
Correlation between Methomyl Insecticide Concentration with Cholinesterase in Blood and Postmortem Interval in Cases Died from Methomyl Ingestion

ความสัมพันธ์ระหว่างความเข้มข้นของสารเคมีกำจัดแมลงชนิดเมทโทมิลกับระดับเอนไซม์โคลีนเอสเตอเรสในเลือดและระยะภายหลังเสียชีวิตในผู้ที่กินเมทโทมิล

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Abstract

Methomyl is the important substance that is used for suicide. Methomyl is a methylcarbamate insecticide which acts as the reversible cholinesterase enzyme inhibitor (ChE inh.). Methomyl concentration in blood and amount of methomyl ingestion might not cause the fatal case of methomyl intoxication. The retrospective study was performed completely with autopsy and laboratory analysis. The analysis of 55 cases showed that blood methomyl concentration (BMC) ranged 0.6-375.5 µg/ml and the mean concentration was 55.71µg/ml Serum cholinesterase (ChEserum) ranged 160-3060 U/l and the mean was 524.6 U/l. Whole blood cholinesterase (ChEWB) ranged 1900-5200 U/l and the mean was 3072.1 U/l. Correlation between BMC with postmortem interval (PI), ChEserum and ChEWB were not different in significant statistic.

Keywords: Metholmlyl, Lannate^R, Pesticide, Cholinesterase (ChE), Pseudocholinesterase, Serum cholinesterase, Whole blood cholinesterase and Postmortem interval.

บทคัดย่อ

เมทโทมิลเป็นสารเคมีกำจัดแมลงในกลุ่มคาร์บาเมทที่ใช้ฆ่าตัวตายเป็นจำนวนมากและมีปัญหาการวินิจฉัยสาเหตุการตายกรณีที่ไม่สัมพันธ์กับปริมาณของเมทโทมิลในเลือดและที่กิน ในการศึกษาแบบย้อนหลังในศพที่เสียชีวิตจากการกินเมทโทมิล จำนวน 55 รายที่มีการชันสูตรพลิกศพอย่างสมบูรณ์พบความเข้มข้นของเมทโทมิลในเลือด 0.6-375.5 ไมโครกรัมต่อมิลลิกรัม เจลลี่ 55.71 ไมโครกรัมต่อมิลลิกรัม ระดับเอนไซม์โคลีนเอสเตอเรสในน้ำเลือดมีระดับ 160-3060 หน่วยต่อลิตร เจลลี่ 524.6 หน่วยต่อลิตร และระดับเอนไซม์ในน้ำเลือดรวม มีระดับ 1900-5200 หน่วยต่อลิตร เจลลี่ 3072.1 หน่วยต่อลิตร ความเข้มข้นของเมทโทมิลในเลือดกับระยะเวลาภายหลังตาย เอนไซม์ในน้ำเลือดและในน้ำเลือดรวม ไม่มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติ

Introduction

Methomyl is an insecticidal substance which is enormously imported to Thailand and is popularly used for agriculture and suicide in northern of Thailand.^[1,2] Methomyl (S-Methyl-N-[(methylcarbamoyl)oxy]-thioacetimidate)^[3-6] is a methylcarbamate insecticide which easily dissolve in water. The chemical structure is $C_5H_{10}N_2O_2S$ ^[3,7] (as picture 1.) and acts as the reversible ChE inh.^[1,3-5,7-12]. At the first time of reaction, it is hydrolyzed within few hours^[3,10] by attaching to serine hydroxyl group which is the active site of ChE in blood stream. The XOH group is leaved in reaction after hydrolysis but carbamate enzyme which is the inactive enzyme is remained. After that, there was decarbamylation reaction of carbamate enzyme that reversed to active ChE again. These reactions are slowly reversed to reactive enzyme^[8,10]. Rate of reversed reaction from carbamate enzyme to active ChE depended on the factor such as.

1. The interval time of methomyl ingestion extended to laboratory analysis.
2. Methomyl concentration in blood.^[3]

The fatal case of methomyl ingestion might be resulted from BMC and amount of methomyl ingestion. This problem hasn't been successfully studied before.

Methomyl is unstably bounded to ChE^[1, 3-5, 7-11] and rapidly disintegrated to carbondioxide and ammonia^[3]. There is a high level of BMC and a low level in ChE of some patients for the first visiting. The BMC decreased while the ChE level returned to normal in many corpses such as

putrefaction cases. Moreover, the methomyl might not be detected and the level ChE in blood was in a high level because of red blood cell hemolysis. Therefore, the correlation between BMC with ChE in blood and PI in case that died from methomyl ingestion has to be studied next.

