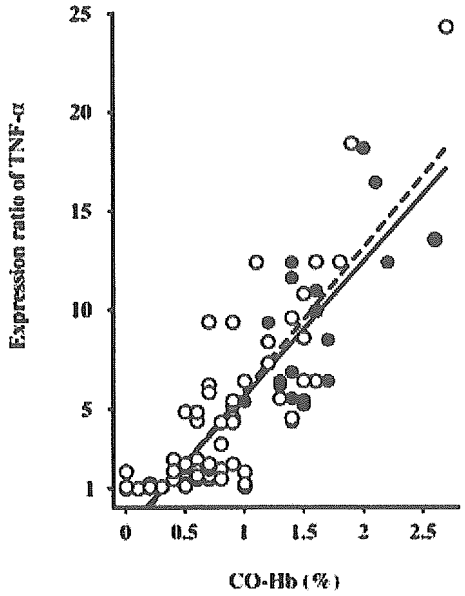

Fig. 6 Relationship between the carboxyhemoglobin concentrations (CO-Hb) and the expression ratio of tumor necrosis factor-α (TNF-α). Open circles = patients with CO-Hb concentrations ≥ 1.0% at the preoperative measurement (n=35); closed circles = patients with CO-Hb concentrations < 1.0% at the preoperative measurement (n=10). Solid line = regression among patients who showed CO-Hb concentrations < 1.0% at the preoperative measurement; dotted line = regression among all patients.

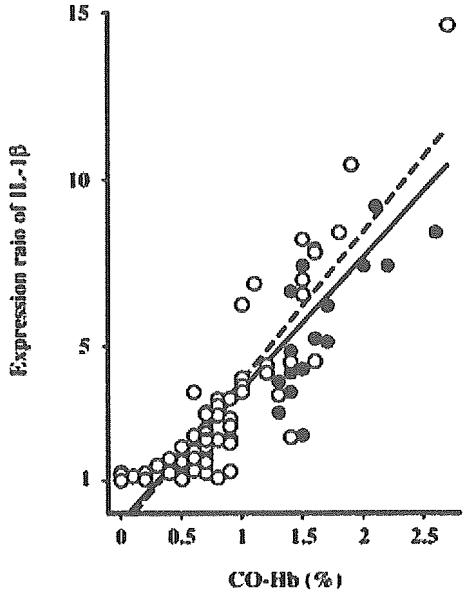

Fig. 7 Relationship between the carboxyhemoglobin concentrations (CO-Hb) and the expression ratio of interleukin-1β (IL-1β). Open circles = patients with CO-Hb concentrations < 1.0% at the preoperative measurement (n=35); closed circles = patients with CO-Hb concentrations ≥ 1.0% at the preoperative measurement (n=10). Solid line = regression among patients who showed CO-Hb concentrations < 1.0% at the preoperative measurement; dotted line = regression among all patients.

glucocorticoids to regulate the CPB-induced inflammatory response also has been shown[3] to attenuate the increase in serum inflammatory cytokines and increased anti-inflammatory cytokines. In this study, we measured the mRNA expression of key inflammatory cytokines such as TNF-α and IL-1β because the mRNA level is thought to be a more sensitive and earlier indicator than the concentration of serum cytokines that follows the expression of mRNA. The results of this study clearly showed that the use of CPB during CABG increased the gene expressions of TNF-α and IL-1β as well as HO-1 in circulating blood, and that anti-inflammatory strategies attenuated those increases. In addition, significant correlations of HO-1 expression with inflammatory cytokines expression indicate that expression changes of HO-1 may reflect surgical stress-induced inflammation. Results of a previous study[25] that measured gene expressions by less quantitative method in off-pump CABG and standard CABG support our results.

Whether CO-Hb is a clinical useful index reflecting the inflammatory response induced by surgical insults remains unknown. In this study, the concentrations of CO-Hb varied before surgery and the change rates were lower than those shown in CO poisoning or sepsis[8]. In addition, the correlation between CO-Hb concentrations and the expression of inflammatory mediators was low in patients who showed high concentrations of CO-Hb before surgery. These results indicate that the usefulness of CO-Hb concentrations as a marker of surgical stress might be limited based on the facts that an excessive inflammatory response can influence

morbidity in more critical patients than those included in this study, and that CO-Hb concentrations before the surgery may vary further in such patients.

In conclusion, the expression of inflammatory mediators such as TNF-α, IL-1β, and HO-1 in circulating blood increased during CABG, especially when CPB was used. Treatment with glucocorticoid therapy attenuated these increases. CO-Hb concentrations also increased during CABG, however, CO-Hb concentrations did not change and were less well correlated with inflammatory mediators in patients who showed CO-Hb values≧ 1% before surgery, despite the fact that we chose patients with no obvious signs of inflammation or respiratory disease, and that the inhalation oxygen concentration and hemoglobin concentration were managed in a uniform fashion. CO-Hb measurements might not be useful in the clinical monitoring of surgical stress because of the limited degree of changes, the variation of baseline values, and the necessity for management under fixed conditions.

References

1. Laffey JG, Boylan JF, Cheng DC: The systemic inflammatory response to cardiac surgery: implications for the anesthesiologist. Anesthesiology 2002; 97: 215–252.
2. Lin E, Calvano SE, Lowry SF: Inflammatory cytokines and cell response in surgery. Surgery 2000; 127: 117–126.
3. Chaney MA: Corticosteroids and cardiopulmonary bypass: a review of clinical investigations. Chest 2002; 121: 921–931.
4. Markewitz A, Lante W, Franke A, Marohl K, Kuhlmann WD, Weinhold C: Alterations of cell-mediated immunity following cardiac operations: clinical implications and open questions. Shock 2001; 16: 10–15.
5. Asimakopoulos G, Gourlay T: A review of anti-inflammatory strategies in cardiac surgery. Perfusion 2003; 18: 7–12.
6. Paparella D, Yau TM, Young E: Cardiopulmonary bypass induced inflammation: pathophysiology and treatment. An update. Eur J Cardiothorac Surg 2002; 21: 232–244.
7. Hunter K, Mascia M, Eudaric P, Simpkins C: Evidence that carbon monoxide is a mediator of critical illness. Cell Mol Biol (Noisy-le-grand) 1994; 40: 507–510.
8. Moncure M, Brathwaite CE, Samaha E, Marburger R, Ross SE: Carboxyhemoglobin elevation in trauma victims. J Trauma 1999; 46: 424–427.
9. Zegdi R, Perrin D, Burdin M, Boiteau R, Tenaillon A: Increased endogenous carbon monoxide production in severe sepsis. Intensive Care Med 2002; 28: 793–796.
10. Takeda R, Tanaka A, Maeda T, Yamaoka Y, Nakamura K, Sano K, Kataoka M, Nakamura Y, Morimoto T, Mukaihara S: Perioperative changes in carbonylhemoglobin and methemoglobin during abdominal surgery: alteration in endogenous generation of carbon monoxide. J Gastroenterol Hepatol 2002; 17: 535–541.
11. De las Heras D, Fernandez J, Gines P, Cardenas A, Ortega R, Navasa M, Barbera JA, Calahorra B, Guevara M, Bataller R, Jimenez W, Arroyo V, Rodes J: Increased carbon monoxide production in patients with cirrhosis with and without spontaneous bacterial peritonitis. Hepatology 2003; 38: 452–459.
12. Maines MD: The heme oxygenase system: a regulator of second messenger gases. Annu Rev Pharmacol Toxicol 1997; 37: 517–554.
13. Keyse SM, Tyrrell RM: Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide, and sodium arsenite. Proc Natl Acad Sci U S A 1989; 86: 99–103.
14. Piantadosi CA: Biological chemistry of carbon monoxide. Antioxid Redox Signal 2002; 4 : 259–270.
15. Meyer J, Prien T, Van Aken H, Bone HG, Waurick R, Theilmeier G, Booke M: Arterio-venous carboxyhemoglobin difference suggests carbon monoxide production by human lungs. Biochem Biophys Res Commun 1998; 244: 230–232.
16. Sedlacek M, Halpern NA, Uribarri J: Carboxyhemoglobin and lactate levels do not correlate in critically ill patients. Am J Ther 1999; 6: 241–244.
17. Chomczynski P: A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. Biotechniques 1993; 15: 532–534, 536–537.
18. Chang JT, Chen IH, Liao CT, Wang HM, Hsu YM, Hung KF, Lin CJ, Hsieh LL, Cheng AJ: A reverse transcription comparative real-time PCR method for quantitative detection of angiogenic growth factors in head and neck cancer patients. Clin Biochem 2002; 35: 591–596.
19. Moncada S, Higgs EA: Endogenous nitric oxide: physiology, pathology and clinical relevance. Eur J Clin Invest 1991; 21: 361–374.
20. Marks GS, Brien JF, Nakatsu K, McLaughlin BE: Does carbon monoxide have a physiological function? Trends Pharmacol Sci 1991; 12: 185–188.
21. Morse D, Choi AM: Heme oxygenase-1: the "emerging molecule" has arrived. Am J Respir Cell Mol Biol 2002; 27: 8–16.
22. Zegdi R, Caid R, Van De Louw A, Perrin D, Burdin M, Boiteau R, Tenaillon A: Exhaled carbon monoxide in mechanically ventilated critically ill patients: influence of inspired oxygen fraction. Intensive Care

Med 2000; 26: 1228–1231.
23. Angelini GD, Taylor FC, Reeves BC, Ascione R: Early and midterm outcome after off-pump and on-pump surgery in Beating Heart Against Cardioplegic Arrest Studies (BHACAS 1 and 2): a pooled analysis of two randomized controlled trials. Lancet 2002; 359: 1194–1199.
24. Fransen E, Maessen J, Dentener M, Senden N, Geskes G, Buurman W: Systemic inflammation present in patients undergoing CABG without extracorporeal circulation. Chest 1998; 113: 1290–1295.
25. Okubo N, Hatori N, Ochi M, Tanaka S: Comparison of m-RNA expression for inflammatory mediators in leukocytes between on-pump and off-pump coronary artery bypass grafting. Ann Thorac Cardiovasc Surg 2003; 9: 43–49.

(Received, August 24, 2004)
(Accepted, October 20, 2004)

Journal of Clinical Anesthesia (2005) 17, 3–7

ELSEVIER

Journal of Clinical Anesthesia

Original contribution

Effects of atrial natriuretic peptide at a low dose on water and electrolyte metabolism during general anesthesia

Mariko Koda MD (Staff Anesthesiologist)*, Atsuhiro Sakamoto MD (Assistant Professor), Ryo Ogawa MD (Professor and Chairman)

Department of Anesthesiology, Nippon Medical School, Tokyo 113-8603, Japan

Received 23 June 2003; accepted 10 March 2004

Keywords:
Atrial natriuretic peptide;
Water metabolism;
Electrolyte metabolism

Abstract

Study Objective: To assess the hemodynamic, renal, and endocrine effects of small continuous doses of atrial natriuretic peptide (ANP) in patients anesthetized with sevoflurane for gastrectomy.

Design: Prospective randomized study.

Setting: Operating room and wards of a university hospital.

Patients: 20 ASA physical status I and II patients scheduled for gastrectomy.

Intervention: Atrial natriuretic peptide (0.05 μg/kg/min; ANP group, n = 10) or saline (control group, n = 10) was infused continuously for 2 hours beginning at the start of the operation.

Measurements: Plasma concentrations of ANP, brain natriuretic peptide, cortisol, angiotensin II, and aldosterone; plasma renin activity; serum and urinary sodium, potassium, and chloride; and urinar output.

Main Results: The ANP group showed much greater urine volume and sodium, potassium, and chloride excretion than the control group, although the ANP group had a lower arterial blood pressure. The infusion did not affect surgery-induced increases in hormones. No patients experienced excessive hypotension, bradycardia, or other perioperative complications.

Conclusions: Continuous intravenous infusion of ANP at 0.05 μg/kg/min during gastrectomy was associated with greater water and electrolyte excretion unaccompanied by changes in potentially interacting hormones. Low-dose infusion may be particularly safe and useful for controlling water and electrolyte metabolism intraoperatively.

1. Introduction

Atrial natriuretic peptide (ANP) is a circulating hormone secreted predominantly by the left atrium. Atrial natriuretic peptide affects diverse biologic functions such as diuresis, natriuresis, and vasodilation [1,2]. Atrial natriuretic peptide also is known to inhibit secretion and actions of several hormones including renin, angiotensin II, aldosterone, arginine vasopressin (AVP), adrenocorticotropic hormone (ACTH), and endothelin [2-5]. Many investigators have suggested that secretion and metabolism of ANP is likely to change radically in patients undergoing surgical interventions [1,6].

