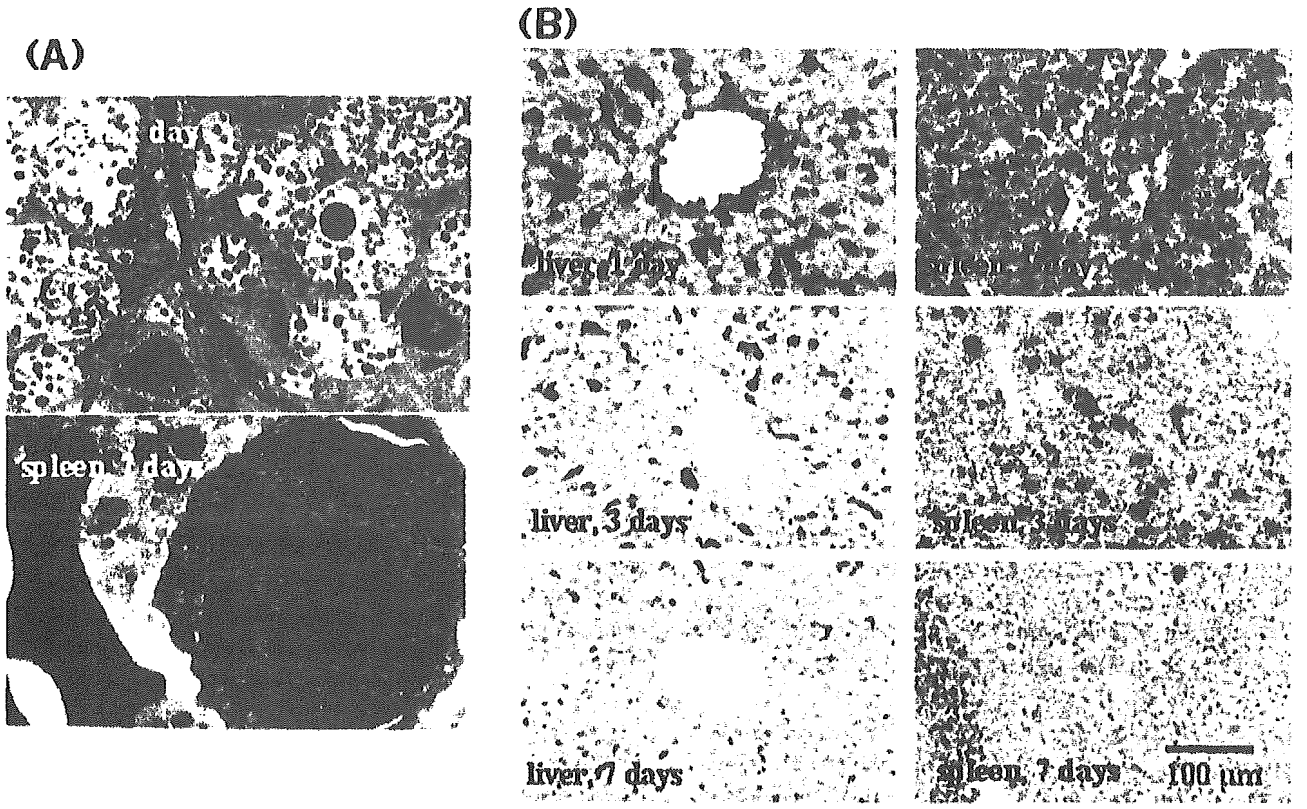

Figure 8. (A) TEM of rat spleen 1 day after the infusion of HbV (20 ml/kg) and after 7 days. Black dots are HbV particles captured in phagosomes in the spleen macrophages, and they disappeared at 7 days. (B) Staining with anti-human Hb antibody showed the presence of HbV in spleen and liver. HbV particles disappeared within 7 days.

showed the time course of biodistribution. After HbV finished playing its role in O_2-transport, a total of 35% of HbV are finally distributed mainly in the liver, spleen and bone marrow. The transmission electron microscopy (TEM) of the spleen 1 day after infusion of HbV clearly demonstrated the presence of HbV particles in macrophages, where HbV particles that appear as black dots are captured by the phagosomes[34] (Fig. 8). RBCs and HbV contain a lot of ferric ion with a high electron density, so that they show strong contrast in TEM. However, after 7 days, the HbV structure cannot be observed. There were no abnormalities in the tissues and no irreversible damages to the organs. A polyclonal anti-human Hb antibody was used as the marker of Hb in the HbV. This antibody does not recognize rat Hb. The red colored parts indicate the presence of Hb in HbV, and they have almost disappeared after 7 days in both the spleen and liver. Therefore, this shows that HbV can be metabolized quite promptly.

One issue of the Hb-based O_2-carriers is that they have a significant influence on clinical laboratory tests. They remain in the plasma phase in hematocrit capillaries after centrifugation of blood samples, and interfere with the colorimetric and turbidimetric measurements. However, HbV can be simply removed from blood plasma either by ultracentrifugation or centrifugation in the presence of a high-molecular-weight dextran to enhance precipitation. A very clear supernatant for accurate analyses can be obtained.[35] This is one advantage of HbV in comparison with acellular Hb solutions. Accordingly, the influence on organ functions by serum clinical laboratory tests after the bolus infusion of HbV at a dose rate of 20 ml/kg was examined. Albumin, alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase, which reflect the liver function, moves their values within normal range.[36] Concentrations of bilirubin and ferric ion are maintained at a low level. The concentration of lipids transiently changed. In particular, the cholesterols increased significantly. And phospholipids slightly increased, however, they returned to the original level after 7 days. These results indicate that the membrane components of HbV, once they reappear from RES, are metabolized on the physiological pathway.

A test of daily repeated infusion is required to evaluate the safety of a new drug. The daily repeated infusion of HbV in Wistar rats at a dose rate of 10 ml/kg/day for 14 days, everyday was tested.[37] The total infusion volume (140 ml/kg) was 2.5 times as much as the volume of the whole blood (56 ml/kg), however, all rats tolerated it well and survived. The body weight showed a monotonous but slightly depressed increase in comparison with the saline. However, after 2 weeks there was no significant difference with the saline control group. All the rats seemed very healthy and active. Histopathological examination 1 day after the final infusion of HbV showed significant accumulation of HbV in spleen macrophages, and liver Kupffer cells, and they mostly disappeared after 14 days. There were no irreversible other morphological abnormalities, and the serum clinical chemistry indicated transient but reversible increases in lipid components. AST and ALT were within the normal range. From these results the authors are confident with the safety of HbV.

DESIGN AND PHYSICOCHEMICAL PROPERTIES OF rHSA-HEME

In this study research on totally synthetic O_2-carriers, or so-called albumin-heme that does not require Hb has been conducted. HSA is the most abundant plasma protein in our blood stream, but its crystal structure has not been elucidated for a long time. In 1998, Dr Stephen Curry of the Imperial College London first elucidated the crystal structure of the HSA complexed with seven molecules of myristic acids.[38] He found that the dynamic conformational changes of albumin take place by the binding of fatty acid. However, in Japan, rHSA is now manufactured on a large scale by expression in the yeast *Pichia pastoris*, and it will appear on the market soon.[39] A large-scale plant, which can produce one million vials per year, has been already established. From the viewpoint of clinical application, O_2-carrying albumin is quite exciting and may be of extreme medical importance. With this background, it has been found that synthetic heme derivative is efficiently incorporated into rHSA, creating a red-colored rHSA-heme hybrid. This rHSA-heme can reversibly bind and release O_2 molecules under physiological conditions in the same manner as Hb. In other words, the rHSA-heme hybrid is a synthetic O_2-carrying hemoprotein, and it is believed that its saline solution will become a new class of RBC substitute.[40–51]

Figure 9 summarizes the structure of the rHSA-heme molecule. The maximal binding numbers of heme to one albumin are eight, and the magnitude of the binding constants ranged from 10^6 to 10^4 (M^{-1}). The isoelectric point of rHSA-heme was found to be 4.8, independent of the binding numbers of heme. This value is exactly the same as that of albumin itself. Furthermore, the viscosity and density did not change after the incorporation of heme molecules, and the obtained solution showed a long shelf life of almost 2 years at room temperature. Since the O_2-binding sites of rHSA-heme are iron-porphyrin, the color of the solution changed in a similar way to Hb. Upon addition of O_2 gas through this solution, the visible absorption pattern immediately changed to that of the O_2-adduct complex. Moreover, after bubbling carbon monoxide gas, rHSA-heme formed a very stable carbonyl complex.

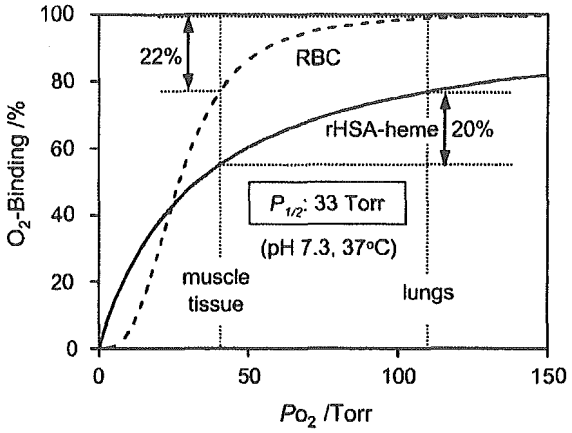

Figure 10. O_2-binding equilibrium curve of albumin-heme.

