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THE 14TH ANNUAL WORKSHOP OF THE REGIONAL NETWORK ON
ASIAN SCHISTOSOMIASIS AND OTHER HELMINTH ZOONOSIS

THE 5TH ANNUAL MEETING OF SOUTH EAST ASIA
VETERINARY SCHOOL ASSOCIATION

THE 3RD SCIENTIFIC MEETING OF INDONESIAN
VETERINARY SCHOOL ASSOCIATION

IPB International Convention Center, Bogor, Indonesia
13-15 October 2014

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SOUTH EAST ASIA VETERINARY SCHOOL ASSOCIATION (SEAVSA)

THE 3RD SCIENTIFIC MEETING OF
INDONESIAN VETERINARY SCHOOL ASSOCIATION (AFKHI)

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Renal Adenocarcinoma with Marked Desmoplasia in a Lion (Panthera leo): Pathomorphological Study

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Key words: adenocarcinoma, Panthera leo, pathomorphology, renal

INTRODUCTION

Adenocarcinoma is a malignant tumor which characterized by epithelial cells, arrange in tubular, papillary or solid structures. The occurrence of this tumor is rarely reported in wild mammals. A 16-year-old female lion had gradually weight loss and lethargy about one year before death. Treatments were done to rise up the health condition but unfortunately she was death with icteric mucosa. A pathological evaluation on internal organs was done as a regular pathological diagnosis. In a careful investigation the irregular masses were characterized and found in evaluated internal organs such as kidney, heart, and lung. Further evaluation was focus on cellular characterization, which was done using special staining and immunostaining method, to visualize the detail of tissues structure and cell's masses identification.

MATERIALS AND METHODS

The female lion was necropsied within 6 hours of death, and representative sections of all major organs were collected, fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned 5 μm, and stained using hematoxylin and eosin (HE). Additional sections of effected kidney, heart and lung were stained using Masson trichrome (MT) and Periodic Acid Schiff (PAS) methods. To characterize neoplasms with Immunohistochemical methods, we used standard immunoperoxidase staining for cytokeratin AE1/AE3, vimentin, and cyclooxygenase 2 (cox-2). Formalin-fixed paraffin embedded sections were deparaffinized, rehydrated, and subsequently blocked with hydrogen peroxide. Pretreatment for all antibodies by steamed it in 0.01M citrate buffer for 20 minutes, followed by 20 minutes of cooling. All steps were followed by a phosphate-buffered saline wash.

Sections were incubated with these primary antibodies; monoclonal mouse anti-cytokeratin AE1/AE3 (Millipore, USA), monoclonal mouse anti-vimentin (DAKO, Denmark), polyclonal rabbit anti-cyclooxygenase-2/cocx-2, (Millipore, USA), and were incubated overnight in refrigerator. All slides were developed with 3,3- diaminobenzidine/DAB chromogen (BioSM,USA) and counterstained with Mayer’s hematoxylin. In all cases, step sections were incubated with isotype-specific irrelevant antibodies for negative controls.

RESULTS AND DISCUSSION

Gross necropsy examination findings showed mild icterus. A white nodular and firm mass was found on surface area of the right kidney. On cross section, the mass was 15 mm in diameter, characterized by infiltrative growth, located in renal cortex and protruded to the renal surface, well demarcated but non-encapsulated. Multinodular and irregular masses were also found in the left myocard of heart. Similar multiple masses located in lung; about 200 nodules, white in color, irregular shape, and were diffusely scattered throughout the lung. These lesions accompanied with inflammatory reactions, necroses and small numbers of cystic formation. The liver revealed highly discoloration with extrahepatic bile duct obstruction.

Histopathological findings indicated that the mass of kidney consisted of neoplastic cells which cubical to low-columnar in shape, arranged in 1 to 3 stratified cells with moderate to severe
the organ that contains the androgen receptor. Microscopic observation of brain tissue of guinea pigs in each treatment group with Hematoxylin & Eosin staining (HE) showed a change in brain neuronal cells. Such changes can be observed in the cortex, temporal lobe, parietal and guinea pig hippocampus of the brain characterized by the degeneration of brain neurons and neuronal cell death (necrosis) accompanied by increased activity of glial cells (gliosis). The changes in neuronal cells of the brain sections were then performed calculations (scoring) cell death (necrosis) of each of the treatment groups. Based on the results of the calculation of the number of cells undergoing cell death (necrosis) of each treatment group showed a trend of decrease in the number of cells undergoing death (necrosis) in the testosterone-injected group compared with the control group (not given the hormone testosterone). The number calculation of neurons to cell death (necrosis) showed that the group who were not given testosterone showed a high level of cell death, whereas in the group given injections of testosterone both low dose and high dose showed a decrease in the amount of neuronal cell death. The steroid hormone (testosterone) is a potential hormone that regulates the function of neurons, the normal development of neurons to damage and aging. Steroid hormones of neuroprotective agents as an effective therapy for the treatment or prevention of neurodegenerative diseases such as Alzheimer's damage [3]. The brain is one organ that responsive to androgens and androgen receptors [4]. The morphology of neurons in the hippocampus, amygdala and cerebral cortex are sensitive to androgen levels. Dendritic synapse density in the CA1 region of the hippocampus increased with DHT treatment in ovariectomized female rats [5]. Consistent with the mechanism of androgen testosterone showed that the neuroprotective against amyloid β toxicity as a result of DHT not estradiol [6]. Furthermore dependent signaling and activation of AR encouraged phosphorylation Rsk kinase and activates protein proapoptosis Bad [7].

CONCLUSIONS

Testosterone injection therapy of low-dose and high dosage may increase plasma testosterone levels in the blood plasma and play a role in lowering the rate of cell death.

