ORIGINAL ARTICLE

Comparisons of Caspase-3 and Caspase-7 Expression from Retinal Ganglion Cells Apoptosis Post Folic Acid and Methylcobalamine Administrations In Methanol Toxic Wistar Rats Models

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ABSTRACT

Aim: To analyse the differences of caspase-3 and caspase-7 expression from retinal ganglion cells apopotosis post folic acid and methylcobalamine administrations in methanol toxic wistar rats model.
Methods: Two group of methanol toxic wistar rats models were divided into group 1 which was administrated with oral 1mg/kgBW folic acid and group 2 which was given intraperitoneal 2500 µg/kgB methylcobalamine. Two control group were included to measure caspase-3 and caspase-7 expression after methanol intoxication. Each group consist of 7 wistar rats. Caspase-3 and caspase-7 expression were assessed using immunohistochemical examination. The difference of caspase-3 expression was assessed using One Way Annova and the difference of caspase-7 expression was assessed using Kruskal Wallis.
Results: Mean value of caspase-3 and caspase-7 expression from group 1 were 6.40 and 6.50, from group 2 were 6.64 and 6.96. When it compared with control, caspase-3, caspase 7 expression in group 1 and caspase-3 expression was not significantly different (p=0.30, p=0.86, p=1.00). While caspase-7 expression in group 2 was significantly different (p=0.04), the value is higher than control.
Conclusion: Folic acid administration reduce the expression of caspase-3 and caspase-7 in methanol toxic wistar rats models. While methylcobalamine admisinistration showed same value in caspase-3 expression and higher caspase-7 expression when it compared with control.
Keywords: Methanol toxicity, caspase-3, caspase-7, folic acid, methylcobalamine

INTRODUCTION

The cases of methanol poisoningare increased along with the increasing of alcoholic beverage taxes. The inability of consumers to buy authentic alcoholic beverages causes many producers of homemade beverages exist. Alcoholic beverages mixed with other ingredients, one of them is methanol. Methanol is often used as a mixture of homemade beverage because of its cheap price. Methanol intoxication is intoxication due to methanol consumption resulting in the formation of acidemia, uncompensated metabolic acidosis, visual impairment, coma and even death[1-4].

The case of methanol intoxication began to appear in America in 1904, with the publication of Wood and Buller's report of 153 cases of blindness caused by methyl alcohol intoxication. World Health Organization (WHO) data in 2011, 2.5 million people around the world died from methanol and 9% death occurs at a young age (15-29 years). The occurrence of methanol intoxication were mostly through oral, consumed as a mixture of alcoholic beverages such as lapen or energy drinks[5].

The abuse of methanol occurring in Indonesia by WHO (WHO SEARO, 2012) from year to year is as follows: from 1999 to 2000, 58% of the crime rate occurred in West Nusa Tenggara due to the influence of liquor. In 2001 there were 39 deaths in adolescence because Hepatitis B is closely related to the impact of methanol consumption occurred in Bali. In 2002 there were 50% of total 65 cases of methanol poisoning died in Manado and Minahasa, in 2008 recorded more than 40 deaths due to alcohol poisoning in Kendari, in 2009 in Surabaya 9 people died in three different locations after consuming homemade beverage. In 2010, 3 shukoi technicians were death in Makasar due to drinking homemade liquor[3,5,6].

Methanol intoxication is caused by the oxidation of methanol by the enzyme dehydrogenase of alcohol to formaldehyde, and subsequently metabolized into formic acid by formaldehyde dehydrogenase. The acid accumulation of this formic acid is toxic metabolites that cause hypoxic toxicity by inhibiting cytochrome c oxidase. Cytochrome c oxidase is a heme protein that acts as an electron carrierwater-soluble in mitochondrial oxidative phosphorylation process. This protein will be released from the mitochondria in response to apoptotic signals. The cytochrome c oxidase that comes out into the cytoplasm then binds to Apaf-1 form CARD (Caspase Recruitment Domain).Some CARDs combine to form apoptosomes and then bind to pro-caspase-9 and activate it into caspase-9 (caspase initiator). Caspase-9 this will activate procaspase-3 into caspase-3 which is an effector caspase carry out apoptosis. Caspase 3 and 7 are apoptotic caspases executor that play a critical role in apoptosis[7,8].

