The Role of Extrinsic Pathway (Death Receptor Pathway) Apoptosis through Caspase-8 in Atherogenesis due to High Fat and High Cholesterol Diet

Yanuartono¹, Hastari Wuryastuti¹, R. Wasito¹, and Sri Raharjo²
¹Faculty of Veterinary Medicine - Gadjah Mada University - Jogjakarta
²Faculty of Agricultural Technology - Gadjah Mada University - Jogjakarta

ABSTRACT

Thirty male rats, strain Sprague Dawley were used as experimental animal to study the role of death receptor pathway apoptosis in atherogenesis due to high fat and high cholesterol diet. The rats were randomly allotted into three group (I, II, III) of 10 each. Group I as control was fed normal diet, group II was fed diet containing high fat diet, and group III was fed containing high fat and high cholesterol diet (atherogenic diet). After 6 and 12 weeks on experimental diet, 15 rats were selected randomly (5 rats of each group). All animal were then killed and the aorta were taken out for caspase-8 immunohistochemical analysis. Based on the present study result it can be concluded that high cholesterol and/or high fat diet induced apoptosis through death receptor pathway via caspase-8.

Key words: Sprague Dawley, apoptosis, caspase-8, death receptor pathway

INTRODUCTION

Apoptosis can be defined as a carefully regulated process, characterized by specific morphologic and biochemical features (Zeiss, 2003). Cells undergoing apoptosis exhibit a series of characteristic morphological changes, including plasma membrane blebbing, cell body shrinkage, and formation of membrane-bound apoptotic bodies, which in vivo are quickly engulfed by neighboring healthy cells (Wyllie et al., 1980; Bennet et al., 1995; Geng et al., 1996). Apoptosis represents a major mechanism by which tissues eliminate unwanted or harmful cells and maintain (Wyllie et al., 1980). During apoptosis, intracellular contents are not released and potentially harmful inflammatory responses are prevented (Chang and Yang, 2000). The recent study demonstrated of high levels of apoptotic cells in atherosclerosis (Geng and Libby, 1995; Han et al., 1995).

Caspase-8/MORT-associated CED-homologue (MACH)/Fas-associated death domain-like IL-1β-converting enzyme (FLICE)/mammalian CED-3 homologue 5 (Mch5) play an essential role in the mediation of apoptosis by several death domain (DD) (Chaudary et al., 1999). Caspase-8 is recruited to these receptors via the interaction of its prodomain with Fas associated death domain (FADD or MORT 1), which leads to formation of the death-inducing signaling complex (DISC) (Chinmaiyan et al., 1995; Boldin et al., 1996). Upon its recruitment to the DISC, caspase-8 is activated by an autoproteolytic mechanism involving the removal of the prodomain and the release of its activated protease subunit into the cytosol. Activated caspase 8 acts as the initiator caspase in the caspase cascade, activation of which eventually

Indonesian Food and Nutrition Progress, 2005 Vol. 12 no. 1
result in cell death (apoptosis) (Chaudary et al., 1999).

Evidence suggesting that pathogenesis of vascular diseases is due to imbalance Evidence suggesting that pathogenesis of vascular diseases is due to imbalance between cell proliferation and apoptosis (Bennet et al., 1995; Gibbon and Dzau, 1994; Bennet and Boyle, 1998; DeBlois et al., 1997). Cell death is a major event occurring during atherosclerotic plaques development both in humans (Thomas et al., 1976; Geng and Libby, 1995; Bjorkerud and Bjorkerud, 1996) and animals (Garrat et al., 1991; Arbustini et al., 1991), accumulating evidence indicates that apoptosis is component of atherosclerotic plaques and its prominent atherosclerotic lesions both in humans (Bjorkerud and Bjorkerud, 1996; Geng et al., 1997; Geng and Libby, 1995; Han et al., 1995; Isner et al., 1995) and animals (Best et al., 1999; Kockx et al., 1996).

Apoptosis, which almost is absent in normal arteries, become barely detectable in fatty streaks and is more abundant in advanced plaque (Mallat and Tedgui, 2000). Apoptosis has been suggested as a prominent atherosclerotic lesions and closely related with unstable and remodeling of atheroma plaques (Libby et al., 1996; Yuan, 1999).

between cell proliferation and apoptosis (Bennet et al., 1995; Gibbon and Dzau, 1994; Bennet and Boyle, 1998; DeBlois et al., 1997). Cell death is a major event occurring during atherosclerotic plaques development both in humans (Geng and Libby, 1995; Bjorkerud and Bjorkerud, 1996) and animals (Garrat et al., 1991; Arbustini et al., 1991), accumulating evidence indicates that apoptosis is component of atherosclerotic plaques and its prominent atherosclerotic lesions both in humans (Bjorkerud and Bjorkerud, 1996; Geng et al., 1997; Geng and Libby, 1995; Han et al., 1995; Isner et al., 1995) and animals (Best et al., 1999; Kockx et al., 1996). Apoptosis, which almost is absent in normal arteries, become barely detectable in fatty streaks and is more abundant in advanced plaque (Mallat and Tedgui, 2000). Apoptosis has been suggested as a prominent atherosclerotic lesions and closely related with unstable and remodeling of atheroma plaques (Libby et al., 1996; Yuan, 1999).