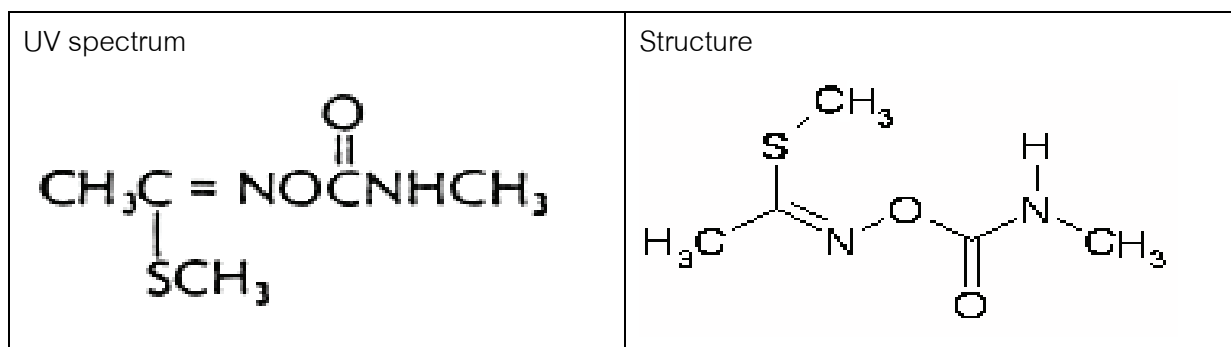


Fig I Chemical structure of methomyl^[3,4,6,7]

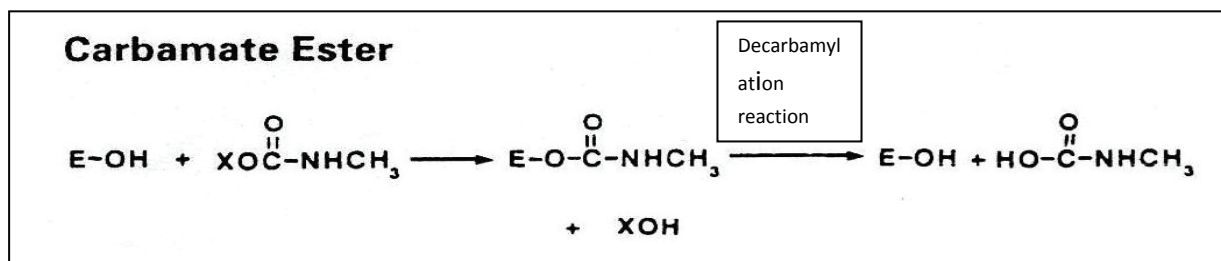


Fig II The interaction between carbamate ester with the serine hydroxyl group (-OH) in the active site of the ChE (E-OH)^[3]

Materials and Methods

The data of corpses who died from methomyl intoxication performing at the Forensic Department of Maharaj Nakorn Chiangmai hospital, Faculty of Medicine, Chiang Mai University, Thailand, were collected since 1 January 2002 to 31 December 2007. The retrospective study was completely collected from an autopsy and a laboratory analysis. The data of sex, age, PI, BMC, level of ChEserum and ChEWB were carried out after the exclusion of unsuitable corpses for the statistical analysis. The methomyl was detected by spectrophotometry. The inclusion criterias included unnatural death due to methomyl intoxication which aged 20-60 years, no decomposition or interval was more than 24 hours, no previous treatment, no other causes of death and no complete data.

Results

The 5994 corpses were performed in this study. In these group there were 112 corpses who died from methomyl ingestion; 74 males (66.1%) and 38 females (33.9%). There were 59 corpses (37 males and 22 females) that matched with inclusion criteria which were studied in detail data. Age group was presented as table 2. However, there were 4 from 55 corpses which could not detected methomyl to receive BMC data. The BMC ranged from 0.6-375.5 µg/ml. A mean and standard deviation of BMC was 55.71µg/ml and 78.84 µg/ml respectively.

