Blood Levels and Cardiovascular Dynamics During Methoxyflurane Inhalation in Dogs

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RECENT advances in gas-liquid adsorption chromatography have permitted rapid, precise, quantitative measurement of a variety of volatile organic substances.[1,2] This technique has proven to be of particular value in determining blood concentrations of various anesthetic agents. Recently, a gas chromatographic method for determining blood levels of methoxyflurane was developed by us.[3] Methoxyflurane* is a fluorinated ether introduced as a useful agent for clinical anesthesia by Artusio and associates.[4] The purpose of the present study was to measure arterial and venous blood levels of methoxyflurane during induction, maintenance and recovery with this agent and to correlate these levels with concurrent changes in mean aortic pressure and ventricular contractile force.

Methods

Twenty-one mongrel dogs were used in these experiments. Ventricular contractile force (strain gauge arch[5,6,7]) and aortic pressure (Statham transducer) were continuously recorded during induction and maintenance of anesthesia in all experiments. Arterial and venous blood samples were taken periodically to determine blood concentrations of methoxyflurane. Each animal was prepared 24 hours prior to the experiment by suturing a strain gauge arch to the right ventricle through a thoracotomy in the interspace between the fourth and fifth ribs and by placing indwelling polyethylene cannulas through a femoral artery and vein into the thoracic aorta and inferior vena cava. The animals were anesthetized with thiamylal sodium for this procedure.

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* 2,2-Dichloro-1,1-difluoroethyl methyl ether (Penthrane–Abbott Laboratories).

Arterial and venous blood levels of methoxyflurane were determined by the previously mentioned gas chromatographic method.[3] Basically, the method involves distillation of a blood sample containing the inert gas, methoxyflurane, and a measured volume of iso-amyl alcohol. The organic fraction is trapped in a small sample container, lifted from the water phase, placed in a small (1 ml.) flacon and dried with anhydrous potassium carbonate. A 20 microliter sample is injected onto a 4-foot hexadecane column in a Beckman GC-2 instrument at a temperature of 70° C. and eluted at a constant helium carrier gas pressure of 40 psi. Quantitation was obtained by peak height analysis compared to methoxyflurane standards.

Inhalation anesthesia was accomplished using a Foregger anesthesia machine equipped with a high-flow 'copper kettle' vaporizer. Anesthesia was induced in 9 animals with methoxyflurane using a semiclosed system. Induction was begun with approximately a 1.00 per cent concentration and gradually increased to 3.00 per cent. Following intubation, the animals were maintained on graded concentrations (0.50–1.25 per cent) using a nonrebreathing system and artificially ventilated with a constant volume respirator.† Anesthesia was induced in 12 animals with thiamylal sodium (15–25 mg./kg.) and maintained with methoxyflurane (0.50 per cent). All data were analyzed statistically by the "test of differences" variation of the Student's *t* test.[8]

Results

Table 1 summarizes the arterial and venous blood concentrations of methoxyflurane in nine experiments in which the animals were induced and maintained with increasing con-

† Harvard Apparatus Co., Cambridge, Massachusetts.

TABLE 1. Blood Levels (mg./100 ml.) of Methoxyflurane During Increasing Concentrations in the Inspired Gas Mixture

Experiments	Induction		Maintenance								Emergence					
			Per Cent Methoxyflurane in Inspired Air								45 Minutes		4 Hours		24 Hours	
	Art.	Ven.	0.50%		0.75%		1.00%		1.25%							
			Art.	Ven.	Art.	Ven.	Art.	Ven.	Art.	Ven.	Art.	Ven.	Art.	Ven.	Art.	Ven.
A	30.2	—	26.5	22.0	38.9	27.6	53.9	35.6	43.7	43.5	—	—	13.0	—	1.7	—
B	15.9	13.0	15.0	15.2	30.9	30.6	41.9	39.3	—	—	10.0	12.6	3.9	3.7	—	—
C	19.9	15.7	13.8	12.9	34.5	33.4	39.9	28.6	43.8	47.2	24.7	16.0	11.4	10.5	4.1	3.9
D	20.2	15.2	26.4	18.3	41.0	27.2	57.0	30.9	—	—	18.0	20.2	4.9	5.1	1.6	—
E	26.1	23.0	25.6	21.3	—	—	42.1	41.6	45.5	36.5	13.2	16.9	7.0	9.3	.9	.6
F	37.9	21.3	21.3	30.9	37.4	24.4	67.4	36.5	56.7	41.3	18.5	13.8	—	—	—	—
H	25.3	21.9	30.1	26.7	51.1	35.7	70.2	44.9	—	—	20.8	20.8	4.8	6.5	—	—
I	36.5	23.6	28.6	25.8	37.4	36.5	54.2	30.9	39.0	41.8	15.2	14.0	6.5	6.5	—	—
Mean	26.5	19.1	23.4	21.6	38.7	30.8	53.3	36.0	45.7	42.1	17.2	16.3	7.4	6.9	2.1	2.3
± SE	2.8	1.6	2.2	2.2	2.4	1.7	1.3	2.0	3.0	1.7	1.9	1.2	1.3	1.0	.7	