* Corresponding author.
E-mail address: marikim@pg7.sonet.ne.jp (M. Koda).

0952-8180/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.jclinane.2004.03.006

Atrial natriuretic peptide is recognized as an important factor for homeostasis of body fluid volume, and many studies have found ANP infusion to be useful for improving cardiorenal regulation in some abnormal states, such as heart failure, renal failure, hypertension, and hypertensive episodes related to anesthesia [1,2,5,7]. Atrial natriuretic peptide has been reported to have additional clinical benefits. Like nitrates, it has a coronary vasodilator action, attenuates myocardial ischemia, and prevents left ventricular remodeling after myocardial ischemia [5,8-10]. Atrial natriuretic peptide reduces pulmonary arterial blood and pulmonary capillary wedge pressures, improves left ventricular function in heart failure [2,5], prevents ischemia and reperfusion injury in the kidney and liver [11,12], and relaxes bronchial smooth muscle [5,13].

Increases in circulating catecholamines, vasopressin, resin, angiotensin II, ACTH, and cortisol occur in patients under surgical stress, promoting renal and peripheral vasoconstriction and salt and water retention [14,15], which may persist for several days. As well, anesthetic agents generally decrease GFR and urine output [16]. The clinical sequelae is postoperative oliguria and edema, and to maintain perioperative urine output is one of the important role of anesthetic management. Atrial natriuretic peptide may solve this problem, however, few reports have examined ANP from the viewpoint of perioperative water and electrolyte metabolism.

Shirakami et al [17] reported that intraoperative ANP infusion may have undesirable effects on the renin-angiotensin-aldosterone system despite its benefits for cardiovascular control. In clinical use, ANP is usually infused at a rate of 0.1 to 0.2 μg/kg/min, a dose at which it can affect other hormone systems. In vitro, even smaller doses of ANP have been found to affect the renin-angiotensin-aldosterone system [18]. Taking these reports into consideration, changes in other hormones may have influence on the net of ANP administration on water and electrolyte metabolisms as well as other aspects of homeostasis. We hypothesized that the smaller dose of ANP than that of previous studies is enough to maintain intraoperative urine output as well as electrolytes secretion.

In this study, we examined the effects of continuous low-dose ANP infusion on hormonal, renal, and hemodynamic function as well as water and electrolyte metabolisms in patients undergoing upper abdominal surgery.

Table 1 Patient characteristic, preoperative medical conditions, duration of surgery, and amount of sevoflurane used

Group	Control	Treatment	(P Value)
No. of patients	10	10	–
Gender (male/female)	5/5	5/5	–
Age (yrs)	61.8 ± 17.8	57.0 ± 3.5	0.460
Body weight (kg)	50.4 ± 9.9	55.7 ± 3.1	0.242
Complicating preoperative condition (no. of patients)			
Hypertension	2	3	0.628
Diabetes mellitus	0	1	0.343
Obesity	2	2	1
Anemia	0	1	0.343
Duration of surgery (min)	175.9 ± 34.5	184.9 ± 13.7	0.607
Total sevoflurane used (MAC · hr)	3.7 ± 1.3	3.6 ± 0.9	0.864

Continuous variables are shown as means ± SD. No significant difference was evident between the two groups.

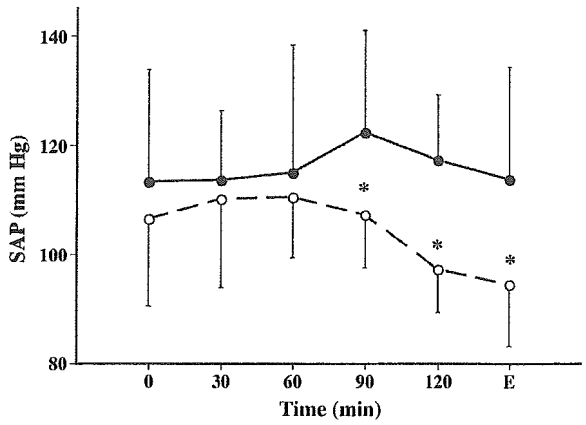

Fig. 1 Changes in systolic blood pressure. ●, control group; ○, treatment group. *P < 0.05 between groups.

2. Materials and methods

Twenty ASA physical status I and II patients without apparent cardiac, pulmonary, or renal disease, who underwent gastrectomy for gastric cancer were included in the study. Approval for this study was obtained from the Ethics Committee of the Nippon Medical School's hospital, and informed consent to participate was obtained from each patient. Patients were assigned to receive either recombinant human α-ANP (Suntory, Osaka, Japan; ANP group, n = 10) or saline vehicle (control group, n = 10) by randomized placebo-controlled double-blind manner.

Patients were premedicated intramuscularly with hydroxyzine (0.5 mg/kg) 1 hour before anesthesia. Epidural catheters were inserted under local anesthesia for pain control in the postoperative period. General anesthesia was induced with intravenous administration of propofol (2 mg/kg) and vecuronium bromide (0.1 mg/kg), plus inhalation of sevoflurane. Anesthesia was maintained with inhalation of 66% nitrous oxide and sevoflurane and supplementary vecuronium. Inhalation of sevoflurane was controlled to maintain systolic pressure at 80 to 150 mmHg. Immediately after induction of anesthesia, a urinary catheter was inserted and a 22-gauge Teflon catheter was inserted percutaneously into the radial artery to monitor blood

Table 2 Water and electrolyte balance during anesthesia

Group	Control	Treatment	(P value)
Fluid infused during anesthesia (A) (mL)	1987 ± 245	2214 ± 702	0.354
Sodium infused during anesthesia (α) (mEq)	263 ± 31	298 ± 100	0.318
Potassium infused during anesthesia (α') (mEq)	8.1 ± 1.0	9.2 ± 3.2	0.313
Chloride infused during anesthesia (α'') (mEq)	221 ± 26	250 ± 85	0.317
Urine output during anesthesia (B) (mL)	139 ± 89	756 ± 609	0.011
Urinary sodium excretion during anesthesia (β) (mL)	24 ± 15	129 ± 94	0.006
Urinary potassium excretion during anesthesia (β') (mL)	10.5 ± 6.2	20.9 ± 7.5	0.003
Urinary chloride excretion during anesthesia (β'') (mL)	23 ± 11	119 ± 89	0.008
Blood loss during surgery (mL) (C)	346 ± 83	316 ± 64	0.785
Fluid balance [(A)-(B)-(C)] (mL)	1503 ± 299	1142 ± 302	0.013
Sodium balance [(α)-(β)] (mEq)	240 ± 29	169 ± 74	0.016
Potassium balance [(α')-(β')] (mEq)	−2.3 ± 5.7	−11.7 ± 7.8	0.007
Chloride balance [(α'')-(β'')] (mEq)	198 ± 24	131 ± 63	0.009

Values are mean ± SD.

pressure and to provide arterial blood samples for gas analysis. No local anesthetic was administered via the epidural catheter during surgery. Ventilation was controlled to result in an end-tidal carbon dioxide concentration of 30 to 35 mmHg. Ringer's acetate solution was infused at a rate of 10 mL/kg/hr throughout the operation. Blood transfusion was given if necessary.

Beginning just after initiation of surgery, ANP [0.05 μg/kg/min intravenously (IV)] was infused in saline at 5 mL/hr for 120 minutes in the ANP group, using an infusion pump. Patients in the control group received an equivalent volume of saline vehicle. Arterial blood (10 mL) was sampled eight times: at 0, 30, 60, 90, and 120 minutes after starting the operation; at the end of the surgery; and on postoperative days 1 and 3. Blood samples were collected in ice-chilled tubes containing sodium ethylenediaminetetraacetic acid. Other blood samples were taken to measure blood gases and serum electrolyte concentrations. Urine samples were collected at 0, 60, and 120 minute after starting the operation; at the end of the surgery; and on postoperative days 1 and 3. Heart rate and arterial blood pressure were monitored continuously during anesthesia.

Plasma concentrations of α-ANP and human brain natriuretic peptide (BNP) were assessed without extraction, using radioimmunometric assays (IR-MA; ShionoRIA ANP and ShionoRIA BNP; Shionogi, Osaka, Japan). Plasma renin activity (PRA) and plasma concentrations of angiotensin II and aldosterine were determined without extraction using radioimmunoassays (PRA and angiotensin II kits, SRL, Tokyo, Japan; and the SPAC-S aldosterone kit, Daiichi Radioisotope Laboratories, Tokyo, Japan). Plasma ADH concentrations were determined by radioimmunoassay after extraction (Yuka AVP RIA, Mitsubishi Kagaku Medical, Tokyo, Japan). Plasma cortisol concentrations were measured by a radioimmunoassay (cortisol kit, TFB, Tokyo, Japan).

Data obtained are expressed as means ± standard deviation. One-way analysis of variance was used to compare control and ANP groups with respect to demographic (age and weight) data, duration of surgery, and sevoflurane dose requirement during anesthetic maintenance. The incidence of preoperative complications was analyzed by Chi-squared tests. Other data were analyzed by two-factor repeated-measures analysis of variance for comparisons of trends over time, followed by Bonferroni's test for comparison of

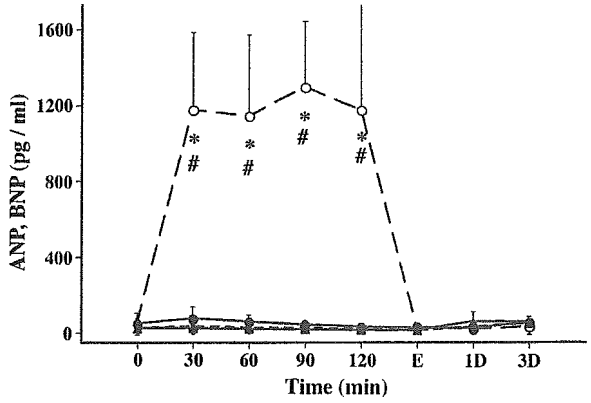

Fig. 2 Changes in the plasma concentrations of atrial (●, ○) and brain (▲, △) natriuretic peptides (ANP and BNP). ● and ▲, control group; ○ and △, treatment group. *P < 0.05 between groups. #P < 0.05 compared to the value at 0 minutes.

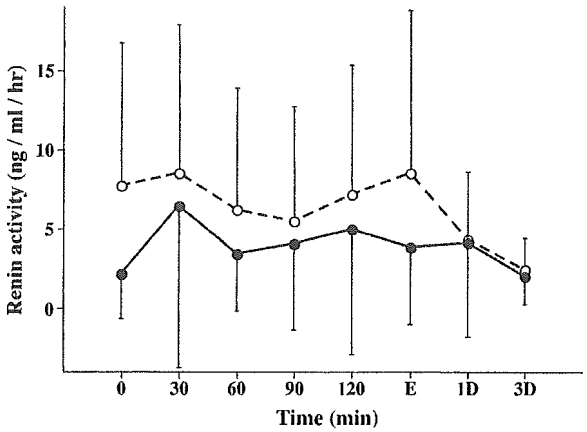

Fig. 3 Changes in rennin activity. ●, control group; ○, treatment group. No significant changes were seen.

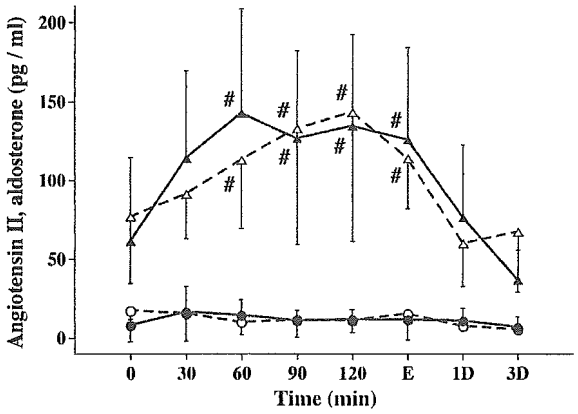

Fig. 4 Changes in the plasma concentrations of angiotensin II (●, ○) and aldoserone (▲, △). ● and ▲, control group; ○ and △, treatment group. #$P < 0.05$ compared to the value at 0 minutes.

time-matched data. Values were considered significantly different when they achieved $P < 0.05$.

3. Results

None of the patients needed blood transfusion or any inotropic drug, and no patient in either group developed any perioperative complication.

No significant differences between groups were noted for age, body weight, complicating preoperative illnesses, duration of surgery, or amount sevoflurane used (Table 1).

