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Research Article

Hemolysis of human blood cells induced by gasohol *in vitro* model: the preliminary study

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ABSTRACT

Background: Ethanol has been shown to inhibit spontaneous cell-mediated cytotoxicity and antibody-dependent cell-mediated cytotoxicity *in vitro* in a dose-dependent manner. A mixture of ethanol in biofuel may show interaction with respect to spontaneous and cell-mediated cytotoxicity.
Methods: The cytotoxicity on Human Red Blood Cells (RBCs) and White Blood Cells (WBCs) was studied in vitro model of 4 types of biofuels (gasohol) (octane 91, octane 95, E 20, and E 80) in serial dilutions. The lysis and abnormal morphology of cells were analyzed by colorimetric and microscopic methods.
Results: Quantitative hemolysis of RBCs was increased with rising ethanol concentration as well as abnormal morphology of RBCs like spherocyte. Moreover, ethanol might effect to lysis and morphology of WBCs.
Conclusion: It is suggested that the gasohol-induced cytotoxicity may be related to concentration of ethanol in gasohol. However, it is possible to future study on mechanism of action leading to cell lysis and kinetic of morphological change in RBCs and WBCs.

Keywords: Hemolysis, Cytotoxicity, *In vitro* model, Gasohol

INTRODUCTION

Gasohol, oxygenated gasoline, is a fuel consisting of a blend usually of ethanol and gasoline which used as an alternative fuel for gasoline. There are variety types of gasohol which depend on percentage of mixing ethanol such as gasohol 91, 95, E20, and E85. Gasohol with higher content of ethanol has more volatility than gasohol with lower ethanol content. Consequently, benzene emissions in unleaded and gasohol vapors may be increased.[1]

These higher benzene and ethanol emission levels may be an indication that volatility increases in toxicity. There is rapidly increasing of gasohol consume in Thailand, especially in big city like Bangkok, following by the policy of ministry of energy since 2009. Therefore, people can easily expose to volatile components. Previous study showed the cytotoxicity of gasohol in an animal model;[2] however, a toxicological study in either human model or human blood cells was lacked. Therefore, this study aimed to compare human cytotoxicity of gasohol on blood cells *in vitro* model.

METHODS

Gasohol dilutions

The four types of gasohols including octane 95, octane 91, E20, and E85 were collected from gasoline stations in Bangkok, Thailand. All of them were diluted with pool human plasma for 6 dilutions of 1:10, 1:20, 1:40, 1:80, 1:160, and 1:320, respectively.

Cytotoxicity assay

Blood sample was collected from healthy man by heparinized vacuum tube. Briefly, 500 µl of blood samples (5% cell suspension of packed red cell group O) were incubated with each 500 µl of gasohol dilutions and incubated at 37°C for 30 min. Then, cell suspension were centrifuged at 3500 x rpm for 5 min and measured percentage of hemolysis by measurement optical density of supernatant at 540 nm which pool human serum was as blank by Biomate 3S UV-Visible spectrophotometer (Thermo Fisher Scientific, USA). The cell precipitate was smeared by Wright stain for RBCs and WBCs morphology and cell lysis by light microscopic method (Nikon Eclipse E200, Japan) and compared to control.

RESULTS

The hemolysis of RBCs showed trend of rising with increasing ethanol content in gasohol 95, 91, E 20, and E 85 which their hemolysis were 34.3%, 30.4%, 44.9%, and 98.5%, respectively (Table 1). The hemolysis of RBCs was decreasing in increasing gasohol dilution (Table 1 and Figure 1). Both gasohol 95 and 91 have 10% of ethanol content with octane number 95 and 91 while gasohol E 20 and E 85 have 20% and 85% of ethanol content. Gasohol E 85 has more strongly effected to RBC hemolysis than that of E 20 while gasohol 95 and 91 were nearly affected. It may be caused of ethanol content in gasohol.

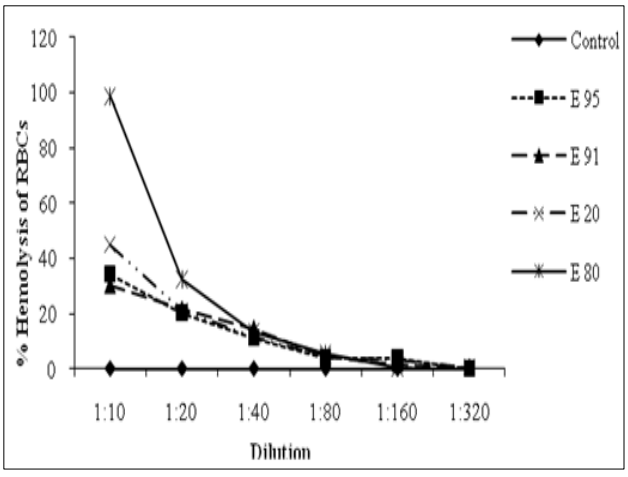

Figure 1: Percentage of RBC hemolysis at serial dilution of gasohol.