centrations of this agent. Arterial and venous blood samples drawn immediately following placement of the endotracheal tube were found to contain mean methoxyflurane concentrations of 26.5 and 19.1 mg./100 ml. respectively. Since the anesthetic was not being administered during the intubation procedure the blood levels may have been reduced somewhat during the insertion of the endotracheal tube. However, muscle relaxation was virtually complete at the time that the samples were taken. Following intubation the animals were maintained on approximately a 0.50 per cent inspired concentration of methoxyflurane for 30 minutes. After this period of stabilization, blood samples were again drawn and the animals placed on the next higher concentration and the procedure repeated. There was little difference between the blood levels following intubation and those obtained after maintenance on 0.50 per cent for 30 minutes. However, with further increments in the inspired concentrations of methoxyflurane both the arterial and venous blood levels progressively increased with the arterial-venous ratio increasing to a certain degree. One exception to this pattern occurred during ventilation with 1.25 per cent of methoxyflurane. The mean arterial concentration at this level was found to be lower than at 1.00 per cent. However, this decrease was not of statistical significance nor was it consistent throughout the individual experiments. Following ventilation with 1.25 per cent methoxyflurane, the anesthetic was withdrawn and the animals allowed to recover. Oxygen was also administered by positive pressure ventilation until the animals were able to breathe on their own. The first postanesthesia blood samples were drawn 45 minutes following the removal of methoxyflurane from the inspired gas. In all instances the animals were still unconscious.

TABLE 2. Mean Changes in Aortic Pressure and Ventricular Contractile Force During Increasing Concentrations of Methoxyflurane

	Mean Aortic Pressure	Ventricular Contractile Force
	Mm. Hg ± SE	Grams ± SE
Control	112.3 ± 6.9 (9)	89.3 ± 5.6 (8)
Intubation	66.4 ± 8.3 (9)	74.0 ± 4.1 (8)
Methoxyflurane		
0.50%	73.4 ± 3.6 (9)	71.8 ± 2.6 (8)
0.75%	58.7 ± 3.1 (7)	59.3 ± 3.7 (6)
1.00%	42.1 ± 3.3 (8)	43.1 ± 2.7 (7)
1.25%	41.0 ± 5.1 (5)	41.0 ± 1.5 (4)
Emergence (45 minutes)	88.9 ± 6.1 (7)	96.8 ± 7.2 (5)
Emergence (4 hours)	94.4 ± 9.3 (5)	87.3 ± 10.4 (3)

( ) Number of observations.

TABLE 3. Blood Concentrations of Methoxyflurane During Prolonged Administration of 0.50 Per Cent Inspired Concentration.

Experiment	Blood Levels in mg./100 ml.							
	½ Hour*		1½ Hours*		2½ Hours*		3½ Hours*	
	Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial	Venous
J	20.5	18.0	24.2	26.7	33.7	15.7	34.3	25.0
K	28.1	30.6	40.4	41.8	51.7	43.8	38.2	37.6
L	32.9	25.3	44.4	46.6	56.7	38.2	67.4	18.3
M	20.2	11.2	33.4	30.3	40.4	37.9	41.3	34.0
N	22.5	13.2	—	—	46.1	23.0	36.2	25.3
O	36.5	27.8	44.4	45.5	53.1	56.7	56.7	35.1
P	28.1	22.2	33.1	37.1	48.0	22.7	28.9	28.1
Q	43.5	25.6	39.9	31.7	39.3	51.7	64.6	31.2
R	30.3	16.8	40.4	25.8	41.8	21.6	33.1	22.5
S	14.9	11.8	19.1	19.1	17.7	15.7	21.9	10.7
T	18.3	8.4	12.4	11.0	18.3	12.1	27.8	13.8
U	16.6	16.9	31.7	25.6	33.7	33.1	33.5	33.5
Mean	26.0	19.0	33.0	31.0	40.0	31.0	40.3	26.3
±SE	2.5	2.1	3.2	3.3	3.8	4.3	4.2	2.5

* Time following introduction of methoxyflurane into the inspired air. Anesthesia was induced in all animals with thiamylal sodium.