Currently, oral and intravenous corticosteroids are given as methanol intoxication therapy and are able to significantly improve vision, but the extent of corticosteroid side effects encourages the study of alternative therapies. The selection of other therapies that is the administration of folic acid and methylcobalamin that will increase the metabolism of formic acid so that the formic acid content in the blood decrease[9,10].

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MATERIALS AND METHODS

This research is a laboratory experimental test with a post-test only randomized controlled group design using a methanol-intoxicationWistar rat. The rat was then given folic acid and methylcobalamine therapy and as experimental units were caspase -3 and caspase-7 expression in retinal ganglion cell apoptosis.

The research was conducted in Biology Laboratory of Faculty of Mathematics and Natural Science (MIPA) State University of Semarang (UNNES) for maintenance and animal treatment. While preparation and immunohistochemical examination were conducted at the Anatomy Pathology Laboratory, Faculty of Medicine, Diponegoro University/ Dr. Kariadi Hospital, Semarang and Waspada Diagnostic LaboratorySemarang. Data collection began at December 2016 to March 2017.

The samples of this study were Wistar rats which had been intoxicated with25% methanol, inclusion criteria were as follows: the heredity factor of rats derived from rat that are genetically same, age selected 6-8 weeks old, male sex, 200-300 gram weight. The sample size for each group was 7 rats. Prior to use in the study all rats were adapted for 1 week in the same environment. During the adaptation period rats were fed and drunk in ad libitum.

All rats were intoxicated with 25% wt/vol methanol in saline with 4 g/KgBW intraperiotenal dose, followed by 2 g/KgBW additional dose at 24 and 48 hours. Then performed randomization and divided into 2 groups. Group 1 was given oral folic acid therapy at a dose of 1 mg/KgBW for 3 days. Group 2 was given methylcobalamine therapy at a dose of 2500 µg/KgBB, daily frequency of 1 time for 3 days intraperitoneally.Two control group were included to measure caspase-3 and caspase-7 expression after methanol intoxication.

After 3 days of therapy, all rats were undergone euthanasia using 10 mg/kgBW pentobarbital intravenous injection, then terminated with cervical dislocation technique and subsequently enucleated. After the enucleation, the eyeball is fixed with 10% formalin buffers, the cornea and lens are separated. The retinal preparation is obtained by a longitudinal retinal slice technique on the optic disc to the peripheral retina within a radius of 2 mm from the optic disc.

Caspase 3 and 7 expression of retinal ganglion cells was examined by immunohistochemical examination by using Bioss polyclonal antibody. Caspase 3 and 7 expression was calculated by using Allred score.

The difference analysis of caspase expression 3 used Oneway Annova test while for caspase 7 expression using Kruskal Wallis test.

This study has received approval from the Medical Research Ethics Commission of the Faculty of Medicine of Diponegoro University in accordance with the recommendations of the Association for Research and Vision in Ophthalmology (ARVO).

RESULTS

The mean value of caspase-3 expression in group 1 was 6.40 while in group 2 was 6.64. Caspase-7 expression in group 1 was 6.50, while in group 2 was 6.96.When it is compared with control, expression of cascpase-3 and caspase-7 in group 1 or group which was administrated with folic acid therapy was found to be lower but not significant (p> 0.05).

In group 2 or group which was administrated with methylcobalamine therapy, caspase-3 expression was found to be the same result when compared with control, while the value of caspase-7 expression showed higher result, and was significantly different (p <0.05).