Figure 10 shows the O_2-binding equilibrium curve of rHSA-heme. The O_2-binding affinity of rHSA-heme is always constant independent of the number of heme, and the O_2-binding profile does not show cooperativity. However, the O_2-transporting efficiency of rHSA-heme between the lungs measuring 110 Torr and muscle tissue measuring 40 Torr increases to 22%, which is identical to the 22% efficiency for RBCs. The O_2-binding property of rHSA-heme can be controlled by changing the chemical structure of heme derivatives incorporated. More recently, it has been found that a protoheme derivative is also incorporated into albumin and can bind and release O_2 as well.[52]

IN VIVO SAFETY AND EFFICACY OF rHSA-HEME

Based on these findings, it can be said that rHSA-heme can become an entirely synthetic O_2-carrier, and satisfy the initial clinical requirements for a RBC substitute. However, there is another problem to solve before this material can be used as an O_2-carrier in the circulatory system. This problem is NO scavenging. Of course, rHSA-heme can bind NO, and it may be anticipated that the injection of rHSA-heme also induce hypertensive action. The authors have evaluated the

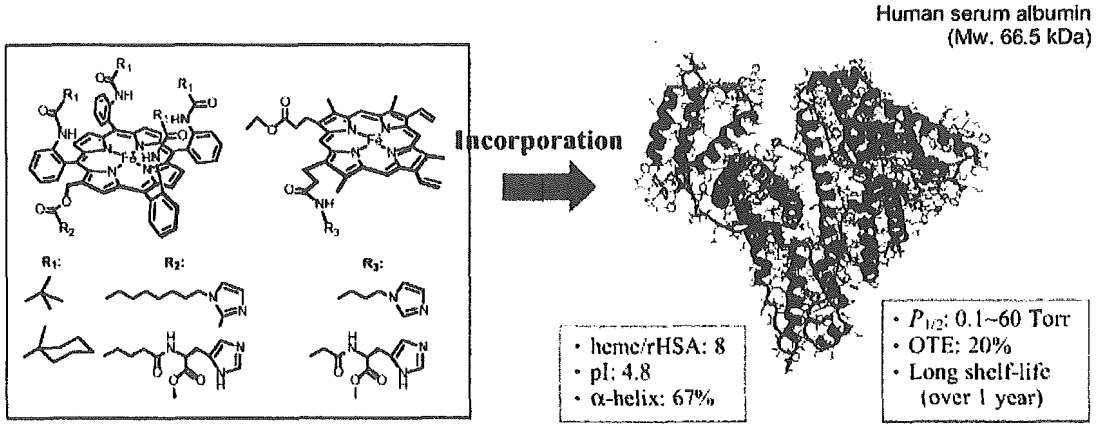

Figure 9. Structure of the albumin-heme molecule.

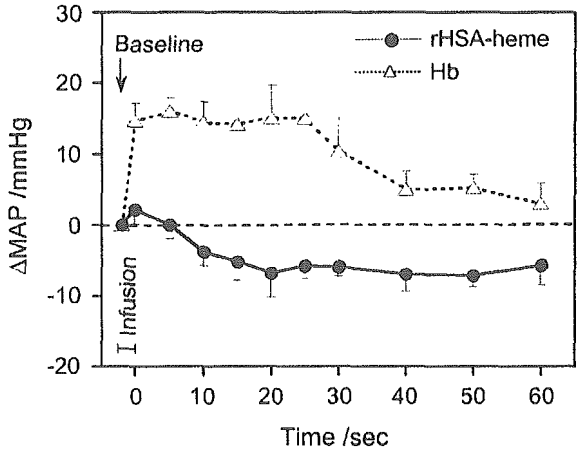

Figure 11. Change of MAP after the administration of rHSA-heme solution in the anesthetized rats ($n=5$). All data are shown as changes from the basal values (ΔMAP) just before the infusion and expressed as mean ± SE. Basal value is 90.1 ± 3.0 mmHg.

efficacy and safety of this rHSA-heme solution with animal experiments.

As described earlier, small Hb molecules extravasate through the vascular endothelium and react with NO, thus inducing vasoconstriction and acute increases in systemic blood pressure. Contrary to the expectations, the observation of the intestinal microcirculation after the infusion of rHSA-heme into an anesthetized rat revealed that the diameters of the venules and arterioles were not deformed at all.[53] Indeed, only a small change in the mean arterial pressure was observed after the administration of the rHSA-heme solution (Fig. 11). In contrast, the infusion of Hb elicited an acute increase in blood pressure. Why does rHSA-heme not induce vasoconstriction or hypertension? The answer probably lies in the negatively charged molecular surface of albumin. One of the unique characteristics of serum albumin is its low permeability through the muscle capillary pore, which is less than 1/100 that for Hb due to the electrostatic repulsion between the albumin surface and the glomerular basement membrane around the endothelial cells.

Thus the authors are now evaluating the O_2-transporting ability of this rHSA-heme molecule in the circulatory system with further animal experiments.[54] First, the physiological responses to exchange transfusion with rHSA-heme solution into rats after 70% hemodilution and 40% hemorrhage was determined (Fig. 12). The declined mean arterial pressure and blood flow after a 70% exchange with albumin and further 40% bleeding of blood showed a significant recovery of up to 90% of the baseline values by the infusion of the rHSA-heme solution. However, all rats in the control group only injected with albumin died within 30 min. Furthermore, muscle tissue O_2-tension significantly increased. These responses indicate the *in vivo* O_2-delivery of the rHSA-heme solution.

More recently, HSA dimer, which can incorporate 16 hemes in its hydrophobic domain has been synthesized.[55] The human serum rHSA-heme dimer solution dissolves 1.3-times more O_2 compared to that of RBC and keeps its colloid osmotic pressure at the same level as the physiological value.

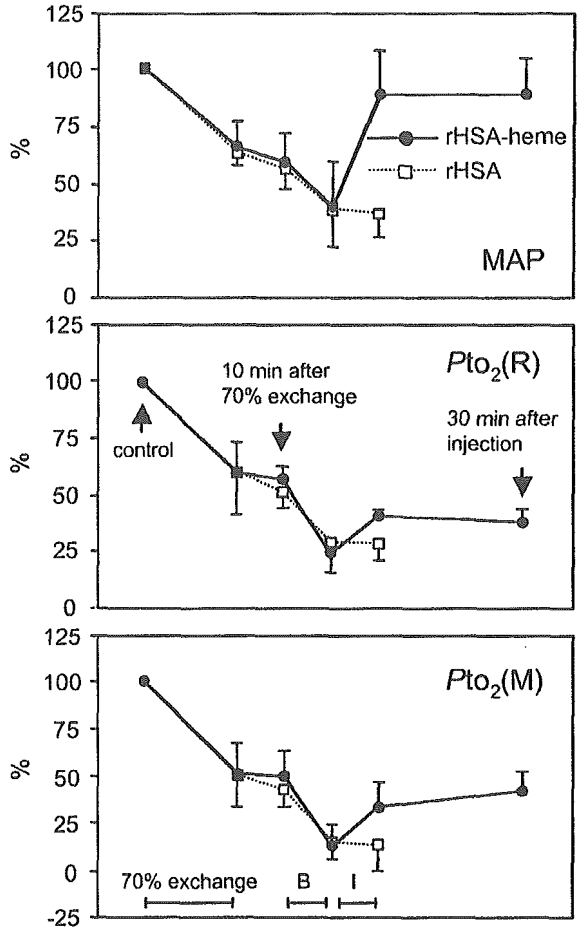

Figure 12. Change of (a) MAP and (b) O_2-tension in renal cortex during the 70% hemodilution with 5 wt% rHSA and further 40% exchange transfusion with rHSA-heme in anesthetized rats ($n=5$). All data are shown as changes from the basal values and expressed as mean ± SE.

POTENTIAL APPLICATIONS OF ARTIFICIAL O_2 CARRIERS

As described earlier the primary application of artificial O_2-carriers would be the resuscitative fluid for hemorrhage. Since some of the characteristics of artificial O_2-carriers overwhelm those of donated blood, there are many potential applications other than blood substitutes.

Tumor oxygenation

Unlike vessels in normal tissues, the development of a vasculature in a tumor lacks regulation and is hence, highly heterogeneous. Consequently, areas of hypoxia are quite common in tumors. In these hypoxic regions, it can be added that tumor cells acquire resistance to treatments such as chemotherapy and radiation. The rHSA-heme was injected into the responsible artery that supplies circulation to an implanted tumor (Fig. 13).[56] O_2-tension of the tumor rises immediately after intra-arterial infusion of albumin heme up to 2.4 times that of the baseline value. The findings in animals indicate that tumor tissue O_2-levels can be elevated by the administration of artificial O_2-carriers due to the

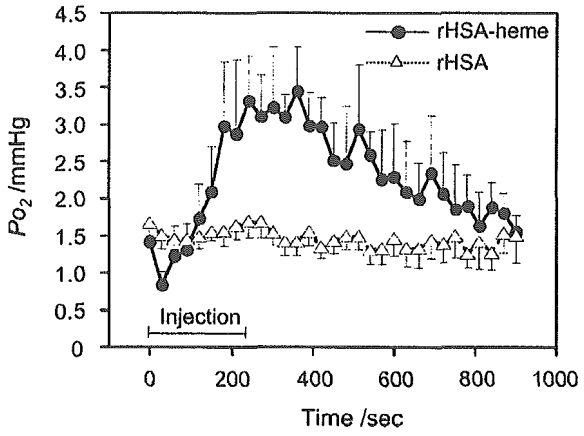

Figure 13. Changes in the O_2 tension of the hypoxic region of the ascites hepatoma LY80 solid tumor after the administration of the O_2 saturated rHSA-heme or rHSA solutions in the anesthetized rats ($n=4$ each). All data are shown as changes from the basal values (P_{O_2}) just before the infusion and expressed as mean ± SE.

difference in O_2-transporting properties from RBCs. Whether this increase in tissue O_2 can potentiate cancer treatment is currently under investigation.

Oxygenation of ischemic tissue

Tissue ischemia can ensue from impairment of peripheral perfusion due to a variety of diseases such as arteriosclerosis obliterans, diabetes, and Burger's disease. The key event in the progression of ischemic diseases is the inability of red cells to flow through the capillaries, beyond which point ulceration and gangrene formation become imminent. It is believed that this critical phase can be avoided or delayed by the application of artificial O_2-carriers, which can be designed to flow even through these damaged capillaries.[27,28]

Organ preservation

One of the most important agenda in transplantation medicine is long-term organ preservation and circumvention of ischemia reperfusion injuries. It is believed that artificial O_2-carriers can be applied as a perfusate for donor tissue in order to overcome these problems. In particular, its O_2 carrying capacity has the potential to significantly extend the preservation period. This will make it easier to transport organs. Also, utilizing the extra time, it may be possible in the future to perform additional organ tests for better compatibility, or even perform genetic modifications during this period. It is believed that through these applications, the concept of organ preservation can be expanded to culture organs, and furthermore to include the preservation of cells derived from donor tissues.

Extracorporeal circulation

Extracorporeal circulation is quite common in cardiac surgery. Improvements are being made in the priming solutions but red cells are often still required to fill the device circuit, particularly in compromised cases and in children.[57] It is believed that the use of artificial O_2-carriers in the priming solution can decrease or completely eliminate the need for a transfusion in such cases, and hence reduce the incidence of infection or graft-versus-host disease (GVHD).

