THE EFFECTS OF ATROPINE ON PHARMACOKINETICS OF THEOPHYLLINE IN RABBITS

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SUMMARY

The effects of atropine on serum concentrations and pharmacokinetics of theophylline were studied in six rabbits (2.07 ± 0.11 kg). Theophylline serum concentration was determined by ultraviolet spectrophotometry. After i.v. administration of aminophylline 12.5 mg/kg, i.m. atropine 1 mg/kg decreased significantly the maximal serum concentration of theophylline from 23.6 ± 1.1 to 19.6 ± 1.1 mg/l, and increased that of theophylline at the time of 6 and 8 hours after injection. After i.v. aminophylline administration without or with atropine, the pharmacokinetic parameters of theophylline calculated using a one compartment open model were: K (elimination rate constant) = 0.23 ± 0.03, 0.19 ± 0.02 /h; $t_{1/2}$ (half life) = 3.04 ± 0.40, 3.66 ± 0.40 h; Cl (clearance) = 0.26 ± 0.04, 0.23 ± 0.03 l/kg/h, respectively ($P < 0.01$). But there was no significant variation in modification of volume of distribution (V_d). The results suggested that there is a significant drug interaction between atropine and theophylline.

KEY WORDS

atropine; theophylline; pharmacokinetics; drug interaction

INTRODUCTION

Theophylline is a commonly used bronchodilator in patients with chronic obstructive lung disease /1,2/. Because the therapeutic range is narrow and the pharmacologic effect and toxicity correlate closely to its concentration in serum, small changes in theophylline clearance may result in insufficient bronchodilation or in the appearance of toxic effects /2,3/. Some of the genetic and pathophysiological factors which can modify theophylline pharmacokinetics have already been reported /4-6/. However, concurrent medications may also have considerable effects on theophylline clearance. Asthmatics often require comedication (e.g., other bronchodilators, antibiotics, corticosteroids, cimetidine), and theophylline dosage should be adjusted in conjunction with plasma concentration monitoring /2,3/. It has been noted that a number of drugs have a significant influence on the pharmacokinetics of theophylline /3,7-9/, but little is known about interaction between theophylline and atropine, an anticholinergic drug. The purpose of this study was to investigate the effects of atropine on serum concentrations and pharmacokinetics of theophylline.

MATERIALS AND METHODS

Apparatus

Ultraviolet spectrophotometer: A model UV-754 spectrophotometer (Analytical Instrument Factory, Shanghai) was used for these studies with quartz cuvettes (2.5 ml capacity; 1 cm path length).

Reagents and chemicals

All reagents were analytical reagent grade and prepared as follows:

NaOH, 0.1 mol/liter
HCl, 0.1 mol / liter
Chloroform/isopropanol, 95/5 (v/v)
Aminophylline and theophylline were obtained from Changzhou Pharmaceutical Laboratory, 880105.

Atropine was obtained from Shanghai Tianfeng Pharmaceutical Laboratory, 860505.

Animal experiments

Six New Zealand White rabbits (4 male, 2 female), weighing 2.07 ± 0.11 kg (mean ± SD) and after overnight fasting, were given a bolus dose of aminophylline 12.5 mg/kg (in 10 ml of 5% glucose) via the marginal ear vein over 2 min. Water was allowed *ad libitum* during fasting and throughout the experiment. Blood samples were collected by cutting another marginal ear vein at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6 and 8 hours after dosing. Serum was separated after centrifugation and analyzed within 6 hours of collection.

Four days later, the second experiment was performed. All animals were administered atropine (1 mg/kg, i.m.), and immediately given aminophylline (12.5 mg/kg, i.v.). Blood sampling was as described above.

Sample preparation and serum theophylline assay

0.5 ml of serum, 0.2 ml of HCl and 5 ml of chloroform/ isopropanol were mixed in a 10 ml test tube and stirred vigorously for 20 s with a vortex-type mixer. After centrifugation, the chloroform layer was back-extracted with NaOH. The extracted phase was removed and the ultraviolet absorption spectrum was determined. If theophylline is present, there is an absorption peak at 274 nm. The concentration of theophylline was calculated by ΔA (A274 - A298). A solution containing 2 ml of distilled water and 4 ml of NaOH was used as the reference solution. The absorption of the NaOH was at 298 nm. In our laboratory, this method had between-run and within-run coefficients of variation of less than 5%. The presence of atropine did not interfere with the determination of serum theophylline.

