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Effect of sample heating on results of therapeutic drug monitoring

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Abstract

Objectives: Heat treatment is a convenient measure for pathogens inactivation. The authors investigated the effects of this method on blood concentrations of six commonly therapeutic drugs.

Methods: Plasma and whole blood were pretreated with or without heating at 56 °C for 30 min, and drug concentrations of vancomycin, methotrexate, valproic acid, digoxin, carbamazepine, and cyclosporine were examined.

Results: Increased valproic acid levels after plasma heating (63.2 ± 30.2 vs. 62.1 ± 29.8 mg/L, mean recovery 102.0%) and whole blood heating (64.5 ± 30.5 vs. 62.1 ± 29.8 mg/L, mean recovery 104.6%) were observed (both $p < 0.05$), but these differences were not considered clinically important. Recoveries of vancomycin in heat treatments varied widely, with an average and significant decrease of 15.8% in value after whole blood heating (11.7 ± 8.1 vs. 13.7 ± 8.6 mg/L, $p < 0.05$).

Conclusions: Plasma or whole blood heating at 56 °C for 30 min are feasible in pathogens inactivation during monitoring methotrexate, valproic acid, digoxin, carbamazepine, and cyclosporine. However, such pretreatment seems inappropriate in monitoring vancomycin concentrations. Those results highlight the need for caution when applying heat treatment for pathogens inactivation in therapeutic drug monitoring.

Keywords: effect; heat treatment; pathogens inactivation; therapeutic drug monitoring; vancomycin.

Introduction

Health care workers (HCWs) are at risk of accidental infections acquired from exposures to biological substances containing pathogens. Universal precautions are available to reduce the risk of transmission, but cannot eliminate them and transmissions do occur. Pedrosa and Cardoso [1] reviewed previous publications on accidental viral infections in hospital and laboratory workers. They showed that the accidental viral infections correlate with characteristics of pathogens and the settings where transmissions occur. For example, for airborne viruses (e.g., severe acute respiratory syndrome coronavirus [SARS-CoV]), aerosol/droplet inhalation is the leading way of infection in laboratory and hospital settings; while for blood-borne viruses, percutaneous exposure is the main route of transmission in hospitals (e.g., human immunodeficiency virus [HIV]) and aerosol inhalation is the primary cause of laboratory infections (e.g., parvovirus B19 [Parvo-B19]). Of note, atypical transmission was also addressed in their study. For example, one laboratory case of accidental HIV infection occurred through aerosol inhalation. On the other hand, in an earlier survey, bacterial and fungal infections were reported by 33% of laboratories [2]. Exposures involving higher volumes of samples or sources with higher amounts of pathogens would also be expected to involve higher risks of transmission. Based on these observations, there should be extra attention on safety issues when handling biological materials (e.g., blood).

Therapeutic drug monitoring (TDM) involves frequent contacting different sources of human biological specimens (e.g. blood, urine, breast milk and saliva) [3], which may contain pathogenic microorganisms and biomolecules that can be harmful to the environment and human health. The possible involved manipulations (e.g. centrifugation, sonication, and vigorous mixing) during sample processing and accidental leakage or spillage also generate high-concentration aerosols. All these issues will raise potential risks to TDM staff and the environment.

One strategy to reduce occupational exposure in laboratories is pathogens inactivation before sample handling. Several studies have noted that a convenient measure, or heat treatment at 56 °C for 30 min, can inactivate the pathogenic viruses, including SARS-CoV-2, SARS-CoV, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), HIV,

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Alkhumra Hemorrhagic Fever Virus (AHFV) [4–6]. For example, infectivity of AHFV was completely lost upon heating for 30 min at 56 °C [6]. However, the impact of this method on TDM results need to be validated.

Dasgupta and Bard [5] & Burger et al. [7] evaluated the effect of heat treatment on results of selected monitored drugs, but there are certain limitations in these studies. For example, some commonly monitored therapeutic drugs, like methotrexate and cyclosporine, had not been studied, and number of observations on certain drugs (e.g. vancomycin) was relatively limited. Therefore, conclusions of these studies should be treated with caution. In this report, we investigated the effect of heat inactivation (56 °C for 30 min) on the concentrations of monitored drugs being currently carried out at our laboratory including vancomycin, methotrexate, valproic acid, digoxin, carbamazepine, and cyclosporine.