Liquid ventilation for acute lung injury

For patients who present acute lung injury or acute respiratory distress syndrome (ARDS), gas exchange in the lung exhibits severe deterioration and sometimes even the newest mechanical ventilation method fails to establish adequate oxygenation of the blood. In this type of critical case, liquid ventilation using an artificial O_2-carrier can establish optimal oxygenation of the blood and may reproduce the integrity of lung parenchyma.[58] Briefly explained, oxygenated liquid ventilation fluid is administered into the lung through trachea and O_2 molecules are transferred through diseased alveolus by diffusion and oxygenate the blood. Currently, this method is thought to be effective for patients with congenital diaphragmatic herniation. Efficacy for adult acute lung injuries is now under investigation. Perfluorochemicals are the main fluid used for clinical use, however, aqueous artificial O_2-carriers may have the potential to be used for liquid ventilation.

FUTURE SCOPE

The research field of the red cell substitutes is moving forward very rapidly, and the paradigm in this field is expanding from red cell substitutes to "O_2 therapeutics". Significant efforts have been made to produce HbV and albumin-heme with a facility of GMP standard, and to start preclinical and finally clinical trials. We look forward to the day that our research will play an effective role in treating patients.

Acknowledgements

This work has been supported by The Ministry of Education, Culture, Sports, Science and Technology and The Ministry of Health, Labor and Welfare.

REFERENCES

1. Tsuchida E (ed.). *Macromolecular Complexes, Dynamic Interactions and Electronic Processes*. VCH: New York, 1991.
2. Ciardelli F, Tsuchida E. Wöhrle D (eds). *Macromolecule-metal Complexes*. VCH: New York, 1996.
3. Matsushita Y, Hasegawa E, Eshima K, Tsuchida E. Synthesis of amphiphilic porphinatoiron complexes having phosphocholine groups. *Chem. Lett.* 1983; 1387–1389.
4. Tsuchida E. Liposome-embedded iron-porphyrins as an artificial oxygen carrier. *Ann. New York Acad. Sci.* 1985; **446**: 429–442.
5. Tsuchida E, Nishide H. Hemoglobin model—artificial oxygen carrier composed of porphinatoiron complexes. *Top. Curr. Chem.* 1986; **132**: 63–99.
6. Komatsu T, Moritake M, Nakagawa A, Tsuchida E. Self-organized lipid-porphyrin bilayer membranes in vesicular form: nanostructure, photophysical properties and dioxygen coordination. *Chem. Eur. J.* 2002; **8**: 5469–5480.
7. Chang TMS. *Blood Substitutes: Principles, Methods, Products, and Clinical Trials*. Karger: Basel, 1997.
8. Riess JG. Oxygen carriers ("blood substitutes")-raison d'etre, chemistry, and some physiology. *Chem. Rev.* 2001; **101**: 2797–2919.
9. Sloan EP, Koenigsberg M, Gens D, Cipolle M, Runge J, Mallory MN, Rodman G Jr. Diaspirin cross-linked hemoglobin (DCLHb) in the treatment of severe traumatic hemorrhagic shock. *JAMA* 1999; **282**: 1857–1864.

10. Tsuchida E. *Blood Substitutes: Present and Future Perspectives*. Elsevier: Amsterdam, 1998.
11. Djordjevich L, Mayoral J, Miller IF, Ivankovich AD. Cardiorespiratory effects of exchange transfusions with synthetic erythrocytes in rats. *Crit. Care Med.* 1987; **15**: 318–323.
12. Takeoka S, Ohgushi T, Yokohama H, Sakai H, Nishide H, Tsuchida E. Preparation conditions of human hemoglobin vesicles covered with lipid membrane. *Artif. Organs Today* 1993; **3**: 129–136.
13. Sakai H, Takeoka S, Yokohama H, Seino Y, Nishide H, Tsuchida E. Purification of concentrated Hb using organic solvent and heat treatment. *Protein Expression Purif.* 1993; **4**: 563–569.
14. Takeoka S, Ohgushi T, Terase K, Ohmori T, Tsuchida E. Layer-controlled hemoglobin vesicles by interaction of hemoglobin with a phospholipid assembly. *Langmuir* 1996; **12**: 1755–1759.
15. Naito Y, Fukutomi I, Masada Y, Sakai H, Takeoka S, Tsuchida E, Abe H, Hirayama J, Ikebuchi K, Ikeda H. Virus removal from hemoglobin solution using Planova membrane. *J. Artif. Organs* 2002; **5**: 141–145.
16. Fukutomi I, Sakai H, Takeoka S, Nishide H, Tsuchida E, Sakai K. Carbonylation of oxyhemoglobin solution using a membrane oxygenator. *J. Artif. Organs* 2002; **5**: 102–107.
17. Sou K, Endo T, Takeoka S, Tsuchida E. Poly(ethylene glycol)-modification of the phospholipid vesicles by using the spontaneous incorporation of poly(ethylene glycol)-lipid into the vesicles. *Bioconjugate Chem.* 2000; **11**: 372–379.
18. Sakai H, Tomiyama K, Sou K, Takeoka S, Tsuchida E. Poly(ethyleneglycol)-conjugation and deoxygenation enable long term preservation of hemoglobin vesicles as oxygen carriers. *Bioconjugate Chem.* 2000; **11**: 425–432.
19. Sou K, Naito Y, Endo T, Takeoka S, Tsuchida E. Effective encapsulation of proteins into size-controlled phospholipid vesicles using the freeze–thawing and extrusion. *Biotechnol. Prog.* 2003; **19**: 1547–1552.
20. Izumi Y, Sakai H, Hamada K, Takeoka S, Yamahata T, Kato R, Nishide H, Tsuchida E, Kobayashi K. Physiologic responses to exchange transfusion with hemoglobin vesicles as an artificial oxygen carrier in anesthetized rats: changes in mean arterial pressure and renal cortical tissue oxygen tension. *Crit. Care Med.* 1996; **24**: 1869–1873.
21. Izumi Y, Sakai H, Kose T, Hamada K, Takeoka S, Yoshizu A, Horinouchi H, Kato R, Nishide H, Tsuchida E, Kobayashi K. Evaluation of the capabilities of a hemoglobin vesicle as an artificial oxygen carrier in a rat exchange transfusion model. *ASAIO J.* 1997; **43**: 289–297.
22. Kobayashi K, Izumi Y, Yoshizu A, Horinouchi H, Park SI, Sakai H, Takeoka S, Nishide H, Tsuchida E. The oxygen carrying capability of hemoglobin vesicles evaluated in rat exchange transfusion models. *Artif. Cells Blood Substitues Immobilization Biotechnol.* 1997; **25**: 357–366.
23. Sakai H, Takeoka S, Park SI, Kose T, Nishide H, Izumi Y, Yoshizu A, Kobayashi K, Tsuchida E. Surface modification of hemoglobin vesicles with poly(ethyleneglycol) and effects on aggregation, viscosity, and blood flow during 90% exchange transfusion in anesthetized rats. *Bioconjugate Chem.* 1997; **8**: 23–30.
24. Sakai H, Tsai AG, Kerger H, Park SI, Takeoka S, Nishide H, Tsuchida E, Intaglietta M. Subcutaneous microvascular responses to hemodilution with a red cell substitute consisting of polyethyleneglycol-modified vesicles encapsulating hemoglobin. *J. Biomed. Mater. Res.* 1998; **40**: 66–78.
25. Sakai H, Tsai AG, Rohlfs RJ, Hara H, Takeoka S, Tsuchida E, Intaglietta M. Microvascular responses to hemodilution with Hb-vesicles as red cell substitutes: influences of O_2 affinity. *Am. J. Physiol.* 1999; **276**: H553–H562.
26. Sakai H, Takeoka S, Wettstein R, Tsai AG, Intaglietta M, Tsuchida E. Systemic and microvascular responses to the hemorrhagic shock and resuscitation with Hb-vesicles. *Am. J. Physiol. Heart Circ. Physiol.* 2002; **283**: H1191–H1199.
27. Erni D, Wettstein R, Schramm S, Contaldo C, Sakai H, Takeoka S, Tsuchida E, Leunig M, Banic A. Normovolemic hemodilution with hemoglobin-vesicle solution attenuates hypoxia in ischemic hamster flap tissue. *Am. J. Physiol. Heart Circ. Physiol.* 2003; **284**: H1702–H1709.
28. Contaldo C, Schramm S, Wettstein R, Sakai H, Takeoka S, Tsuchida E, Leunig M, Banic A, Erni D. Improved oxygenation in ischemic hamster flap tissue is correlated with increasing hemodilution with Hb vesicles and their O_2 affinity. *Am. J. Physiol. Heart Circ. Physiol.* 2003; **285**: H1140–H1147.
29. Goda N, Suzuki K, Naito M, Takeoka S, Tsuchida E, Ishimura Y, Tamatani T, Suematsu M. Distribution of heme oxygenase isoform in rat liver: topographic basis for carbon monoxide-mediated micorvascular relaxation. *J. Clin. Invest.* 1998; **101**: 604–612.
30. Sakai H, Hara H, Yuasa M, Tsai AG, Takeoka S, Tsuchida E, Intaglietta M. Molecular dimensions of Hb-based O_2 carriers determine constriction of resistance arteries and hypertension in conscious hamster model. *Am. J. Physiol. Heart Circ. Physiol.* 2000; **279**: H908–H915.
31. Wakamoto S, Fujihara M, Abe H, Sakai H, Takeoka S, Tsuchida E, Ikeda H, Ikebuchi K. Effects of PEG-modified hemoglobin vesicles on agonist induced platelet aggregation and RANTES release *in vitro*. *Artif. Cells Blood Substitues Immobilization Biotechnol.* 2001; **29**: 191–201.
32. Kyokane T, Norimizu S, Taniai H, Yamaguchi T, Takeoka S, Tsuchida E, Naito M, Nimura Y, Ishimura Y, Suematsu M. Carbon monoxide from heme catabolism protects against hepatobiliary dysfunction in endotoxin-treated rat liver. *Gastroenterology* 2001; **120**: 1227–1240.
33. Ito T, Fujihara M, Abe H, Yamaguchi M, Wakamoto S, Takeoka S, Sakai H, Tsuchida E, Ikeda H, Ikebuchi K. Effects of poly(ethyleneglycol)-modified hemoglobin vesicles on *N*-formyl-methionyl-leucyl-phenylalanine induced responses of polymorphonuclear neutrophils *in vitro*. *Artif. Cells Blood Substitues Immobilization Biotechnol.* 2001; **29**: 427–438.
34. Sakai H, Horinouchi H, Tomiyama K, Ikeda E, Takeoka S, Kobayashi K, Tsuchida E. Hemoglobin-vesicles as oxygen carriers: influence on phagocytic activity and histopathological changes in reticuloendothelial systems. *Am. J. Pathol.* 2001; **159**: 1079–1088.
35. Sakai H, Tomiyama K, Masada Y, Takeoka S, Horinouchi H, Kobayashi K, Tsuchida E. Pretreatment of serum containing Hb-vesicles (oxygen carriers) to avoid their interference in laboratory tests. *Clin. Chem. Lab. Med.* 2003; **41**: 222–231.
36. Sakai H, Horinouchi H, Masada Y, Takeoka S, Kobayashi K, Tsuchida E. Metabolism of hemoglobin-vesicles (artificial oxygen carriers) and their influence on organ functions in a rat model. *Biomaterials* 2004; **25**: 4317–4325.
37. Sakai H, Masada Y, Horinouchi H, Ikeda E, Sou K, Takeoka S, Suematsu M, Kobayashi K, Tsuchida E. Physiologic capacity of reticuloendothelial system for degradation of homoglobin-vesicles (artificial oxygen carriers) after massive intravenous doses by daily repeated infusions for 14 days. *J. Pharmacol. Exp. Ther.* 2004; **311**: 874–884.
38. Curry S, Mandelkow H, Brick P, Franks N. Crystal structure of human serum albumin complexes with fatty acid reveals an asymmetric distribution of binding sites. *Nature Struct. Biol.* 1998; **5**: 827–835.
39. Sumi A, Ohtani W, Kobayashi K, Ohmura T, Yokoyama K, Nishida M, Suyama T. Purification and physicochemical properties of recombinant human serum albumin. In *Biotechnology of Blood Proteins*, Rivat C, Stoltz JF (eds). John Libbey Eurotext: Montrouge, 1993; vol. 227, 293–298.
40. Komatsu T, Ando K, Kawai N, Nishide H, Tsuchida E. O_2-transport albumin: a new hybrid-haemoprotein incorporating tetraphenylporphinatoiron(II) derivative. *Chem. Lett.* 1995; 813–814.
41. Tsuchida E, Ando K, Maejima H, Kawai N, Komatsu T, Takeoka S, Nishide H. Properties of oxygen binding by albumin-tetraphenylporphyrinatoiron(II) derivative complexes. *Bioconjugate Chem.* 1997; **8**: 534–538.
42. Wu J, Komatsu T, Tsuchida E. Resonance raman studies of O_2-binding to *ortho*-substituted tetraphenyl- and tetranaphthyl-porphinatoiron(II) derivatives with a covalently linked axial imidazole. *J. Chem. Soc., Dalton Trans.* 1998; 2503–2506.
43. Komatsu T, Hamamatsu K, Wu J, Tsuchida E. Physicochemical properties and O_2-coordination structure of human serum albumin incorporating tetrakis(*o*-pivalamido)phenylporphinatoiron(II) serivatives. *Bioconjugate Chem.* 1999; **10**: 82–86.
44. Tsuchida E, Komatsu T, Mastukawa Y, Hamamatsu K, Wu J. Human serum albumin incorporating tetrakis(*o*-pivalamido)phenylporphinatoiron(II) derivative as a totally