Pharmacokinetic parameters

The calculation of pharmacokinetic parameters was based on a one-compartment open model, using a computerized program NONLIN ("PKBP-N1", General Hospital of Armed Forces of Nanjing) /12/. The clearance (Cl) was calculated by use of the following equation:

$$Cl = V_d \cdot K$$

Statistics

Statistical analysis was performed using the paired t-test. The level of probability used to determine statistical significance was 0.05. All data are reported as the mean ± one standard deviation.

RESULTS

Serum concentrations of theophylline

The mean serum concentration-time data of theophylline are summarised in Table 1. After the i.v. dose of aminophylline, the

TABLE 1

Serum theophylline concentration (mg/l) - time data following administration of aminophylline (12.5 mg/kg, i.v.) alone (AM) and after treatment with atropine (1 mg/kg, i.m.) (AT+AM) in rabbits. n = 6, mean ± SD. * P < 0.05, ** P < 0.01

	Time (h)	No. 1	2	3	4	5	6	mean	± S.D.
	0.25	24.3	24.3	24.9	23.2	22.2	22.6	23.6	1.1
	0.5	21.3	21.5	20.2	21.1	20.2	19.2	20.6	0.9
	1.0	19.8	17.5	16.8	17.9	17.2	17.0	17.7	1.1
	1.5	18.0	15.7	14.4	16.4	16.2	15.0	16.0	1.2
AM	2.0	14.9	13.8	14.0	15.3	14.9	14.2	14.5	0.6
	3.0	12.8	12.6	11.5	12.9	10.3	12.2	12.0	1.0
	4.0	11.6	12.5	8.6	7.8	7.8	8.0	9.4	2.1
	6.0	8.0	5.9	5.9	5.2	4.1	5.0	5.7	1.3
	8.0	5.4	3.9	3.3	3.7	3.1	3.9	3.9	0.8
	0.25	20.9	18.3	19.6	21.1	19.2	18.7	19.6	1.1**
	0.5	20.2	17.9	18.8	19.6	18.8	17.2	18.8	1.1**
	1.0	19.1	15.0	16.1	18.7	17.6	15.8	17.0	1.7
AT	1.5	18.1	13.6	14.7	17.9	16.8	14.9	16.0	1.9
+	2.0	16.0	12.9	14.2	15.3	14.9	14.0	14.6	1.1
AM	3.0	14.9	11.6	13.6	13.8	12.5	11.7	13.0	1.3
	4.0	12.4	9.8	10.5	11.4	8.8	9.4	10.4	1.3
	6.0	8.6	6.5	5.9	6.5	5.9	5.6	6.5	1.1*
	8.0	6.5	4.4	4.8	5.2	3.5	4.8	4.9	1.0**

maximal serum concentration of theophylline was 23.6 ± 1.1 mg/l at 0.25 h. Then theophylline serum concentrations progressively decreased. They were 5.7 ± 1.3 and 3.9 ± 0.8 mg/l, respectively, at 6 and 8 hours. After pretreatment with atropine in these animals, the maximal serum concentration of theophylline was significantly decreased. The value observed was 19.6 ± 1.1 mg/l at 0.25 h ($P < 0.01$). Theophylline serum concentration was significantly increased at 6 and 8 hours after dosing (6.5 ± 1.1 and 4.9 ± 1.0 mg/l, $P < 0.05$ and $P < 0.01$, respectively).

