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An experimental study on investigating the postmortem interval in dichlorvos poisoned rats by GC/MS-based metabolomics

Xinhua Dai, Fei Fan, Yi Ye, Xiang Lu, Fan Chen, Zhigui Wu, Linchuan Liao*

West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University, Chengdu, 610041, Sichuan, China

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ABSTRACT

The estimation of the postmortem interval (PMI) is always a key issue in forensic science. Although many attempts based on metabolomics approaches have been proven to be feasible and accurate for PMI estimation, there have been no reports regarding the determination of the PMI in acute dichlorvos (DDVP) poisoning. In this study, all rats were killed by acute DDVP poisoning at a dose three fold the oral LD_{50} (240 mg/kg). Gas chromatography-mass spectrometry (GC/MS) was applied to investigate the metabolic profiling of blood samples at various times after death up to 72 h. A total of 39 metabolites were found to be associated with PMI, and the combinations of various numbers of metabolites were used to establish support vector regression (SVR) models to investigate the PMI. The SVR model constructed by 23 metabolites had a minimum mean squared error (MSE) of 5.49 h for the training set. Then, the SVR model was validated by prediction set with an MSE of 10.33 h, suggesting good predictive ability of the model for investigating the PMI. The findings demonstrated the great potential of GC/MS-based metabolomics combined with the SVR model in determining the PMI of DDVP poisoned rats and provided an experimental basis for the application of this approach in investigating the PMI of other toxicants.

1. Introduction

The accurate estimation of the postmortem interval (PMI) is critically important in excluding or including suspects and delimiting the investigation range in forensic science. The examination of the development of postmortem changes, including hypostasis [1], algor mortis [2] and rigor mortis [3,4], is the traditional method for estimating PMI in forensic practice. Nevertheless, only an approximate estimate can be derived from traditional methods [5].

Many biochemical changes continue occurring in blood after death because of the absence of oxygen, the changes in enzymatic reactions, cellular autolysis, and the cessation of synthetic pathways [6]. These changes mean that metabolomics, a holistic approach for quantitative measuring comprehensive systemic fluctuations of low-molecular-weight metabolites [7], can be used to investigate the metabolic changes that occur following death. A study by Donaldson provided a comprehensive overview of the metabolic changes in blood after death and a total of 26 metabolites was detected to have the potential to estimate the PMI [8]. This outcome suggests that the PMI can be determined by overall change in biochemical markers. Recently, several studies investigated metabolic profiling in corpses using nuclear magnetic resonance (NMR) [9–11] and mass spectrometry (MS) [12–14] to determine the PMI. Compared with NMR, the MS technique, especially GC/MS, is considered an ideal tool for PMI estimation due to its accessible standard database as well as its excellent separating and qualitative abilities. The GC/MS-based metabolomics analytical platform can be used for simultaneous qualitative and quantitative analyses of multiple compounds within a single injection [14]. In our previous study, we have successfully applied GC/MS to explore the whole metabolome of suffocated rats for PMI estimation, indicating its effectiveness and potentiality [15]. Overall, GC/MS-based metabolomics can be considered a feasible and accurate approach for estimating the PMI. Nevertheless, the causes of death in previous studies are simple, and most are suffocation or cervical dislocation. There are very few reports regarding the estimation of the PMI by metabolic profiling in poisoning models.

Recently, metabolomics has been applied to investigate the changes in metabolites after administration of poisons, including dichlorvos (DDVP) [16], paraquat [17], brodifacoum [18] and aconitine [19]. These studies suggested that normal homeostasis could be disturbed by toxicants, resulting in changes in metabolites in the body. The presence of poisons may also influence postmortem metabolic changes. As a preliminary study, we selected DDVP to induce poisoning death in rats due to the high frequency of DDVP poisoning in rural areas of China

* Corresponding author.
E-mail address: linchuanliao@scu.edu.cn (L. Liao).

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Table 1
Seventy-two metabolites identified in blood of DDVP poisoned rats by GC/MS and the RSD of each metabolite across the QC samples.