Table 1.Expression different test of caspase-3 and 7 between groups

	Control	Folic Acid	p
Caspase-3	6.70	6.40	0.30[1]
Caspase-7	6.70	6.50	0.86[2]
	Control	Methylcobalamine	
Caspase-3	6.64	6.64	1.00[1]
Caspase-7	6.40	6.96	0.04[2*]

[1]One Way Annova [2]Kruskal-Wallis *significant p<0.05

Fig..1 Immunohistochemical examination of Caspase-3 and Caspase-7 Express in in Control group (A) Folic acid therapy group (B)

Group A

Group B

DISCUSSION

Expression of caspase-3 and caspase-7 increased in the methanol intoxication group because in the body methanol was oxidized to formaldehyde by alcohol dehydrogenase and immediately split into formic acid. Formic acid causes hypoxic toxicity through cytochrome c oxidase. On histopathologic examination, swelling and mitochondrial damage to the retina were observed. The opening of the outer membrane of the mitochondria causes the release of cytochrome c and caspase activity including caspase-3 and caspase-7. Caspase-3 and caspase-7 are apical caspases in apoptosis. There after caspase contributes to cell death by degradation of enzymes that repair DNA and structural elements[11,12].

Mitochondrial damage caused by methanol intoxication will create apoptotic signals and then activate procaspase-3 into caspase-3 which is the effector caspase that carries out apoptosis.Caspase is a group of enzymes

that have a major role in apoptosis and can be used as an indicator of apoptosis in a cell. This was supported by previous research by Tezel Gulgun et al (2004), mentioned that increased apoptosis and caspase-3 activity in retinal ganglion due to exposure of TNF alpha and hypoxia for 48 hours in methanolintoxication rats. Similar studies have also been conducted by Chaudary P et al (1999) who found the involvement of caspase in retinal ganglion cell death by the optic nerve axotomy and N-methyl-D-aspartate. Other studies by Kurokawa et al (1999) found that the involvement of caspase 1,2,3, and 8 by induction of retinal ischaemia[13-15].

Observations in the folic acid treatment group showed lowerexpression of caspase-3 and caspase-7compared with control group. This is according to previous research by Seskoati Prayitnaningsih, comparing folic acid with ginkgo biloba extract, and methyl cobalamin, resulted in a significant decrease in apoptotic index after administration of folic acid compared with the control group and other treatments which was using the TUNEL assay method to assess the apoptotic index.[16]Another study by Shahriari (2005) demonstrated the effect of giving folic acid and vitamin B12 to β-ERG waves on the intoxication methanol rabbit retinas. This study showed that acombination of vitamin B12 and folic acid can prevent retinopathy caused by methanol[17].

In the methylcobalamin treatment group there was no decrease in caspase-3 expression, which was not statistically significant (p=0.19) but an increase in caspase-7 expression in that group (p=0.04). The results of this study differ from Shahriari et al research which showed the decreased ofβ wave amplitude of ERG rabbit due to methylcobalamine effect. This study did not assess β wave of ERG. The results of this study contradict the results of in-vitro studies of Hemendinger et al who investigated cell deaths because homosisteine which mentioned methylcobalamin decreased caspase-3 and caspase-7[17,18].

The results of this study also contradicts with the results of research Seskoati et al who got the results of caspase-3 decrease in the provision of metilkobalamine 500 µg/kg BW.

The cause of increased caspase-7 expression in this study might be related to the administration of ultra high doses of methylcobalamine. Ultra high doses in humans are declared safe, non-toxic, minimal side effects, but caution should be given to conditions of renal failure. Methanol intoxication can lead to systemic complications such as metabolic acidosis and renal failure. In this study, the rats in the intoxication of methanol was not assessed the condition of its renal function. The condition of renal failure can lead to accumulation of methylcobalamine dose. The administration of methylcobalamine with ultra high doses in rats in methanol intoxication should be investigated further whether causing toxicity or not.

The limitation of this study is that the determination of methylcobalamine dose had not been adjusted to the specific conditions of methanol intoxication, the length of treatment is too short to assess the decrease in caspase activity. Suggestions for further research can be done by giving treatment for 7 days or 1 month.

CONCLUSION

The administration of folic acid therapy could decrease caspase-3 and caspase-7 expressions in the methanol toxicity rat model, although the results were not significantly different when compared with the control groups. However, this study can be used as a preliminary study to find alternative therapies in cases of methanol intoxication.

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