The mean arterial systolic blood pressure in the ANP group was lower than that in the control group during the period from 90 minutes after the start of IV infusion to end of the operation (Fig. 1).

The patients of the ANP group excreted more urine and electrolytes, such as sodium, potassium, and chloride, as compared with the patients of the control group (Table 2). Fig. 2 shows changes in ANP and BNP concentrations. Plasma ANP in the ANP group were significantly different

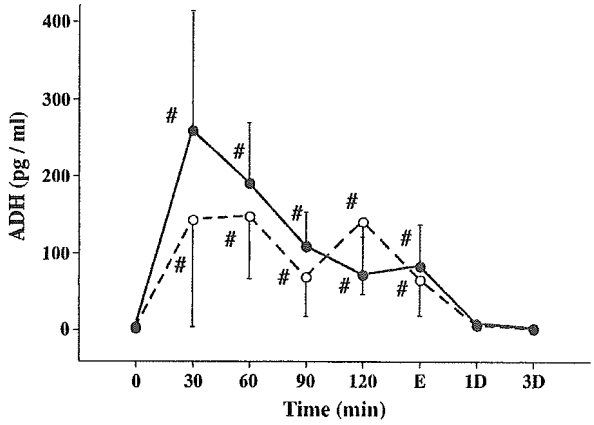

Fig. 5 Changes in the plasma concentration of antidiuretic hormone (ADH). ●, control group; ○, treatment group. #$P < 0.05$ compared to the value at 0 minutes.

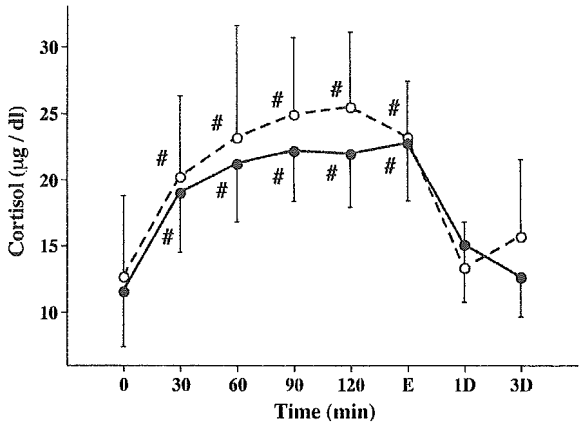

Fig. 6 Changes in the plasma concentration of cortisol. ●, control group; ○, treatment group. #$P < 0.05$ compared to the value at 0 minutes.

from corresponding control group values. The serum BNP concentration did not differ between groups.

Renin activity did not show a significant change in either group during the study period (Fig. 3), nor did the plasma concentration of angiotensin II (Fig. 4). Plasma concentrations of aldosterone were significantly increased from 60 minutes after initiating the infusion to the end of surgery in both groups; however, concentrations did not significantly differ between groups (Fig. 4). Although plasma concentrations of ADH and cortisol were significantly increased from the start of the intravenous infusion to the end of surgery, they did not show significant differences between groups (Figs. 5 and 6).

4. Discussion

Blood pressures in the ANP group were lower than those in the control group from 90 minutes after beginning the infusion to the end of the operation. Atrial natriuretic peptide reduces blood pressure not only by directly relaxing vascular smooth muscle, but also by reducing cardiac output via a decrease in circulating blood volume. Reduced cardiac output may have been the major cause of decreased blood pressure in the ANP group, because no significant differences were evident between groups in the dose of sevoflurane or in intravenous fluid infusion volumes during the operation.

Our study clearly demonstrated that urinary output and electrolyte excretion in the ANP group during gastric surgery were greater than in the control group. Surgical injury activates the renin-angiotensin-aldosterone system, ADH secretion, ACTH-cortisol system; these are involved in renal vasoconstriction, antidiuresis, and fluid and sodium retention [14,15]. Atrial natriuretic peptide increases salt and water excretion mediated by an increase in the glomerular filtration rate, reduction of tubular sodium reabsorption, and inhibition of the tubular actions of angiotensin II, aldosterone, and ADH [1,2].

Atrial natriuretic peptide infusion at a dose of 0.1 μg/kg/min during major surgery reduces blood pressure, causes diuresis and natriuresis, and inhibits the renin-angiotensin-aldosterone system. This ANP dose has been reported to require the treatment for excessive bradycardia, hypotension, and hypoxemia in some cases [17]. In normal subjects under no surgical stress, even smaller doses of ANP such as 0.75 pmoL/kg/min (i.e., 0.0023 μg/kg/min) increase renal electrolyte excretion and decrease activity of the renin-angiotensin-aldosterone system [18]. In our intraoperative study, we used ANP at a dose of 0.05 μg/kg/min, which brought about sufficient diuresis and electrolyte excretion without affecting other hormones. Our data suggested that against the background change in the renin-angiotensin-aldosterone system and other hormones occurring during surgery, little hormone interaction from ANP infused at this low dose could be discerned. Further study is necessary to clarify these issues more fully.

Although this study revealed the usefulness of ANP administration on water and electrolytes managements during surgery, it is necessary to consider the balance between potential benefit of a prophylactic measure and the cost of the intervention because human ANP is more expensive than fluid treatments and standard diuretics. If adequate hydration and normal hemodynamics are maintained, many patients who show intraoperative oliguria do not show postoperative complications. The further study is necessary to solve this issue.

In summary, we demonstrated that ANP infusion at a dose of 0.05 μg/kg/min, during gastrectomy causes diuresis and electrolyte excretion, with no accompanying changes in other hormones, excessive bradycardia, or hypotension. The results suggest that low-dose ANP administration during surgery is likely to be safe and useful for improving intraoperative water and electrolyte homeostasis.

References

[1] Espiner EA. Physiology of natriuretic peptides. J Intern Med 1994;235: 527-41.

[2] Levin ER, Gardner DG, Samson WK. Natriuretic peptides. N Eng J Med 1998;339:321-8.

[3] Brenner BM, Ballermann BJ, Gunning ME, et al. Diverse biological actions of atrial natriuretic peptide. Physiol Rev 1990;70:665-99.

[4] Imura H, Nakao K, Itoh H. The natriuretic peptide system in the brain: implications in the central control of cardiovascular and neuroendocrine functions. Front Neuroendocrinol 1992;13:217-49.

[5] Struthers AD. Ten years of natriuretic peptide research: a new dawn for their diagnostic and therapeutic use? BMJ 1994;308:1615-9.

[6] Shirakami G, Mori K. The natriuretic peptide system and surgical stress. Anasthesiol Intensivmed Notfallmed Schmerzther 1994;29:135-6.

[7] Goto F, Kato S, Sudo I. Treatment of intraoperative hypertension with enflurane, nicardipine, or human atrial natriuretic peptide: haemodynamic and renal effects. Can J Anaesth 1992;39:932-7.

[8] Hayashi M, Tsutamoto T, Wada A, et al. Intravenous atrial natriuretic peptide prevents left ventricular remodeling in patients with first anterior acute myocardial infarction. J Am Coll Cardiol 2001;37: 1820-6.

[9] Okawa H, Horimoto H, Mieno S, et al. Preischemic infusion of alpha-human atrial natriuretic peptide elicits myoprotection through a nitric oxide-dependent mechanism. J Cardiol 2002;39:299-304.

[10] Takagi G, Kiuchi K, Endo T, et al. Alpha-human atrial natriuretic peptide, carperitide, reduces infarct size but not arrhythmias after coronary occlusion/reperfusion in dogs. J Cardiovasc Pharmacol 2000; 36:22-30.

[11] Shaw SG, Weidmann P, Hodler J, et al. Atrial natriuretic peptide protects against acute ischemic renal failure in the rat. J Clin Invest 1987;80:1232-7.

[12] Bilzer M, Witthaut R, Paumgartner G, et al. Prevention of ischemia/reperfusion injury in the rat liver by atrial natriuretic peptide. Gastroenterology 1994;106:143-51.

[13] Angus RM, McCallum MJ, Hulks G, et al. Bronchodilator, cardiovascular, and cyclic guanylyl monophosphate response to high-dose infused atrial natriuretic peptide in asthma. Am Rev Respir Dis 1993;147:1122-5.

[14] Segawa H, Mori K, Kasai K, et al. The role of the phrenic nerves in stress response in upper abdominal surgery. Anesth Analg 1996;82: 1215-24.

[15] Espiner EA. The effects of stress on salt and water balance. Baillieres Clin Endocrinol Metab 1987;1:375-90.

[16] Salden RN. Renal physiology. In: Miller RD, editor. Anesthesia. 5th ed. New York: Churchill-Livingstone; 2000. p. 663-93.

[17] Shirakami G, Segawa H, Shingu K, et al. The effects of atrial natriuretic peptide infusion on hemodynamic, renal, and hormonal response during gastrectomy. Anesth Analg 1997;85:907-12.

[18] Richards AM, McDonald D, Fitzpatrick MA, et al. Atrial natriuretic hormone has biological effects in man at physiological plasma concentrations. J Clin Endocrinol Metab 1988;67:1134-9.

tool for brainstem death in patients with brain injury should be done with care.

Tomasz Gaszynski, MD, PhD
Department of Anaesthesiology and Intensive Therapy
Medical University of Lodz
Barlicki University Hospital
Lodz, Poland
tomgaszyn@poczta.onet.pl

Dr. Myles does not wish to respond.

References
1. Myles PS, Cairo S. Artifact in the Bispectral Index in a patient with severe ischemic brain injury. Anesth Analg 2004;98:706–7.
2. Gaszynski T, Wieczorek A, Krupowczyk W, Gaszynski W. BIS for recognition of brain-death in potential organ donors. Crit Care 2002;6(suppl 1):S26.
3. An Overview: The Effects of Electromyography (EMG) and Other High-Frequency Signals on the Bispectral Index (BIS). Aspect Medical Systems.

DOI: 10.1213/01.ANE.0000141278.95547.D4

Multiple Tracheobronchial Foreign Bodies in an Infant

To the Editor:

Foreign bodies in the upper aerodigestive tract of an infant continue to be a diagnostic and therapeutic challenge (1,2). The initial choking incidents may not be witnessed and delayed symptomatology mimics common respiratory pathology (3). Despite significant advances in endoscopic technology and use of digital substraction fluoroscopy (DSF) (4), accurate diagnosis is not always easy. A 7-month-old boy with persistent cough underwent diagnostic bronchoscopy. After an inhaled induction of anesthesia, a ventilating bronchoscope with optical forceps (Karl stortz-3.5 mm) was introduced after administration of succinylcholine. The pediatric surgeon removed small particles from the right bronchus. However, mask-bag ventilation could not be resumed. Direct laryngoscopy revealed a large whitish substance sitting over the glottis. It was removed with Maggill's forceps, and positive pressure ventilation was instituted. Meanwhile, hemoglobin desaturation occurred, and atropine was administered for bradycardia. The removed particle was identified as a whole-wheat kernel with grains and bristles (Fig. 1). It probably entered by ciliary movement of the bristles. Pediatric bronchoscope provides a narrow view, so a single grain was visualized and extracted. The rest of the kernel was also pulled and obstructed the airway, necessitating subsequent removal to restore spontaneous respiration. Check bronchoscopy ruled out other foreign bodies. Recovery was uneventful. In conclusion, speedy and safe retrieval of tracheobronchial foreign bodies requires a team effort with the anesthesiologist and surgeon working out a technique to prevent life-threatening respiratory sequelae.

Indu Sen, MD
K. L. Narsimhan, MCh (Ped Surg), FRCS Ed, FRCS (Glasgow)*
P. Chari, MD, DAc, MAMS, FAMS
*Department of Anaesthesia and Intensive Care and *Department of Paediatric Surgery*
Post Graduate Institute of Medical Education and Research
Chandigarh, India
senramesh@rediffmail.com

References
1. Donato L, Weiss L, Bing J, Schwarz E. Tracheobronchial foreign bodies. Arch Pediatr 2000;7(suppl 1):56–61S.
2. Friedman EM. Tracheobronchial foreign bodies. Otolaryngeal Clin North Am 2000;33: 179–85.
3. Yamamoto S, Suzuki K, Itaya T, et al. Foreign bodies in the airway: eighteen-year retrospective study. Acta Otolaryngol Suppl 1996;525:6–8.
4. Ikeda M, Himi K, Yamauchi Y, et al. Use of digital substraction fluoroscopy to diagnose radiolucent aspirated foreign bodies in infants and children. Int J Pediatr Otorhinolaryngol 2001;61:233–42.