but were beginning to show physical signs of diminished muscle relaxation. Blood samples were taken again 4 hours following termination of anesthesia, and at this time the animals were awake but tranquil. Twenty-four hour samples were drawn in a few experiments and only a trace of methoxyflurane could be found at this time. Aortic pressure and ventricular contractile force measurements taken simultaneously with the blood samples are summarized in table 2. During induction of anesthesia with methoxyflurane, mean aortic pressure was depressed to a greater degree than ventricular contractile force. When the animals were placed on a maintenance concentration of 0.50 per cent, there were no significant changes in either parameter from the values obtained on intubation. This correlates with the similar blood levels of methoxyflurane at these concentrations (table 1). As the inspired concentration was increased, there was a significant depression of both aortic pressure and contractile force from the preceding level of anesthesia. The only deviation from this general depression occurred when the inspired concentration was increased to 1.25 per cent. The values obtained at this level were quite similar to those at 1.00 per cent. However, it should be mentioned that 3 animals were depressed to such a degree when ventilated with a 1.00 per cent concentration that they were not exposed to 1.25 per cent. Therefore, if only the data from the 5 animals exposed to both concentrations are used in comparing the degree of depression between these two concentrations, the mean aortic pressure at 1.00 per cent is increased to 53.6 mm. of mercury as compared to 42.1 mm. of mercury when data from all 7 animals are considered. The difference between the mean pressures at the two levels then becomes significant, but we believed, after examining the data, that values from all 7 animals ventilated with 1.00 per cent should be used in calculating the mean pressure at this level. Fortunately, this was the only place in which variation in the number of animals caused a significant change in the results obtained. During emergence from anesthesia, contractile force had returned to control levels 45 minutes following withdrawal of the anesthetic while aortic pressure was still significantly depressed at this time. After four hours both parameters had returned to near control levels.

Table 3 summarizes the arterial and venous

TABLE 4. Mean Changes in Aortic Pressure and Ventricular Contractile Force in 12 Animals During Prolonged Administration of 0.50 Per Cent Methoxyflurane

	Mean Aortic Pressure	Ventricular Contractile Force
	Mm. Hg±S.E.	Grams±S.E.
Control	108.7 ± 6.8	112.0 ± 17.1
½ Hour	81.3 ± 6.4	77.6 ± 7.1
1½ Hours	73.4 ± 6.0	76.0 ± 6.5
2½ Hours	76.3 ± 2.9	80.0 ± 4.1
3½ Hours	67.5 ± 1.9	73.3 ± 4.1

blood levels of methoxyflurane in 12 animals in which anesthesia was induced with thiamylal and was then maintained with 0.50 per cent methoxyflurane for three and one-half hours. The arterial and venous concentrations of methoxyflurane after the first one-half hour of anesthesia were quite similar to the values obtained in the animals respired for 30 minutes with this concentration in the first series of experiments (table 1). As anesthesia was prolonged, the blood levels increased to some extent during the following two hours and then began to level off during the last hour. However, only the increases in the venous concentrations from one-half to one and one-half hours and the arterial concentrations from one and one-half to two and one-half hours were of statistical significance. Table 4 summarizes the changes in mean aortic pressure and ventricular contractile force during prolonged anesthesia with a 0.50 per cent concentration of methoxyflurane. The most significant changes in both parameters occurred during the first one-half hour of anesthesia. During the next three hours only small decrements in contractile force occurred while blood pressure was significantly depressed again between two and one-half and three and one-half hours.

Discussion

Arterial concentrations of methoxyflurane in dogs between 35 mg./100 ml. during light anesthesia and 45 mg./100 ml. during surgical levels have been reported.‡ The venous concentrations at the same levels were found to be 15 and 35 mg./100 ml., respectively. A considerable difference between arterial and venous concentrations during induction and maintenance of anesthesia was also shown but little difference found during the recovery period. Walts *et al.*§ have reported human arterial blood concentrations of 9 mg./100 ml. during light methoxyflurane anesthesia and 15 mg./100 ml. in deeper levels.

In the present experiments the mean arterial and venous concentrations of methoxyflurane following insertion of the endotracheal tube were found to be approximately 26.5 and 19.1 mg./100 ml. respectively. It was also observed that mean aortic pressure was depressed to a greater extent than contractile force during induction of anesthesia with this agent. However, we have shown in a previous study[4] that the myocardial depressant effect of methoxyflurane is not secondary to peripheral vasodilation since ventricular contractile force remains depressed even when arterial pressure is supported by the administration of methoxamine. As the inspired concentration of methoxyflurane was progressively increased there was a corresponding increase in the arterial and venous blood levels resulting in depression of both aortic pressure and ventricular contractile force. The maximum blood levels and depressions of both parameters with this agent occurred with an inspired concentration of approximately 1.00 per cent. Forty-five minutes after discontinuing anesthesia ventricular contractile force had returned to control levels while aortic pressure was still significantly depressed. This indicates that the peripheral effects of methoxyflurane are also more prolonged than the myocardial depressant action. Consideration might also be given to the possible influence of variations in blood pH and P_{CO_2} on the cardiovascular effects of methoxyflurane. However, other investigators have shown the changes in these parameters to be minimal during anesthesia with methoxyflurane as long as respiration is supported[10,11] as in the present experiments.

