

Depth of Halothane Anesthesia Potentiates Citrate-induced Ionized Hypocalcemia and Adverse Cardiovascular Events in Dogs

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The purpose of this study was to determine if depth of halothane anesthesia contributed to the adverse cardiovascular effects of citrate-induced ionized hypocalcemia. Six mongrel dogs were monitored with arterial, central venous, and pulmonary artery flow-directed catheters. Measured end-tidal halothane assured a constant depth of anesthesia, while controlled ventilation and arterial blood gas analysis provided constant acid-base status. Each dog received sodium citrate (USP Fenwal) equivalent to fresh frozen plasma, 1.0 ml · kg⁻¹ · min⁻¹, during both deep (D) and light (L) halothane anesthesia. Three dogs received the infusion during L halothane anesthesia first; after a 1-h stabilization period (2.5 h after first infusion) they received a second equivalent infusion during D halothane anesthesia. Three other dogs were studied first with D, then with L halothane. Mean expired halothane (±SEM) for group D was 1.52 ± 0.08%, for group L, 0.85 ± 0.07%. Significantly greater adverse cardiovascular effects were seen during D halothane anesthesia; four of the six dogs that received citrate during D halothane anesthesia required cessation of the infusion or suffered cardiac arrest. All six infusions during L halothane anesthesia were tolerated. In both groups, significant reductions in ionized calcium [Ca⁺⁺] ($P < 0.0001$) and mean arterial pressure (MAP) ($P < 0.005$) were observed; greater reductions in both parameters occurred in group D ($P < 0.0036-0.0005$). In group D, but not in group L, cardiac output was depressed compared to baseline ($P < 0.005$). Pulmonary vascular resistance and central venous pressure were increased ($P < 0.001$) in group D but not in group L; pulmonary artery occlusion pressure and heart rate were increased in both groups compared to baseline ($P < 0.005$). The author concludes that a deeper plane of halothane anesthesia was associated with both greater myocardial depression and greater ionized hypocalcemia following citrate infusion in dogs. (Key words: Anesthetics, volatile: halothane. Ions, calcium, citrate: cardiovascular effects.)

WE HAVE OBSERVED a number of children who have developed severe hypotension, occasionally requiring resuscitation, during the rapid administration of fresh frozen plasma (FFP); we postulated that these effects were due in part to binding of ionized calcium [Ca⁺⁺] by citrate, so-called "citrate toxicity." An extensive clinical study of children who had suffered severe thermal injury found that marked reductions in [Ca⁺⁺] consis-

tently follow rapid FFP administration. There was no relationship, however, between the development of hypotension and rate or duration of FFP infusion, or degree of ionized hypocalcemia.† A review of operating room records at the Shriners Burns Institute revealed that, in intraoperative resuscitations which were temporally related to the rapid administration of FFP, seven patients were receiving halothane anesthesia at the time of eight resuscitations. The purpose of this laboratory study was to determine if depth of halothane anesthesia affects the development of the adverse cardiovascular events that are observed during citrate-induced ionized hypocalcemia.

Methods and Materials

Anesthesia was induced in six mongrel dogs with intravenous thiopental (15–20 mg/kg). Following endotracheal intubation, anesthesia was maintained with halothane in oxygen and sufficient metubine hydrochloride to completely suppress the train-of-four response to peripheral nerve stimulation.¹ Arterial, central venous, and pulmonary artery flow-directed catheters were percutaneously inserted. The electrocardiogram lead II and hemodynamic variables were recorded on a Hewlett-Packard strip-chart recorder (Model 7758A) using transducers calibrated to mercury. Controlled ventilation and measured arterial blood gases provided normal acid-base status (Harvard Small Animal Ventilator, Harvard Apparatus, South Natick, MA). End-tidal halothane concentrations were measured with a Beckman LB 2 analyzer (Beckman Instruments, Inc., Irvine, CA). Animals were randomly assigned to first receive either deep halothane anesthesia (group D, approximately 1.5% end-tidal) or light halothane anesthesia (group L, approximately 0.8% end-tidal). Each animal was scheduled to receive two 20-min infusions of sodium citrate (CPD, Formula A, USP‡) administered through an upper extremity intravenous catheter (Harvard constant infusion pump). Sodium citrate, 4.6 ml/kg, was diluted to 180 ml with 0.9% saline in two large syringes;§ this dose delivered

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‡ Supplied by Fenwal Laboratories, Division of Travenol Laboratories, Inc., Deerfield, IL 60015.