Table 1: Parameters of cytotoxicity and serial dilution of gasohols.

Parameter	Gasohol dilution					
	1:10	**1:20**	**1:40**	**1:80**	**1:160**	**1:320**
RBC hemolysis (%)						
Control	0.0	0.0	0.0	0.0	0.0	0.0
Gasohol 95	34.3	20.2	11.3	3.9	3.7	0.05
Gasohol 91	30.4	21.7	14.7	4.4	1.6	0.6
E 20	44.9	21.0	11.5	4.6	3.4	0.7
E 85	98.5	32.5	13.5	5.6	0.3	0.0
Abnormal RBC morphology*(%)						
Control	0.0	0.0	0.0	0.0	0.0	0.0
Gasohol 95	All lysed	All lysed	50.0	0.0	0.0	0.0
Gasohol 91	All lysed	All lysed	100.0	40.0	0.0	0.0
E 20	All lysed	All lysed	100.0	20.0	0.0	0.0
E 85	All lysed	All lysed	100.0	40.0	0.0	0.0
Lysis of WBC (%)						
Control	0.0	0.0	0.0	0.0	0.0	0.0
Gasohol 95	100.0	99.0	76.0	0.0	0.0	0.0
Neutrophil	52.0	77.0	65.0	0.0	0.0	0.0
Monocyte	-	1.0	1.0	0.0	0.0	0.0
Lymphocyte	48.0	21.0	10.0	0.0	0.0	0.0
Basophil	-	-	-	-	-	-
Gasohol 91	100.0	95.0	96.0	81.0	0.0	0.0
Neutrophil	58.0	75.0	76.0	40.0	0.0	0.0
Monocyte	1.0	-	-	1.0	0.0	0.0
Lymphocyte	41.0	20.0	20.0	40.0	0.0	0.0
Basophil	-	-	-	-	-	-
E 20	100.0	100.0	100.0	80.0	0.0	0.0
Neutrophil	55.0	57.0	54.0	46.0	0.0	0.0
Monocyte	3.0	8.0	2.0	2.0	0.0	0.0
Lymphocyte	42.0	34.0	43.0	32.0	0.0	0.0
Basophil	-	1.0	1.0	-	0.0	0.0
E 85	100.0	100.0	95.0	0.0	0.0	0.0
Neutrophil		64.0	65.0	0.0	0.0	0.0
Monocyte		-	1.0	0.0	0.0	0.0
Lymphocyte		36.0	29.0	0.0	0.0	0.0
Basophil		-	-	-	-	-

*Abnormal RBC morphology as spherocyte

Most morphology of RBCs was highest changed to spherocyte at gasohol dilution of 1:40 and all of them were lysed at dilution of 1:10 and 1:20 (Figure 2 and Figure 4).

The lysis of WBCs was also decreasing in increasing dilution of each gasohol. Most of gasohol effect to WBCs lysis by nearly 100% between dilution of 1:10 and 1:40 (Table 1 and Figure 3) and the rapid decreasing of WBCs lysis were occured between the dilution of 1:40 and 1:160 to zero.

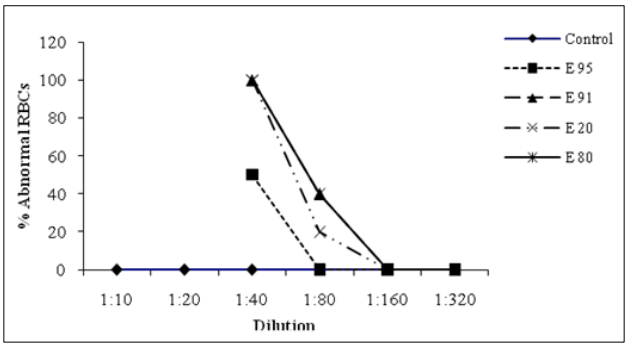

Figure 2: Percentage of abnormal RBC at serial dilution of gasohol.

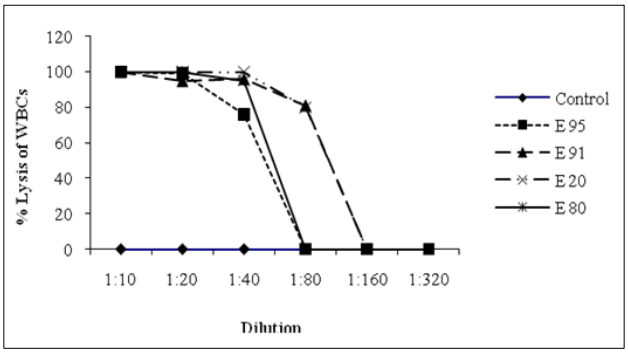

Figure 3: Percentage of WBC lysis at serial dilution of gasohol.