synthetic O_2-carrying hemoprotein. *Bioconjugate Chem.* 1999; **10**: 797–802.
45. Komatsu T, Matsukawa Y, Tsuchida E. Kinetics of CO and O_2 binding to human serum albumin-heme hybrid. *Bioconjugate Chem.* 2000; **11**: 772–776.
46. Komatsu T, Matsukawa Y, Tsuchida E. Reaction of nitric oxide with synthetic hemoprotein, human serum albumin incorporating tetraphenylporphinatoiron(II) derivatives. *Bioconjugate Chem.* 2001; **12**: 71–75.
47. Nakagawa A, Komatsu T, Tsuchida E. Photoreduction of autooxidized albumin-heme hybrid in saline solution: revival of its O_2-binding ability. *Bioconjugate Chem.* 2001; **12**: 648–652.
48. Komatsu T, Okada T, Moritake M, Tsuchida E. O_2-Binding properties of double-sided porphyrinatoiron(II)s with polar substituents and their human serum albumin hybrids. *Bull. Chem. Soc. Jpn* 2001; **74**: 1695–1702.
49. Wu Y, Komatsu T, Tsuchida E. Electrochemical studies of albumin-heme hybrid in aqueous media by modified electrode. *Inorg. Chim. Acta* 2001; **322**: 120–124.
50. Komatsu T, Matsukawa Y, Tsuchida E. Effect of heme structure on O_2-binding properties of human serum albumin-heme hybrids: intramolecular histidine coordination provides a stable O_2-adduct complex. *Bioconjugate Chem.* 2003; **13**: 397–402.
51. Tsuchida E, Komatsu T, Yanagimoto T, Sakai H. Preservation stability and *in vivo* administration of albumin-heme hybrid solution as an entirely synthetic O_2-carrier. *Polym. Adv. Technol.* 2002; **13**: 845–850.
52. Nakagawa A, Komatsu T, Ohmichi N, Tsuchida E. Synthetic dioxygen-carrying hemoprotein: human serum albumin including iron(II) complex of protoporphyrin IX with an axially coordinated histidylglycyl-propionate. *Chem. Lett.* 2003; **32**: 504–505.
53. Tsuchida E, Komatsu T, Matsukawa Y, Nakagawa A, Sakai H, Kobayashi K, Suematsu M. Human serum albumin incorporating synthetic heme: red blood cell substitute without hypertension by nitric oxide scavenging. *J. Biomed. Mater. Res.* 2003; **64A**: 257–261.
54. Tsuchida E, Komatsu T, Hamamatsu K, Matsukawa Y, Tajima A, Yoshizu A, Izumi Y, Kobayashi K. Exchange transfusion of albumin-heme as an artificial O_2-infusion into anesthetized rats: physiological responses, O_2-delivery and reduction of the oxidized hemin sites by red blood cells. *Bioconjugate Chem.* 2000; **11**: 46–50.
55. Komatsu T, Hamamatsu K, Tsuchida E. Cross-linked human serum albumin dimers incorporating sixteen (tetraphenylporphinato)iron(II) derivatives: synthesis, characterization, and O_2-binding property. *Macromolecules* 1999; **32**: 8388–8391.
56. Kobayashi K, Komatsu T, Iwamaru A, Matsukawa Y, Horinouchi H, Watanabe M, Tsuchida E. Oxygenation of hypoxic region in solid tumor by administration of human serum albumin incorporating synthetic hemes. *J. Biomed. Mater. Res.* 2003; **64A**: 48–51.
57. Kobayashi K, Izumi Y, Yamahata T, Sakai H, Takeoka S, Nishide H, Tsuchida E. Efficacy of synthetic oxygen-carrying substances. In *Int. Congr. Ser. 1995, 1102 (Shock: from Molecular and Cellular Level to Whole Body)*. Elsevier: Amsterdam, 1996; 305–310.
58. Horinouchi H, Tajima A, Kobayashi K. Liuid ventilation using artificial oxygen carrier. *Artif. Blood* 2001; **9**: 2–5 (in Japanese).

The Pain Clinic, Vol. 16, No. 4, pp. 389–392 (2004)

Also available online - www.vsppub.com

Research paper

Efficacy of oral pranlukast hydrate, a leukotriene receptor antagonist, in the treatment of postherpetic neuralgia

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summary The purpose of this study is to investigate the analgesic effect of pranlukast hydrate (PH), a leukotriene receptor antagonist, in patients with postherpetic neuralgia (PHN). Twenty patients with PHN whose pain reached a plateau after 3 or more months received oral administration of PH at doses of 450 mg/day for 1 month. Visual analogue scales of rest pain, tactile allodynia, and paroxysmal pain were significantly reduced at 1 week, 2 weeks, and 1 month after the beginning of oral administration. The analgesic effect was better in patients suffering from PHN for 1 year or more than in the others. This suggests that leukotrienes may play a role in PHN.

Key words: Pranlukast; leukotriene; postherpetic neuralgia.

introduction

Postherpetic neuralgia (PHN) is one of the most painful neuropathic pain syndromes, and also is one of the commonest intractable conditions seen in pain clinics. The mechanism of PHN remains unclear, and there is no established preventive or therapeutic regimen.[1] Although systemic and local administrations of steroids have been reported to be effective for PHN,[2,3] there may be a risk in using steroids because of their adverse effects. On the other hand, nonsteroidal anti-inflammatory drugs are ineffective.[4] Phospholipase A2 inhibitors such as steroids may exert an analgesic effect in patients with PHN. The aim of our research was to examine the efficacy of pranlukast hydrate, an antagonist of leukotriene receptors, in the treatment of PHN.

methods

Among patients with neuropathic pain following herpes zoster infection, 20 patients whose pain reached a plateau after 3 or more months were selected as subjects for this study after they had given informed consent. They comprised 10 males and 10 females with age 72 ± 7 years, height 156 ± 10 cm, and body weight 54 ± 8 kg. Patients who had received pranlukast hydrate in the previous 3 months were excluded.

The subjects received oral administration of pranlukast hydrate (Onon®, oral pranlukast hydrate) at doses of 450 mg/day for 1 month. When adverse events appeared, the pain severely worsened, or any other problems affecting the continuous application of the drug occurred, the study was discontinued. The levels of rest pain, tactile allodynia, and paroxysmal pain before and at

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1 week, 2 weeks, and 1 month after the oral administration were scored using a visual analogue scale (VAS).

All values were recorded as mean ± SE. The Wilcoxon t-test was used to compare the data of before, during and after administration. Differences were considered statistically significant at $p < 0.05$.

results

Only one patient interrupted the treatment as he got a cold. Only one of the remaining 19 patients suffered from slight nausea, but it was possible to continue the oral treatment for one month. No other adverse events were observed.

