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Determination of theophylline concentration in serum by chemiluminescent immunoassay

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Abstract: Objective: This study aimed to establish chemiluminescent immunoassay (CLIA) for quantitative determination of theophylline levels in human serum. Methods: To measure the concentration of theophylline (n=122) and evaluate the assay. Results: The linear range of the CLIA method was 0.51~40 mg/L (Y=1.02X+0.44, r=0.995). The intra and inter CV (coefficient variance) of CLIA were 3.20% and 3.57%, respectively. The average recovery rate was 102.3%. This method was free from interference by brilirubin (<200 μmol/L), hemoglobin (<10 g/L), and triglycerides (<15 mmol/L). Conclusion: This method is simple, convenient and precise for clinical pharmacokinetics study of theophylline.

Key words: Theophylline, Chemiluminescent immunoassay (CLIA), Fluorescence polarization immunoassay (FPIA)
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INTRODUCTION

Theophylline is one of the antiasthmatic drugs commonly used in a clinic. The individual difference of its bio-availability and the elimination rate in vivo is very large (Vergin *et al*., 2003). Its valid blood drug level is 10~20 mg/L, and it has a narrow safety range (Dawson and Whyte, 1999; Li *et al*., 2000). As it is apt to result in the serious side-effect if the blood drug level exceeds 20 mg/L, monitoring the blood drug level of theophylline is necessary. In many different methods for theophylline detection, radioimmunoassay (RIA) has radioactivity, many interference factors, and requires a long run time (Yu and Lin, 2003). The high-performance liquid chromatography method needs skilled staff and more quantity of samples for detection than immunoassay. In the fluorescence polarization immunoassay (FPIA) method, several blood drug levels cannot be measured at the same time, which required expensive reagent (Solnica, 2004). It is necessary to establish a new excellent method. In recent years, chemiluminescent immunoassay (CLIA) has been widely applied to the detection of drug concentration (Feng *et al*., 2005; Gupta *et al*., 2005; Shukla and Krag, 2005; Zamora *et al*., 1999). A study showed that CLIA detected chloramphenicol successfully (Lin *et al*., 2005). We measured theophylline concentration in the serum of 122 patients by CLIA and evaluated the method.

MATERIALS AND METHODS

Subject

The research covered 122 patients (72 men and 50 women; mean age, 62±12 years) with diagnosis of asthmatic bronchitis, pneumonectasis, chronic bronchitis, pneumosilicosis. They were outpatients or inpatients of our hospital from February, 2003 to May, 2004 and took theophylline by common dose (0.1~0.2 g p.o.b.i.d. or 0.25~0.5 g ivgtt.t.i.d.). All samples collected were observed with observing routine pre-

caution for venipuncture. Stored samples were tightly stoppered, at room temperature (15~30 °C) for no longer than eight hours.

Instruments

Chemiluminescent immunoassay Instrument was Access analyzer manufactured by the Beckman-Coulter Company in USA in cooperation with Pasture Academe in France. Fluorescence polarization immunoassay Instrument was TDx manufactured by the Abbott Company, USA.

Reagents

The Beckman-Coulter Company, USA offered reagents of CLIA, quality control serum, and calibrators. R1: Paramagnetic particles coated with goat anti-mouse IgG suspended in Tris buffered saline, with surfactant, bovine serum albumin (BSA), 0.1% sodium azide, and 0.1% ProClin^{TM} 300; R2: Theophylline-alkaline phosphatase (bovine) conjugate diluted in Tris buffered saline, with surfactant, BSA, 0.1% sodium azide, and 0.25% ProClin^{TM} 300; R3: Mouse monoclonal antibody to theophylline diluted in Tris buffered saline, with surfactant, bovine serum albumin (BSA), 0.1% sodium azide, and 0.1% ProClin^{TM} 300. Substrate: Lumi-Phos® 530 (AMPPD). Wash buffer: Tris buffered saline. Calibrators: Theophylline in human serum at levels of approximately 0.0, 5.0, 10.0, 15.0, 20.0, and 40.0 mg/L respectively, with 0.1% sodium azide, and 0.5% ProClin^{TM} 300. Reagents of FPIA were bought from Abbott Company, USA.

