma</td>
<td>94</td>
</tr>
<tr>
<td>6</td>
<td>Mixed breed, ♀, age 13</td>
<td>Molar teeth right maxilla</td>
<td>Fibrosarcoma</td>
<td>97</td>
</tr>
<tr>
<td>7</td>
<td>Mixed breed, ♂, age 16</td>
<td>Premolar teeth left mandible</td>
<td>Amelanotic melanoma</td>
<td>96</td>
</tr>
<tr>
<td>8</td>
<td>Cocker spaniel, ♂, age 11</td>
<td>Molar teeth left maxilla</td>
<td>Amelanotic melanoma</td>
<td>77</td>
</tr>
<tr>
<td>9</td>
<td>Shnauzer, ♂, age 11</td>
<td>Molar teeth right maxilla</td>
<td>Amelanotic melanoma</td>
<td>125</td>
</tr>
<tr>
<td>10</td>
<td>Dobermann, ♂, age 6</td>
<td>Incisive teeth right maxilla</td>
<td>Amelanotic melanoma</td>
<td>82</td>
</tr>
</tbody>
</table>
Discussion

Malignant tumours of the oral cavity are more and more commonly diagnosed in animals. As calculated by Morris and Dobson (11), they correspond to roughly 6% of all neoplastic lesions diagnosed in dogs. Risk factors that can influence the development of tumours include increased exposure to environmental factors - particularly in urban areas - chronic inflammatory conditions within the dental and periodontal areas, and chronic irritation of oral mucosa (6).

Clinical symptoms accompanying oral tumours can be observed relatively early. The increasing tumour mass often hampers ingestions and can sometimes damage teeth roots or force them out of the alveoli (12). Bad breath and excessive amounts of saliva are also among the most characteristic symptoms. Neoplastic hyperplasia in the mandibular and maxillary regions is rapid and highly invasive. Within a short period, the tumours attack the splanchnocranium and metastases are observed in the lymph nodes and lungs (8, 18).

Three types of malignant tumours were diagnosed in the collected histopathological samples: amelanotic melanoma, squamous cell carcinoma, and fibrosarcoma. Many researchers mention these types as the most common malignant oral tumours observed in dogs, with melanoma being the most invasive (1, 3, 10, 16, 18).

In human medicine, the correlation between the size of the tumour, the presence of metastases, the patient’s life expectancy, and angiogenesis has been observed. An intensive angiogenesis has been noted to increase the risk of metastases in cancers of the breast, lung, prostate, bladder, head, and neck, as well as the large intestine (7).

Jeleń-Krzeszewska et al. (9) observed that the number of blood vessels in the oral cavity increased with the progression of lesions in the subsequent stages of invasive tumours. They also found that the vascular density was higher in non-metastatic tumours, while the processes of neo-angiogenesis and metastasis only involved vessels of 10µm in diameter or more.

As already mentioned, one of the factors conditioning tumour growth and metastasis is the creation of a vascular network in the process of angiogenesis. Research is currently being done into the possible method of neoplastic angiogenesis assessment and its potential as a prognostic factor (7, 14). It is estimated that a solid tumour will not exceed the size of several mm³ without a network of newly-created vessels. It has also been observed that the density of capillary tubes in such tumours may constitute a prognosis of invasive neoplastic disease with metastases.

The results obtained in our study indicate that the most vascularised of the analysed tumours was squamous cell carcinoma. It can be assumed that the high vascular density (218-322/mm²) observed in this type of tumour constitutes a marker of the degree of its malignancy. Consequently, it seems justified to expect that it is also type of growth, which is most likely to develop metastases earlier than the other types of neoplasms.

In the case of oral cavity tumours, the treatment typically involves radical surgery removing the lesion, along with a clear margin of healthy tissue (1, 2, 8, 10, 16, 17). Angiogenesis assessment may provide a valuable indication of the potential risk of postoperative metastases.

References