Table 1 Number of methomyl ingestion corpses during 2002-2007

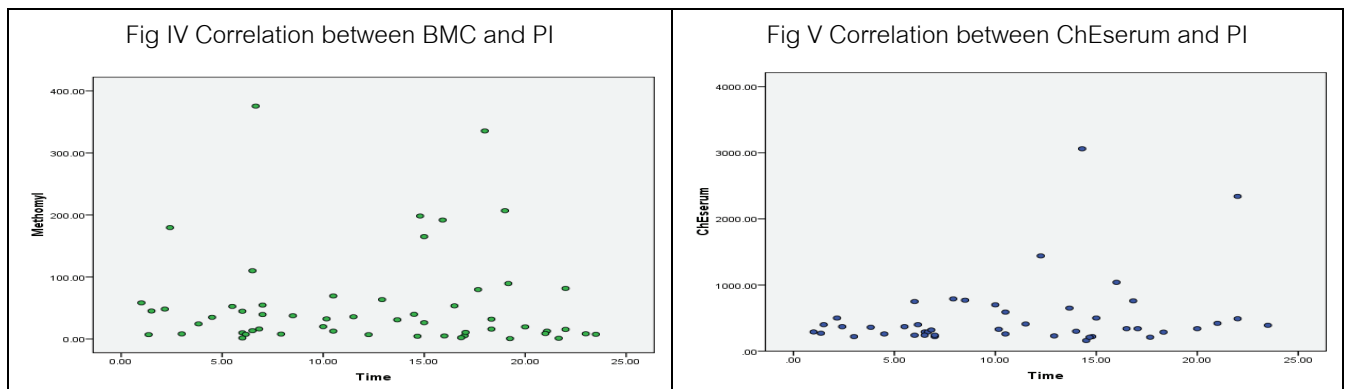
year	2002	2003	2004	2005	2006	2007
No of methomyl ingestion	24	28	17	13	16	14

Table 2 Cases in age group

Age range (year)	21-30	31-40	41-50	51-60
Corpses (number)	14 (23.7%)	16 (27.1%)	15 (25.4%)	14 (23.7%)

Table 3 The result of BMC, ChEserum and ChEWB

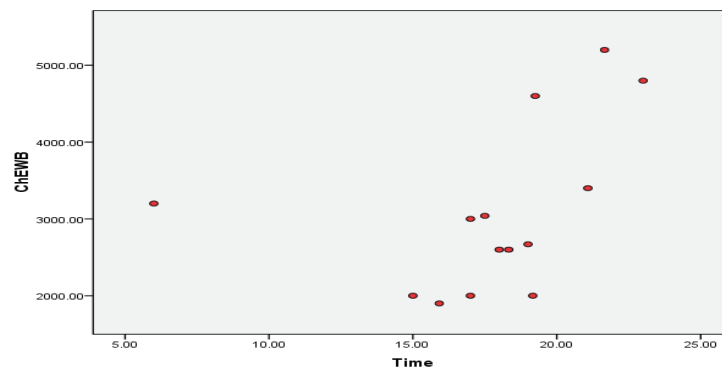
	BMC (µg/ml)	ChEserum (U/l)	ChEWB (U/l)
Mean+/-sd	55.7 +/- 78.8	524.6 +/- 540.7	3072.1 +/- 1088.7
Minimum - Maximum	0.6 - 375.5	160 - 3060	1900 - 5200



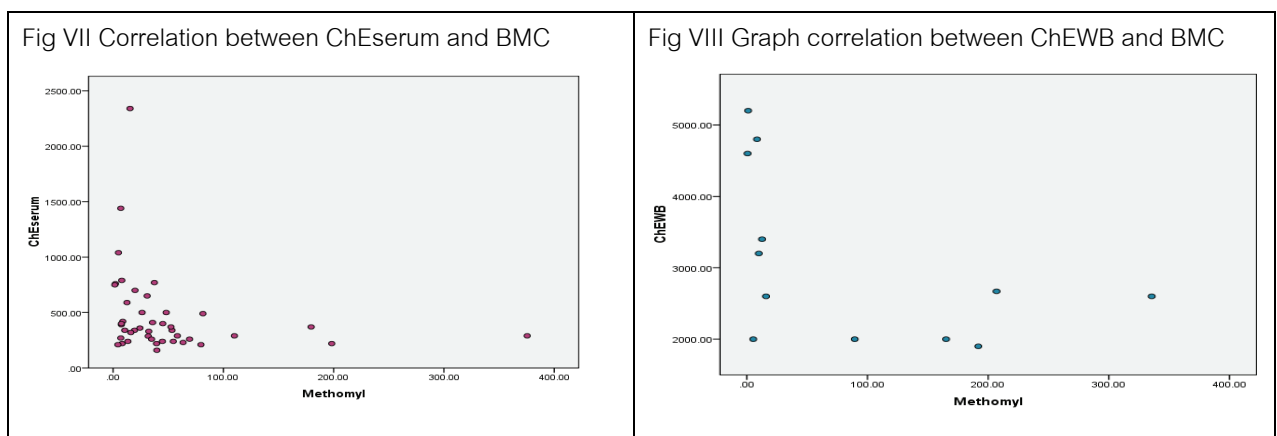
Correlation between BMC and PI were not different in significant statistic. $P = 0.938$ and Pearson correlation $R = -0.11$.