Biological principles of the procedure

Theophylline assay is a competitive binding immunoenzymatic assay. A sample is added to a reaction vessel with mouse monoclonal antibody to theophylline, theophylline-alkaline phosphatase (ALP) conjugate, and paramagnetic particles coated with goat anti-mouse capture antibody. Theophylline in the sample competes with the theophylline-alkaline phosphatase conjugate for binding sites on a limited amount of specific anti-theophylline antibody. Resulting antigen-antibody complexes bind to the goat anti-mouse antibody on the solid-phase. Separation in a magnetic field and washing removes materials not bound to the solid phase. A chemiluminescent substrate, AMPPD was added to the reaction vessel and light generated by the reaction is measured with a luminometer. The photon production is inversely proportional to the concentration of theophylline in the sample. The amount of analyte in the sample determined by means of a stored, multi-point calibration curve.

Statistical analysis

Data were expressed as means±standard deviation. Statistical differences were determined by *t*-test and linear correlation analysis.

RESULTS

Calibration

We measured 6 calibrators with different concentrations 0.0, 5.0, 10.0, 15.0, 20.0 and 40.0 mg/L, respectively and traced the standard curve by the measured value, and stored them in the computer. Recalibration was required every 28 d. Fig.1 is the standard curve.

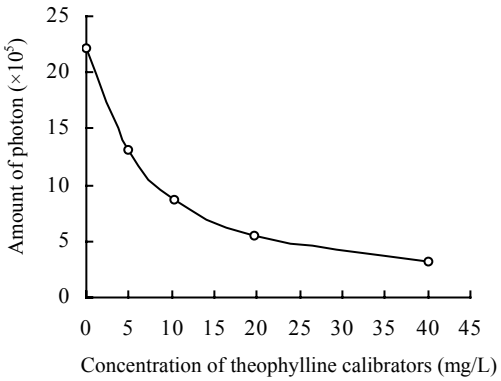

Fig.1 The standard curve of theophylline

Precision

Precision was achieved by measuring three groups of quality control serum with different concentrations. Comparison of theophylline values using CLIA and FPIA showed that there was no statistical significance between CLIA and FPIA (P>0.05). The intra-CV and inter-CV of CLIA were lower than those of FPIA. Statistical data are shown in Table 1.

Linearity

Two calibrators at concentrations of 5 mg/L and 40 mg/L were used. Then we obtained 9 samples

Table 1 Precision comparison CLIA method with FPIA method

Controls (mg/L)	Determined concentration (mg/L)		Intra-CV (%)		Inter-CV (%)	
	CLIA	FPIA	CLIA	FPIA	CLIA	FPIA
5.00	4.91±0.26	4.99±0.31	3.99	5.56	4.79	6.25
12.00	12.18±0.53	11.86±0.69	3.28	4.37	3.57	5.08
20.00	19.50±0.66	18.93±0.85	2.33	3.55	2.34	4.01

at concentrations of 5.00, 12.00, 13.75, 16.67, 22.50, 28.33, 31.25, 33.00, 40.00 mg/L, respectively, by mixing the two calibrators in proportion of 1:0, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 0:1. The measurement was performed in two sequences, from high to low and from low to high. Linear regression analysis on the measured value (Y) and the theoretical value (X) yielded a relationship Y=1.02X+0.44 (r=0.995). The upper limit determined from the measurement value was 40.00 mg/L. The value of SD was calculated by repeating 10 times the measurement at the concentration of 5 mg/L as the lower limit of detection. We got the result 0.51 mg/L regarding 3SD as the lower limit of detection.

Recovery

For this experiment, we added three calibrators with different concentrations (high, medium, low) of one sample with known concentration. At the same time, the sample was made up to the same volume of distilled water as the blank control. Then we repeated measuring each sample for 3 times and calculated the ratio of recovery as 102.1%, 100.6%, 104.2%, respectively. The mean ratio of recovery was 102.3%.