DOI: 10.1213/01.ANE.0000141279.16282.9D

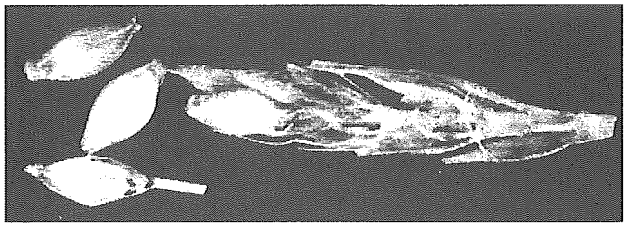

Figure 1. Wheat kernel with grains and bristles: tracheobronchial foreign body in an infant.

Successful Management of Tachycardiac Atrial Fibrillation in a Septic Patient with Landiolol

To the Editor:

In sepsis patients, management of tachycardia accompanied by hypotension is difficult. A 75-year-old man was admitted for urgent laparotomy because of septic peritonitis by superior mesenteric artery obstruction. General anesthesia was induced with propofol 60 mg, fentanyl 0.1 mg, and was maintained with supplementary fentanyl and sevoflurane. Although IV fluid administration and catecholamine infusion were continued from the admission, heart rate (HR) increased to 150 bpm, pulmonary artery pressure (PA) increased to 54 mm Hg, and systolic blood pressure (SBP) decreased to 90 mm Hg after the start of operation. Landiolol was started at the rate of 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 15 min, and was maintained 2 to 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$. In 15 min after landiolol infusion, HR and PA decreased and stabilized at around 80 bpm, around 30 mm Hg, respectively (see Fig. 1 on page 295). SBP increased and stabilized at about 120 mm Hg. This case demonstrates that landiolol attenuated the tachycardia-induced low cardiac stroke volume and stabilized the hemodynamics in a patient with sepsis. Landiolol was reported to cause neither excessive hypotension nor cardiac decompression and is thought to be useful for treating tachycardia in sepsis patients (1,2).

Yuko Yoshida, MD
Takashi Hongo, MD
Atsuhiro Sakamoto, MD
Ryo Ogawa, MD
Department of Anesthesiology
Nippon Medical School
Tokyo, Japan
s7100@nms.ac.jp

References
1. Atarashi H, Kuruma A, Yashima M, et al. Pharmacokinetics of landiolol hydrochloride, a new ultra-short-acting beta-blocker, in patients with cardiac arrhythmias. Clin Pharmacol Ther 2000;68:143–50.
2. Sasao J, Tarver SD, Kindscher JD, et al. In rabbits, a new ultra-short-acting beta-blocker, exerts a more potent negative chronotropic effect and less effect on blood pressure than esmolol. Can J Anaesth 2001;48:985–9.

DOI: 10.1213/01.ANE.0000140814.28118.6F

ECG Monitoring Is Essential for Echocardiographic Analysis

To the Editor:

We have read with great interest the case report published by Glas et al. (1), concerning an unusual cause of left ventricular outflow tract obstruction after mitral valve repair diagnosed using transesophageal echocardiography (TEE). Indeed, a such technique would be a precious tool in order to evaluate, in real time, the dynamic working heart in the postsurgical scene.

I was surprised not to see a recorded electrocardiogram (ECG) in the illustrations of the TEE, assuming that Figure 4 in their article was

CASE REPORT

Unmasking of Brugada Syndrome by an Antiarrhythmic Drug in a Patient with Septic Shock

Katsuyuki Terajima, MD, Takesi Yamamoto, MD, Hidetaka Onodera, MD, Shinhiro Takeda, MD, Keiji Tanaka, MD, and Atsuhiro Sakamoto, MD

Division of Intensive Care and Coronary Care Unit, Nippon Medical School, Tokyo, Japan

Asymptomatic Brugada syndrome patients often display concealed Brugada-type electrocardiogram patterns that result in under-diagnosis of this syndrome. These patients include individuals of both genders and a wide range of ages. They are as likely as non-Brugada patients to have normal longevity or to suffer from a critical illness. Here we report a case of septic shock in which Brugada-type electrocardiogram patterns were induced by pilsicainide administration for the treatment of atrial fibrillation. This case report suggests that some drugs used in the treatment of septic shock can unmask the Brugada-type electrocardiogram pattern and induce lethal ventricular tachyarrhythmia.

(Anesth Analg 2006;102:233–6)

Brugada syndrome is thought to be a major cause of sudden death and of syncope and idiopathic ventricular tachyarrhythmia in young people with structurally normal hearts (1). However, this syndrome occurs in both genders and all age groups, including infants (2) and the elderly (3), and shows no correlation with the health of the patient. The syndrome is characterized by a right bundle-branch block with ST elevation in leads V1 to V3, as shown by an electrocardiogram (ECG). The Brugada-type ECG can transiently normalize for a period of time leading to an under-diagnosis of the disease (4). Here we report a case of septic shock with undiagnosed asymptomatic Brugada syndrome that was unexpectedly unmasked by an antiarrhythmic drug for atrial fibrillation (AF).

Case Report

An 84-yr-old male patient at the Nippon Medical School, Japan, was suffering from septic shock after aspiration pneumonia. Two months before the incidence of septic shock, a total gastrectomy and partial esophagectomy had been performed to remove the remnants of a previous gastric cancer. A preoperative 12-lead ECG revealed no significant ST-T change (Fig. 1) and an echocardiograph showed no structural abnormalities, with a left ventricular ejection fraction of 65%. The patient had neither a history of syncope nor a family history of sudden death. Before septic shock, the patient had experienced a loss of appetite for 2 days, accompanied by sudden vomiting.

On the morning after he vomited, the patient was febrile, hypotensive, and hypoxemic, with an oxygen saturation of 86%. He was immediately transferred to the intensive care unit (ICU). The patient was tracheally intubated, and the intratracheal fluid contents were suctioned with bronchoscopy. Despite aggressive IV fluid resuscitation, the patient remained hypotensive. Infusion of dopamine at 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ and norepinephrine at 0.4 $\mu g/kg$ subsequently commenced. After catecholamine administration, his heart rate was 100 to 120 bpm with sinus rhythm. Blood samples taken from the patient and grown in culture revealed *Klebsiella pneumoniae*. Hemoperfusion with polymyxin B-immobilized fibers and continuous hemodiafiltration (CHDF) were performed to treat septic shock with acute renal failure; however, catecholamine administration was still required.

On day 2 in the ICU, the patient developed AF (Fig. 2). We subsequently administered 0.6 mg/kg of pilsicainide IV. The AF was converted to sinus rhythm, but the ECG revealed a Brugada-type pattern (Fig. 3). Twelve hours after the administration of pilsicainide, the patient developed ventricular tachycardia (Fig. 4). The serum electrolyte concentrations when the patient showed this ECG pattern and the polymorphic ventricular tachycardia were within normal limits, but the metabolic acidosis was persistent (Table 1). In view of the unmasked characteristic ECG features after administration of pilsicainide IV, the induced ventricular tachyarrhythmia, and the absence of underlying structural heart disease according to echocardiography, the patient was diagnosed with Brugada syndrome. External defibrillation pads were attached to his chest to prevent recurrence of the lethal arrhythmias. The serum creatinine level of the patient was 0.6 to 0.97 mg/dL during his time in the ICU. The 12-lead ECG

Accepted for publication July 11, 2005.

Address correspondence and reprint requests to Katsuyuki Terajima, 1–1–5 Sendagi, Bunkyo-ku, Tokyo, 113–8603, Japan. Address e-mail to terajima.katsuyuki@nifty.com.

DOI: 10.1213/01.ANE.0000184258.64205.9B

0003-2999/06

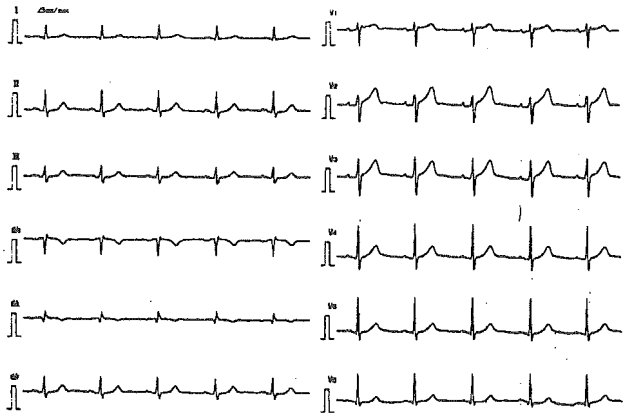

Figure 1. The preoperative electrocardiogram shows sinus rhythm with no significant ST segment elevation.

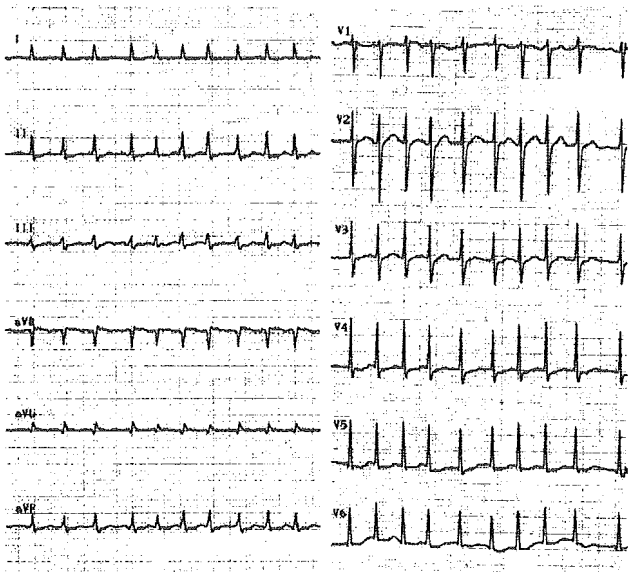

Figure 2. On the second day in the intensive care unit, the patient developed atrial fibrillation.

showed recovery to sinus rhythm after polymorphic ventricular tachycardia, with a Brugada-type pattern (Fig. 5) that persisted 3 days after pilsicainide administration. The creatine kinase isoenzyme MB levels were not increased at 0 and 6 h and the troponin T was not detected (TROPT sensitive™, Roche Diagnostics Inc., Basel, Switzerland) at 0, 12, and 18 h after the recovery to sinus rhythm from AF. Asynergy of the left ventricle was not detected by echocardiography 0 and 12 h after the recovery to sinus rhythm. Unfortunately, the patient died on his fifth day in the ICU as a result of sepsis.

Discussion

A previous study has shown that the incidence of AF increases in critically ill patients who have received significantly more fluids and catecholamines

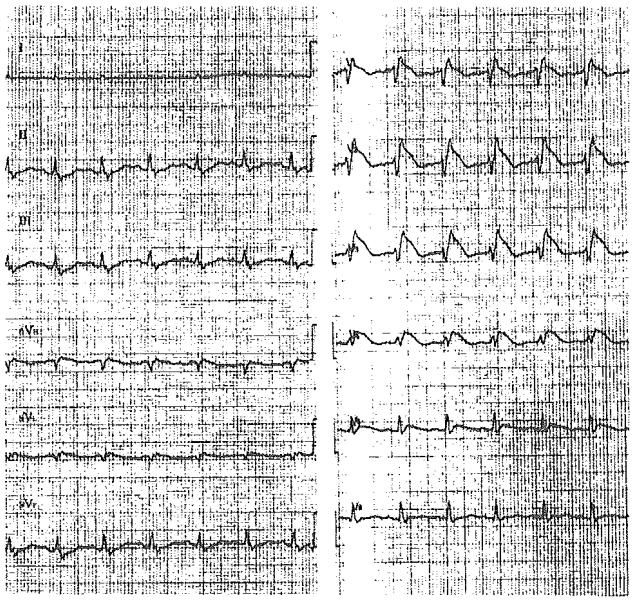

Figure 3. The 12-lead electrocardiogram shows right bundle-branch block and "coved" type ST elevation in leads V1 to V4 (Brugada-type electrocardiogram) after pilsicainide administration.

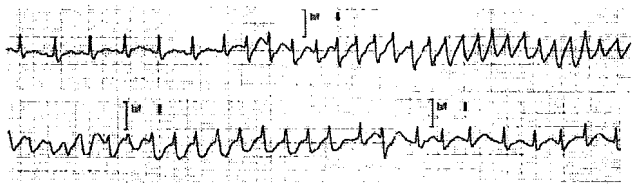

Figure 4. The electrocardiogram 12 h after pilsicainide administration shows polymorphic ventricular tachycardia.

than other patients in an effort to treat septic shock and acute renal failure (5). Therefore, the occurrence of AF in this case was not surprising, as the onset of AF appears to reflect the severity of the critical illness. Moreover, it has been shown that AF occurs in 10% to 39% of all patients with Brugada syndrome (6–8).

