International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.8, No.6, pp 599-602, 2015

Time response to Cytochrome C, Caspace 8 and PARP-1 Expression of Traumatic Brain Injury (TBI) in Rats *(Rattus norvegicus)* Model

Imam Hidayat[1,] *, RullyRoesli[2], ZafrullahArifin[3], Aulanni'am Aulanni'am[4]

[1]General Surgery University of Syiah Kuala, Banda Ace, Indonesia
[2,3] Medical Faculty, PadjajaranUniversity Bandung, West Java, Indonesia
[4] Faculty of Sciences, BrawijayaUniversity Malang East Java, Indonesia

Abstract: Cytochrome C, Caspase-8 and Poly (ADP-ribose) polymerase (PARP) are essentials proteases of the extrinsic and intrinsicapoptotic pathways. Understanding the proteases expression profile and its relationship to time period of cerebral damage post traumatic brain injury (TBI) become an important step in managing TBI. The aimed of this research was to evaluate the response of family protease enzymes CytochomeC and PRP (intrinsic pathway) and Caspase8(extrinsic pathway) afterTBI on rats. Rats were devided into 4 groups : observation of TBI after1h, 3 h, 6 h and 12 h. This study analyzed the expression of Cytochrome C, Caspase-8 and PARP byImmunohistochemistry technique. The results showed Cytocthe increasing of Cytochrome C tobe25%, 60%, 80% and 80%for 1 h, 3 h, 6 h and 12h after TBI, respectively. Meanwhile the Caspase-8 increased up to25%, 40%, 50% and 65% and PARP increases up to 40%, 65%, 78% and 85% in 1 h, 3 h, 6 h and 12 h after TBI, respectively. This findingsshowed traumatic brain injury (TBI) resulted different responses in proteases as an apoptotic marker. The study also showed that expression of Cytochrome C and PARP (intrinsic pathway) higher compared by Caspase-8 expression (intrinsic pathway).
Keywords: Traumatic brain injury, apoptosis, Cytochrome C, Caspase-8, PARP.

Introduction

The incidence Traumatic Brain Injury (TBI) increase with development of transportation. The more opportunity of the traffic flow lead to increasingly high incidence of traffic accidents, which are common causes to TBI.Some studies estimate that more than 80.000 individuals become disabled from TBI each year. TBI contributed greatly to morbidity, neuropsyachiaric and disability[1,2].The mechanisms of neural injury associated with TBI have been categorized as primary and secondary. Primary TBI occur at the time of the impact, while secondary TBI occur minutes to days after the injury and are broadly the consequences of the response [3].

Interestingly, distinct modes of cell death dominate these two phases. In particular, though the main cell death event observed immediately after impact is necrosis, the second phase of cellular death is mainly because of apoptosis. Apoptotic cell death can be triggered by a variety of molecular signals. More specifically, apoptosis can be induced through mitochondrial (intrinsic) or the receptor-mediated (extrinsic) pathways. Both pathways lead to activation of proteases which have a critical role in brain cell death [4]

The proteases which are present of a multitude of cell death pathways which have both overlapping and

distinct molecular mechanisms induced neuronal cell death include cytochrome c, caspase-8 and Poly (ADP-ribose) polymerase (PARP). These proteases known as apoptotic marker [5].

This study analyzed changes in the expression of these proteases; Cytochrome c, Caspase-8 and PARP and its relationship to time period of cerebral damage post traumatic brain injury.

Materials and Methods

Rats. Three to four months old rats (*Ratusnorvegicus*) were selected. There were 8 rats which were classified in four different groups; 1 h, 3 h, 6 h and 12 h after traumatic brain injury (TBI).Rats were anesthetized with ketamine. The head was shaved and cleaned with 10% povidone-iodine. Aseptic technique adjusted to the surgical procedures. Craniectomy performed with high speed drill with 5 – 7 mm diameters. The animal used in this research was approved by Animal Used Ethic Committee, Brawijaya University

Immunohistochemistry.Brain tissueswere immersed in 3% hydrogen peroxide, washed with pH 7.4of Phosphate Buffer Saline (PBS) for 3 times, 5 min. The following antibodies were used were rabbit anti rat of PARP, caspase-8 and of cytochrome C. These antibodies were added on glass slide, incubated at 4°C overnight, washed with PBS for 3 times for each of 3 min, and added with Strep Avidin-Horseradish Peroxide (SA-HRP) for 10-20 min and washed with PBS for 3 times, for each of 3 min. Chomogen DAB (3,3-diaminobenzidine tetrahydrochloride) were added and incubated for 5-10 min in room temperature, followed by washing with PBS. Counterstained was done with methylene blue for 5 minutes in room temperature, washed and dried. The tissues analyzed microscopically.