Interference

We added hematoglobin, bilirubin, triglyceride, at different concentration, to three samples with low, medium, high concentration of theophylline. The interference substances were as follows: hematoglobin, bilirubin, triglyceride at concentration of 10 g/L, 200 μmol/L, 15 mmol/L, respectively. When they were added to the samples, we found no significant difference in the measurement values.

Analytical specificity

Drugs are commonly used in clinic, such as 1,3-dimethyluric acid, theobromine, caffeine, xanthine, phenobarbital, prednisone and phenacetin are similar in structure to theophylline. Within the blood drug level for therapy, the influence of those drugs on the measured value was little. The variance was less than 1%.

Comparison of two methods

The CLIA and the FPIA were compared by measuring the theophylline concentration of 30 samples. We obtained Y from the results of CLIA and X from the results of FPIA. The statistical analysis of results between CLIA and FPIA yielded the correlation coefficient r=0.993, Y=1.01X+0.30. This indicated that they have significant correlation. Fig.2 shows their correlation.

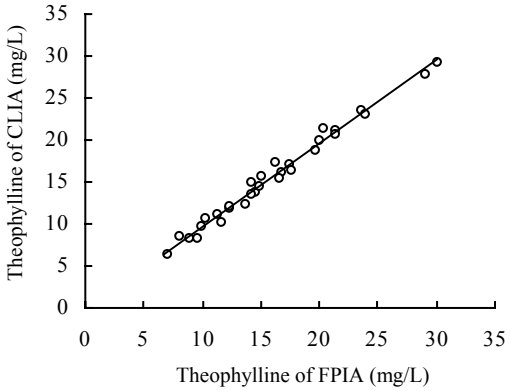

Fig.2 Correlation of CLIA method with FPIA method

Antibody and reagent

The antibody contained monoclonal antibody coated paramagnetic micropaticles and polyclonal antibody ALP-labelled conjugate. We used the chemiluminescent substrate AMPPD. The reagents were good for use for about 12~18 months stored at 2~8 °C.

Clinical application

We measured the concentration of theophylline concentration in 122 inpatients or outpatients in our hospital. The concentration range was 3.8~32.6 mg/L, the mean concentration was 14.67 mg/L. The difference between the low concentration and the upper

concentration was 28.8 mg/L. For those patients whose blood drug levels were higher, clinical emergency treatments were applied to them immediately. For patients with blood drug levels less than therapy level, we could regulate the drug program and use effective methods to control the asthma symptom in time. There were 8.2% of patients who were sensitive to theophylline, which has good effect when the blood drug level is less than 10 mg/L, which is probably caused by individual variance.

DISCUSSION

Schack and Waxler (1949) described a method for measuring theophylline in blood and tissue by means of ultraviolet spectrophotometry. More recent and improved methods include gas liquid chromatography, high pressure liquid chromatography (HPLC), radioimmunoassay (RIA), and enzyme immunoassay (EIA). CLIA measuring theophylline is an enzyme immunoassay that combines many of the favorable characteristics found in HPLC, RIA and EIA: accuracy, sensitivity, extended shelf life, and the potential for automation (Ogbonna *et al*., 1995; Terrier *et al*., 2004). CLIA is a new technology, which involves the luminescence analysis and immunoassay. So it has both high sensitivity similar to that of luminescence analysis and high specificity similar to that of the reaction of antigen and antibody (Amal and Bergmann, 2004). In addition, it is easy to prepare label with long period of validity. The reagent is steady and the reaction time is short. Therefore it is recommended for in clinic measurement of a large number of samples. For this experiment, the CLIA method provides a more precise result and better linearity. Also it has good correlation with the FPIA method (r=0.993). The CLIA has high specificity, in which there is low interference caused by similar substance. And it also has powerful anti-interference to hematoglobin, bilirubin, triglyceride (Dasgupta and Datta, 2004). Moreover, compared with FPIA method, CLIA does not need exciting light, so that the influence of the stray light in FPIA can be avoided, so that higher sensitivity and accuracy can be obtained. And CLIA requires less investment. Also unlike RIA, CLIA does not cause damage to the environment and human health. We have achieved the desirable effects during the clinical administration of this drug. In short, CLIA is distinguished for its rapid and convenient procedure as well as for its wider measurement spectrum (Zamora *et al*., 1999). It is really an excellent quantitative method of theophylline.