Several studies with both animals and humans, involving in vivo and in vitro assay or administration of dietary lipids, have recently described an important role of several fatty acid in the induction of apoptosis (dePablo et al., 2002). Different mechanisms of action have been proposed in order to explain the action of fatty acids on apoptosis modulation.

Alright free cholesterol is non toxic to the cell, however, high levels of oxidized cholesterol would trigger foam cell death through apoptosis (Kellner-Weibel et al., 1999). Studies in atheroma and advanced atherosclerotic lesions in rabbit aorta due to high cholesterol diets showed the incidence of apoptosis (Kockx et al., 1996, 1998; Lutgens et al., 1999; Harada et al., 1997). Cholesterol and esterified cholesterol itself has a light pro-apoptotic, however, it turn to be cytotoxic after oxidation (Chisolm et al., 1994; Sevanian et al., 1995), and moreover, the Ox-cholesterol could induced apoptosis (Harada-Shiba et al., 1998).

**MATERIALS AND METHODS**

**Preparing and maintaining rats as an experimental animals**

Thirty male Sprague Dawley rats, 100 grams average of body weights and a month of age were used in this research. Before this research began, rats were adapted for a week and were fed basal diet. The rats were then randomly allotted into three groups. Group I as a control was fed normal diet, group II was fed diet containing high fat, and group III was fed diet containing high fat and high cholesterol (atherogenic). The rat had free access to water during the experimental periods. After 6, and 12 weeks on experimental diets, 15 rats were selected randomly (5 rats of each group). All animals were then killed and the heart was taken out for immunohistochemical analyses.

**Imunohistochemical analysis for caspase-8**

Immunohistochemistry was carried out on 5 μm section of formalin-fixed paraffin-embedded
tissue using streptavidin-biotin technique. The technique was divided into 4 steps, (1) the tissue sections were deparaffinized and rehydrated (2) washed with H₂O₂ to remove endogenous peroxidase, and the incubated in microwave for 10 minutes. After washed with phosphate buffer saline (PBS) for 10 minutes, tissue sections were incubated with blocking serum (Santa Cruz, Biotechnology, USA) solution for 10 minutes. Removes excess serum from tissue section, and applied primary antibody (antibody anti-caspase-8) (BioVision Research Products) without washing, followed by incubation at room temperature for 45 minutes, then washed with phosphate buffer saline (PBS) for 10 minutes, (3) tissue section were incubated with biotinylated secondary antibody (Santa Cruz, Biotechnology, USA) at room temperature for 10 minutes, washed with PBS for 10 minutes, and incubated with streptavidin-peroxidase conjugate for 5 minutes. After washed with PBS for 10 minutes, tissue sections were incubated with 3,3' diaminobenzidin (Santa Cruz, Biotechnology, USA) solution for 15 minutes, washed with aquadest for 10 minutes, and (4) applied counterstain hematoxyline-eosin (Zymed Laboratory Inc, Carlton Court, San Francisco, USA) for 3 minutes, washed with aquadest and applied mounting medium Mayer’s egg albumin (Zymed Laboratory Inc, Carlton Court, San Francisco, USA) for microscopic examination (Microscope digital camera system, Olympus DP 12) (Wasito, 1997; Trieb et al., 2003)

**RESULTS AND DISCUSSION**

The result after 6 and 12 weeks on experimental diets on immunohistochemical analysis of caspase-8 are presented on table 1.

Immunohistochemical analysis of aorta showed positive result of caspase-8 on the rats fed high fat diet (group II) and fed high fat and high cholesterol (group III). Caspase-8 were found on atheromatous plaque rats number 7 and 8 (high fat diet for 6 weeks) (fig. 1), rats number 24 and 25 (high fat diet for 12 weeks) (fig. 2), rats number 14, 15

<table>
<thead>
<tr>
<th>Group</th>
<th>Time periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No 6 weeks</td>
</tr>
<tr>
<td>Group I</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>Group I</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>Group I</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>Group I</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>Group I</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>Group II</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>Group II</td>
<td>Positive (+)</td>
</tr>
<tr>
<td>Group II</td>
<td>Positive (+)</td>
</tr>
<tr>
<td>Group II</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>Group II</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>Group III</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>Group III</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>Group III</td>
<td>Positive (+)</td>
</tr>
<tr>
<td>Group III</td>
<td>Positive (+)</td>
</tr>
</tbody>
</table>

(high fat and high cholesterol diet for 6 weeks) and rats number 27, 28, 29 (high fat and high cholesterol diet for 12 weeks) (fig. 3).

