THE PROFILE OF ANTIOXIDANT SUPEROXIDE DISMUTASE (SOD) IN THE TISSUES OF MACAQUES (*Macacafascicularis*) : AN IMMUNOHISTOCHEMICAL STUDY

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Introduction

Superoxide dismutase (SOD) is one of the free radical scavenger which catalyzes superoxide anion radical generated intracellularly. SOD thus eliminates O_2^- from virtually all types of human and animal tissues and prevents cellular injury caused by free radicals (Halliwell and Gutteridge, 1999).

There are three isoforms of superoxide dismutase (SOD); copper, zinc (Cu,Zn)-SOD, manganese (Mn)-SOD, and iron (Fe)-SOD. It was reported that SOD was immunohistol cytochemically localized in the human and rat tissues (Dobashi *et al.* 1989; Wresdiyati & Makita 1997). SOD was also reported plays important role in physiological processes. Some cases of failed pregnancy in human was caused by the decreasing level of SOD (Sugino et *al.* 2000). Profile of SOD was also reported in pathophysiological condition such as stress, diabetes mellitus and hypercholesterolemia (Wresdiyati et *al.* 2002; Wmsdiyati 2003; Wresdiyati *et al.* 2003; Wresdiyati *et al.* 2006a; Wresdiyati et *al.* 2006b), in neoplastic tissues (Keller *et al.* 1991), and neuron of hypocampus in Alzheimer and Down's syndrome patient (Furuta *et al.* 1995).

It was reported the profile of Cu,Zn-SOD in the liver tissues of diabetic experimental *Macaca fascicularis* (Wresdiyati et al., 2003). However there is e few report on the profile and distribution of SOD in the tissues of *Macaca fascicularis*. The present study was conducted to observe the profile and distribution of Cu,Zn-SOD in the liver, kidney, testis, adrenal gland, heart, lung, and pancreatic tissues of Macaca fascicularis.

Materials and Methods

The liver, kidney, testis, adrenal gland, heart, lung, and pancreatic tissues of Macaca fascicularis were obtained and fixed in Bouin's solution for 24 hr. After fixation, the tissues were then dehydrated in a series of alcohols and cleared in xylol. The tissues were then embedded in paraffin before microtome sectioning (4 μm thickness). Tissue sections were immunohistochemically stained for Cu,Zn-SOD as described previously (Dobashi et *al.* 1989; Wresdiyati et al., 2008) with modifications. The tissue sections were washed for 15 min with 3 changes of PBS between each step. After deparaffinization and rehydration, the tissue sections were exposed to 3% H_2O_2 for 10 min to inactivate endogenous peroxidase activity and then to 10% normal goat serum to block nonspecific binding. Following rinsing with PBS, the tissue sections were incubated with the primary antibody of Cu,Zn-SOD (Sigma S2147) at 4°C. The tissues were then incubated with enhanced labelled polymer peroxidase (Dako K1491). The reaction product of antigen-antibody was visualized using diamino benzidine (DAB). The tissue sections were then counterstained with haematoxylin, followed by dehydrated through a series of alcohols, and cleared with xylol. Finally, the sections were mounted on glass slides with entelan, For the control of staining, tissue sections were incubated with PBS instead of Cu,Zn-SOD antibody. The tissue sections of control staining showed a negative reaction with minimal background staining.

The distribution and frequency of positive reaction product of immunohistochemical staining on the tissues was observed qualitatively by means of light microscopy. It was based on the brown colour intensity in the calls and the distribution of the reaction product.

Results and Discussion

The positive reaction products of immunohistochemically localization to the Cu,Zn-SOD showed brown colour in the observed tissues (Figure 1). The liver, kidney, testis, adrenal gland, heart, lung, and pancreatic tissues of Macaca *fascicularis*

showed contain the enzyme. The nuclear and cytoplasm of hepatocytes showed high content of Cu,Zn-SOD. These findings related to the liver function as a main gate for detoxification. It was well known that the liver produce more free radicals, by redox reactions in mitochondria, peroxisomes, and xenobiotic coumpond detoxification (Langseth, 1995) compared to the others tissues. Kidney tissues also showed high content of Cu, Zn-SOD especially In the proximal tubule cells, while distal tubule cells contain less Cu,Zn-SOD, and there is no Cu,Zn-SOD in the glomerulus. The proximal tubule cells consist more cellular organel peroximes than other part of kidney tissues (Wresdiyati and Makita, 1995), so more redox reaction take place In the cells. The high Cu,Zn-SOD content in the cells may to prevent from all those danger free radical generated in the cells.

In the adrenal tissues. there is different content of Cu,Zn-SOD in each part of the tissues. The highest content showed in the zona reticularis, followed by zona fasciculata, and zona glomerularis, respectively. Medulla area of adrenal gland showed negative content of Cu,Zn-SOD. While testicle tissues showed high content of Cu,Zn-SOD in spermatocytes and early spermatid cells, less content in late spermatid cells, and there is no Cu,Zn-SOD in spermatogia cells. *SOD* activity was also reported in spermatocyte and early spermatid of rat testis (Peltola et al., 1992). Heart tissues especially heart muscles showed high content of Cu,Zn-SOD, but some of muscles showed less content. It may related to the degenerative processing of the cells. Cu,Zn-SOD showed high levels in the Langerhans island of pancreatic tissues, by contrast there is almost no content of the enzyme in the acinar cells both nuclear and cytoplasm. It may related to the function of oxidations in the a, β, δ, and gama cells of Langerhans island. These findings also showed that oxidations may less take place in acinar cells compared to the cells in Langerhans island.

The present study concluded that Cu,Zn-SOD was localized in the liver, kidney, adrenal gland, lung, testis, heart, and pancreatic tissues, except in kidney glomerulus. medulla of adrenal gland, spermatogonia calls, and acinar cells of pancreatic tissues.

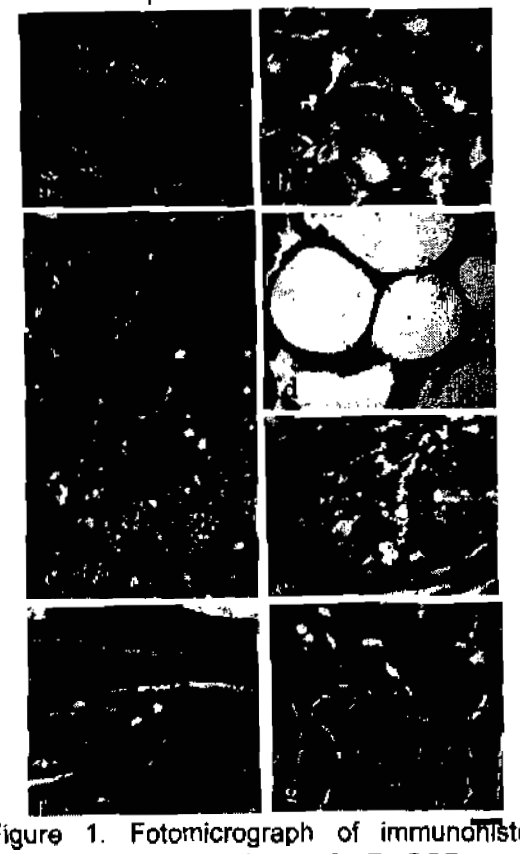

Figure 1. Fotomicrograph of immunohistochemical localization of Cu,Zn-SOD in the (a) liver, (b), kidney, (c) adrenal gland, (d) lung, (e) testis, (f) heart, and (g) pancreatic tissues. Scale = 25 μm

References

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