§ Supplied by Monoject, Division of Sherwood Medical, A. Brunswick Co., St. Louis, MO 63103.

citrate roughly equivalent to that in $1.0 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ FFP. One infusion was administered during deep and one during light halothane anesthesia. A 1-h stabilization period at the desired expired halothane concentration was allowed prior to beginning each infusion. The second infusion of citrate was begun a minimum of 2.5 h after the first infusion. Heart rate, central venous pressure (CVP), mean arterial pressure (MAP), mean pulmonary artery pressure (PAP), pulmonary artery occlusion pressure (PAOP), thermodilution cardiac output (CO), and $[\text{Ca}^{++}]$ were measured at 2-min intervals for 20 min. Blood samples for later citrate determination were obtained at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 21, 22, 23, 24, 25, 30, and 45 min.

Data were analyzed by analysis of variance with allowance for repeated measures. Variables are expressed as mean \pm SEM. Significance of planned comparisons was judged according to Bonferroni *t* tests, using a (simultaneous) level of 0.05; *i.e.*, 0.05 was divided by the number of comparisons in order to obtain the individual level that a *P* value must satisfy to achieve significance.^{2,3}

The analysis of each hemodynamic variable involved ten comparisons: the difference between the two treatments at 6, 8, and 10 min *versus* baseline within each treatment; and the difference in the change from baseline to 6, 8, and 10 min between the treatments. Hemodynamic data were analyzed at these time intervals because our previous experience found that 5 min was the time interval when cardiovascular effects became evident. In addition, the analysis of $[\text{Ca}^{++}]$ compared the mean at 2 and 4 min against baseline within each treatment (for a total of 14 comparisons). Ionized calcium was analyzed at all time intervals because I anticipated these changes to occur immediately. Thus, for example, with ten comparisons, each would have to have a *P* value smaller than $0.05/10 = 0.005$ in order to be judged significant at the simultaneous 0.05 level. This allowance for simultaneous testing ensures that one or more significant differences can arise by chance alone with probability no greater than 0.05.

Results

The mean weight (\pm SEM) of the animals was $23.0 \pm 1.04 \text{ kg}$; the mean (\pm SEM) expired halothane was $1.52 \pm 0.08\%$ for group D and $0.85 \pm 0.07\%$ for group L. For group D, the mean \pm SEM pH was 7.49 ± 0.03 , PaCO_2 24.2 ± 1.4 , PaO_2 526 ± 24 , while, for group L the mean pH was 7.46 ± 0.03 , PaCO_2 27 ± 1.6 , and PaO_2 550 ± 25 . There was no difference in temperature or acid-base balance between study groups. Three animals received the first infusion during D halothane anesthesia and the second infusion during L halothane anesthesia;

the three other animals were treated in the reverse order.

The infusion of citrate produced significantly greater adverse cardiovascular effects during D halothane anesthesia. Two of the animals that received citrate during D halothane as the first part of the experiment required cessation of the citrate infusion between the 11th and 14th min because the systolic blood pressure had fallen to $<50 \text{ mmHg}$, and cardiac arrest seemed imminent; the $[\text{Ca}^{++}]$ had fallen to 0.64 and 0.36 mM/L. The three dogs who received citrate during D halothane as the second half of the experiments were allowed to receive the full 20-min citrate infusion; two suffered cardiac arrest prior to the end of the infusion. The $[\text{Ca}^{++}]$ had fallen to 0.20 and 0.27 mM/L. For these reasons, statistical analysis examined only data from the first 10 min of each infusion. All animals who received citrate infusions during L halothane anesthesia tolerated the