Approximately 20%–30% of Brugada patients have mutations in the *SCN5A* gene on chromosome 3 (9). The *SCN5A* gene encodes the α subunit of the human cardiac voltage-gated sodium channel. This disorder can be inherited through autosomal dominant transmission, although there is some degree of genetic heterogeneity. Sodium-channel blockers and an autonomic imbalance have previously been thought to help unmask the Brugada-type ECG. Administration of ajmaline, flecainide, or procainamide accentuates ST segment elevation (1) and assists in the diagnosis of Brugada syndrome (10). Pilsicainide, which is a class IC drug, has been found to induce similar ST segment elevation in

Table 1. Blood Gas Values and Serum Electrolyte Concentrations

	Before ECG	After ECG	After PVT
pH	7.205	7.169	7.26
Pco_2 (mm Hg)	42.3	40.5	48.6
Po_2 (mm Hg)	85.5	88.6	85.2
HCO3-(mmol/L)	17.4	15.4	20.4
BE (mmol/L)	−10.4	−12.8	−5.6
Na+ (mmol/L)	136	137	138
K+ (mmol/L)	4.3	4.3	4.3
Cl− (mmol/L)	106	107	110
Ca2+ (mmol/L)	1.01	1.01	1.06
Glucose (mg/dL)	76	91	137
Lactate (mg/dL)	151	146	169
Hb (g/dL)	7.8	8	10.1
SaO_2 (%)	95	95.7	96.5

Before ECG = before the Brugada-type electrocardiogram findings; After ECG = after the Brugada-type electrocardiogram findings; After PVT = immediately after the polymorphic ventricular fachycardia.

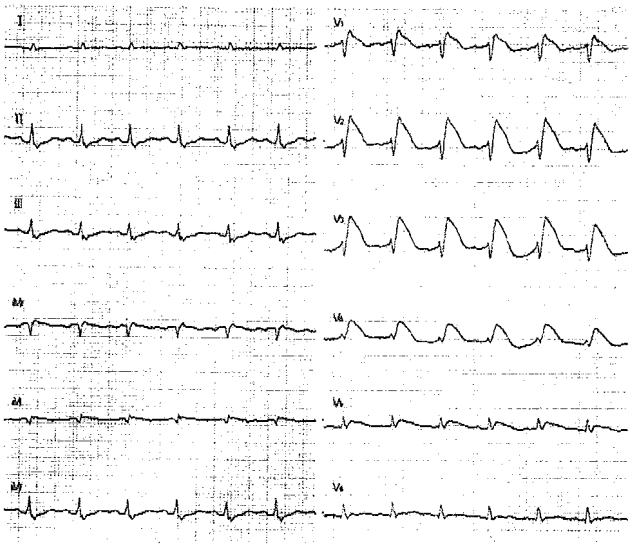

Figure 5. The electrocardiogram after polymorphic ventricular tachycardia shows recovery to sinus rhythm with a Brugada-type pattern.

response to administration of flecainide (11). In patients with severe renal failure, the half-life of elimination for pilsicainide is prolonged to 23.7 hours (12). The elimination and total body clearance of pilsicainide are not always linearly correlated with the creatinine clearance when CHDF is performed. Alpha-adrenergic, but not β-adrenergic, stimulation has been shown to augment the ST segment elevation in Brugada syndrome (13). The Brugada-type ECG might be preserved by continuous IV administration of norepinephrine and/or insufficient clearance of pilsicainide. Although selective stimulation of muscarinic receptors can augment the ST segment, tachycardia alone cannot prevent a Brugada-type ECG pattern in this situation. The febrile status *per se* might unmask Brugada syndrome (14). The Brugada-like ECG pattern can also be caused by electrolyte disturbance and ketoacidosis (15), but flecanide drug challenge and electrophysiological studies can exclude an underlying Brugada syndrome. If the Brugada-type ECG shows ST elevation during treatment for sepsis and arrhythmias, Brugada syndrome should be considered. If characteristic Brugada-type ECG patterns are observed in the absence of evidence for ischemia or underlying structural heart disease according to echocardiography, the causes of ST-T elevation should be avoided, defibrillation should be available for lethal ventricular tachyarrhythmias, and methods such as dialysis to remove the causative drugs should be considered.

In summary, the drugs used for treating septic shock, such as norepinephrine and antiarrhythmic drugs, might unmask Brugada syndrome. Asymptomatic patients with concealed Brugada-type ECGs might have an increased risk of developing lethal arrhythmia when treated for septic shock, and this might require external defibrillation. We suggest that on recovery from sepsis, the implantation of an internal defibrillator should be considered for these patients.

References

1. Brugada J, Brugada P, Brugada R. The syndrome of right bundle branch block ST segment elevation in V1 to V3 and sudden death: the Brugada syndrome. Eurace 1999;1:156–66.
2. Priori SG, Napolitano C, Giordano U. Brugada syndrome and sudden cardiac death in children. Lancet 2000;355:808–9.
3. Huang MH, Marcus FI. Idiopathic Brugada-type electrocardiographic pattern in an octogenarian. J Electrocardiol 2004;37:109–11.
4. Gussak I, Antzelevitch C, Bjerregaard P, et al. The Brugada syndrome: clinical, electrophysiologic and genetic aspects. J Am Coll Cardiol 1999;33:5–15.
5. Seguin P, Signouret T, Laviolle B, et al. Incidence and risk factors of atrial fibrillation in a surgical intensive care unit. Crit Care Med 2004;32:722–6.
6. Anzelevitch C, Brugada P, Brugada J, et al. The Brugada syndrome. In: Camm AJ, ed. Clinical approaches to tachyarrhythmias. Armonk, NY: Futura Publishing Co., 1999:30–2.
7. Itoh H, Shimizu M, Ino H, et al. Hokuriku Brugada Study Group: arrhythmias in patients with Brugada-type electrocardiographic findings. Jpn Circ J 2001;65:483–6.
8. Morita H, Kusano-Fukushima K, Nagase S, et al. Atrial fibrillation and vulnerability in patients with Brugada syndrome. J Am Coll Cardiol 2002;40:1437–44.
9. Naccarelli GV, Antzelevitch C, Wolbrette DL, Luck JC. The Brugada syndrome. Curr Opin Cardiol 2002;17:19–23.
10. Brugada R, Brugada J, Antzelevitch C, et al. Sodium channel blockers identify risk for sudden death in patients with ST-segment elevation and right bundle branch block but structurally normal hearts. Circulation 2000;101:510–5.
11. Fujiki A, Usui M, Nagasawa H, et al. ST segment elevation in the right precordial leads induced with class IC antiarrhythmic drugs: insight into the mechanism of Brugada syndrome. J Cardiovasc Electrophysiol 1999;10:214–8.

12. Takabatake T, Ohta H, Yamamoto Y, et al. Pharmacokinetics of SUN 1165, a new antiarrhythmic agent, in renal dysfunction. Eur J Clin Pharmacol 1991;40:411–4.
13. Miyazaki T, Mitamura H, Miyoshi S, et al. Autonomic and antiarrhythmic drug modulation of ST segment elevation in patients with Brugada syndrome. J Am Coll Cardiol 1996;27:1061–70.
14. Wakita R, Watanabe I, Okumura Y, et al. Brugada-like electrocardiographic pattern unmasked by fever. Jpn Heart J 2004;45: 163–7.
15. Kovacic JC, Kuchar DL. Brugada pattern electrocardiographic changes associated with profound electrolyte disturbance. Pacing Clin Electrophysiol 2004;27:1020–3.

SHOCK, Vol. 25, No. 2, pp. 2006

FLUID RESUSCITATION WITH HEMOGLOBIN VESICLES IN A RABBIT MODEL OF ACUTE HEMORRHAGIC SHOCK

Katsuyuki Terajima, Takaya Tsueshita, Atsuhiro Sakamoto, and Ryo Ogawa

Department of Anesthesiology, Nippon Medical School, Tokyo, Japan

Received 19 Aug 2005; first review completed 8 Sep 2005; accepted in final form 12 Oct 2005

ABSTRACT—Several hemoglobin (Hb)-based oxygen carriers are available for use in clinical situations, but their use risks inducing cardiovascular dysfunction as a result of Hb interacting with nitric oxide. Hb vesicles (HbV) are liposome-encapsulated purified human Hb with polyethylene glycol chains at the surface. This study evaluated the effects of HbV on hemodynamics, tissue and systemic oxygenation, and osmotic pressure after fluid resuscitation in an acute hemorrhagic shock model. Hemorrhagic shock was induced in 24 anesthetized mechanically ventilated male rabbits by withdrawing blood to a mean arterial blood pressure (MAP) of 30 to 35 mmHg over 15 min and maintaining this state for 30 min. The animals were resuscitated by replacing the blood with equal volumes of HbV in recombinant human albumin solution (HbV/rHSA), rHSA alone, or Ringer lactated solution (RL), or with three times the withdrawn volume of RL and observed for 2 h. Fluid resuscitation restored MAP, central venous pressure, and cardiac index values, but these fell again within 2 h in rabbits treated with RL. Fluid resuscitation using HbV/rHSA immediately increased MAP and cardiac index but not systemic vascular resistance, maintained a high level of oxygen consumption, and reduced the blood glucose level, which increased after hemorrhage. Fluid resuscitation using HbV/rHSA did not disturb microoxygenation in the brain, kidneys, liver, or muscle; allowed an immediate recovery of tissue oxygenation without decreasing cardiac output or increasing systemic vascular resistance, and increased the oxygen consumption. HbV solution offers the advantages of systemic oxygenation without impairing microcirculation in the treatment of hemorrhagic shock.

KEYWORDS—Encapsulated hemoglobin, hemoglobin-based oxygen carriers, nitric oxide, osmotic pressure, oxygen consumption, tissue oxygenation, vasoconstriction

ABBREVIATIONS—BE, base excess; CI, cardiac index; CVP, central venous pressure; Hb, hemoglobin; HBOC, hemoglobin-based oxygen carrier; HbV, hemoglobin vesicles; MAP, mean arterial blood pressure; NO, nitric oxide; Po_2, oxygen partial pressure; RBC, red blood cell; rHSA, recombinant human albumin solution; RL, Ringer lactated solution; SVR, systemic vascular resistance

AQ1

INTRODUCTION

When a patient suffers hemorrhagic shock, fluid resuscitation using extracellular fluid replacement or a plasma substitute is necessary to restore hemodynamics and the transfusion of red blood cells (RBCs). Alternatively, an erythrocyte substitute can be used to restore the oxygen-carrying capacity of the blood. The use of artificial oxygen carriers can avoid the need for clinical tests such as blood typing and cross-matching required before transfusion and can avoid some side effects of transfusion (such as transfusion reaction, immunomodulatory reactions including graft-vs.-host disease, and bacterial, viral, and prion infections). In addition, artificial oxygen carriers have longer shelf lives than normal cells and are convenient if an emergency transfusion of RBCs is needed in a patient having massive hemorrhage caused by trauma or surgery.

Fluorocarbon is used as an oxygen carrier because of its gas-dissolving capacity, but ventilation with supplemental oxygen is essential to obtain an adequate level of tissue oxygenation (1). Several hemoglobin (Hb)-based oxygen carriers (HBOCs), including polyhemoglobin (poly-Hb) (2–4), conjugated Hb (5), and liposome-encapsulated Hb (6), are currently being tested in preclinical or clinical trials to evaluate their potential as RBC substitutes in blood loss, and some modified Hbs are now available for use in the clinic. The side effects of native (7, 8) and some modified Hbs (9) include renal dysfunction and and pulmonary hypertension. In animal experiments and clinical trials, HBOCs have caused vasoconstriction, mainly due to nitric oxide (NO) scavenging and, to a lesser extent, reactive vasoconstriction resulting from precapillary oxygen off-loading. The vasopressor response has been attributed to NO scavenged by modified Hbs, which might penetrate the vascular lining (1). Hb vesicles (HbV) have been developed as oxygen carriers and consist of liposome-encapsulated, highly purified Hb. They have a particle size of approximately 250 nm, an Hb concentration of 10 $g \cdot dL^{-1}$, and an oxygen affinity, P50, restricted to 32 mmHg (6). The diameter of HbV is larger than those of cross-linked poly-Hb, conjugated Hb, cross-linked Hb, or recombinant Hb, but is small relative to the diameter of microvessels (6, 10).

