A COMPARATIVE STUDY OF SERUM GELATINASE IN NORMAL AND TUMOUR AFFECTED DOGS THROUGH GELATIN ZYMOGRAPHY

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Abstract: A comparative study was undertaken to assess the gelatinase activity in the sera of normal and tumour affected dogs through gelatin zymography. In gelatin zymography, the presence of three major bands 220, 92kDa of MMP-9 and 72kDa of MMP-2 were confirmed. The intensity of active form of 92kDa of MMP-9 was comparatively thinner in sera of tumour affected than in normal dog. The intensity of 220, 92 kDa of MMP-9 were 3-4 times higher than that in normal dog. But the intensity of 72kDa MMP-2 band was slightly higher in sera samples of tumour affected than normal one. Latent form of 220 kDa band was comparatively thinner than 92kDa of MMP-9. The latent form 72 kDa of MMP-2 was observed in the serum of normal dog samples. There was more up-regulation of MMP-9 mediated through MMP-2 activity was observed in tumour affected dog. It was concluded that the intensity of MMP-9 and MMP-2 was higher in tumour affected than in normal dogs and it could be used as a potential early diagnostic method/marker in the determination of canine tumour progression.

Key words: Serum Gelatinase, Tumour, Dog

INTRODUCTION

Matrix metalloproteinases (MMPs) are a family of zinc-containing, proteolytic enzymes implicated in the degradation of extracellular matrix [1]. These proteolytic enzymes have been involved in a variety of physiologic processes. Similarly, they are also implicated in pathologic processes such as tumor invasion and metastasis in animals [2] and human beings [3]. Moreover, increased MMPs activities are associated with invasion, metastasis and prognosis in human and animal malignancies [4] especially breast cancer [5]. Recently, tissue MMP-2 and -9 were documented in canine tumors and a high level of pro-MMP-9, pro- MMP-2 and active MMP-2 were detected in most canine tumors. In addition, high levels of MMP-9 activity was found in the sera of canine with mammary adenocarcinoma indicating that MMP-9 plays an important role in the progression of a canine mammary tumor and that serum MMP-9 analysis provides as early diagnosis of adenocarcinoma [6]. In human, plasma MMP-9 is a useful marker in the follow-up and in the assessment of prognosis in breast cancer patients [7]. Hence, the present study was carried out to find out the existence of MMP-2 and MMP-9 in the serum samples of tumour affected dogs and further it was compared with normal dogs.
MATERIALS AND METHODS

The proposed study was carried out at the Department of Veterinary Physiology and Biochemistry, TANUVAS-Veterinary College and Research Institute, Orathanadu, Tamilnadu, India.

Five healthy and three tumor affected dogs were selected for the study. The dogs have been properly vaccinated and dewormed. Blood samples were collected, transported to the laboratory immediately and centrifuged at 3000rpm for 15 minutes. The serum samples were stored at -20°C for further analysis and protein content was estimated with standard method.

The serum samples were subjected to modified SDS-PAGE carried out by Heussen and Dowdle [8] by the addition of co-polymerizing substrate of gelatin (0.3%) (final concentration was 0.15% to the resolving gel 8%). The samples were electrophoresed at 100V for 20 min. Renaturation was carried out with 2.5% Triton X-100 for 3 hrs on a mechanical shaker with a mild agitation. Then developing was done by incubating the gel in 10mM CaCl2, 0.15 M NaCl and 50mM Tris pH 7.5 for 18 hrs at 37°C. The gel was stained with 0.25% coomassie brilliant blue for 2 hrs, followed by destaining with destaining solution for 1 hr and finally the gel was washed with distilled water.

Human capillary blood gelatinase was used as the standard marker for comparing the zymogram bands as per the procedure was carried out by Makowski and Ramsby [9]. Using a finger stick puncture, the blood was collected from a capillary and weighed in a tarred polypropylene tube using analytical balance. Samples were added with 20X volume of Laemmli sample loading buffer and thoroughly mixed. Then the aliquots were stable for 3 months at -20°C.

RESULTS AND DISCUSSION

All the serum samples were subjected to gelatin zymography and the results were depicted in figure 1. Based on previous reports [10], the bands with 225, 72 and 62 kDa were corresponded to dimer-MMP-9, proMMP-2 and active MMP-2. On gelatin zymography, the presence of major bands at 220, 135, 92 kDa of MMP-9 and 72 kDa of MMP-2 bands were observed in tumour affected dog sera samples (Lane 1, 2 and 6). Except 135 kDa of MMP-9 band, all the other three bands were present in normal dog sera samples (Lane 3, 4, 5, 8 and 9). The intensity of active form of 92kDa in tumour groups is 3-4 times higher than the normal dog sera sample and it was also thicker than 72kDa of MMP-2 band in tumour group. The variation in molecular weights (by 10 to 15 kDa) of gelatinases of different origins can be a result of different glycosylation. It might be inferred that the minor variation in the bands could be due different source of tumour, degree and stage of tumour.