Results

The expression of Cytochrome c, Caspase 8 and PARP-1 increase after administration traumatic brain injuries (TBI) in different times treated(Table 1, Fig.1)).The strong levelexpression of CytochromeC occurred at 3 h after TBI, while Caspase-8 and PARP at 1 h after TBI already expressed moderately and high expression at 3,6,and 12 h after TBI (Table 1). The category of level expression in 3 levels with value of category ie : >20% isweak,>20-50ismoderateand>50is astrong level.

This study showed proteases expression depend on the longer time after TBI. It also showed expression of Caspase-8 slow than cytochrome c and PARP-1.

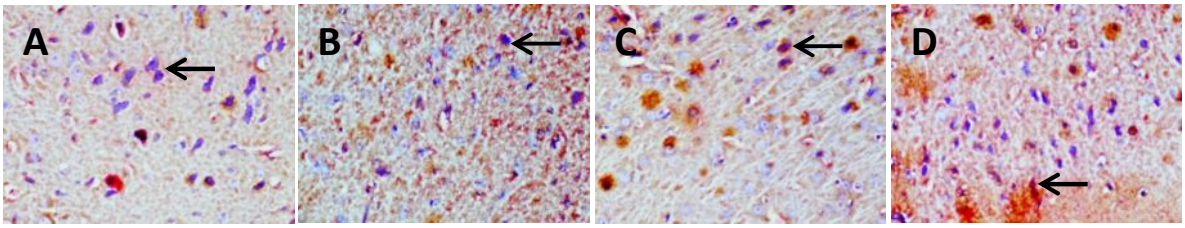

Figure1. Immunohistochemistry result for caspase-8 (fast red/red) co-staining with Cytochrome-C (DAB/brown).
A: 1 h after TBI, B: 3 h after TBI, C: 6 h after TBI, D: 12 h after TBI.

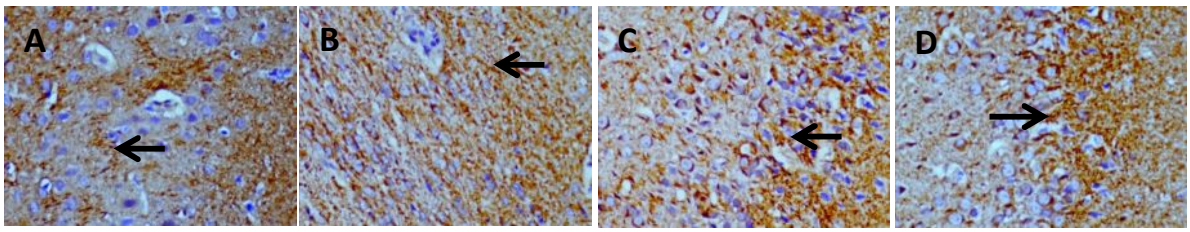

Figure2.Immunohistochemistry result for PARP (DAB/brown).
A: 1 h after TBI, B: 3 h after TBI, C: 6 h after TBI, D: 12 h after TBI.

Table 1. Expression Cytochrome c, Caspase 8 and PARP-1 by Immunohistochemistry Technique

Indicators (expression	Groups (hours after TBI)			
	1	3	6	12
Cytochrome c	25%	60%	80%	80%
Caspase-8	25%	40%	50%	65%
PARP-1	40%	65%	78%	85%

*<20% weak, 20-50% moderate, >50% strong

Discussions

Traumatic Brain Injury(TBI)is defined as abrain injury caused by collisons that occur directly or indirectly on the head, causing wounds in the scalp, skull fracture, tear the lining of the brain, and the brain tissue damage that causes neurogical disorders. TBI in human caused by a severe hit in the head. Some experimental models have built to initiate pathogenic characteristic different from TBI. Thre are two main kinds of TBI, that are closed and permeable TBI. Based on the use of permeable TBI model, this study characterized the destruction probability other than in the cirtex area. The central neural system consist of the neuron and glial cells. Neuron send the information to all parts of the body in from of electrical signals through the axon and release the neurotransmitter in the synaps. Glia are composed of various cells which have the function to maintain and protect the neuron. Astrocytes and oligodenrocytes are the two main cells composing glia. Oligodendrocytes produce myelin, a membrane which is rich of lipid, to wrap axon. The function of myelin is to reduce the signal capasitance passing the membrane, and to increase the rate of electrical conduction in the axon. Oligodendocytes are extending the length of myelin by some bump, constructing the segments of meylin. Myelin membrane has a lot of lipid content and cholesterol as the main elements. Rats having defect in cholesterol synthesis showed retardation of myelinization [6,7]. The interesting toic in TBI research is the patophysiology process after traumatic condition or tissue recovery and neuronal cell regeration.