Materials and methods

The reagents were purchased from Siemens Healthcare Diagnostics (Newark, DE, USA) and listed as follows: enzyme multiplied immunoassay technique (EMIT) vancomycin assay (Lot: 4W019UL-M7); EMIT methotrexate assay (Lot: 6L119UL-L2); EMIT valproic acid assay (Lot: 4G019UL-M2); EMIT digoxin assay (Lot: 4H019UL-M2); EMIT carbamazepine assay (Lot: 4F019UL-M1); EMIT cyclosporine specific assay (Lot: 6R079UL-M2); EMIT cyclosporine sample pretreatment reagent (Lot: 6R719UL-M1). Lyphochek TDM control (Lot: 57370) was purchased from Bio-Rad Laboratories (Irvine, CA, USA). Ethylenediaminetetraacetic acid (EDTA)-containing vacutainer tubes were obtained from Kangjian Medical Apparatus (Taizhou, Jiangsu, China). The Viva-E Drug Testing System was purchased from Siemens Healthcare Diagnostics (Newark, DE, USA).

Ethical approval for this study and oral informed consent procedures were obtained from Ethical Review Committee of First Affiliated Hospital of Guangxi Medical University with the approval No. 2020(KY-E-165). The EDTA-anticoagulated whole blood samples for TDM at the First Affiliated Hospital of Guangxi Medical University in February and July 2020 were collected. According to the specifications of assay kits, plasma or whole blood were used as the specimen matrix. Cyclosporine was determined in whole blood, and the other drugs (vancomycin, methotrexate, valproic acid, digoxin, and carbamazepine) were tested in plasma. The samples were prepared as described below and assayed using the EMIT method on a Siemens Viva-E drug testing system at the pharmacy laboratory. The system was controlled by performing internal quality control over all assay runs.

For TDM of vancomycin, methotrexate, valproic acid, digoxin, and carbamazepine, each sample was centrifuged at 4,000 rpm for 5 min and the supernatant (i.e. plasma) was transferred into two aliquots (100 µL each). One aliquot was kept untreated and served as reference, while the other one and the remainder of the sample were incubated in a water bath at 56 °C for 30 min (denoted as plasma heating and whole blood heating, respectively). Then, they were cooled to room temperature (25 °C) and centrifuged again as above, and the supernatants were assayed simultaneously with the reference.

As for cyclosporine, every whole blood sample was mixed thoroughly and transferred into two tubes (100 µL each). One tube was left untouched and set as reference. The other tube was placed in a 56 °C water bath for 30 min and then cooled to room temperature (whole blood heating). After that, 300 µL of cyclosporine sample pretreatment reagent were added to both tubes and mixed thoroughly and kept at room temperature for 10 min. Both tubes were then centrifuged at 13,000 rpm for 5 min, and the supernatants were harvested for cyclosporine assay on the same system. Samples for calibration of cyclosporine were performed using the same approach as described above.

Statistical analysis was performed with SPSS 22.0 software. Drug concentrations were expressed as mean $\pm$ standard deviation (SD). The paired t-test was used to analyze the differences between the reference and heat-treated groups, and p-value of less than 0.05 was considered significant.

Results and discussion

A total of 394 samples were received in the TDM laboratory during the study period and the main results are shown in Table 1. In accord with the outcomes reported by Burger et al. [7] no significant changes were observed in the concentrations of digoxin and carbamazepine when treated with either plasma heating or whole blood heating (all $p > 0.05$).

In this study, we investigated the drugs like cyclosporine and methotrexate which were not evaluated previously. As the two medications have the immunosuppressive properties and can weaken the immune system, patients taking them are more susceptible to infection with pathogens, which may be present in the body fluids, e.g. blood. We found no considerable effects of plasma heating or whole blood heating on concentrations of methotrexate and whole blood heating on levels of cyclosporine (all $p > 0.05$).

As for valproic acid, compared with the reference, concentrations after plasma heating and whole blood heating were significantly elevated (both $p < 0.05$), and the mean recovery were 102.0 and 104.6%. Although such changes may seem small, the paired t-test confirmed that the differences between heat treatments with the reference were indeed statistical significant. To examine whether these changes were clinically meaningful, we furthered compared the proportion of values that fall within the therapeutic ranges (40–100 mg/L) using the McNemar test. As a result, compared with reference, the ratios in plasma heating and whole blood heating showed no significant differences (both $p > 0.05$, data not shown), suggesting these differences were not clinically important.