REFERENCES


Potency of Testosterone Hormone Therapy in the Guinea Pig (Cavia porcellus) as an Alzheimer’s Disease Model

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Key words: Alzheimer’s disease, testosterone, amyloid β

INTRODUCTION

Alzheimer’s disease (AD) is an irreversible disease, and is characterized by loss of memory and cognitive progressive. Steroid hormones are hormones that regulate the potential of living nerve cells in the central nervous system (CNS) of normal development to nerve damage. There is no definitive therapy or treatment in patients with Alzheimer’s until now. Treatment is only intended to reduce the symptoms caused by Alzheimer’s disease. It is very possible that testosterone is a strategic step in addressing neurodegenerative disorders such as Alzheimer’s disease [1]. Testosterone and its active metabolite dihydrotestosterone (DHT) has important actions on the brain. The brain is the most responsive tissue to androgen which induces some important brain functions, such as improving the condition of the body and support the cognitive aspects including behavior [2]. The principle of treatment of Alzheimer’s disease is basically reduce and eliminate the production of amyloid β plaques (βA). This study aims to investigate the influence of the hormone testosterone in the guinea pig plasma testosterone levels in the blood plasma and pathological changes in the brain with Hematoxylin & Eosin staining (HE).

MATERIAL AND METHODS

The eighteen old male guinea pigs (32-48 months) with body weight ranging from 950-1300 g were divided randomly into three groups of 6 animals each. Group I, six guinea pigs were done castration and testosterone injection Sustanon®250 given a low dose (DR), group II, six guinea pigs were done castration and testosterone injection Sustanon®250 given a high dose (DT), group III, six guinea pigs were done castration without given injection of testosterone (K). Castration was done by lifting the testicles (orchidectomy) using general anesthesia. Testosterone injections were performed every 4 weeks. Each group were performed euthanasia of two animals in the first, third, and fifth month after treatment. This study was conducted with the approval and supervision of the animal ethics committee of Animal Care and Use Committee (ACUC) No. 12-IA-ACUC-001, PT. Indonesia, Kencana Park, Bogor. Blood sampling was done two times that prior to castration and when euthanasia at first, the third and fifth month after the treatment. Blood plasma was used for analysis of testosterone levels using enzyme-linked immunosorbent assay (ELISA) (DGR EIA 1559), after castrated, all organs were weighed and put in neutral buffered formalin solution (BNF) 10% for 24 hours. Brain organ was cut at 3-5 mm with transversal cutting in the cortex, parietal lobe, temporal lobe and hippocampus for histopathological analysis with Hematoxylin & eosin staining (HE).

RESULTS AND DISCUSSION

The results of measurements of plasma testosterone levels of guinea pigs from each treatment group showed that administration of high-dose injection of either testosterone or low doses cause an increase in blood plasma levels of testosterone significantly. Increased testosterone levels were high in the blood plasma due to injection of the hormone testosterone. It was likely to affect the function of the organs of the brain as an organ that is responsive to testosterone levels. The brain is...
anisocytosis and anisokaryosis. Sometimes the nuclei were 2 to 4 times larger than those of normal epithelial cells. These cells arranged in distinct and indistinct tubular structures surrounded by fibrous stroma with produced collagen fibers (Masson trichrome stain) but negative result for carbohydrate (PAS stain). The neoplasm was infiltratively growth through the renal cortex, had indistinct border between the mass and renal parenchyma. Neoplastic cells has eosinophilic cytoplasm and large prominent nuclei with 1 to 2 central or eccentrically nucleoli. Mitotic figures were various in averaged 0 to 1 per high power field. The neoplastic tissue was protruded from cortex to the renal surface, consisted of the tubular structures of epithelial cells, surrounding with prominent fibroblastic desmoplastic cells. Similar morphology and structures of neoplastic cells were identified in myocardial and lung. Renal carcinomas in mammals often invade the renal vein, ascend the caudal vena cava, and frequently metastasize to the lungs. Secondary metastases of renal carcinoma may be widespread with involvement of many organs, including the liver and heart [1]. Icteric condition of the lion was caused by obstruction of extrahepatic bile duct which producing hyperbilirubinemia.

Immunohistochemical works demonstrated that neoplastic cells were positive for cytokeratin, vimentin and cox-2 (Table 1). Previous study indicated that acquisition of vimentin staining by neoplastic cells of epithelial origin is correlated with a higher metastases rate and lower survival time in cervical and breast carcinoma in human [2]. Other study explained that vimentin expression in feline mammary carcinoma has been proposed as indicative of malignancy [3], and another study demonstrated that atypical vimentin was also expressed in feline salivary gland adenocarcinoma [4]. The fibroblastic desmoplastic cells in neoplastic nodule were positive immunoreactivities for vimentin. Collagen fiber proliferations (using Masson trichrome stain), so called desmoplasia, were often seen around neoplastic cells in the primary and metastatic lesions [5]. In addition, the image of desmoplastic areas contained scanty tubular structures of neoplastic cells giving an appearance of scirrhoues carcinoma. The present tumor revealed that desmoplastic areas were stained blue by Masson trichrome stain, and spindle cells present in these areas reacted to vimentin, these findings suggestive of fibroblastic nature of desmoplastic cells.

<table>
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<th>Cytokeratin</th>
<th>Vimentin</th>
<th>Cox-2</th>
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<tr>
<td>Kidney</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Heart</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lung</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
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---(strong) +++ moderate, + mildly positive immunoreactivities

CONCLUSION

In conclusion, although vimentin expression in the present tumor could be somehow misleading, histopathological and immunohistochemical results confirmed diagnosis of renal adenocarcinoma (tubular type) with marked desmoplasia, and metastasized to the heart and lung.

REFERENCES