The data on the treatment with pranlukast hydrate are summarized in Fig. 1. All the VAS of rest pain, tactile allodynia, and paroxysmal pain were significantly decreased by the treatment, and the analgesic effects persisted at least for one month.

No significant differences were found in VAS with regard to gender, age or affected dermatomes. The patients were divided into two groups according to PHN duration (Table I). The long duration group (LDG: $n = 10$) and the short duration group (SDG: $n = 9$) had PHN for more than or less than one

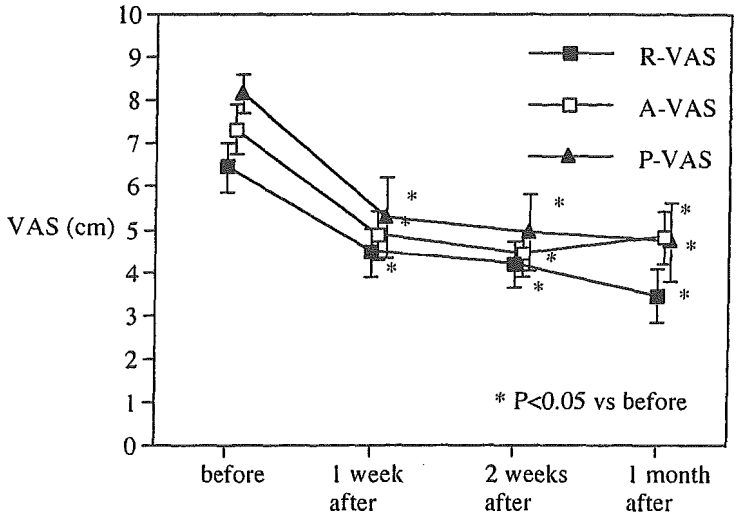

Figure 1. Visual analogue scales (VAS) of rest pain (R-VAS), allodynia (A-VAS), and paroxysmal pain (P-VAS) before and after the oral administration of pranlukast hydrate. All the VAS were significantly decreased after the treatment with pranlukast hydrate.

Table I.
The comparison between SDG and LDG in visual analog scales (VAS) of rest pain, allodynia, and paroxysmal pain before and after the oral administration of pranlukast hydrate

		Before	1 week after	2 week after	1 month after
Rest pain	SDG	6.3 ± 1.7	4.7 ± 1.9*	4.6 ± 3.0*	4.0 ± 2.9**
	LDG	6.6 ± 2.2	4.3 ± 2.3**	3.9 ± 1.8**	3.0 ± 2.3**
Allodynia	SDG	6.8 ± 2.1	5.2 ± 2.4*	4.9 ± 2.8*	4.5 ± 3.1*
	LDG	7.8 ± 2.6	4.6 ± 2.5**	4.0 ± 1.7**	5.1 ± 2.3**
Paroxysmal pain	SDG	8.4 ± 1.4	6.5 ± 3.7	6.5 ± 3.7	5.1 ± 4.5
	LDG	8.0 ± 2.1	4.4 ± 4.2*	3.8 ± 3.6*	3.7 ± 3.6*

SDG: short duration group (less than 1 year, $n = 9$), LDG: long duration group (more than 1 year, $n = 10$).

*$p < 0.05$, **$p < 0.01$ *vs.* before.

year, respectively, before starting this study. VAS values in LDG decreased slightly more than those in SDG.

discussion

There are two types pain in PHN: persistent pain described as burning, raw, severe aching or tearing, and a superimposed paroxysmal pain expressed as stabbing or lancinating.[5] Pranlukast hydrate may relive all the types of pain, as suggested by the present study.

The precise mechanism of herpes zoster virus in producing pain is not known, but acute herpetic neuralgia is considered a variant of PHN. In the patients with PHN, inflammation and necrosis of dorsal root ganglia have been well documented in pathological reports of herpes zoster.[6] The commonest cause of viral recrudescence is probably due to a decline in cell mediated immunity, which is often related to age.[7]

The leukotriene receptor antagonists, such as pranlukast, montelukast, and zafirlukast, do not improve reduced immunity. Sheftell *et al.* have shown that montelukast is effective in relieving pain associated with migraine.[8] Pranlukast is an antagosist of C4, D4 and E4 leukotriene receptors, whereas montelukast is a specific D4 antagonist. The efficacy of pranlukast in PHN is possibly due to suppression of the inflammation in dorsal root ganglia.

Therapeutic agents commonly used to treat PHN include tricyclic antidepressants, antiepileptic drugs and opioids.[9] However, severe side effects can occur with these drugs. One of the most well established risk factors for PHN is old age.[7] Many patients over the age of 50 also have other diseases. Severity of the pain and risk factors for complications must be considered in the assessment of the risk/benefit ratio of different therapeutic strategies. Pranlukast hydrate may provide sufficient pain relief with minor adverse events.

Watson *et al.* have found that in some patients with long duration of PHN their pain gradually worsened, despite may attempts to obtain pain relief.[6] Established PHN generally tends to be intractable and lead to considerable disability and suffering in terminal patients. In patients with PHN over 50 years, 20% continue to complain of pain six months after onset of the rush despite adequate antiviral therapy.[7] Pranlukast hydrate may be an analgesic drug especially for those patients.

In conclusion, the results of the present study indicate that oral pranlukast hydrate produces analgesia in patients with PHN and its analgesic effect is greater in patients suffering from PHN for more than 1 year.

Acknowledgements

This study was supported by Grant-in-Aid for Young Scientists B-14770789 and Scientific Research B-14370494, Ministry of Education, Science, Sports and Culture, Japan.

references

1. Opstelten W, van Wijk AJM, Stolker RJ, Interventions to prevent postherpetic neuralgia: cutaneous and percutaneous techniques, *Pain* **3**, 202–6 (2004).
2. Keczkes K, Basheer AM, Do corticosteroids prevent post-herpetic neuralgia? *Brit J Dermatol* **102**, 551–5 (1980).
3. Kotani N, Kushikata T, Hashimoto H, *et al.*, Intrathecal methylprednisolone for intractable postherpetic neuralgia, *New Engl J Med* **343**, 1514–9 (2000).
4. Max MB, Schafer SC, Culnane M, *et al.*, Association of pain relief with drug side effects in postherpetic neuralgia: a single-dose study of clonidine, codeine, ibuprofen, and placebo, *Clin Pharmacol Ther* **43**, 363–71 (1988).
5. Watson CP, Morshead C, Van der Koog D, *et al.*, Post-herpetic neuralgia: post mortem analysis of a case, *Pain* **34**, 129–38 (1988).
6. Watson CP, Watt VR, Chipman M, *et al.*, The prognosis with postherpetic neuralgia, *Pain* **46**, 195–9 (1991).

7. Johnson RW, Dworkin RH, The treatment of herpes zoster and postherpeticneuralgia, *Brit Med J* **326**, 748–50 (2003).
8. Sheftell F, Rapoport A, Weeks R, *et al.*, Montelukast in the prophylaxis of migraine: a potential role for leukotriene modifiers, *Headache* **40**, 158–63 (2000).
9. Raja SN, Haythornthwaite JA, Pappagallo M, *et al.*, Opioids versus antidepressants in postherpetic neuralgia: a randomized, placebo-controlled trial, *Neurology* **59**, 1015–21 (2002).

Faster oscillometric manometry does not sacrifice the accuracy of blood pressure determination

Masaru Sugimachi[a], Hirotsugu Okamoto[b], Sumio Hoka[b] and Kenji Sunagawa[a]

Faster oscillometry enables one to track rapid pressure changes. We therefore examined whether it was possible to shorten the measurement time without sacrificing accuracy. We accelerated and linearized cuff deflation and determined systolic and diastolic pressure values by the appearance and disappearance of oscillometric waves based on the interpolated cuff pressure-oscillometric wave amplitude relationship. The accuracy of faster oscillometry was examined by comparing correlations between invasive radial and oscillometric brachial pressure with either the conventional or the faster oscillometry in 23 patients (32 ± 16 measurement pairs). Faster oscillometry shortened the measurement time from 27.7 ± 3.5 s to 17.1 ± 2.6 s. Neither pressure levels nor heart rate altered the time required for measurement. Bland-Altman analysis indicated that mean and standard deviation of difference between oscillometric and invasive systolic pressure was comparable (conventional, 2.1 ± 7.5 mmHg; faster, 1.4 ± 7.3 mmHg) without correlations between difference and average of systolic pressure. Similar differences (conventional, 5.0 ± 6.8 mmHg; faster, 4.9 ± 5.8 mmHg) and lack of correlations were also found for diastolic pressure. In conclusion, we succeeded in shortening the oscillometric measurement time to approximately 60% of the original time without sacrificing accuracy. This was achieved by acceleration and linearization of cuff deflation and by interpolation of the relationship between cuff pressure and oscillometric wave amplitude. *Blood Press Monit* 9:135–141 © 2004 Lippincott Williams & Wilkins.

Blood Pressure Monitoring 2004, 9:135–141

Keywords: measurement time, measurement error, accelerated linear deflation, algorithm with interpolation

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Received 5 December 2003 **Revised** 15 March 2004
Accepted 19 March 2004

Introduction

Blood pressure is one of the most important and ubiquitous variables for demonstrating the vital status of patients and its measurements are required almost everywhere in medical service. Although the invasive intra-arterial pressure measurements are taken as the gold standard due to its accuracy, introduction of non-invasive sphygmomanometric measurements dramatically widened the clinical use of blood pressure. In addition, the introduction of automatic oscillometric manometry greatly reduced the costs for human resources such as paramedical staff.

Despite its popularity, oscillometric manometry sometimes fails to meet the clinical requirements. One of the limitations is that this type of oscillometry is not intended to track rapid changes in pressure for example in operations and/or emergency rooms. Development of faster oscillometry ameliorates this limitation and improves the quality of medical services, especially in operating theatres, emergency rooms and intensive care units. Introduction of faster oscillometry is advantageous not only for such special medical services but also for regular wards and outpatient clinics. Faster oscillometry may enhance the merit of automatic manometry by saving measurement time, especially in the setting where many patients require measurement.

We therefore examined in this study whether faster oscillometry is feasible. We developed a faster oscillometry method by linearizing the deflation rate and by introducing a new pressure determination algorithm based on interpolated data from a small number of beats. We then determined the accuracy of this faster oscillometry and compared it with that of conventional oscillometry.