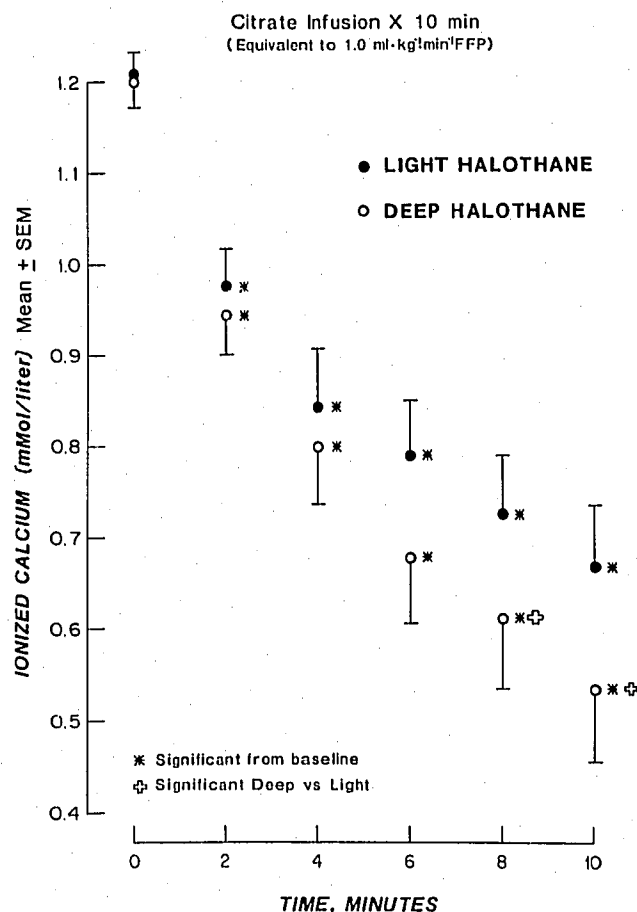


FIG. 1. Ionized calcium $[\text{Ca}^{++}]$ is plotted *versus* time for equivalent infusions of citrate. A highly significant decrease in $[\text{Ca}^{++}]$ was observed in both groups; however, a significantly greater decrease in $[\text{Ca}^{++}]$ was noted in the deeply anesthetized compared to the lightly anesthetized animals.

TABLE 1. Cardiovascular Events Produced by Sodium Citrate Infusion in Six Dogs During Deep (D) and Light (L) Halothane Anesthesia (Mean \pm SEM)

		Time (Min)					
		0	2	4	6	8	10
[Ca ⁺⁺]	D	1.20 \pm 0.03	0.94 \pm 0.04*	0.80 \pm 0.06*	0.67 \pm 0.07*	0.61 \pm 0.08*†	0.53 \pm 0.08*†
(mM/L)	L	1.21 \pm 0.02	0.97 \pm 0.04*	0.84 \pm 0.06*	0.78 \pm 0.06*	0.72 \pm 0.06*	0.66 \pm 0.07*
MAP	D	76.1 \pm 6.6	70.8 \pm 6.2	56.5 \pm 7.7	45.8 \pm 7.5*†	41.7 \pm 7.5*†	35.6 \pm 8.8*†
(mmHg)	L	98.3 \pm 11.4	96.6 \pm 10.7	90.3 \pm 10.4	88.4 \pm 10.4	88.9 \pm 11.4	80.0 \pm 9.9*
CO	D	2.89 \pm 0.74	2.53 \pm 0.83	2.53 \pm 0.83	2.52 \pm 1.33	1.99 \pm 1.10*	1.73 \pm 1.16*
(l/min)	L	4.46 \pm 0.41	4.14 \pm 0.41	4.42 \pm 0.63	4.11 \pm 1.09	4.26 \pm 1.35	3.83 \pm 1.40
PAP	D	11.4 \pm 0.8	10.9 \pm 1.0	10.8 \pm 1.0	10.4 \pm 0.9	11.5 \pm 0.6	11.2 \pm 0.7
(mmHg)	L	11.4 \pm 1.6	11.8 \pm 1.5	12.3 \pm 1.3	12.7 \pm 1.2	12.8 \pm 1.4	12.8 \pm 1.3
PAOP	D	6.5 \pm 1.1	7.2 \pm 1.0	8.0 \pm 1.3	7.7 \pm 1.2	8.7 \pm 0.9*	9.0 \pm 1.1*
(mmHg)	L	6.2 \pm 1.1	6.7 \pm 1.0	7.2 \pm 1.0	8.0 \pm 1.0	8.0 \pm 0.9	8.5 \pm 1.1*
CVP	D	3.5 \pm 2.7	3.2 \pm 2.5	4.5 \pm 2.9	5.5 \pm 2.9	5.8 \pm 2.8	7.3 \pm 3.0*
(mmHg)	L	4.2 \pm 2.1	4.3 \pm 2.7	4.8 \pm 2.8	4.7 \pm 2.8	4.8 \pm 2.5	4.8 \pm 3.0
HR	D	110.2 \pm 7.9	111.0 \pm 6.7	115.7 \pm 6.3	116.2 \pm 5.1	122.0 \pm 6.2*	121.7 \pm 5.7*
(b/min)	L	113.5 \pm 6.4	121.7 \pm 5.9	129.3 \pm 7.1	130.7 \pm 6.6*	134.5 \pm 5.8*	132.7 \pm 5.2*
PVR	D	4.1 \pm 0.4	4.6 \pm 0.6	4.8 \pm 0.5	5.3 \pm 1.3	7.2 \pm 1.6*	8.5 \pm 1.8*†
(PRU)	L	2.6 \pm 0.4	2.9 \pm 0.4	2.8 \pm 0.3	3.2 \pm 0.4	3.2 \pm 0.5	3.6 \pm 0.5
SVR	D	28.2 \pm 4.1	30.1 \pm 4.3	24.7 \pm 3.5	21.2 \pm 3.8	23.6 \pm 4.0	24.9 \pm 6.9
(PRU)	L	22.3 \pm 2.8	23.3 \pm 2.4	20.6 \pm 2.4	22.3 \pm 3.0	23.0 \pm 4.3	21.0 \pm 2.9