Recoveries of vancomycin in plasma heating (mean [range], 102.3 [73.2–184.2]) and whole blood heating (84.2 [0–157.9], %) varied widely, and concentrations in whole blood heating were significantly decreased when compared

Table 1: Effects of plasma heating and whole blood heating on therapeutic drugs.

Drug	Detection range	Subgroup	Units	n	Reference Concentration	Plasma heating		Whole blood heating	
						Concentration	Mean recovery, % (range)	Concentration	Mean recovery, % (range)
Vancomycin	2–50		mg/L	83	13.7 ± 8.6	13.6 ± 8.7	102.3 (73.2–184.2)	11.7 ± 8.1 ^a	84.2 (0–157.9)
		<10	mg/L	28	5.8 ± 2.3	6.1 ± 2.1	110.6 (73.9–184.2)	4.8 ± 2.4 ^a	81.3 (0–157.9)
		10–20	mg/L	38	13.7 ± 2.7	13.3 ± 3.0	96.6 (73.2–118.2)	11.5 ± 2.5 ^a	84.2 (53.4–106.2)
		≥20	mg/L	17	26.5 ± 8.2	26.7 ± 8.8	101.3 (74.1–126.6)	23.6 ± 8.6 ^a	88.9 (60.1–115.9)
Methotrexate	0.3–2		μmol/L	67	0.71 ± 0.51	0.72 ± 0.51	101.0 (93.3–116.7)	0.73 ± 0.52	102.5 (92.9–121.4)
Valproic acid	1–150		mg/L	59	62.1 ± 29.8	63.2 ± 30.2 ^a	102.0 (90.4–114.1)	64.5 ± 30.5 ^a	104.6 (93.1–134.1)
Digoxin	0.3–5		μg/L	63	1.49 ± 0.91	1.51 ± 0.96	99.5 (83.8–114.8)	1.53 ± 0.94	101.8 (78.7–131.8)
Carbamazepine	0.5–20		mg/L	50	7.34 ± 3.06	7.42 ± 3.09	100.8 (93.4–114.5)	7.22 ± 3.05	98.3 (86.2–114.6)
Cyclosporine	40–500		μg/L	72	138.5 ± 68.7			139.0 ± 68.5	100.8 (83.1–130.5)

^aSignificant difference compared with the reference ($p < 0.05$).

with the reference ($p < 0.05$). The distribution of vancomycin results was analyzed and presented in Table 1. The results showed that concentrations of vancomycin in whole blood heating at sub-therapeutic (<10), therapeutic (10–20) and toxic (≥20) ranges were all reduced significantly ($p < 0.05$), when compared with the reference, and –18.7, –15.8, and –11.1% of mean decrease in value were shown, which all exceed the bounds of acceptable deviation in routine control assays (i.e. 10%). The imprecision of the vancomycin assay was evaluated at three concentration levels by repeated measurements of quality control. As shown in Table 2, the variation of vancomycin at low (5.2 ± 0.4), medium (13.1 ± 0.6), and high (27.3 ± 0.9) dose were 6.8, 4.3 and 3.2%, which were all lower than the mean decrease of drug concentration in whole blood heating.

Dasgupta and Bard [5] observed no significant change in the concentrations of vancomycin and valproic acid using serum separator tubes (SST) for blood collection. This discrepancy with our results may be attributed to our larger sample size or to the different blood collection container used in our study. As barrier gel in SSTs can prevent passage of hemoglobin from hemolyzed cells to serum and may absorb certain degree of drugs, findings from their research cannot be applied directly when using different blood collection container. In this study, we used blood specimen collected in EDTA tubes, which were commonly applied in TDM. As is known, accurate

quantification of drug concentrations is essential in clinical practice, and insufficient sample size may bias the analysis of results and influence further clinical decision making. Herein, we enlarged the sample size of drugs (e.g. vancomycin, from 2 to 83) to increase confidence in the findings.