Correlation between ChEserum and PI were not different in significant statistic. $P = 0.091$ and Pearson correlation $R = 0.255$.

Fig VI Correlation between ChEWB and PI



Correlation between ChEWB and PI were not different in significant statistic. $P = 0.155$ and Pearson correlation $R = 0.401$.



Correlation between ChEserum and BMC were not different in significant statistic. $P = 0.136$ and Pearson correlation $R = 0.231$.

Correlation between ChEWB and BMC were not different in significant statistic. $P = 0.092$ and Pearson correlation $R = -0.507$.

As a result, data analysis is divided in 2 groups. Group 1 There were 45 cases which were collected ChEserum (76.27%). The lowest and highest was 160 U/l and 3060 U/l. Mean and Standard deviation was 524.6 U/l and 540.7 U/l. Group 2 There were 14 cases which were collected ChEWB (23.73%). The lowest and highest was 1900 U/l and 5200 U/l. Mean and Standard deviation was 3072.1 U/l and 1088.67 U/l.

Discussion

The result of the study was no correlation between BMC, ChEserum or ChEWB and PI. BMC ranged 0.6-375.5 µg/ml. In just one case, BMC was 0.6µg/ml and ChEWB is 4600 U/l with methomyl detection in gastric content. Shinji Ito has explained that laboratory detection of BMC was difficult because methomyl is rapidly degraded by heat, base, UV light and putrefaction or decomposition of body remains. The study presented that concentration of methomyl in biological fluids decreased in the period of postmortem changes of decedent^[6]. The oral lethal dose and lethal concentration was 5-50 mg/kg^[7] and 0.56-6.35 µg/ml^[6,9,13-15]. The result showed that serum Butyryl-ChE or pseudo-ChE ranged 160-3060 U/l. However, acetyl-ChE or true-ChE in red blood cell or nerve tissue will be inhibited more than butyryl-ChE or pseudo-ChE. Moreover, acetyl-ChE predicted the severity of neurotoxicity better than butyryl-ChE^[3,7,10]. Nevertheless, the method of butyryl-ChE analysis was easier and it could predict the severity and following up the result of treatment. Normal activity was 5200-12800 U/l^[10] and lethal activity was 11-22 % of normal activity^[2]. The level of ChEserum decreased in all decedents but wasn't as low as the lethal activity in two cases. It was reversible reaction of methomyl reversible bound of ChE and the enzyme activity increased in human or postmortem^[8, 10]. ChEWB ranged 1900-5200 U/l. It was the result of summation between red blood cell ChE (normal 18-29 U/l)^[10] and pseudo-ChE (normal 5200-12800 U/l)^[10]. Hemolysis of red blood cells was indeed occurred in postmortem and it was increasing with more longer than PI. BMC and PI was no significant correlation. Shinji Ito has kept blood for methomyl analysis in 25°C for 1 and 2 weeks. The BMC was 33.33% and 1% of first analysis, respectively^[6]. Differentiation between the BMC in each PI was not cleared. Because the exact time of death cannot be detected in all cases. However, the BMC depended on amount of methomyl absorption, bioactivity of each person and PI etc. However, the correlation between ChEserum and ChEWB with PI were not

significant in our study. The reason was similar to correlation between BMC and PI. There was no correlation between ChEserum ChEWB and BMC. But most case of high BMC was the lethal activity of ChE cases. There was one corpse which had PI 59.5 hours but BMC and ChEWB was 5.8µg/ml and 2106 U/l. The other one had PI 40 hours but BMC and ChEserum was 6.1µg/ml and 290 U/l. Fumio Moriya, et al. has analyzed methomyl concentration in tissue at PI, 40 hours. The BMC, methomyl in CSF and vitreous concentration was 0.03-0.08 µg/ml, 2.26µg/ml and 2.68µg/ml, respectively. Methomyl was detected at low level in other tissue. ChEserum activity of this case was 11-45% of normal. So they cannot conclude the cause of death because of the degradation of methomyl in blood and tissue after death, especially putrefaction case^[9]. Methomyl metabolism was catalyzed by carboxylesterase^[8,9] hydrolysis, hydroxylation and conjugation in liver and intestine, 90% excretion in urine within 3 day^[3,8,10,11]. The metabolites were carbon dioxide, acetonitrile and urine polar metabolites in ratio 1:2:1^[7].

Conclusion

BMC, pseudo-ChE and PI have no correlation, in each group. The most important consideration is the diagnosis of a cause of death which doesn't depend on only one parameter but it should be collected for all data, including of history, pathological autopsy, microscopic finding, laboratory investigation of toxic substance, decomposition, post treatment and the other course of death exclusion.

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