The effectiveness of tissue oxygenation depends on the level of microcirculation achieved by the oxygen carrier. It is therefore important to establish the efficacy of HBOC in restoring tissue oxygenation and the osmotic pressure of the blood as well as hemodynamics. The aim of the current study was to simultaneously evaluate the initial effects of HbV on hemodynamics, multiple organ oxygenation, and osmotic pressure after fluid resuscitation in a rabbit model of acute hemorrhagic shock.

MATERIALS AND METHODS

Animals

This study was approved by the Ethics Committee for Animal Experiments at Nippon Medical School, Tokyo, Japan. A total of 26 male New Zealand white

Address reprint requests to Katsuyuki Terajima, Department of Anesthesiology, Nippon Medical School, 1-1-5, Sendagi, Bunkyo-ku, Tokyo, 113-8603, Japan. E-mail: terajima.katsuyuki@nifty.com.

DOI: 10.1097/01.shk.0000192118.68295.5d

rabbits, aged 12 to 14 weeks and weighing 2.26 ± 0.25 kg (mean ± SD), were anesthetized and mechanically ventilated during the study. Two animals died within 2 h of fluid resuscitation using Ringer lactate solution (RL) and were excluded from the analysis. One received an infusion of the same volume of RL, whereas the other received three times the volume of RL. The results are reported for the remaining 24 animals.

Anesthesia and surgical preparation

Anesthesia was induced by a bolus injection of ketamine (Ketalar, Sankyo Yell Yakuhin Co. Ltd., Tokyo, Japan; 10 mg · kg^{-1} body weight) intramuscularly, followed by continuous intravenous injection of sodium thiamylal (Isozole, Mitsubishi Pharma Co., Osaka, Japan; 10 mg · kg^{-1} · h^{-1}) via the left ear vein. No muscle relaxants were used. Tracheotomies were performed on the animals, which were mechanically ventilated with oxygen in air (fraction of inspired oxygen = 0.40) in a volume-controlled mode to maintain normocapnia and an arterial pH of around 7.4. The core body temperature was maintained at 38°C using heating blankets. RL was infused perioperatively at 10 mL · kg^{-1} · h^{-1} to maintain normovolemia, as measured by central venous pressure (CVP).

A central venous catheter was inserted from the right internal jugular vein into the right atrium, and both femoral arteries were catheterized to allow measurements of the mean arterial blood pressure (MAP) and the withdrawal of blood to induce hemorrhagic shock. A pulse dye-densitometry probe (DDG-3300, Nihon Kohden Co., Tokyo, Japan) was attached to the base of the tail to measure cardiac output, using indocyanine green infused intravenously. Through a burr hole and a midline laparotomy, four microelectrode sensors (PO2-150M, Eikou Kagaku Co., Ltd., Tokyo, Japan) were inserted into the left cerebral cortex, the right lobe of the liver, and the renal cortex, to measure tissue oxygenation simultaneously. A tissue oxygen microelectrode sensor was also inserted into the muscle of one hind leg. After surgical preparation, the RL infusion was stopped, and baseline measurements were made. Blood gases were analyzed using an ABL 700 (Radiometer A/S, Copenhagen, Denmark), crystalloid osmotic pressure was measured using a vapor pressure osmometer (5520 Vapro, Wescor Inc., Logan, Utah), and colloid osmotic pressure was measured using a 4420 Colloid (Wescor Inc.).

Hemorrhagic shock and fluid resuscitation

Hemorrhagic shock was induced by withdrawing blood to give a MAP of 30 to 35 mmHg and then maintaining this state for 30 min. Animals were resuscitated by infusing the same volume of either HbV (Advanced Research Institute for Science and Engineering, Waseda University) suspended in 5% recombinant human serum albumin (HbV/rHSA), rHSA alone, or RL, or by infusing three times the volume of RL (3 × RL). Animals were observed over 2 h. Systemic vascular resistance (SVR) was calculated using standard equations based on pressures and flows. Oxygen consumption was calculated using oxygen equilibrium curves measured using rabbit Hb and HbV (Fig. 1).

Formulas for calculated values

SVR and oxygen consumption values were calculated from the measured values. The formulas used were as follows:

$SVR = 80\ (MAP - CVP)/CO$
$\dot{V}O_2i = CI \times 10\ (Cao_2 - Cvo_2)$
$CO_2 = 1.34\ (nHbcon \times nSo_2 + HbVcon \times HbVSo_2) + 0.003\ Po_2$
SVR = systemic vascular resistance (dyne · s · cm^{-5})
MAP = mean arterial pressure (mmHg)
CVP = central venous pressure (mmHg)
CO = cardiac output (L · min^{-1})
CI = cardiac index (L · min^{-1} · m^{-2})
$\dot{V}O_2i$ = oxygen consumption index (mL · min^{-1} · m^{-2})
Cao_2 = arterial blood oxygen content (mL oxygen per 100 mL blood)
Cvo_2 = venous blood oxygen content (mL oxygen per 100 mL blood)
nHbcon = native (rabbit) Hb concentration (g · dL^{-1})
nSo_2 = native (rabbit) Hb oxygen saturation (%)
HbVcon = HbV concentration (g · dL^{-1})
$HbVSo_2$ = HbV Hb oxygen saturation (%)
Po_2 = oxygen partial pressure (mmHg)

Statistical analyses

All data are expressed as mean ± SD. The tissue oxygen partial pressure (Po_2) and blood osmotic pressure values are expressed as percentages of both the absolute levels and the changes relative to baseline. All statistical analyses were performed using Statview version 5.0 for Macintosh software (Abacus Concepts Inc., Berkley, Calif). Differences between groups were analyzed using the Mann-Whitney *U* test, and differences within groups were analyzed using the Wilcoxon signed rank test, with *P* values less than 0.05 taken as statistically significant.

RESULTS

Baseline measurements of hemodynamic variables were similar for all groups (Table 1). The volumes of blood withdrawn to induce hemorrhagic shock were 22.5 ± 3.7, 24.7 ± 4.7, 18.5 ± 4.8, and 21.4 ± 7.4 mL · kg^{-1} for the HbV/rHSA, rHSA, RL, or 3 × RL groups, respectively; there were no significant differences between these values. Inducing hemorrhagic shock by withdrawing blood reduced CVP and the cardiac index (CI) significantly and tended to increase the SVR. Fluid resuscitation increased MAP, CVP, and CI in all groups, but these values declined again within 2 h in the RL and 3 × RL groups. Importantly, fluid resuscitation using HbV/rHSA immediately increased MAP and CI without increasing SVR.

Hemorrhagic shock increased the blood lactate level and decreased the arterial base excess (BE). Resuscitation with HbV/rHSA caused a greater decline in blood lactate levels and a greater increase in the arterial BE than resuscitation with RL (Table 2). The drop in the percentage mixed venous blood oxygen saturation recovered after resuscitation using rHSA, but not using RL. Oxygen consumption in the HbV/HSA group was significantly higher than in the HAS group after fluid resuscitation. In addition, BE was higher, and blood lactate level was lower in the HbV/HSA group compared with the HSA group, although these differences were not significant.

Colloid osmotic pressure dropped in response to hemorrhagic shock, but recovered after fluid resuscitation with rHSA solution, whereas it dropped further after resuscitation with RL solution (Table 3). Crystalloid osmotic pressure consistently increased after hemorrhagic shock, even after fluid resuscitation, in all groups. The rise in blood glucose level induced by hemorrhagic shock was only reduced by resuscitation with HbV/rHSA. Therefore, the glucose level

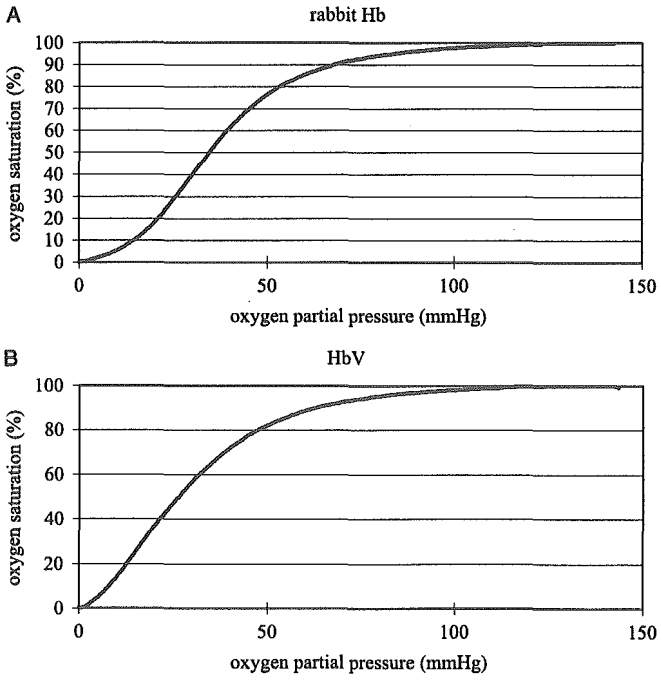

FIG. 1. **The oxygen equilibrium curves for (a) rabbit Hb and (b) HbV.**

TABLE 1. **Hemodynamic variables in New Zealand white rabbits after inducing hemorrhagic shock by withdrawing blood (T1) and stabilization for 30 min (T2)**

	Baseline	T1	T2	T3	T4	T5	T6
MAP (mmHg)							
HbV/rHSA	85 ± 10	33 ± 1*	43 ± 3*	88 ± 10†	90 ± 16†	90 ± 17†	90 ± 7†
rHSA	89 ± 11	32 ± 3*	36 ± 2*	68 ± 14	76 ± 9†	77 ± 9†	80 ± 9†
RL	78 ± 10	34 ± 1*	41 ± 9*	69 ± 9	56 ± 13*	51 ± 10*	54 ± 20*
3 × RL	80 ± 17	31 ± 3*	38 ± 7*	67 ± 12	62 ± 17	48 ± 20*	46 ± 16*
CVP (mmHg)							
HbV/rHSA	4.5 ± 1.2	3.0 ± 0.9*	2.8 ± 1.5*	5.8 ± 1.5	5.8 ± 1.5	4.2 ± 1.2	4.7 ± 1.2
rHSA	5.2 ± 1.7	3.2 ± 1.2*	3.0 ± 1.3*	5.0 ± 1.7	4.8 ± 1.5	4.7 ± 0.5	4.2 ± 0.8
RL	6.2 ± 1.3	2.7 ± 0.8*	2.8 ± 0.4*	4.7 ± 1.4	4.0 ± 1.7*	3.8 ± 0.8*	3.5 ± 1.0*
3 × RL	4.3 ± 1.0	2.8 ± 1.0*	2.8 ± 1.2*	5.5 ± 0.8	4.8 ± 1.2	3.7 ± 0.5	2.8 ± 1.0*
CI ($L \cdot min^{-1} \cdot m^{-2}$)							
HbV/rHSA	2.9 ± 0.7		1.2 ± 0.4*		2.9 ± 0.2†	3.2 ± 0.8†	2.8 ± 0.4†
rHSA	2.7 ± 0.8		1.0 ± 0.2*		2.7 ± 0.7	2.3 ± 0.5†	2.4 ± 0.6†
RL	2.6 ± 0.9		1.1 ± 0.4*		1.8 ± 0.4	1.3 ± 0.3	1.1 ± 0.3*
3 × RL	2.9 ± 0.5		1.1 ± 0.2*		2.6 ± 0.9	1.9 ± 0.6	1.4 ± 0.4*
SVR ($dyne \cdot s \cdot cm^{-5}$)							
HbV/rHSA	1,579 ± 738		1,839 ± 420		1,516 ± 294	1,498 ± 440	1,604 ± 268
rHSA	1,834 ± 494		1,956 ± 394		1,600 ± 569	1,820 ± 549	1,912 ± 655
RL	1,559 ± 564		2,012 ± 695		1,578 ± 509	1,993 ± 587	2,445 ± 1,096
3 × RL	1,575 ± 465		1,868 ± 370		1,374 ± 334	1,420 ± 717	1,848 ± 618

Animals were resuscitated using the same volume of HbV/rHSA, rHSA, or RL, or using 3 × RL, over 15 min (T3). The hemodynamic variables were measured again after 15 min (T4), 1 h (T5), and 2 h (T6). All values are presented as mean ± SD (n = 6).
*Significant difference from baseline ($P < 0.05$).
†Significant difference from the RL group ($P < 0.05$).

might not be the main factor influencing the crystalloid osmotic pressure.