The latent form of 220 kDa band was comparatively fainter than 92kDa of MMP-9. Minor catalytic breakdown products of 135kDa band were observed. All the three forms are proteolytic active as they fully degraded the gelatin. The relative amount of 92kDa of MMP-9 to that of 72kDa of MMP-2 was at least 4-5 times higher. The ratio of MMP-9 and MMP-2 was higher comparing to the human marker. The 220 and 72 kDa bands were found to be catalytically active and TIMP-1 free. Below 72kDa band, there was no bands of MMP-2 were observed [10,11].

Several studies have been documented the enhanced expression of these MMP’s with tumor progression and metastasis [1] suggesting that increased expression of MMP and progression of endometrial carcinoma are closely related with active gelatinoma suppression in endometrial carcinoma resulting in alteration. The microenvironment that promotes tumour cell was the main one for MMP-2, MMP-9 and MT1 protein. Lana et al. [12] observed that MMP-2, MMP-9 concentration were increased in malignancy of squamous cell carcinoma compared to that healthy subjects. Daniel et al. [11] observed that the serum MMP-2 and MMP-9 concentration were higher in patients diagnosed with or without metastatic breast cancer compared to the healthy women. MMP-9 had higher concentration levels in patients with breast cancer than in healthy volunteers. Similarly, the intensity of MMP-9 (92 kDa) and MMP-2 (72 kDa) was higher in tumour group than the normal dogs group.

Akkoc et al. [13] found that expression of MMP-9 could be an important indication for tumour progression and the possibility of metastatic nature in feline tubule papillary carcinomas. Further, they
demonstrated the active and latent MMP-9 clear bands were proteolytic active as they degraded the gelatin and it was further revealed that both normal and mammary tumour cells expressed MMP-2, MMP-9 but gelatinolytic activity of MMP-9 was greater than MMP-2. The up-regulation of MMPs was reported in a number of canine tumors [14] and human tumors [15]. MMPs are explicitly involved in the degradation and remodeling of the ECM under various physiological conditions and in tumor progression and invasiveness [16]. MMP-2 and MMP-9 are well known gelatinases for their ability to degrade type IV collagen, a major component in the BM [17].

MMP activity in tumor cells local environment results in proteolytic cleavage of membrane-associated extracellular matrix metalloproteinase inducer releasing soluble EMMPRIN. Soluble EMMPRIN in turn acts in a paracrine fashion on stroma cells that are both adjacent and distant to tumor sites to further stimulate the production of MMPs and additional EMMPRIN, which consequently contributes to tumor angiogenesis, tumor growth, and metastasis [18].

The high levels of MMP-2 and MMP-9 documented in this study indicated the level of tumor which may be associated with the angiogenesis and inhibition of immune cell proliferation. In the micro-environment of a tumor, high levels of MMP-2 and MMP-9 are associated with the angiogenesis [19], an important process in tumor metastasis, growth and tumor cells entering general circulation [20]. In addition, the expression of vascular endothelial growth factor can be affected by the MMP-9 during angiogenesis. Besides the angiogenesis, MMP-2 and MMP-9 can also inhibit the proliferation of T-cells allowing tumor cells to escape immune surveillance [21].

In human, it has been documented that high serum MMP-9 levels are present in patients with lung cancer, but no association has been reported with the tumor stage and malignancy [22]. On contrary, the plasma level of MMP-9 has a positive correlation with the status of patients with breast cancer [7]. Thus, the implementation of serum MMP-2 and MMP-9 levels is used as a useful parameter in correlation with tumor malignancy is warranted. MMP-9 has been demonstrated to play an important role in both normal and tumor affected animal. Therefore, in gelatin zymography, the active and latent form of MMP-9 was detected in both tumor and normal dogs. Moreover, the amount of inflammatory cells contained in the tissue may reflect the quantity of both active and latent forms of MMP-9. Further, the concentration of MMP-2 and MMP-9 was estimated and it should be correlated with clinical state of the animal to confirm the stage of the tumour. Though high serum

**Fig. 1.** Comparative gelatin zymogram of sera samples of normal and tumour dog
level of MMP-2 and MMP-9 was observed in dogs with malignancy in this study, only serum MMP-9 levels were reflected the activity of MMP-9 from tumour tissues. It was concluded that the intensity of MMP-9 and MMP-2 was higher in tumour affected dogs than normal one. It depends upon the degree and severity of tumour which indicates the metastasis stage of tumour. Serum MMP-9 analysis is a potential early diagnostic tool/method in the determination of canine tumour progression.

REFERENCES