In the animals maintained on a 0.50 per cent concentration of methoxyflurane for a prolonged period of time, the blood levels of methoxyflurane reached peak concentrations after about two and one-half hours of anesthesia. These blood levels agree with those

‡ Chenoweth, M. B., and others, from brochure made available to investigators.

§ Walts, L. F., Lewis, E. H., and Sweet, E. F., from brochure made available to investigators.

reported by Chenoweth‡ during surgical levels of anesthesia. It was also noted that the blood levels of methoxyflurane obtained after prolonged administration of the 0.50 per cent concentration were comparable to those obtained by administering higher concentrations for shorter periods of time. However, the greatest depression of aortic pressure and contractile force occurred during the first half hour of anesthesia with constant 0.50 per cent concentration with only minimal changes occurring during the next two hours, while in the experiments in which the inspired concentration was increased there was a progressive depression of both parameters which resulted in cardiac arrest if the anesthetic was not removed. Therefore, maximum blood levels of methoxyflurane can be attained by prolonged anesthesia with a minimal inspired concentration without the development of severe cardiovascular depression. This may be a significant safety factor to consider when choosing an anesthetic for lengthy procedures which require deep levels of anesthesia.

Summary

Arterial and venous blood concentrations of methoxyflurane were determined by gas-liquid adsorption chromatography during induction and/or maintenance and recovery from anesthesia with methoxyflurane. The blood levels of this agent were correlated with simultaneous measurements of ventricular contractile force and aortic pressure.

Mean methoxyflurane concentrations of 26.5 and 19.1 mg./100 ml. were found in the arterial and venous blood, respectively, immediately following placement of the endotracheal tube. Following induction of anesthesia, aortic pressure was found to be depressed to a greater degree than contractile force. On increasing the concentration of methoxyflurane in the inspired air, there was a corresponding increase in the blood levels resulting in a depression of both aortic pressure and contractile force. However, it was also observed that maximum blood levels of methoxyflurane could be obtained by maintaining the animals on an inspired concentration of 0.50 per cent for about two and one-half hours. When this procedure was used, the greatest depressions of both parameters occurred during the first half hour of anesthesia with only minimal changes occurring during the next two hours. When anesthesia was discontinued, contractile force returned to control levels within 45 minutes, while aortic pressure was still significantly depressed. At this time, the animals were still unconscious but showing physical signs of diminished muscle relaxation. Four hours after termination of anesthesia, all animals were awake and both parameters were back to control levels. The following day only trace amounts of methoxyflurane could be found in the arterial and venous blood.

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References

1. Summers, F. W., and Adriani, J.: Gas chromatography: an analytical method for anesthesiology research, ANESTHESIOLOGY 22: 100, 1961.
2. Noehren, T. H., and Cudmore, J. W.: Ethyl ether content in blood as determined by gas chromatography, ANESTHESIOLOGY 22: 519, 1961.
3. Gadsden, R. H., McCord, W. M., Woods, E. F., and Bagwell, E. E.: Estimation of methoxyflurane (Penthrane) in biological fluid by gas chromatography, In preparation.
4. Artusio, J. F., Jr., Van Poznak, A., Hunt, R. E., Tiers, F. M., and Alexander, M.: Clinical evaluation of methoxyflurane in man, ANESTHESIOLOGY 21: 512, 1960.
5. Boniface, K. J., Brodie, O. J., and Walton, R. P.: Resistance strain gauge arches for direct measurement of heart contractile force in animals, Proc. Soc. Exp. Biol. Med. 84: 263, 1953.
6. Cotten, M. DeV.: Circulatory changes affecting measurement of heart force in situ with strain gauge arches, Amer. J. Physiol. 174: 365, 1953.
7. Cotten, M. DeV., and Bay, E.: Direct measurement of changes in cardiac contractile force, Amer. J. Physiol. 187: 122, 1956.
8. Snedecor, G. W.: Statistical Methods, Fifth Edition. Ames, Iowa, The Iowa State College Press, 1956, Chapter 2, p. 49.
9. Bagwell, E. E., and Woods, E. F.: Cardiovascular effects of methoxyflurane, ANESTHESIOLOGY 23: 51, 1962.
10. Dobkin, A. B., and Fedoruk, S.: Comparison of cardiovascular, respiratory and metabolic effects of methoxyflurane and halothane in dogs, ANESTHESIOLOGY 22: 355, 1961.
11. Millar, R. A., and Morris, M. E.: Study of methoxyflurane anesthesia, Canad. Anaesth. Soc. J. 8: 210, 1961.