References

Amal, L., Bergmann, P., 2004. Evaluation of a chemiluminescence immunoassay for the determination of intact parathyroid hormone using the ADVIA Centaur. *Clin. Lab.*, **50**(11-12):695-702.

Dasgupta, A., Datta, P., 2004. Rapid detection of oleander poisoning using digoxin immunoassays: comparison of five assays. *Ther. Drug Monit.*, **26**(6):658-663. doi:10.1097/00007691-200412000-00012.

Dawson, A.H., Whyte, I.M., 1999. Therapeutic drug monitoring in drug overdose. *Br. J. Clin. Pharmacol.*, **48**(3):278-283. doi:10.1046/j.1365-2125.1999.00033.x.

Feng, Z., Li, Z., Sui, B., Xu, G., Xia, T., 2005. Serological diagnosis of infectious mononucleosis by chemiluminescent immunoassay using capsid antigen p18 of Epstein-Barr virus. *Clin. Chim. Acta*, **354**(1-2):77-82. doi:10.1016/j.cccn.2004.11.010.

Gupta, S.V., McGowen, R.M., Callewaert, D.M., Brown, T.R., Li, Y., Sarkar, F.H., 2005. Quantitative chemiluminescent immunoassay for NF-kappaB-DNA binding activity. *J. Immunoassay Immunochem.*, **26**(2):125-143.

Li, J., Mo, H., Huang, H., 2000. Effect of low dose of inhaled corticosteroid combined with small dose of oral theophylline on treatment of bronchial asthma. *Zhonghua Jie He He Hu Xi Za Zhi*, **23**(6):336-339 (in Chinese).

Lin, S., Han, S.Q., Liu, Y.B., Xu, W.G., Guan, G.Y., 2005. Chemiluminescence immunoassay for chloramphenicol. *Anal. Bioanal. Chem.*, **382**(5):1250-1255. doi:10.1007/s00216-005-3273-6.

Ogbonna, G., Caines, P.S., Catomeris, P., 1995. A competitive chemiluminescent immunoassay for the sensitive measurement of apolipoprotein B100. *Clin. Biochem.*, **28**:117-122. doi:10.1016/0009-9120(95)00006-U.

Schack, J.A., Waxler, S.H., 1949. An ultraviolet spectrophotometric method for the determination of theophylline and theobromine in blood and tissues. *J. Pharmacol. Exp. Ther.*, **97**(3):283-291.

Shukla, G.S., Krag, D.N., 2005. Sensitive and rapid chemiluminescence ELISA for filamentous bacteriophages. *J. Immunoassay Immunochem.*, **26**(2):89-95.

Solnica, B., 2004. Comparison of serum digoxin concentration monitoring by fluorescence polarization immunoassay on the TDxFLx and dry chemistry enzyme immunoassay on the Vitros 950. *Clin. Chem. Lab. Med.*, **42**:958-964. doi:10.1515/CCLM.2004.156.

Terrier, N., Bonardet, A., Descomps, B., Cristol, J.P., Dupuy, A.M., 2004. Determination of beta2-microglobulin in biological samples using an immunoenzymometric assay (chemiluminescence detection) or an immunoturbidimet-

ric assay: comparison with a radioimmunoassay. *Clin. Lab.*, **50**(11-12):675-683.

Vergin, H., Mahr, G., Winterhalter, B., Wigand, R., 2003. Relative bioavailability and bioequivalence study of theophylline sustained release formulations. *Arzneimittelforschung*, **53**(9):635-639.

Yu, J.Y., Lin, Y., 2003. Clinical value comparison between chemiluminescent immunoassay and immunoradiometric assay in detecting TSH in serum. *Hunan Yi Ke Da Xue Xue Bao*, **28**:275-277 (in Chinese).

Zamora, B.M., Hartung, M., Hildebrandt, G., Kasbohrer, A., 1999. Detection of antibodies to S. enteritidis in broilers by means of indirect ELISA and chemiluminescent immunoassay (CLIA). *Zentralbl Veterinarmed B*, **46**(1):9-23.