In this report, we did not make any changes in assay matrix validated by the reagent manufacturer in all drugs. Matrix used in reference, plasma heating, and whole blood heating for quantification of vancomycin, methotrexate, valproic acid, digoxin and carbamazepine were all plasma, and matrix employed in reference and whole blood heating for determination of cyclosporine were both whole blood. The pretreatment of whole blood heating mentioned in our study for monitoring plasma drugs meant these drugs were determined in plasma after the samples (whole blood) were centrifuged, heated, cooled and centrifuged again. Therefore, matrix used for detection in this process was also plasma and consistent with the instructions by the reagent manufacturer.

To minimize the risk of potentially infectious materials to the environment and employees, we validated the heat inactivation using whole blood concurrently with plasma. Compared with the plasma heating, the pretreatment of whole blood heating eliminates the steps of sample transfer before inactivation and therefore is more effective in preventing pathogen infection. However, as is known, heating the blood can result in hemolysis due to disruption

Table 2: Results of within-batch imprecision analysis (n=30).

Drug	Subgroup	Units	Concentration (range)	Within-batch imprecision, %
Vancomycin	Low	mg/L	5.2 ± 0.4 (4.6–5.9)	6.8
	Medium	mg/L	13.1 ± 0.6 (12.3–14.1)	4.3
	High	mg/L	27.3 ± 0.9 (26.0–31.1)	3.2
Methotrexate		μmol/L	1.14 ± 0.05 (1.03–1.26)	4.4
Valproic acid		mg/L	81.5 ± 2.43 (76.2–87.4)	3.0
Digoxin		μg/L	1.43 ± 0.03 (1.26–1.57)	2.1
Carbamazepine		mg/L	8.21 ± 0.32 (7.58–9.16)	3.9
Cyclosporine		μg/L	87.3 ± 3.6 (78.2–96.4)	4.1

of membrane integrity of red blood cells, and thus cause the release of hemoglobin and other intracellular components, which may generate alteration of drug concentrations in blood samples [5, 7–9]. In order to minimize the impact of hemolysis on the TDM results of plasma drugs, we centrifuged the samples before whole blood heating and handled gently during the entire procedure. Our results indicate that, when pretreated with heating, the presence of endogenous substances and concurrent medications in blood samples does not influence the outcome of investigated drugs except vancomycin.

Though we studied heating on results of TDM, this does not mean that safety precautions are not important. Instead, the main measure to increase biosafety is to perform the analyses in appropriately equipped laboratories with appropriately trained technicians. As an additional approach, heating can be taken into account just if more effective and well established measures cannot be followed due to any emergency reasons and after careful evaluation of the risk to benefit ratio. Since we did not validate which pathogens can be inactivated by heating, this pretreatment is therefore likely applicable to cases that have been proven effective against specific pathogens. It should also be noted that, even after heating, the possible existence of non-inactivated pathogens in samples remain a risk for transmission. Therefore, if conditions are met, HCWs should comply with multiple safety precautions as much as possible to minimize these accidental infections.

There are some limits for this study. First, TDM is known to be performed for many other drugs, limited by the resources currently available at our laboratory, we only recruited six drugs for investigation. We will expand this validation to more drugs in future studies. Second, for most of the drugs investigated in this study there are numerous analytical methods which enable analysis without any direct contact of the technician with the sample material. These methods are highly automated and sometimes even there is no need to open the blood collection tube manually. In addition, as for cyclosporine, there is one method (e.g.,

antibody-conjugated magnetic immunoassay [ACMIA]) that does not require a manual sample pretreatment before analysis. Therefore, heating used in this study may not be universally of relevance for different assays.

In conclusion, plasma or whole blood heating at 56 °C for 30 min prior to analysis does not alter the drug concentrations of methotrexate, valproic acid, digoxin, carbamazepine, and cyclosporine in general. As for vancomycin, given that recoveries vary widely and concentrations fall overall, the pretreatment with whole blood heating seems inappropriate as a strategy to reduce the risk of transmission in TDM.

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Author contributions: Zhen-Guang Huang conceived and designed the study. Dao-Hai Cheng performed the experiments and wrote the paper. Jing-Bing Zhu analyzed the data and edited the manuscript. All authors read and approved the manuscript.

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Informed consent: Not applicable.

Ethical approval: Ethical approval for this study and oral informed consent procedures were obtained from Ethical Review Committee of First Affiliated Hospital of Guangxi Medical University with the approval No. 2020(KY-E-165).

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