[Ca⁺⁺] = ionized calcium; MAP = mean arterial pressure; CO = cardiac output; PAP = mean pulmonary artery pressure; PAOP = pulmonary artery occlusion pressure; CVP = central venous pressure; HR = heart rate; PVR = pulmonary vascular resistance; SVR = systemic

vascular resistance; PRU = peripheral resistance units.

* Significant from baseline.

† Significant deep vs. light.

full 20-min infusion; one animal developed severe hypotension (systolic blood pressure 45 mmHg), but survived.

The infusion of citrate resulted in highly significant reductions in [Ca⁺⁺] in both groups ($P < 0.0001$) at all

time intervals (fig. 1); greater reductions were observed in group D than in group L at 8 and 10 min ($P < 0.0036$, table 1). Mean arterial pressure was reduced from baseline in group L at 10 min and group D at 6, 8, and 10 min ($P < 0.005$); however, the fall in MAP was greater in group D compared to group L at 6, 8, and 10 min ($P < 0.0005$) (fig. 2). Cardiac output was reduced in group D at 8 and 10 min ($P < 0.005$), but not in group L. Pulmonary artery occlusion pressure increased in group D at 8 and 10 min and group L at 10 min compared to baseline ($P < 0.005$); heart rate increased compared to baseline at 8 and 10 min for group D and 6, 8, and 10 min for group L ($P < 0.005$). These changes were not significantly different between groups ($P = NS$). Pulmonary vascular resistance (PVR) was increased at 8 and 10 min and CVP was increased at 10 min in group D ($P < 0.001$), but not in group L. There were no significant changes in pulmonary artery pressure or systemic vascular resistance (SVR) in either group (table 1).

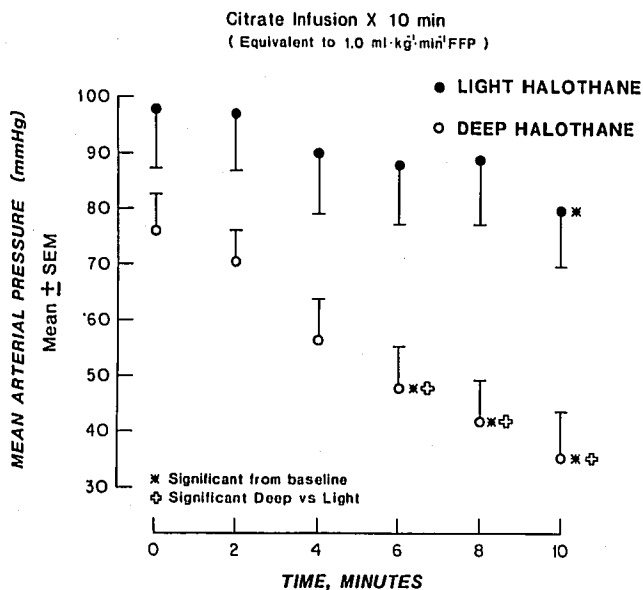


FIG. 2. Mean arterial pressure (MAP) is plotted versus time for infusions of sodium citrate equivalent to $1.0 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ of fresh frozen plasma during both deep and light halothane anesthesia. A significant decrease in MAP was observed in both groups; a significantly greater decrease in MAP was observed during deep compared to light halothane anesthesia.