The finding caspase-8 indicate that apoptotic pathway in this research through extrinsic pathway (death receptor pathway), and may caused by high fat and high cholesterol diet. The increased of cholesterol concentration may caused apoptosis through caspase-8 as an initiator caspase. Although free cholesterol is non toxic to the cell, however, high levels of oxidized cholesterol would trigger

*Indonesian Food and Nutrition Progress, 2005 Vol. 12 no. 1*
foam cell death through apoptosis (Kellnér-Weibel et al., 1999). Studies in atheroma and advanced atherosclerotic lesions in rabbit aorta due to high cholesterol diets showed the incidence of apoptosis (Kockx et al., 1996, 1998; Lutgens et al., 1999; Harada et al., 1997). Cholesterol and esterified cholesterol itself has a light pro-apoptotic, however, it turn to be cytotoxic after oxidation (Chisolm et al., 1994; Sevian et al., 1995), and moreover, the Ox-cholesterol could induced apoptosis (Harada-Shiba et al., 1998).

Fig. 1. Aorta from Sprague dawley rats fed high fat diet 6 weeks on experimental diet. Colored brown of caspase-8 was observed (A) in atheromatosus plaques (SB staining, 500 X.).

Fig. 2. Aorta from Sprague dawley rats fed high fat diet 12 weeks on experimental diet. Colored brown of caspase-8 was observed (A) in atheromatosus plaques (SB staining, 250 X)

Fig. 3. Aorta from Sprague dawley rats fed high fat and high cholesterol diet 12 weeks on experimental diet. Colored brown of caspase-8 was observed (A) in atheromatosus plaques (SB staining, 500 X)

Apoptotic signal from extrinsic pathway in this research probably from interaction between Fas and Fas ligand. Fas and Fas ligand would expressed on arterial tissue, including in atherosclerotic plaque (Geng et al., 1997; Cai et al., 1997; Geng et al., 1998). According to Schneider et al. (2000), overexpressed of Fas ligand in arterial from hypercholesterolemic rabbit could accelerated the formation of atherosclerotic lesion.

This results similar with previous studies that 7α-hydroxycholesterol (7α-OH) and 25-hydroxycholesterol (25-OH) could induced apoptosis via extrinsic pathway through interaction between Fas and Fas Ligand (Lee and Chau., 2001; Geng et al., 1997). Another possibility of extrinsic pathway in this study may through interaction between tumor necrosis factor-receptor 1 (TNF-R1) and TNF-related apoptosis inducing ligand (TRAIL). The interaction between tumor necrosis factor-receptor 1 (TNF-R1) and TNF-related apoptosis inducing ligand (TRAIL) then activated caspase-8 to initiate apoptosis. This result is supported by Varfolomeev and Ashkenazi (2004) and Micheau and Tschoop (2003), showed that TNF-R1 is a signal of extrinsic pathway to activated caspase-8. The result also supported the previous studies that oxysterol could induced apoptosis through intrinsic pathway. Migue-Alfonsi et al.
(2002) have found that 7α-hydroxycholesterol (7α-OH) and ketchuposterone could induce apoptosis in vitro through loss of mitochondrial potential membrane. The loss of mitochondrial potential membrane followed by the release of cytochrome c (Leonarduzzi et al., 2004; Seye et al., 2004).

CONCLUSION

Based on the results of study, it can be concluded that high cholesterol and/or high fat diet induced apoptosis through death receptor pathway via caspase-8.

ACKNOWLEDGEMENTS

The author would like to thank to Mr Dhirgo Adji for their co-assistance, Mr Yuly, Mr Dalio for their technical assistance. This research was funded by BPPS, Directorate General of Higher Education, Department of Education and Culture.

REFERENCES


Bjorkerud, S., and Bjorkerud, B. 1996. Apoptosis is abundant in human atherosclerotic lesions, especially in inflammatory cells (macrophages and T cells), and may contribute to the accumulation of gruel and plaque instability. Am. J. Pathol. 149: 367-380.


Miguet-Alfonsi, C., Prunet, C. and Monier, S. 2002. Analysis of Oxidative Processes and of Myelin Figure Formation Before and After the Loss of Mitochondria Transmembrane Potential During 7α-Hydroxycholesterol (7α-OH) and 7-Ketocholesterol-Induced Apoptosis: Comparison with Various Pro-Apoptotic Chemicals. Biochem. Pharmacol. 64: 527-541.


