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Short Communication

Evaluation of a high-performance thin-layer chromatographic technique for the determination of salbutamol serum levels in clinical trials☆

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ABSTRACT

Salbutamol concentrations were determined by high-performance thin-layer chromatography in the sera of two sets of ten volunteers at hourly intervals for 6 h after taking one 8-mg slow-release tablet. The influence of time lapse in processing of serum samples, *i.e.* centrifugation, extraction and chromatography, was studied. A statistical significant instability of salbutamol in the sera of patients was found which was not present in standard drug-free serum samples spiked with salbutamol and used for construction of standard curves.

INTRODUCTION

Salbutamol (albuterol) is widely used in oral or inhaled forms for the treatment of reversible bronchospasm in children and adults because of its relatively selective β_2-adrenergic agonism [1]. For prophylaxis of asthma, however, it was found that the inhaled form is used incorrectly by a significant proportion of patients [2]. Consequently the slow-release oral forms of salbutamol need to be employed as an alternative. Because of the zero order absorption characteristic of slow-release formulations any clinical study on effectivity of prophylactic treatment ideally needs inclusion of blood level measurements together with lung function tests after bronchial provocation [3].

Studies on therapeutic monitoring of salbutamol serum concentrations have included almost all techniques routinely employed by analytical laboratories, *i.e.* gas chromatography–mass spectrometry [4], high-performance liquid chromatography with fluorescence [5–7], amperometric [8,9] and electrochemical [10] detection, radioimmunoassay [11] and high-performance thin-layer chromatography (HPTLC) [12]. Colthup *et al.* [12] described the HPTLC technique in 1985 and we extended their observations on relatively large

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numbers of blood samples which were collected during study days from volunteers enrolled in a clinical study protocol [3]. A batch of twenty samples can be extracted simultaneously followed by chromatography on one thin-layer plate. In contrast to the batch operation character of this technique, most other dictate repetitive chromatographic separations.

When we instituted the method of Colthup *et al.* [12] our first finding was a significant inter-patient variation in the salbutamol time–concentration relationship [3] which was in contrast with other studies [13,14]. We therefore investigated the effects of the time lapse between drawing of blood and separation of the serum and the time lapse between extraction of salbutamol from serum and HPTLC determination. Since we found a significant influence of these factors on the final serum concentrations we describe our results in this communication in order to bring it to the attention of other investigators.

EXPERIMENTAL

The HPTLC method described by Colthup *et al.* [12] for determination of salbutamol concentrations in serum was instituted in our laboratory without any alternations.

Volunteers who suffer from atopic asthma participated in this study. Ethical approval from our local committee and written informed consent was obtained. On the morning of a study day each patient received one 8-mg slow-release salbutamol tablet (Volmax). They remained ambulatory for at least 2 h after the dose. All other forms of therapy was stopped at least 12 h prior to onset of the study while the subjects also fasted for this period.

The study was conducted in two phases. In both phases control blood samples (3 × 10 ml from each patient) were collected immediately before dosing and therafter at hourly intervals for 6 h. The samples from ten patients who participated in the first phase were handled as follows: two groups of blood samples were centrifuged 15 min after collection of blood and the serum was kept at 4°C until extracted. One of these groups was extracted on a C_{18} silica gel column (Sep-Pak C_{18}) at the end of the study day and one the following morning. The third group of samples was kept at 4°C until the end of the study day and then centrifuged and extracted.

Aliquots of drug-free serum (Hyland Diagnostics) spiked with salbutamol standards (1, 5, 10, 15, 20, 30 and 40 ng/ml) were included in each batch of extractions. All extracts were kept at −20°C until chromatographed the next day.

Statistical analysis was performed by using standard methods.

RESULTS AND DISCUSSION

Fig. 1 (line A) shows that the procedure of immediate centrifugation and extraction at the end of the study day consistently yielded the highest salbutamol levels. Each time point represents the average of the values obtained from ten patients. Both an increase in time lapse before extraction (line B) and centrifugation (line C) resulted in levels 20–50% lower than A. Statistical comparison of the average levels at each time point show a significant difference after 2 h ($P < 0.05$) between line A and those of lines B and C.