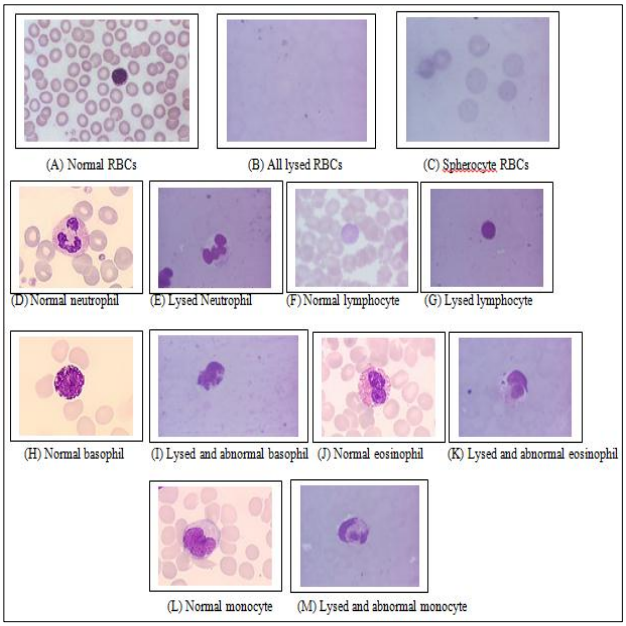

Figure 4: Microscopy of Wright stain RBC and WBC.

DISCUSSION

Most ethanol studies were done by ingestion. There has no clinical report on gasohol-induced toxic effects in human yet. However, the effect might be due to exposure to the gasohol at the gasoline station. In addition, increasing dilution of gasohol was showed the decreasing of abnormal RBCs. At dilution of 1:10 and 1:40 all gasohol effected to all RBCs lysed. Thus, ethanol might effect by changing the RBCs morphology to spherocyte which was depended on the concentration of alcohol in gasohol which high concentration of alcohol in gasohol (at dilution of 1:160 and 1:320) was not affected.

Similar to RBC hemolysis, WBCs were also lysed in relation to the concentration of alcohol in gasohol. The microscopic exam of this study showed the membrane lysis of WBCs which might be resulted from an alcohol-induced damage of WBC pores The result of RBC hemolysis was supported by the previous study of the action mechanism of ethanol leading to the creation of pores in RBC membranes which might involve in the deranged cytoskeletal network of RBC membranes that finally results in the lysis of the cells.[3]

Moreover, the previous study in animal model showed that ethanol consumption alone has shown a significant decrease ($P < 0.01$) in RBC count and an apparent decrease in platelet count compared to control.[4]

Our previous study showed that 8.8% lower hemoglobin level were found in gasoline station workers in 2009 even gasohol was substituted for Methyl Tert-Butyl Ether (MTBE) in 2001.[5] The other study demonstrated the most readily recognizable abnormalities red blood cell associated with alcohol abuse.[6]

The previous study suggested that ethanol promotes T-lymphocyte apoptosis through the activation of intrinsic or mitochondrial pathway.[7]

CONCLUSION

In summary, our study suggested that the gasohol-induced cytotoxicity of blood cells might be related to concentration of ethanol in gasohol. However, the mechanism of gasohol on blood cell damage and lysis remain unclear. The future study on mechanism of action leading to cell lysis and kinetic of morphological change in RBCs and WBCs is still required.

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REFERENCES

1. Mukerjee S. Benzene emission comparisons by gas chromatography of leaded versus unleaded and "gasohol" versus straight-hydrocarbon fuel vapors. Chromatographia. 1900;299:603-05.
2. Poon R, Yagminas A, Singh A, Valli VE, Chu I. Short-term oral toxicity of gasohol in female rats. J Appl Toxicol. 2001;21:461-7.
3. Chi LM, Wu WG. Mechanism of hemolysis of red blood cell mediated by ethanol. Biochim Biophy Acta. 1991;1062:46-50.
4. Nagvekar M, Abraham ME. Effect of cigarette smoking and 3.2% ethanol alone or together on RBC and platelet counts in rats. Indian J Physiol Pharmacol. 2002;46:367-70.
5. Tunsaringkarn T, Soogarun S, Rungsiyothin A, Zapuang K, Chapman RS. Health status of gasoline station workers in Pathumwan area, Bangkok, Thailand, in 2004 and 2009. J Health Res. 2011;25(1):15-9.
6. Heermans ES. Booze and blood: The effects of acute and chronic alcohol abuse on the hematopoietic system. Clin Lab Sci. 1998;11:229-32.
7. Kapasi AA, Patel G, Goenka A, Nahar N, Modi N, Bhaskaran M, et al. Ethanol promotes T cell apoptosis through the mitochondrial pathway. Immunol. 2003;108:313-20.

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