Patients and methods

Conventional oscillometric manometry

In conventional oscillometric manometry, the pulsatile components (oscillometric waves) can be detected in the gradually decreasing pressure measurements within a cuff that compresses the upper arm. Based on the relation between cuff pressure values and oscillometric wave amplitudes thus obtained, systolic, mean and diastolic pressure values are determined (Figure 1). Systolic pressure values are identified as the cuff pressure values at the rising edge of oscillometric wave amplitude, mean

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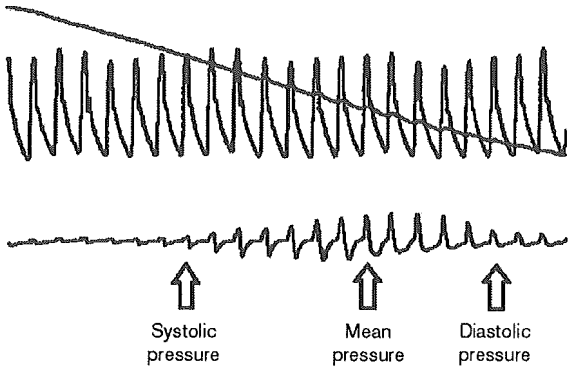
DOI: 10.1097/01.mbp.0000130430.78823.42

Fig. 1



Cuff pressure tracing during oscillometric manometry from a patient, superimposed on simultaneously obtained invasive pressure tracing (top) and the oscillometric wave extracted from cuff pressure (bottom). The systolic, mean, and diastolic pressure values can be obtained from the cuff pressure values for oscillometric wave appearance, peak, and disappearance, respectively. Reproduced and modified with permission from Sugimachi M, Sunagawa K, Okamoto H, Hoka S. New algorithm for oscillometric non-invasive automatic arterial pressure measurement in patients with atrial fibrillation. *Masui* 2002; **51**:784–790.

pressure values as the cuff pressure values at its maximum amplitude, and diastolic pressure values as the cuff pressure values at the falling edge. The conventional oscillometric device is designed to determine these pressure values based on discrete cuff pressure values at each beat. Therefore, after identifying the beat at the rising edge, the beat for the maximal amplitude, and the beat at the falling edge the device determines the systolic, mean, and diastolic pressure values as the cuff pressure values at the corresponding beat. To warrant the number of beats for pressure determination analysis during each measurement, the conventional device is designed to adjust the deflating rate in the late deflation phase.

New faster oscillometric manometry

Besides the acceleration of cuff deflation (from 4–7 mmHg/s [conventional oscillometry at initial deflation] to 11–13 mmHg/s [our faster oscillometry]), to create faster oscillometry, we modified a conventional oscillometric device (BX-10, Colin Corporation, Komaki, Japan) by first, fixing the deflation rate but did not try to adjust the deflating rate to warrant the number of beats. We used a constant deflation rate because, in our preliminary study, changes in cuff deflation rates seem to distort the relationship between cuff pressure and oscillometric wave amplitude. Second, to compensate for the decrease in the number of beats, we interpolated the cuff pressure-oscillometric wave amplitude relationship between the discrete data obtained for each beat. Using the interpolated relationship, we determined the exact cuff pressure values at the time of the appearance of the oscillometric wave, its peak, and subsequent disappearance, rather than choosing cuff pressure values from several beats. We then adopted these cuff pressure values for oscillometric systolic, mean, and diastolic pressure values.

Data collection

To compare the accuracy of the faster oscillometric method with the conventional one, we conducted a clinical study using 36 patients who underwent scheduled surgery at Kitasato University Hospital and required invasive blood pressure monitoring for clinical reasons. There were 16 male and 20 female patients, and they were 56.1 ± 16.2 (16–82) years old. Of these 36 patients, three were excluded from the study for frequent arrhythmias, one for prominent pressure fluctuations synchronous to ventilation, and four for technical reasons that prohibited accurate invasive manometry.

In reference to Figure 2, a 20-gauge intra-arterial catheter was inserted into the radial artery of patients to monitor invasive arterial pressure. We used a commercially available manometer system (MP5200 [TW], Nihon-Kohden, Tokyo, Japan) for invasive pressure with the frequency response (100 Hz) sufficient for the determination of systolic and diastolic pressure values. This pressure served as the gold standard. In order to monitor blood pressure non-invasively simultaneously using the oscillometric method, a cuff was attached around the

Fig. 2

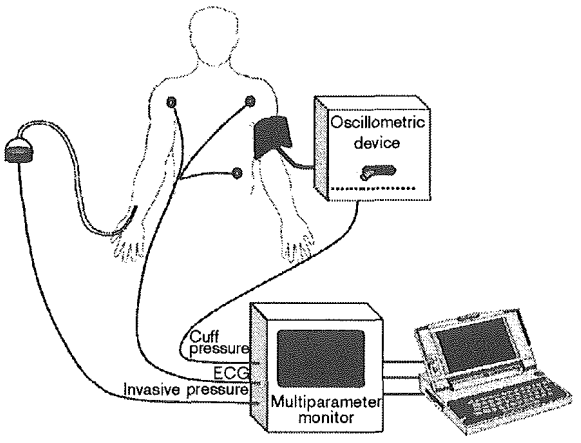


Illustration of an experimental set-up. Blood pressure was measured by a modified oscillometric device every 5 min, with simultaneous invasive pressure recording at the contralateral radial artery. The oscillometric device was switched alternately between the conventional and the faster deflation mode. Signals of invasive pressure, electrocardiogram, and cuff pressure with superimposed oscillometric wave were transferred to a multiparameter monitor and converted to a digital form for the offline analysis.

contralateral upper arm. Oscillometric measurements were performed at 5-min intervals. The cuff was connected to the custom oscillometric device modified for the above requirements based on the conventional oscillometric device (BX-10, Colin Corporation, Komaki, Japan). The device was switched alternately between the conventional and the faster deflation mode every 5 min so that both conventional and faster oscillometry were performed every 10 min.

Although we did not measure blood pressure with the conventional and the faster oscillometry at the same time, we measured invasive arterial pressure throughout all the oscillometric measurements so that one could compare the invasive and non-invasive pressure values. All satisfactory measurements during the surgery were used for analysis. Throughout the operation, signals of electrocardiography, invasive blood pressure, and cuff pressure (with imposed oscillometric wave) were transmitted to a multiparameter monitor (Model 86S, Agilent Technologies, Palo Alto, California, USA), converted to digital signals (1 kHz, 12 bits) (DAQ-CARD-700, National Instruments Corp., Austin, Texas, USA) and stored on a hard disk of a dedicated laboratory computer (Latitude HX500T, Dell Inc., Round Rock, Texas, USA) for the offline analysis. We did not use the electrocardiographic signal to determine blood pressure values.

Data analysis

In each patient, we compared invasive systolic and diastolic pressure values with those obtained by conventional oscillometry, and with those obtained using the faster oscillometry technique, using different pressure readings (average of 28 ± 17 readings) occurring throughout the operation. At this time, a further five patients with < 10 pressure readings were excluded from the analysis. This resulted in 23 patients (32 ± 16 pressure readings, range: 10–68 readings) for final analysis. Both systolic (87 ± 18 mmHg to 145 ± 17 mmHg, range: 75 ± 20 mmHg) and diastolic pressure (48 ± 9 mmHg to 79 ± 9 mmHg, range: 31 ± 10 mmHg) changed considerably during the operation.

Using these comparisons, we quantified the accuracy of the pressure values by the mean and standard deviation of the difference between the invasive and oscillometric pressure values. Invasive pressure values were obtained from the raw pressure signals stored on the hard disk, and all systolic and diastolic pressure values were averaged, respectively, during the corresponding oscillometric manometry.

To identify the possible influence of pressure levels (systolic, diastolic, and pulse pressure) and heart rate on the measurement time and the accuracy of the faster oscillometric technique, we investigated the correlations between these variables.

Statistical analysis

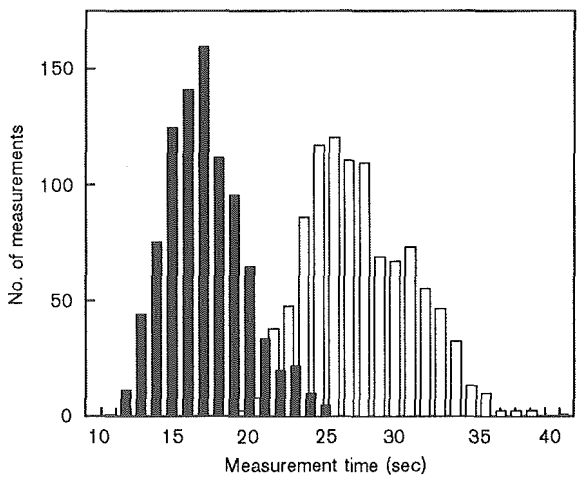
Data were expressed as mean ± standard deviation. Correlations between variables were obtained by linear regression analysis. Coefficients of determination (r^2) were reported.

Results

Figure 3 shows the comparison between measurement time with conventional oscillometry (open bars) and that with the faster oscillometry (solid bars). As expected the faster oscillometry shortened the time needed for pressure measurement to 62% that of the original (conventional, 27.7 ± 3.5 s; faster, 17.1 ± 2.6 s). We examined whether pressure level (one of systolic, diastolic, pulse pressure values) or heart rate serves as an obstacle to shorten pressure measurements. Figure 4 described the relation between one of these factors and measurement time of the faster oscillometric technique. Poor correlations between these factors and measurement time (systolic pressure, $r^2 = 0.08$; diastolic pressure, $r^2 = 0.04$; pulse pressure, $r^2 = 0.07$; heart rate, $r^2 = 0.02$) indicated that none of these factors served as hindrance factors to shorten pressure measurements.