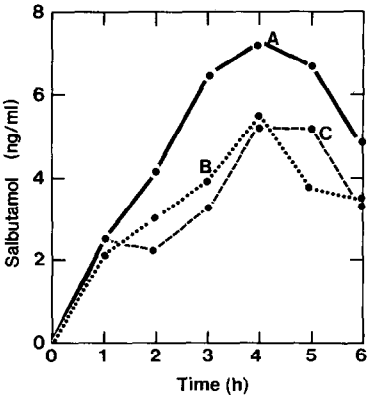

Fig. 1. Influence of time lapse between blood collection, centrifugation and serum extraction on serum salbutamol levels (n = 10). In line A the samples were centrifuged immediately, followed by extraction at the end of the study day. The samples in line B were also centrifuged immediately followed by extraction the next morning. In line C the samples were centrifuged and extracted at the end of the study day.

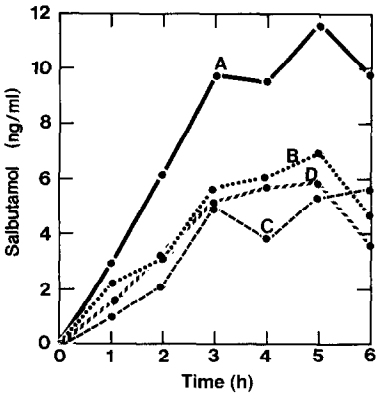

Fig. 2. Influence of time lapse between serum extraction and HPTLC determination of serum salbutamol levels (n = 10). Line A represents chromatography after two days, line B after one week, line C after two weeks and line D after four weeks.

The samples collected in the second phase of the study were from another selection of ten patients. They were centrifuged 15 min after drawing the blood, kept at 4°C and the extraction made at the end of the study day together with four sets of standards. The extracts were kept at −20°C and chromatography was performed two days, one, two and four weeks later. Fig. 2 depicts the statistical significant ($P < 0.05$) higher levels (line A) obtained when chromatography was performed after two days in comparison to one to four weeks later (lines B, C and D, respectively). There was no statistical significant difference between lines B, C and D. The higher average maximum level of 11.7 ng/ml at 5 h in Fig. 2 in comparison to only 7.5 ng/ml at 4 h in Fig. 1 is probably allied to the noted inter-patient variation [3] and the fact that a different set of ten patients participated in phase 2 of the study.

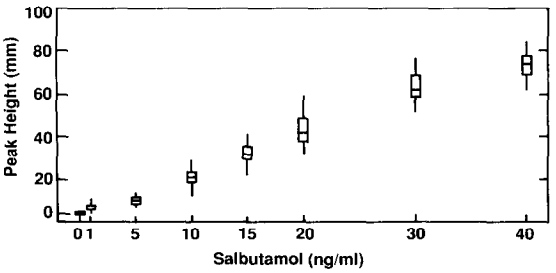

Fig. 3. Repeatability of the salbutamol standard curve obtained by HPTLC (n = 20).

More important, however, is the repeatability of the standard curve shown in Fig. 3. An inter-assay variability of 13.2% was calculated for all the curves that were constructed (n = 20) in this study. This is clearly much less than the decrease in salbutamol concentrations observed when the samples are not centrifuged, extracted and chromatographed as soon as possible. Since a standard curve was included for each batch of samples stored and analyzed it implies that the salbutamol in the sera of the patients was more unstable than in the spiked serum samples. We cannot explain this difference directly except to speculate that some enzymic activity is present in the sera of the patients which is inactive in the standard drug-free serum. Because salbutamol reaches only ng/ml concentrations with normal therapeutic doses we feel that investigations which attempt to correlate its therapeutic effect with serum levels should take into account this apparent instability phenomenon.

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