Pharmacokinetic parameters of theophylline

The parameters obtained from the data that were fitted with the one compartment open model were tabulated (Table 2). After pretreatment with atropine, pharmacokinetic parameters of theophylline were significantly different: K and Cl decreased from 0.23 ± 0.03 to 0.91 ± 0.02 /h, and from 0.26 ± 0.04 to 0.23 ± 0.04 l/kg/h respectively ($P < 0.01$); $t_{½}$ was prolonged from 3.04 ± 0.40 to

TABLE 2

Pharmacokinetic parameters of theophylline following administration of aminophylline (12.5 mg/kg, i.v.) alone (AM) and after treatment with atropine (1 mg/kg, i.m.) (AT+AM) in rabbits. n=6, *P < 0.01

No.	V_d (l/kg)		K(/h)		$t_{½}$ (h)		Cl(l/kg/h)	
	AM	AT +AM	AM	AT +AM	AM	AT +AM	AM	AT +AM
1	1.08	1.09	0.18	0.16	3.77	4.22	0.19	0.17
2	1.10	1.31	0.23	0.18	3.08	3.89	0.25	0.24
3	1.23	1.30	0.24	0.20	2.93	3.52	0.30	0.26
4	1.12	1.12	0.24	0.19	2.85	3.64	0.27	0.21
5	1.14	1.18	0.27	0.23	2.58	3.00	0.31	0.27
6	1.08	1.15	0.23	0.19	3.01	3.69	0.25	0.22
mean	1.13	1.19	0.23	0.19*	3.04	3.66*	0.26	0.23*
± SD	0.06	0.09	0.03	0.02	0.40	0.41	0.04	0.04

3.66 ± 0.41 h. There was no significant variation in the modification of V_d; they were 1.13 ± 0.06 and 1.19 ± 0.09 l/kg respectively ($P > 0.05$).

DISCUSSION

In most published reports on plasma theophylline concentrations and pharmacokinetic studies /13-15/, the spectrophotometric method of Schack and Waxler /16/, or a modification of it, has been used /10/. Theophylline can be measured by ultraviolet spectrophotometry, because of its strong ultraviolet absorption in alkaline solution /10,11/.

The present study was designed to test whether there is an interaction between atropine and theophylline. Our finding demonstrates that atropine, after i.m. administration, significantly modifies the serum concentrations and pharmacokinetics of theophylline.

The results obtained in this work indicate that the maximal theophylline serum concentration was 23.6 ± 1.1 mg/l after i.v. administration of aminophylline. This value is about 75% of that obtained by El-Yazigi and Sawchux /11/. A reason for this difference in theophylline serum concentration is that the dosage of intravenous aminophylline in their experiments was as high as 24.33 mg/kg for the rabbits.

As shown in Table 1, the maximal serum concentration of theophylline significantly decreased after pretreatment with atropine. This effect is probably related to the high concentration of atropine. The mechanism of reduction in serum theophylline concentration is not known. One might suspect that perhaps it is related to dilation of cutaneous blood vessels or to a direct vasodilator action unrelated to cholinergic blockade /17/, which induced a more extensive distribution of theophylline.

It has been reported that atropine is rapidly absorbed and the peak time of its blood concentration appears at 15 - 30 min after i.m. administration to rats /18/ and to humans /19/. On the other hand, theophylline serum concentrations at 6 and 8 h after dosing with aminophylline were significantly increased compared with control values ($P < 0.05$ and $P < 0.01$). The mechanism for this effect is as yet unknown.

For all animals whose data were fitted to a one compartment open system model, there appeared to be a rapid elimination of

theophylline with a mean elimination rate constant of 0.23 ± 0.03 /h, and half life of 3.04 ± 0.40 h (Table 2). These results are in agreement with data in the literature /11/, which showed that after rapid intravenous administration of aminophylline in rabbits, pharmacokinetic parameters of theophylline were: K = 0.26 ± 0.08 /h, $t_{\frac{1}{2}}$ = 2.80 ± 0.73 h, respectively.

However, after pretreatment with atropine the pharmacokinetic parameters of theophylline were significantly different in comparison with control values ($P < 0.01$) (Table 2). The elimination rate constant and the clearance of theophylline after i.m. administration of atropine decreased by 17.4% and 13.0% respectively, and half life prolonged by 20.4%. These modifications of the pharmacokinetic parameters were primarily related to the decrease of theophylline elimination after dosing with atropine. Since up to 90% of theophylline is biotransformed /3,5/, the effects of atropine on theophylline may involve an influence on the microsomal enzyme systems in the liver or other mechanisms.