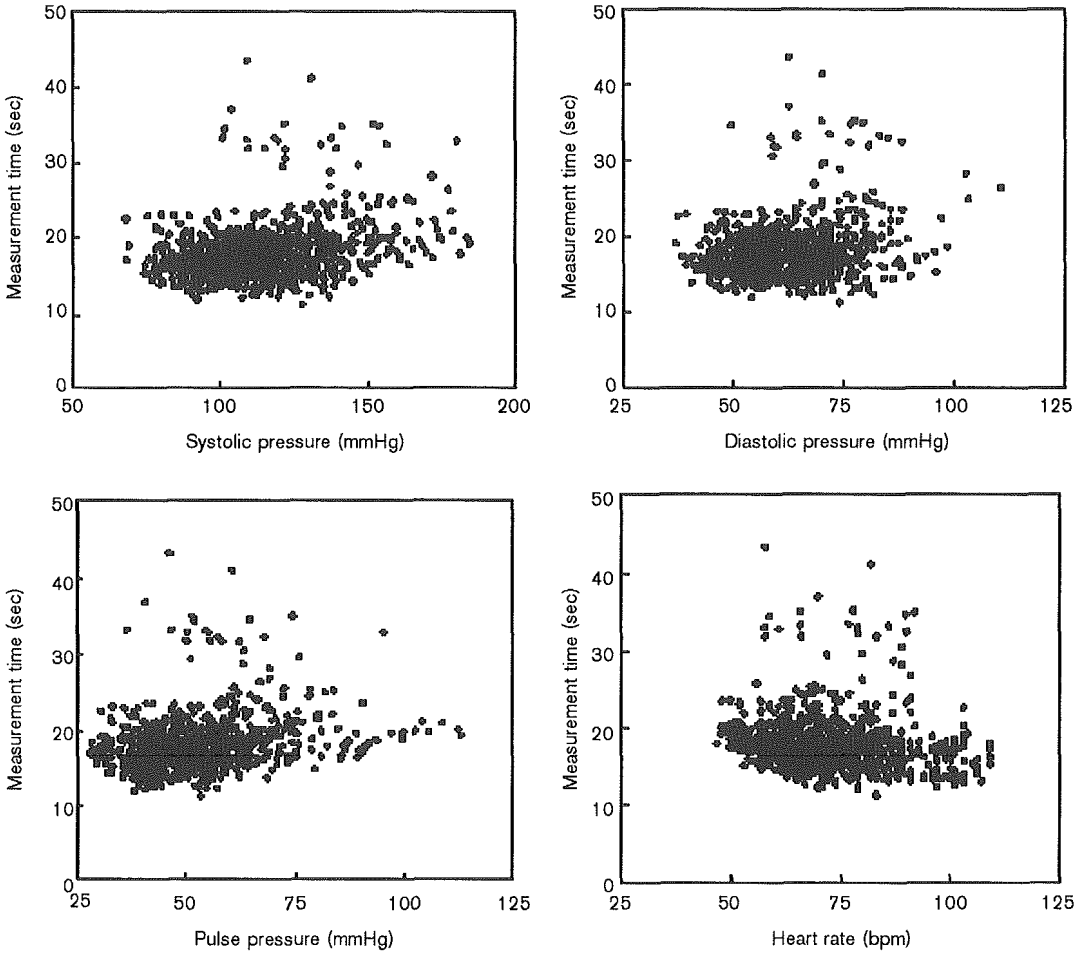
In Figure 5 we compared systolic (left panel) and diastolic (right panel) pressure distribution obtained from pooled measurements by conventional (open bars)

Fig. 3



Comparison between pressure measurement time of conventional oscillometry (open bars) and that of the faster oscillometric method (solid bars). Measurement time was tabulated into 1 s bins and shown by a histogram.

Fig. 4



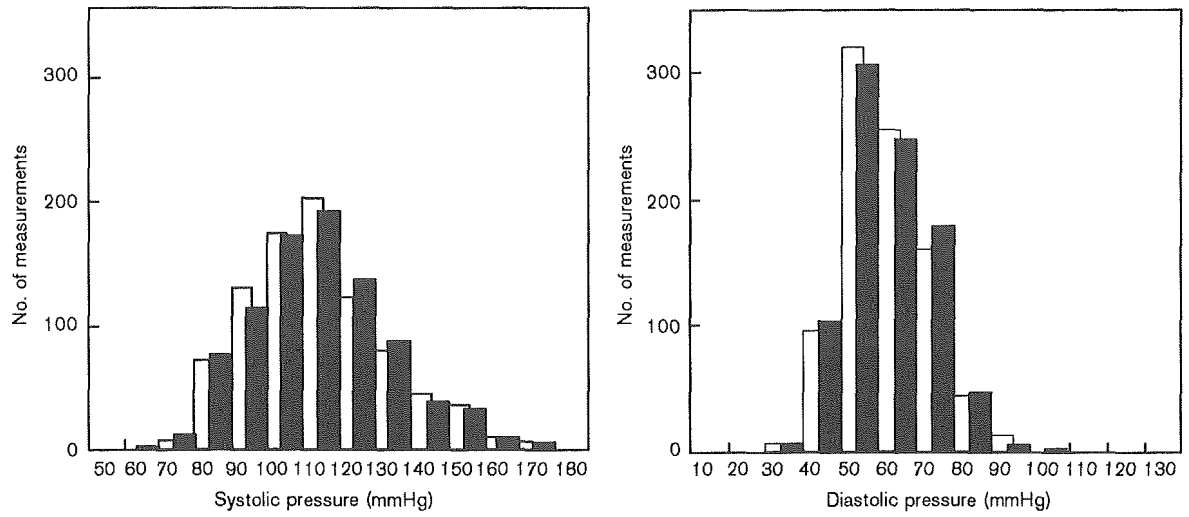
Effects of pressure values (systolic, diastolic and pulse pressure), and heart rate on pressure measurement time were examined by scattergrams. Invasive pressure values were used for independent variables.

and faster (solid bars) oscillometry. These histograms show the similarity of pressure values obtained by the two different oscillometric methods as a whole, though the corresponding two measurements were 5 min apart.

Figure 6 illustrates the accuracy of the conventional (bottom panels) and the faster (top panels) oscillometry by plotting measurement error (oscillometric minus invasive pressure) against the average invasive and oscillometric pressure (Bland-Altman method) [1–2]. The left panels show Bland-Altman plots for systolic pressure measurements. The limit of agreement (mean ± SD of the error, dashed and dotted lines) was comparable between the conventional (2.1 ± 7.5 mmHg) and the faster oscillometry (1.4 ± 7.3 mmHg). Correlation between the error and systolic pressure level was weak (r^2 = 0.08). The right panels show Bland-Altman plots for diastolic pressure measurements. The limit of agreement (dashed and dotted lines) was also comparable between the conventional (5.0 ± 6.8 mmHg) and the faster oscillometry (4.9 ± 5.8 mmHg), and correlation between the error and diastolic pressure level was also weak (r^2 = 0.04).

In Figure 7, we examined possible influences of other pressure levels (average of invasive and oscillometric pressure) or heart rate on measurement error of systolic and diastolic pressure, respectively, determined by the faster oscillometric method. Poor correlations were found between measurement errors for systolic pressure and

Fig. 5



Comparison between measured pressure values (systolic and diastolic pressure) from conventional oscillometry (open bars) with those of the faster oscillometry (solid bars). Pressure values were tabulated into 10 mmHg bins and shown by a histogram.

diastolic (r^2 = 0.07), pulse (r^2 = 0.05) pressure levels, or heart rate (r^2 = 0.008). Measurement error for diastolic pressure correlated poorly with systolic (r^2 = 0.06), pulse (r^2 = 0.04) pressure levels or heart rate (r^2 = 0.06). Poor correlations between errors and pressure levels or heart rate were also found for the conventional oscillometry.

Discussion

We have shown that, by accelerating and linearizing cuff deflation, and by interpolating the relationship between cuff pressure and oscillometric wave amplitude, we were able to shorten pressure measurement time by approximately 40%, without increasing measurement error.

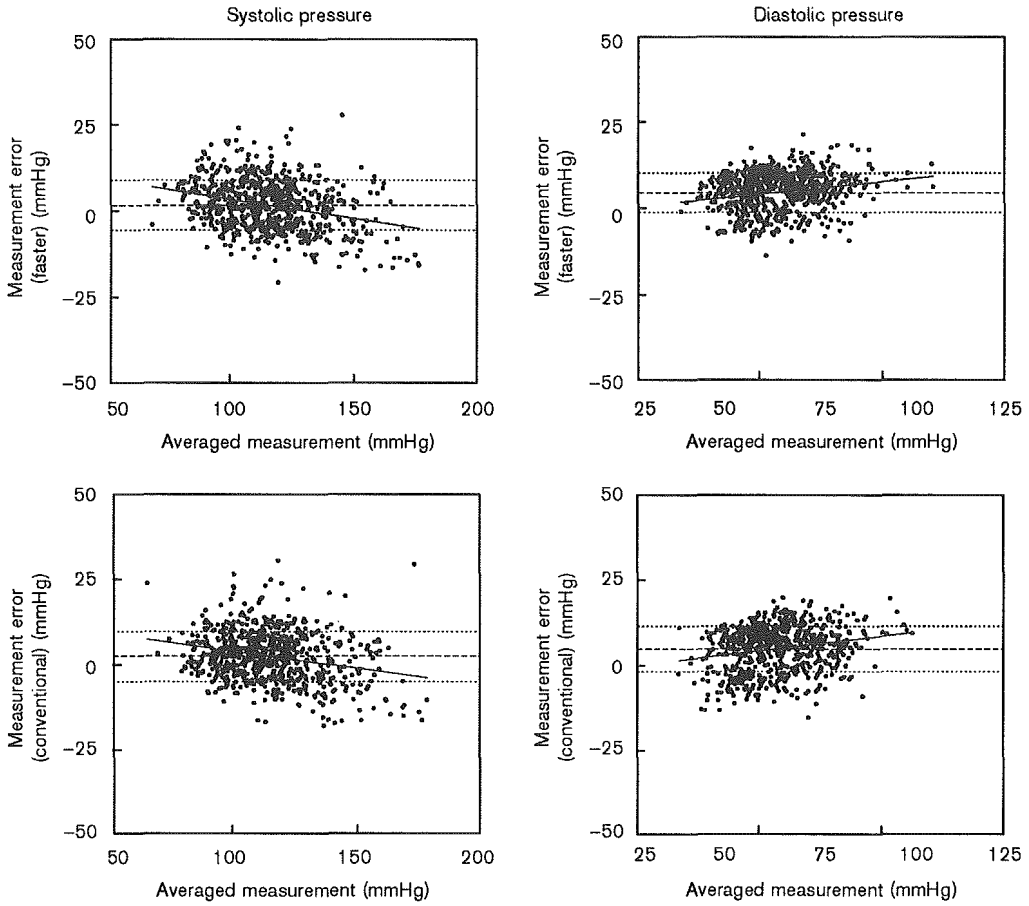
In our results, limits of agreement (oscillometric versus invasive pressure) were comparable between conventional and the faster oscillometric technique for systolic as well as diastolic pressure values. Both the conventional and the faster oscillometry have similar measurement bias (mean error, systole: 2.1 versus 1.4 mmHg, diastole: 5.0 versus 4.9 mmHg). We conjectured that the difference in pressure measurement site (radial artery versus brachial artery) partly accounted for this bias. In addition, the degree of discrepancy between radial and brachial pressure values varies depending on cardiovascular conditions [3], resulting in the increased variability (SD of error). A recent paper investigating the accuracy of oscillometry in critically ill patients indicated that radial invasive and brachial oscillometric pressures were different despite device and cuff size adjustment [4]. Although pressure differences between the right and left arms might contribute to reduce any correlation, this contribution seems small judging by the poor relation between the invasive-oscillometric pressure differences and the right-left oscillometric pressure differences (data not shown).