Tissue oxygen tensions measured by microelectrode sensors in the brain, kidney, liver, and muscle all dropped simultaneously as a result of hemorrhagic shock (Fig. 2) and recovered after fluid resuscitation. In particular, oxygenation in the brain, kidney, and liver recovered immediately after resuscitation using HbV/rHSA. Fluid resuscitation using rHSA and HbV/rHSA maintained this restored level of tissue oxygenation for more than 2 h, whereas the tissue oxygenation in the RL and 3 × RL groups fell again within the 2-h study period. Over the 2-h resuscitation period, rHSA and HbV/rHSA showed superior performance to RL and 3 × RL for restoring oxygenation in the brain and kidney.

TABLE 2. **Oxygenation and respiratory variables in New Zealand white rabbits after inducing hemorrhagic shock by withdrawing blood and stabilization for 30 min (T2)**

	Baseline	T2	T4	T5	T6
BE ($mmol \cdot L^{-1}$)					
HbV/rHSA	0.4 ± 1.5	−7.8 ± 2.9*	−3.3 ± 2.3	0.2 ± 2.6†	0.9 ± 2.5†
rHSA	0.3 ± 3.8	−8.4 ± 3.7*	−6.7 ± 4.3*	−0.9 ± 3.5	−0.7 ± 2.8
RL	0.9 ± 3.8	−7.7 ± 2.8*	−6.2 ± 3.3*	−4.2 ± 4.2*	−5.7 ± 5.2*
3 × RL	−0.9 ± 2.2	−8.4 ± 3.7*	−9.1 ± 6.3*	−8.9 ± 9.3*	−10.2 ± 9.1*
Blood lactate ($mg \cdot dL^{-1}$)					
HbV/rHSA	29.2 ± 16.3	89.3 ± 28.8*	51.0 ± 17.3†	26.0 ± 11.5†	25.7 ± 11.7†
rHSA	25.2 ± 8.8	93.2 ± 44.4*	67.7 ± 39.6*	45.5 ± 25.5	41.2 ± 27.9
RL	33.0 ± 14.1	91.0 ± 16.6*	79.7 ± 16.2*	72.2 ± 24.6*	81.5 ± 37.3*
3 × RL	40.9 ± 27.3	103.8 ± 43.1*	111.0 ± 37.2*	94.8 ± 35.8*	107.3 ± 37.8*
Mixed venous blood oxygen saturation (%)					
HbV/rHSA	68 ± 9	39 ± 8*	62 ± 13	74 ± 11†	66 ± 8†
rHSA	67 ± 18	49 ± 10*	58 ± 11	64 ± 5†	63 ± 4†
RL	71 ± 14	50 ± 14*	51 ± 19	43 ± 12*	39 ± 11*
3 × RL	68 ± 9	38 ± 14*	61 ± 23	45 ± 17*	58 ± 25
Oxygen consumption ($mL \cdot min^{-1} \cdot m^{-2}$)					
HbV/rHSA	146 ± 64	83 ± 29*	154 ± 45†	122 ± 21†	148 ± 31†
rHSA	124 ± 46	55 ± 19*	103 ± 32	77 ± 9	84 ± 16
RL	113 ± 22	72 ± 36*	97 ± 16	84 ± 6*	79 ± 10*
3 × RL	130 ± 55	82 ± 21*	82 ± 23*	106 ± 30	57 ± 27*

Animals were resuscitated using the same volume of HbV/rHSA, rHSA, or RL, or using 3 × RL over 15 min. Observations were made 15 min (T4), 1 h (T5), and 2 h (T6) after the fluid resuscitation. All values are presented as mean ± SD (n = 6).
*Significant difference from baseline ($P < 0.05$).
†Significant difference from the RL group ($P < 0.05$).

TABLE 3. **Osmotic pressures and blood glucose levels in New Zealand white rabbits after inducing hemorrhagic shock by withdrawing blood and stabilization for 30 min (T2)**

	Baseline	T2	T4	T5	T6
Colloid osmotic pressure (mmHg)					
HbV/rHSA	14.0 ± 0.9	11.9 ± 1.1*	13.5 ± 1.0†	14.0 ± 1.1†	14.0 ± 0.9†
rHSA	13.6 ± 1.5	10.2 ± 0.9*	11.9 ± 1.9†	12.4 ± 1.3†	12.9 ± 1.3†
RL	13.5 ± 1.9	11.0 ± 1.2*	9.8 ± 1.5*	9.9 ± 1.1*	10.1 ± 1.2*
3 × RL	13.6 ± 1.2	10.9 ± 1.9*	8.1 ± 1.5*†	8.7 ± 1.9*	9.1 ± 1.8*
Crystalloid osmotic pressure (mmol · kg^{-1})					
HbV/rHSA	288 ± 5	299 ± 4*	300 ± 7*	297 ± 5*	299 ± 4 *
rHSA	291 ± 9	299 ± 11*	301 ± 11*	299 ± 5*	298 ± 4*
RL	287 ± 9	299 ± 11*	297 ± 10*	299 ± 10*	301 ± 10*
3 × RL	292 ± 7	302 ± 11*	297 ± 7*	298 ± 9	303 ± 9*
Blood glucose level (mg · dL^{-1})					
HbV/rHSA	216 ± 58	416 ± 135*	382 ± 134*	308 ± 151	238 ± 129†
rHSA	212 ± 37	447 ± 61*	404 ± 77*	363 ± 122*	312 ± 156
RL	196 ± 49	400 ± 110*	358 ± 78*	390 ± 93*	393 ± 99*
3 × RL	196 ± 36	393 ± 120*	294 ± 85*	352 ± 118*	372 ± 80*

Animals were resuscitated using the same volume of HbV/rHSA, rHSA, or RL, or using 3 × RL over 15 min. Further measurements were made after 15 min (T4), 1 h (T5), and 2 h (T6). All values are presented as mean ± SD (n = 6).
*Significant difference from baseline ($P < 0.05$).
†Significant difference from the RL group ($P < 0.05$).

DISCUSSION

The delivery of oxygen is the primary function of HBOCs. When they are administered to hemorrhagic patients, their purpose is to restore oxygen utilization at the microcirculatory level and to normalize cell and organ function. However, some HBOCs were withdrawn from clinical development because their undesirable side effects led to increased morbidity and mortality. The main reason for their failure in basic and clinical studies was their interaction with NO, which resulted in vasoconstriction and subsequent pathophysiological events, ultimately leading to multiple organ failure (1).

The vasoactivity of HBOCs is attributed to their ability to scavenge NO (11–13), to facilitate the diffusion of oxygen and thus its autoregulation (14), and to exert a direct pharmacological effect on smooth muscle mediated by the formation of oxygen-free radicals as the molecules extravasate and by the release of heme in the interstitium (15). However, it has been reported that, although even a small volume was effective as a resuscitation fluid in response to hemorrhagic shock, the HBOC tested did not improve mortality levels and caused acidosis (16). A variety of chemical modifications of the Hb molecule has been developed in attempts to reduce the toxicity of HBOCs (17). Some HBOCs that blunt vasoactivity can be used in a clinical situation. Pyridoxalated glutaraldehyde cross-linked polymerized human Hb is at an advanced stage of phase III clinical trials (18), whereas glutaraldehyde cross-linked bovine poly-Hb has been approved for routine clinical applications in South Africa (19). However, the segmental hydraulic vascular resistance across the microcirculation was consistently above the control levels for cross-linked Hb and polymerized bovine Hb (20). Furthermore, poly-Hb lacks some of the functions of RBCs required for certain clinical conditions (21).

Our results showed that fluid resuscitation using HbV did not impair the microcirculation and microoxygenation and improved oxygen consumption and CI compared with fluid resuscitation using rHSA alone. These findings suggest that using HbV initially might be advantageous in patients having massive hemorrhages, although they cast doubt on the coagulation influence that has been suggested previously (22). Encapsulated Hb, which is one of the next generation of HBOCs, includes some RBC enzymes (23) and is permeable to glucose and other small hydrophilic molecules. In the future, encapsulated recombinant Hb, which was prepared by Hoffman et al. (24) and developed to prevent vasoactivation (25), might be used in a clinical setting. HbV have a lower colloidal osmotic pressure that can be controlled, to some extent, by the solvent used. If a large volume of HbV solution is administered to resuscitate hemorrhagic shock patients, HbV should be suspended in an adequately colloidal solution, such as 5% albumin solution, to simultaneously provide plasma expansion and oxygen-carrying capacity.

Another potential problem for HbV is their metabolism in the reticuloendothelial system and the transient increase in transaminases (26). By contrast, polyethylene glycol is biodegraded in the body into water and carbon dioxide. HbV have the potential to replace most of the RBCs and are effective for the treatment of massive hemorrhages in rats (27, 28), maintaining both microcirculation and microoxygenation. These preliminary results warrant future studies of the safety of HbV for organ functions, including the liver.

Our study period was shorter than those used in previous investigations, as a longer observation period was expected to increase mortality in the RL and 3 × RL groups. Recently, it has been suggested that some HBOC may offer anti-immunoinflammatory effects, reducing the risk of postinjury multiple organ failure (29). An investigation of the effects of HBOCs on long-term mortality after hemorrhagic shock would be of interest in developing a less invasive procedure for the treatment of organ failure.

In summary, fluid resuscitation using HbV solution increased oxygen consumption without impairing microoxygenation or CI in anesthetized rabbits with controlled

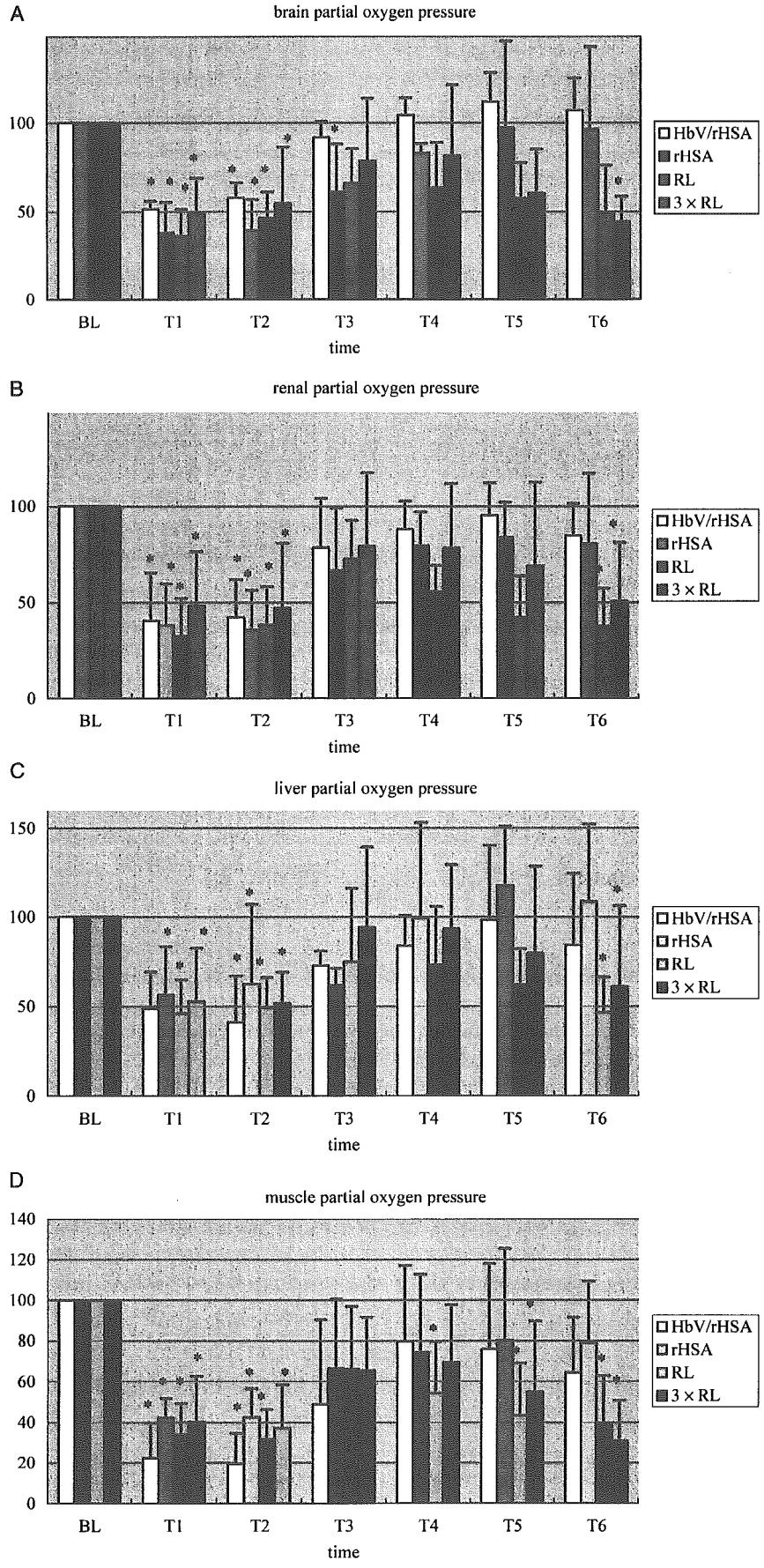

FIG. 2 **Tissue Po_2 values in (a) brain, (b) kidney, (c) liver, and (d) muscle were measured simultaneously using microelectrode sensors at baseline (BL), after inducing hemorrhagic shock by withdrawing blood (T1) and after stabilization for 30 min (T2).** Animals were resuscitated using the same volume of HbV/rHSA, rHSA, or RL, or using 3 × RL over 15 min (T3). Further measurements were taken 15 min (T4), 1 h (T5), and 2 h (T6) after fluid resuscitation. *Significant difference from BL ($P < 0.05$). †Significant difference from the RL group ($P < 0.05$). All values are presented as mean ± SD.

hemorrhagic shock. The use of HSA solution for hemorrhagic shock was shown to improve colloid osmotic pressure and preserve the improvement in hemodynamics for at least 2 h without death. Furthermore, BE was increased, and blood lactate level was decreased by the immediate use of HbV solution. The prognosis of uncontrolled hemorrhagic shock patients after fluid resuscitation with high volumes of HbV solution but no supplementation with coagulation factors remains an open question. Future studies must take this into account when selecting subjects and clinical situations.