The result showed an increase in expression of Cytochrome c, Caspase 8 and PARP-1 is directly proportional to the length of time TBI. Increased expression of PARP-1 was a sign of apoptosis. Excessive activation of PARP-1caused DNA damage and apoptosis. Previous study had shown that PARP is an apoptotic marker which can lead to severe depletion of cellular energy and increase the progressivity of the neural death [5]. So far there has been no single chemical compound that is able to effectively inhibit or prevent the whole process of secondary injury. This is due to still not know the mechanism of the body's response to the TBI at the cellular level fundamentally [6,7].

This apoptotic pathway interfere CD95 protein or reseptor (TNF receptor family) with fas ligand, namely TNF-receptor Apoptotic Inducing Ligand(TRAIL) which lead to apoptosis extrinsic parhway. In the beginning, ischaemic injury in TBI caused by energy failure, the increase of intracellular calcium, and the release of amino acid with exitotoxicity. This process will activate ischaemic failure mediators, including free radicals production, peroxynitrite, calpain, phospholipases, and PARP. Simultaneous with the beginning of apoptotic pathway, the inflammation gives the contribution to the development of tissue failure. Secondary phase of cell death may include the long term changes in macromolecules and the other important metabolite. The whole process may become a potential target to therapeutic intervention.

Apoptotic can be initiated by internal events (intrinsic pathway) involving the disruption of mitochondria and the release of cytochrome c, which leads to do downstream activation of caspaces. Apoptotic process that occurs through the intrinsic pathway involve the mitochondria. All these processes will cause oxidative stress, triggering the mitochondrial damage through increases cytochrome c expression in cytosol as an early sign of the onset of apoptosis in the intrinsic pathway. The release of Cytochrome C is an early sign of the caspase activity until the process of apoptosis or cell necrosis.

Result of this study showed that expression of CytochromeC, Caspase 8 and PARP-1 increase after administration traumatic brain injuries in different times treated. Cytochrome C is an apoptotic factors released by mitochondria, which serves as one of the molecule that play a role in the pathogenesis of cell death. Caspase-8, which is an apical protease in the extrinsic apoptotic pathway, activated at the plasma membrane by various TNF-family death receptors. Stimulation of these receptor by proapoptotic ligands causes receptor clustering followed by the recruitment of Fas-associated Death domain Protein(FADD), which is turn binds

Death Effectors Domains (DEDs) in the perform of caspase-8, a protease that then cleaves and activates downstream effectorscaspaces responsible apoptosis [7,9]. This seems that extrinsic pathway needs lots of way to damage the neuronal than intrinsic pathway. We suggested that this is why in our study caspase-8 expression change slower than the cytochrome c and PARP which are include in intrinsic pathway. For that we need to do further research on the potency of Δ-9-THC as neuroptotective which can serves as neuroprotectan that protect brain cell damage due to ischemia cascade which gives satisfactory results.

Conclusion

It has become increasingly clear that there are many complex signaling pathway involved in apoptosis after TBI, that confirmed by expression of cytochrome C, caspase-8 and PARP-1. Although some specific molecular pathways involved have been described, but still needed an integrated understanding of the molecular processes that contribute to the onset of apoptosis to obtain the relevant therapy

References

1. Bigler, ED. Traumatic Brain Injury. 2009. In: The American Psychiatric Publishing textbook of Alzheimer Disease and Other Dementias. USA: American Psychiatric Publisher. 2009. pp. 229-246.
2. Critchley G & Memon, J. 2009. Epidemiology head injury. Dalam Whitfield,PC., Thomas,EO., Summers, F., Whyte M., dan Hutchinson, PJ.. (ed.)."*Head Injury: A Multidisciplinary Approach* "Selected Reading, hlm. 1-11. London: Cambridge University Press.
3. Risdall JE, Menon DK.2011. Traumatic Brain Injury. *Phil Trans R Soc B*. 2011; 360: 241-250.
4. Umschwief G, Snein NA, Alexanravich AG, Trembovler V, Horowitz M, Shohami E. Heat Acclimation Provides Sustained Improvement in Functional Recovery and Attenuates Apoptosis After Traumatic Brain Injury. *Journal of Cerebral Blood Flow and Metabolism*. 2010; 30: 616-627.
5. Stoica BA, Faden AI. 2010. Cell Death Mechanism and Modulation in Traumatic Brain Injury. *Neurotherapeutics*. 2010; 7(1): 3-12.
6. Warner, C & Engelhard, K. 2007. *Phathophysiology of traumatic brain injury*. British Journal of Anaesthesia 99 (1): 4-9
7. Murthy TVSP BP, Sandhu K, Prabhakar T, Gogna RL. 2005. *Secondary brain injury: prevention and intensive care management.* IJNT. 2(1).
8. Broughton BRS, Reutens DC, Sobey CG. 2009. Apoptotic Mechanisms After Cerebral Ischemia. *Stroke*. 2009; 40: e331-e339.
9. Micheau O, Tschopp J.2003. Induction of TNF Receptors I-mediated Apoptosis via Two Sequential Signaling Complexes. *Cell*. 2003; 144: 181-190.

*****