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Short Communication

Influence of sample preparation and storage on theophylline concentrations in biological fluids

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Monitoring serum concentrations in order to obtain optimum efficacy from theophylline in the management of asthma, occurs routinely nowadays in many hospitals (Hendeles et al., 1978; Ogilvie, 1978; Hendeles and Weinberger, 1980; Weinberger and Hendeles, 1980). However, it may happen frequently that assaying the sample is not possible immediately after the sampling or not even at the same location. Up until now it has been assumed that short-term storage or transportation of samples does not influence the theophylline concentration, but the correctness of this hypothesis has never been verified.

Therefore the results of the theophylline bio-assay were compared after preparing and storing the biological fluid in various ways, simulating some situations frequently occurring in practice (clinical pharmacy or clinical pharmacokinetic studies).

A blood sample of about 150 ml was withdrawn by vein puncture from a healthy volunteer 1.5 h after intake of 3 microcrystalline theophylline-containing tablets of 125 mg each (Theolair = Nuelin, Riker Laboratories, Loughborough, U.K.).

The sample was divided in 5 portions of equal volumes. Serum samples were prepared from those 5 whole blood samples using 5 methods differing in storage time and storage temperature (methods 1–5 in Table 1).

The first portion (1) of whole blood was immediately centrifuged to give serum. This serum sample was divided into 4 equal-volume portions (1a–1d). One of those serum samples (1a) was assayed immediately. Another serum sample (1b) was stored in the refrigerator (at 6°C) for 1 week and then assayed. The two other serum samples were stored in the freezer (−20°C) for 1 month (1c) or 3 months (1d), respectively. The organic extract obtained from the serum sample that was assayed immediately (1a) was divided into 3 portions. The first portion of the extract was promptly injected into the liquid chromatograph (1e) whereas the other portions of the extract were stored at 6°C for 1 day (1f) or 1 week (1g) before quantitation.

The second part of the whole blood sample (2) was stored at 6°C for 1 day, then

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TABLE I

THEOPHYLLINE CONCENTRATIONS AS FOUND IN A SAMPLE OF WHOLE BLOOD WHICH WAS CONVERTED TO SERUM BY VARIOUS METHODS, FOLLOWED BY STORAGE OF THE OBTAINED SERUM AND THE EXTRACT IN DIFFERENT WAYS

Method of preparing the serum	Serum				Extract		
	Method of storage:						
	(a) assayed promptly	(b) stored at 6°C for 7 days	(c) stored at −20°C for 30 days	(d) stored at −20°C for 90 days	(e) assayed promptly	(f) stored at 6°C for 1 day	(g) stored at 6°C for 7 days
Whole blood was:							
(1) centrifuged promptly	7.9	8.1	8.0	7.5	7.9	7.7	8.1
(2) centrifuged after storage at 6°C for 1 day	7.9	8.0	8.0	7.4	7.9	–	8.0
(3) centrifuged after storage at 6°C for 7 days	7.2	7.3	7.3	7.3	7.2	7.6	7.2
(4) centrifuged after storage at 25°C for 1 day	7.5	7.5	8.0	7.2	7.5	–	7.8
(5) centrifuged after storage at 25°C for 7 days	7.0	7.6	7.6	6.8	7.0	7.4	7.3

centrifuged to give serum and the serum divided into 4 portions (2a–2d) which were treated as described for the samples 1a–1d. The extract of serum sample 2a was either promptly injected into the HPLC (2e) or stored as described for 1f and 1g.

The third part of the whole blood (3) was also stored at 6°C, but for 1 week and then centrifuged to serum. This serum sample, and the extract that was obtained from it, were stored and assayed as described above.

The preparation and treatment of the fourth and fifth part of the whole blood (4 and 5) were identical to that of samples 2 and 3, respectively, but before centrifugation storage occurred at 25°C (room temperature) for 1 day and 1 week, respectively.

All samples and extracts were stored in glass tubes with screw caps (Sovirel, France).

Temperature control was obtained at 25 ± 1°C (mean ± S.D.) by use of a thermostatic water-bath. The mean temperature in the refrigerator was 6°C (range 2–7°C); in the freezer the mean temperature was −20°C (range −19 to −22°C).

The assay procedure was as follows: from each tube, two 0.5-ml samples were taken and extracted with 10.0 ml of a chloroform–isopropanol (95:5 v/v) mixture. Quantitation of theophylline in each extract was performed by using a rapid and selective HPLC procedure (duplicate injection; Jonkman et al., 1980). The results of the assays in the various samples are summarized in Table 1.

In order to establish whether significant differences were obtained in the outcomes of the quantitation as a result of the treatment of the whole blood and/or the way in which the serum samples or extracts were stored, analysis of variance (ANOVA) was applied to the observations. The results of these calculations, which are given in Table 2, show that there is a significant difference between the various ways of preparing the serum as well as a significant difference between the various ways of storage of the serum or extract samples.

Concerning the methods of preparing the serum (methods 1–5 in Table 1) no significant difference ($P = 0.25$) could be found between methods 1 and 2, which means that whole blood can be stored for 1 day at 6°C without there being deterioration of the theophylline concentration. The same was found for storage for 1 day at 25°C (methods 1 and 4).

However, there was a significant difference ($P < 0.001$) between methods 1 and 3

TABLE 2

TWO-WAY ANOVA ANALYSIS OF THE OBSERVATIONS AS GIVEN IN COLUMNS (a), (b), (c), (d) AND (g) IN TABLE 1

Source	Variation	Degrees of freedom	Variance	E ratio	Probability value
Between whole blood samples	1.996	4	0.499	16.23	$P<0.001$
Between serum/extract samples	0.932	4	0.233	7.58	$P\simeq0.001$
Residual	0.492	16	0.031		
Total	3.42	24			

and also between methods 1 and 5. This means that a decrease in the theophylline concentration will occur when the whole blood sample is stored for 7 days at either 6°C or 25°C. Therefore such a treatment of the samples should be avoided.

Concerning the methods of storage of the serum (methods a–d in Table 1), no significant differences ($P = 0.25$) could be found between method (a) compared with (b) and (c), indicating that theophylline-containing serum samples can be stored for 1 week at 6°C or for 1 month at −20°C without there being a decrease in concentration. However, method (d) showed significant deviation ($P < 0.05$) from the methods (b and c) but not from method (a) ($P = 0.25$), indicating that storage of theophylline-containing serum samples for 3 months at −20°C might lead to a small decrease in concentration. The decision of whether or not such a storage is allowed depends on the required accuracy in a particular study. These aspects are now being subjected to further investigations.

As far as it concerns the storage of the extract (methods e–g in Table 1), no conclusion could be made due to insufficient data, but apparently the theophylline concentration is not much influenced by these methods of storage.

From these results it can be concluded that theophylline is rather stable in biological fluids like whole blood or serum. Storage of the sample as whole blood at either 6°C or 25°C for 1 day, or as serum at 6°C for 1 week or at −20°C for 1 month, does not significantly influence the accuracy of the final theophylline serum assay.

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