Discussion

Results from this study suggest that the depth of halothane anesthesia influences the adverse cardiovascular responses resulting from citrate-induced ionized hypocalcemia. Citrate by itself, in the presence of normal ionized calcium values, has been demonstrated to have no cardiovascular effects in both isolated and intact dog models.^{4,5}

The myocardial depressant effects of halothane are well documented; it has been postulated that halothane may alter intracardiac $[Ca^{++}]$ homeostasis.⁶⁻⁹ Recently, the actions of the potent anesthetic agents halothane and enflurane have been described as having "calcium channel-blocking activity."^{7,10,11} Indeed, because of this myocardial depressant effect, halothane may, in certain circumstances, be beneficial since myocardial work is decreased.¹²⁻¹⁴ The combination of halothane anesthesia and the acute administration of cardioactive calcium channel-blocking drugs, however, has been demonstrated to cause regional myocardial dysfunction;¹⁵ in some cases, this combination of drugs would be undesirable. The chronic administration of calcium channel blocking drugs and potent anesthetic agents appears to be less dangerous.¹¹

Citrate causes myocardial depression in animals and humans when administered in concentrations similar to those used in this study.^{4,5,16} Citrate-induced ionized hypocalcemia is associated with increased PAOP, decreased CO, and systemic hypotension. Beta adrenergic blockade potentiates the left ventricular dysfunction produced by citrate-induced ionized hypocalcemia in the animal model.⁵ It is not surprising, therefore, that the greater concentrations of halothane anesthesia in our study would result in greater myocardial depression, and that this depression would be potentiated by a drug (citrate or calcium channel-blockers) which causes the sudden reduction of available extracellular $[Ca^{++}]$. This is supported by the observation that halothane-induced myocardial depression is reversed following the administration of exogenous calcium.^{17,18} The presumed mechanism is that, by elevating the $[Ca^{++}]$ in blood, one is able to increase the transport of $[Ca^{++}]$ to the myocardium. This is also consistent with our clinical experience which has been that the hypotension, which occasionally accompanies the rapid infusion of FFP, is readily reversed by the administration of exogenous calcium.

Citrate in low concentrations produces vasodilation; the mechanism of action producing the peripheral vasodilation is unknown.^{5,16} Calcium channel-blocking drugs act on the smooth muscle of peripheral arterioles by a slightly different mechanism than on cardiac muscle; however, the end result is similar, *i.e.*, smooth muscle relaxation and vasodilation.¹⁹ It is possible that the brief but rapid reduction in $[Ca^{++}]$ produced by citrate upsets the normal balance between extracellular and intracellular $[Ca^{++}]$ in vascular smooth muscle, resulting in transient smooth muscle relaxation, although we did not observe changes in SVR.

The rapid administration of citrated blood products in humans has been demonstrated to consistently produce ionized hypocalcemia.^{16,20,21}† The role of ionized

hypocalcemia in the development of systemic hypotension has been debated for many years.²⁰⁻²² Ionized hypocalcemia has been observed in adults transfused with citrated whole blood at rates equivalent to $1.0 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; however, with the current widespread use of component therapy, it is rare to administer large amounts of citrated blood products to adults.²⁰ In citrated packed red blood cells, there is minimal citrate and, therefore, ionized hypocalcemia is infrequently observed. Although FFP has the highest concentration of citrate per unit volume of any blood product, and is easy to administer rapidly because of its low viscosity, it is very unusual to administer FFP to adults at sufficient volume or rate to cause severe ionized hypocalcemia. It is possible, however, to achieve these critical rates and volumes of infusion in children.† The inconsistency of observed adverse hemodynamic effects in patients may well be related to a combination of factors, such as site of infusion (central *vs.* peripheral), temperature of the patient, ability to metabolize citrate, available calcium reserves (neonate *vs.* older child or adult), and relative depth of anesthesia, especially with potent anesthetic agents. Altered citrate kinetics as a result of changes in cardiac output (liver perfusion) and/or hepatic blood flow due to decrease in splanchnic blood flow, as well as reduced renal blood flow, may be a factor. Unfortunately, the planned determination of plasma citrate values was not possible in this study, because most of the specimens were lost in a laboratory accident. However, in a subsequent study, we have found an excellent correlation between $[Ca^{++}]$ and serum citrate ($r = 0.99$, $P = 0.0001$) for 224 determinations made in duplicate.²³ It certainly is possible that a greater depth of halothane anesthesia altered hepatic metabolism of citrate resulting in lower $[Ca^{++}]$ values during deep halothane anesthesia.

In summary, a greater depth of halothane anesthesia was associated with both greater myocardial depression and greater ionized hypocalcemia following citrate infusion in dogs. These data suggest that the depth of halothane anesthesia may be an important consideration when anesthetizing children in whom massive, rapid administration of citrated blood products (*i.e.*, whole blood, FFP) is anticipated.

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