In summary, atropine significantly modified the serum concentrations and pharmacokinetics of theophylline in rabbits. The results appear to indicate that special care should be taken in comedication with these two drugs. Further studies of this interaction in patients taking theophylline with atropine will confirm our findings in animals.

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REFERENCES

1. Paterson JW, Woolcook AJ, Shenfield GM. Bronchodilator drugs. Am Rev Respir Dis 1979; 120: 1149-1188.
2. Advenier C, Cerrina J, Durroux P. Bronchodilatateurs. Sem Hop (Paris) 1984; 60: 179-193.
3. Jonkman JHG, Upton RA. Pharmacokinetic drug interactions with theophylline. Clin Pharmacokin 1984; 9: 309-334.
4. Jusko WJ, Gardner MJ, Mangione A, Shentag JJ, Koup JR, Vance JW. Factors affecting theophylling clearance: Age, tobacco, marijuana, cirrhosis, congestive heart failure, obesity, oral contraceptives, benzodiazepines, barbiturates and ethanol. J Pharmaceut Sci 1979; 68: 1358-1366.
5. Bauer LA, Bauer SP, Blouin RA. The effect of acute and chronic renal failure

on theophylline clearance. J Clin Pharmacol 1982; 22: 65-68.
6. Manjione A, Imhoff TE, Lee RV, Shum LY, Jusko WJ. Pharmacokinetics of theophylline in hepatic disease. Chest 1978; 73: 616-622.
7. Branigan TA, Robbins RA, Cady WJ, Nickols JG, Ueda CT. The effects of erythromycin on the absorption and disposition kinetics of theophylline. Eur J Clin Pharmacol 1981; 21: 115-120.
8. Reed RC, Schwartz HJ. Phenytoin-theophylline-quinidine interactions. New Eng J Med 1983; 308: 724-725.
9. Tonatore KM, Kanarkowski R, McCarthy TL, Gardner MJ, Yurchak AM, Yusko WJ. Effect of chronic oral contraceptive steroids on theophylline disposition. Eur J Clin Pharmacol 1982; 23: 129-134.
10. Jatlow P. Ultraviolet spectrophotometry of theophylline in plasma in the presence of barbiturates. Clin Chem 1975; 21: 1518-1520.
11. El-Yazigi A, Sawchuk RJ. Theophylline absorption and disposition in rabbits: Oral, intravenous, and concentration-dependent kinetic studies. J Pharmaceut Sci 1981; 70: 452-456.
12. Yang YC, Chen G, Yuan L. A non-linear method and its program for calculating pharmacokinetic parameters. Acta Pharmacol Sin 1983; 4: 217-220.
13. Jenne JW, Wyze E, Rood F, MacDonald FM. Pharmacokinetics of theophylline. Application to adjustment of the clinical dose of aminophylline. Clin Pharmacol Ther 1972; 13: 349-360.
14. Mitenko PA, Ogilvie RI. Rapid achieved plasma concentration plateaus, with observations on theophylline kinetics. Clin Pharmacol Ther 1972; 13: 329-335.
15. Mitenko PA, Ogilvie RI. Pharmacokinetics of intravenous theophylline. Clin Pharmacol Ther 1973; 14: 509-513.
16. Schack JA, Waxler SH. An ultraviolet spectrophotometric method for the determination of theophylline and theobromine in blood and tissue. J Pharmacol Exp Ther 1949; 97: 283-291.
17. Weiner N. Atropine, scopolamine, and related antimuscarinic drugs. In: Gilman AD, Goodman LS, Rall TW, Muurad F, eds, The Pharmacological Basis of Therapeutics. New York: Macmillan Inc., 1985; pp 130-144.
18. Huang RH, Xu X. Phosphorimetric method for determination of atropine and its application to pharmacokinetic studies. Chin J Clin Pharmacol 1988; 4: 144-148.
19. Berghem L, Bergman U, Schildt B, Sorbo B. Plasma atropine concentration determined by radioimmunoassay after single-dose i.v. and i.m. administration. Br J Anaesth 1980; 52: 597-601.