No.	Metabolites	RT (min)	RSD in QC (%)	No.	Metabolites	RT (min)	RSD in QC (%)
Amino Acids				*Organic acids*			
1	Valine	6.352	7.75	36	Pyruvate	5.755	8.47
2	Alanine	6.559	28.20	37	Lactate	5.938	5.70
3	Leucine	7.362	14.80	38	Glycolate	6.157	9.25
4	Proline	7.643	9.26	39	2-Hydroxybutyric acid	6.900	7.40
5	Isoleucine	7.679	27.62	40	Oxalate	7.119	20.32
6	Glycine	9.505	13.40	41	3-Hydroxybutyric acid	7.411	5.67
7	Serine	10.236	15.54	42	Malonate	8.531	15.56
8	Threonine	10.577	12.39	43	Succinate	9.627	17.04
9	Methionine	10.930	13.05	44	3-Aminoisobutyric acid	11.064	5.51
10	Pyroglutamate	12.318	7.72	45	4-Aminobutyric acid	11.539	14.99
11	Hydroxyproline	12.391	16.84	46	Aminomalonate	11.673	25.38
12	Asparagine[#]	13.244	39.08	47	Niacin	11.819	7.79
13	Glutamate	13.524	9.14	48	Malic acid	11.904	26.75
14	Phenylalanine	13.597	10.40	49	α-Ketoglutaric acid	16.02	11.27
15	Glutamine	15.253	24.75	50	Gluconate	17.213	12.20
16	Ornithine	15.728	7.70	51	Mannonate	17.335	10.06
17	Lysine	16.811	10.51	52	Pantothenate	17.493	8.64
18	Tyrosine	16.982	7.43	53	Indole-3-propionic acid	18.370	24.40
19	Tryptophan	19.478	21.87	54	Uric acid	18.504	20.66
Carbohydrates				*Alcohols*			
20	Xylose	14.169	23.02	55	2,3-Dihydroxybutanol	5.646	13.86
21	Sorbose	16.276	7.59	56	Glycerol	9.055	14.47
22	Fructose	16.373	12.16	57	Threitol	12.148	13.00
23	Talose	16.422	19.95	58	Pentitol	13.378	23.48
24	Glucose	16.543	3.88	59	Xylitol	14.486	16.50
25	Allose	16.738	6.29	60	Ribitol	14.705	6.91
26	Mannose	17.834	9.50	61	1,5-Anhydrohexitol	16.081	4.88
27	Galactose	18.869	13.83	62	Mannitol	16.933	7.43
28	Maltose[#]	23.764	38.36	63	Inositol	18.443	7.25
Lipids				*Others*			
29	Palmitic acid	18.053	15.77	64	Dimethyl phosphate	5.999	10.18
30	Linoleic acid	19.575	10.74	65	Urea	8.641	6.68
31	Oleic acid	19.624	10.21	66	Ethanolamine	8.958	13.32
32	Stearic acid	19.855	24.70	67	Uracil	9.932	6.26
33	Arachidonic acid	20.927	22.92	68	Creatinine	12.732	23.66
34	Oleamide[#]	21.280	70.74	69	Hypoxanthine	15.594	23.79
35	Cholesterol	26.650	21.11	70	Xanthine	17.737	12.65
				71	Pseudouridine[#]	20.866	44.08
				72	Uridine	21.536	19.98

RT: retention time; RSD: relative standard deviation; QC: quality control.
[#] Metabolites removed due to RSD > 30% in the QC samples.

[20]. Then, GC/MS technology was performed to investigate the differences in blood metabolic patterns among various PMI groups. This study attempted to construct a reliable model for investigating the PMI of acutely DDVP poisoned rats using GC/MS-based metabolomics and support vector regression (SVR) algorithm.

2. Materials and methods

2.1. Chemicals and materials

DDVP was obtained from Runde Pesticides Co., Ltd (Cangzhou, China). Pyridine and n-heptane (both analytical grade) were purchased from Kelong Chemical Company (Chengdu, China). HPLC-grade acetonitrile was purchased from Fisher Scientific (Waltham, MA, USA). Methyl stearate, methoxyamine hydrochloride and N, O-bis-(trimethylsilyl)-trifluoroacetamide with 1% trimethylchlorosilane (BSTFA + 1% TMCS) were supplied by Sigma-Aldrich (St. Louis, MO, USA).

2.2. Animal experiments

2.2.1. Training set

Thirty-six male Sprague-Dawley (SD) rats aged 8 weeks and weighted 212 ± 5 g were obtained from Dossy Experimental Animals Co., Ltd (Chengdu, China). The rats were housed and acclimatized for three days prior to the experiment under a controlled humidity (50 ± 10%) and temperature (23 ± 2 °C) with a 12 h light-dark cycle. Food and water were provided ad libitum. All animal experiments were approved by the Institutional Animal Management and Use Committee. The experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

All rats were orally administered with 240 mg/kg (three fold dose of oral LD_{50} [21]) of DDVP which was dissolved in corn oil. This dose could ensure that the death was reliably induced by acute DDVP poisoning. Administration volume was 1 mL. After exposure to the DDVP, the antemortem symptoms were observed and the time from administration to death was recorded. The cadavers were randomly assigned to six groups according to the time interval after death (0 h, 6 h, 12 h, 24 h, 48 h and 72 h). The cadavers were placed under constant ambient conditions with the temperature of 12 ± 2 °C and the humidity of 50 ± 10%. The cardiac blood was collected by dissection at the predesignated PMIs. The blood samples were rapidly frozen under liquid nitrogen and stored at −80 °C until analysis.

2.2.2. Prediction set

Another six male SD rats aged 8 weeks and weighted 204 ± 3 g were regarded as the blind test to validate the predictive accuracy of