ACKNOWLEDGMENTS

This research was supported by a Health Science Research Grant for Research on Pharmaceutical and Medical Safety, Artificial Blood Project, from the Ministry of Health, Labor and Welfare of Japan.

REFERENCES

1. Cabrales P, Tsai AG, Frangos JA, Briceno JC, Intaglietta M: Oxygen delivery and consumption in the microcirculation after extreme hemodilution with perfluorocarbons. *Am J Physiol Heart Circ Physiol* 287:H320–H330, 2004.
2. Gould SA, Moore EE, Hoyt DB, Ness PM, Norris EJ, Carson JL, Hides GA, Freeman IH, DeWoskin R, Moss GS: The life-sustaining capacity of human polymerized hemoglobin when red cells might be unavailable. *J Am Coll Surg* 195:445–452, 2002.
3. Sprung J, Kindscher JD, Wahr JA, Levy JH, Monk TG, Moritz MW, O'Hara PJ: The use of bovine hemoglobin glutamer-250 (Hemopure) in surgical patients: results of a multicenter, randomized, single-blinded trial. *Anesth Analg* 94:799–808, 2002.
4. Bjorkholm M, Fagrell B, Przybelski R, Winslow N, Young M, Winslow RM: A phase 1 single blind clinical trial of a new oxygen transport agent (MP4), human hemoglobin modified with maleimide-activated polyethylene glycol. *Haematologica* 90:505–515, 2005.
5. Winslow R: Abstract Volume, Fourth International Symposium on Current Issues in Blood Substitute Research. Kjellstrom T, Lowe K, eds. *Artif Cells Blood Substit Biotechnol* 31:479–523, 2003.
6. Sakai H, Takeoka S, Wettstein R, Tsai AG, Intaglietta M, Tsuchida E: Systemic and microvascular response to hemorrhagic shock and resuscitation with Hb vesicles. *Am J Physiol* 283:H1191–H1199, 2002.
7. Amberson WR: Blood substitute. *Biol Rev* 12:48, 1937.
8. Savitsky JP, Doozi J, Black J, Arnold JD: A clinical safety trial of stroma free hemoglobin. *Clin Pharmacol Ther* 23:73, 1978.
9. Standl T: A new oxygen transport agent. *Haematologica* 90:437c–8, 2005.
10. Buehler PW, Alayash AI: Toxicities of hemoglobin solutions: in search of *in-vitro* and *in-vivo* model systems. *Transfusion* 44:1516–1530, 2004.
11. Winslow RM: Crosslinked hemoglobin: was failure predicted by preclinical testing? *Vox Sang* 79:1–20, 2000.
12. Doherty DH, Doyle MP, Curry SR, Vali RJ, Fattor TJ, Olson JS, Lemon DD: Rate of reaction with nitric oxide determines the hypertensive effect of cell-free hemoglobin. *Nat Biotechnol* 16:672–676, 1998.
13. Smani Y, Faivre B, Audonnet-Blaise S, Labrude P, Vigneron C: Hemoglobin-based oxygen carrier distribution inside vascular wall and arterial pressure evolution: is there a relationship? *Eur Surg Res* 37:1–8, 2005.
14. McCarthy MR, Vandegriff KD, Winslow RM: The role of facilitated diffusion in oxygen transport by cell-free hemoglobins: implications for the design of hemoglobin-based oxygen carriers. *Biophys Chem* 92:103–117, 2001.
15. Alayash AI: Effects of intra- and intermolecular crosslinking on the free radical reactions of bovine hemoglobins. *Free Radic Biol Med* 18:295–301, 1995.
16. Handrigan MT, Bentley TB, Oliver JD, Tabaku LS, Burge JR, Atkins JL: Choice of fluid influences outcome in prolonged hypotensive resuscitation after hemorrhage in awake rats. *Shock* 23:337–343, 2005.
17. Cabrales P, Tsai AG, Winslow RM, Intaglietta M: Effects of extreme hemodilution with hemoglobin-based O_2 carriers on microvascular pressure. *Am J Physiol Heart Circ Physiol* 288:H2146–H2153, 2005.
18. Gould SA, Moore EE, Hoyt DB, Burch JM, Haenel JB, Garcia J, DeWoskin R, Moss GS: The first randomized trial of human polymerized hemoglobin as a blood substitute in acute trauma and emergent surgery. *J Am Coll Surg* 187: 113–120, 1998.
19. Lok C: Blood product from cattle wins approval for use in humans. *Nature* 410:855, 2001.
20. Cabrales P, Tsai A, Winslow R, Intaglietta M: Effects of extreme hemodilution with hemoglobin-based O_2 carriers on microvascular pressure. *Am J Physiol Heart Circ Physiol* 288:H2146–H2153, 2005.
21. Chang TMS: New generations of red blood cell substitutes. *J Intern Med* 253:527–535, 2003.
22. Amaud F, Hammett M, Asher L, Philbin N, Rice J, Dong F, Pearce B, Flournoy WS, Nicholson C, McCarron R, Freilich D: Effects of bovine polymerized hemoglobin on coagulation in controlled hemorrhagic shock in swine. *Shock* 24:145–152, 2005.
23. Chang TM: Artificial cells for cell and organ replacements. *Artif Organs* 28: 265–270, 2004.
24. Hoffman SJ, Looker DL, Roehrich JM, Cozart PE, Durfee SL, Tedesco JL, Stetler GL: Expression of fully functional tetrameric human hemoglobin in *Escherichia coli*. *Proc Natl Acad Sci U S A* 87:8521–8525, 1990.
25. Doherty DH, Doyle MP, Curry SR, Vali RJ, Fattor TJ, Olson JS, Lemon DD: Rate of reaction with nitric oxide determines the hypertensive effect of cell-free hemoglobin. *Nat Biotechnol* 16:672–676, 1998.
26. Sakai H, Horinouchi H, Masada Y, Takeoka S, Ikeda E, Takaori M, Kobayashi K, Tsuchida E: Metabolism of hemoglobin-vesicles (artificial oxygen carriers) and their influence on organ functions in a rat model. *Biomaterials* 25:4317–4325, 2004.
27. Philips WT, Klpper RW, Awasthi VD, Rudolph AS, Cliff R, Kwasiborski V, Goins BA: Polyethylene glycol-modified liposome-encapsulated hemoglobin: a long circulating red cell substitute. *J Pharmacol Exp Ther* 288:665–670, 1999.
28. Tsuchida E, ed.: *Blood Substitutes: Present and Future Perspectives*. Amsterdam: Elsevier, 1998.
29. Moore EE, Johnson JL, Cheng AM, Masuno T, Banerjee A: Insight from studies of blood substitutes in trauma. *Shock* 24:197–205, 2005.

Circ J 2005; **69:** 1035–1040

Plasma Matrix Metalloproteinase-8 Concentrations are Associated With the Presence and Severity of Coronary Artery Disease

Ryuichi Kato, MD; Yukihiko Momiyama, MD; Reiko Ohmori, PhD; Hiroaki Taniguchi, MD; Haruo Nakamura, MD*; Fumitaka Ohsuzu, MD

Background Interstitial collagen, especially type I, is a major component of atherosclerotic plaques and the matrix metalloproteinases (MMP) 1, 8 and 13 can initiate collagen breakdown. MMP-8 degrades type I collagen preferentially and more potently than MMP-1 or MMP-13. Although MMP-8 was thought to be produced only by neutrophils, it was recently reported to also be produced by endothelial cells, smooth muscle cells and macrophages in plaques.

Methods and Results Plasma MMP-8 concentrations were measured in 250 patients undergoing coronary angiography for coronary artery disease (CAD: >50% stenosis), which was found in 181 patients, of whom 69 had 1-vessel, 66 had 2-vessel, and 46 had 3-vessel disease. Compared with 69 patients without CAD, the 181 with CAD had higher MMP-8 concentrations (3.5 vs 3.0 ng/ml, $p<0.001$). There was a stepwise increase in MMP-8 concentration depending on the number of stenotic vessels: 3.2 in 1-vessel, 3.6 in 2-vessel, and 4.3 ng/ml in 3-vessel disease ($p<0.001$). Multivariate analysis showed that MMP-8 concentration was independently associated with CAD. The odds ratio for CAD was 1.22 (95%confidence interval=1.07–1.39) for a 1 ng/ml increase in MMP-8 concentration.

Conclusions Plasma MMP-8 concentration is associated with the presence and severity of CAD. (*Circ J* 2005; **69:** 1035–1040)

Key Words: Atherosclerosis; Coronary artery disease; Metalloproteinase

Interstitial collagen, especially type I, is a major component of atherosclerotic plaques[1–3] and although collagen synthesis is generally upregulated in atherosclerotic plaques[3] it is the balance between collagen synthesis and breakdown that is important for plaque growth, plaque vulnerability, and vascular remodeling[4–6] The matrix metalloproteinases (MMP) 1, 8 and 13 initiate the collagen breakdown[7] and MMP-8 in particular degrades type I collagen preferentially and more potently than MMP-1 or MMP-13[8,9] However, MMP-8, known as neutrophil collagenase, was previously thought to be produced only by neutrophils, which are not commonly present in plaques, but recently, it has been found that endothelial cells, smooth muscle cells, and macrophages in atherosclerotic plaques express and produce MMP-8[10] Therefore, the present study aimed to elucidate whether or not plasma MMP-8 concentrations are associated with the presence and severity of coronary artery disease (CAD).

(Received March 28, 2005; revised manuscript received May 23, 2005; accepted June 30, 2005)

*First Department of Internal Medicine, National Defense Medical College, Saitama, *Mitsukoshi Health and Welfare Foundation, Tokyo, Japan*

Mailing address: Yukihiko Momiyama, MD, First Department of Internal Medicine, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan. E-mail: momiyama@ndmc.ac.jp

Methods

Study Patients

We measured the plasma MMP-8 concentration in 250 consecutive patients (mean age 65±8 years, range 40–80) who underwent elective coronary angiography for suspected CAD at the National Defense Medical College Hospital. Patients with myocardial infarction within the past 6 months, those with unstable angina at rest within the past 48 h, or those with a history of percutaneous coronary intervention or coronary artery bypass surgery were excluded, as were those with heart failure, cardiomyopathy or valvular heart disease.

Hypertension was defined as blood pressure ≥140/90 mmHg or on relevant medication (122 (49%) patients); hyperlipidemia was defined as total cholesterol >240 mg/dl or relevant medication (65 (26%) patients taking statins); diabetes mellitus was defined as fasting plasma glucose ≥126 mg/dl or on insulin or hypoglycemic drugs.

Our study was approved by the institutional Ethics Committee. After giving written informed consent, each patient had a fasting blood sample taken on the morning of the day that angiography was performed.

Plasma MMP-8 Measurement

Blood samples were collected in EDTA-containing tubes and the plasma was stored at –80°C. Plasma MMP-8 concentrations were measured by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (MMP-8 Human Biotrak ELISA System, Amersham