Since we did not compare brachial pressure using both oscillometric methods, this study does not comply with the guidelines for faster oscillometry set by the British Hypertension Society (BHS) [5].

Standards issued by Association for the Advancement of Medical Instrumentation (AAMI) [6], however, do provide a way to examine the accuracy against invasive pressure. Although AAMI requires ipsilateral invasive manometry at the arteries proximal to the cuff, the accuracy of systolic and diastolic pressure by the faster oscillometry is compliant with the AAMI standard. The mean error for the systolic pressure (1.4 mmHg) was < 5 mmHg, and SD of error (7.3 mmHg) was < 8 mmHg. Similarly, the mean error for the diastolic pressure (4.9 mmHg) was < 5 mmHg, and SD of error (5.8 mmHg) was < 8 mmHg. Similar accuracy of the faster oscillometry and the AAMI compliant conventional oscillometric device [7] further supported this.

Although attempts to improve the accuracy of oscillometry by fitting curve to cuff pressure-oscillometric wave amplitude are not new [8,9], we are the first to show that such attempts are effective in maintaining the accuracy of oscillometry in accelerated cuff deflation. It is natural that acceleration of cuff deflation

Fig. 6



Bland-Altman plots examining the measurement errors (oscillometric minus invasive pressure) of systolic (left) and diastolic (right) pressure and their dependence on pressure values (average of oscillometric and invasive pressure). Plots are shown for the conventional (bottom) and the faster (top) oscillometry. Solid lines indicate the regression line, dashed lines indicate the mean error, and dotted lines the mean ± SD of the error.

decreases the number of heartbeats available for pressure determination. Without interpolation or curve fitting, oscillometric accuracy would have drastically worsened.

On the other hand, we consider that the linearization of the cuff deflation somewhat contributed to the accuracy. In fact, the mean error for the systolic pressure and the SD error for the diastolic pressure are smaller using the faster oscillometric technique.

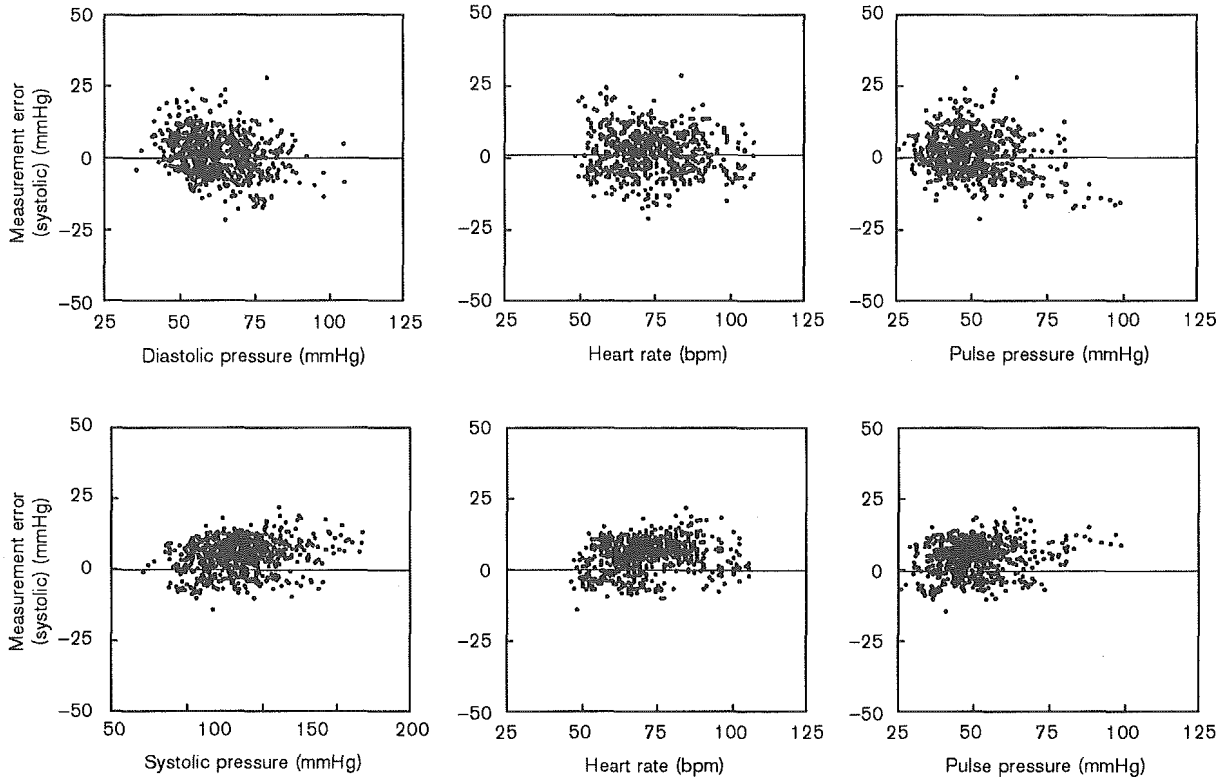
Besides the fact that faster oscillometry can track rapid pressure changes it is favourable from the viewpoint of patients' comfort. Some patients might complain of pains in the upper extremities with sustained arm compression by cuff; the accelerated deflation would be favourable in these patients. Our oscillometry did not increase cuff inflation speed because this is likely to increase pain.

In conclusion, by acceleration and linearization of cuff deflation and by interpolation of the relationship between cuff pressure and oscillometric wave amplitude, we succeeded in shortening the pressure measurement time to approximately 60% of the original without sacrificing measurement accuracy.

Acknowledgements

This study was supported by the Program for Promotion of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research of Japan, and a Health and Labour Sciences Research Grant (Research on Advanced Medical Technology, H14-nano-002) from the Ministry of Health, Labour and Welfare of Japan.

Fig. 7



Scatterplots examining the dependence of the measurement errors for systolic (top) and diastolic (bottom) pressure using the faster oscillometry on other pressure values and heart rate. The average oscillometric and invasive pressure values were used for the independent variables.

References

1 Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; 1:307–310.

2 Bland JM, Altman DG. Comparing methods of measurement: why plotting difference against standard method is misleading. *Lancet* 1995; **346**: 1085–1087.

3 O'Rourke MF, Kelly R, Avolio A. *The Arterial Pulse*. Philadelphia, PA. Lea & Febiger; 1992.

4 Bur A, Herkner H, Vlcek M, Woisetschlager C, Derhaschnig U, Delle Karth G, *et al.* Factors influencing the accuracy of oscillometric blood pressure measurement in critically ill patients. *Crit Care Med* 2003; **31**:793–797.

5 O'Brien E, Petrie J, Litter W, de Swiet M, Padfield OL, Altman DG, *et al.* The British Hypertension Society protocol for the evaluation of blood pressure measuring devices. *J Hypertens* 1993; **11(Suppl 2)**:S43–S62.

6 ANSI/AAMI SP10: 2002. *Manual, Electronic or Automated Sphygnomanometers*. Arlington, VA. Association for the Advancement of Medical Instrumentation. 2003.

7 Ling J, Ohara Y, Orime Y, Noon GP, Takatani S. Clinical evaluation of the oscillometric blood pressure monitor in adults and children based on the 1992 AAMI SP-10 standards. *J Clin Monit* 1995; **11**:123–130.

8 Hersh LT, Friedman B, Medero R. Method for oscillometric blood pressure *determination employing curve fitting*. United States Patent 5704362, January 6, 1998, assigned to Johnson & Johnson Medical, Inc.

9 Friedman B, Hersh LT, Medero R. Calculation of quality and its use in determination of indirect non-invasive blood pressure. United States Patent 6358213, Mar 19, 2002, assigned to Critikon Company, LLC.

原 著

Migration of neutrophils elicited by leukotriene B_4 is inhibited by fasudil, a Rho-kinase inhibitor, in the microvasculature of hamster cheek pouch

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Abstract

This study was designed to investigate possible effects of a Rho-kinase inhibitor, fasudil, on migration of neutrophils induced by leukotriene B_4. The neutrophil behavior was observed in the microvasculature of hamster cheek pouch using a trans-illumination microscope. Superfusion of lekotrience B_4 caused an increase in the number of neutrophils adhering the endothelium and migrating through the endothelium outside the venules. The migration induced by lekotrience B_4 was significantly attenuated in hamsters receiving intravenous infusion of 10 and 30mg/kg of fasudil prior to the leukotriene B_4 superfusion. These results suggest that inhibition of Rho-kinase by fasudil produces an inhibition of neutrophil migration and represents a new therapeutic strategy for neutrophil-mediated tissue damage.

Introduction

Fasudil is a Rho-kinase inhibitor that has shown clinical effectiveness in patients with subarachnoid hemorrhage[1] and that was launched for clinical use after subarachnoid hemorrhage in Japan. Rho-kinase contributes to the reorganization of the actin cytoskeleton and to the formation of stress fibers, and is thought to be one of the critical elements involved in a variety of cytoskeleton-dependent cell functions such as cell migration[2]. It has been shown that neutrophil chemotaxis as well as neutrophil and macrophage infiltration is inhibited by fasudil and its active metabolite, hydroxyfasudil[3~6]. In the present study, therefore, we investigated if the inhibition of Rho-kinase with fasudil would reduce neutrophil migration in vivo in a leukotriene-induced inflammatory model.

Materials and Methods

Ethical committee for animal experimentation of our institution approved this study. Thirty-one male Golden hamsters, weighing 110-160g at 10-16weeks old (Nihon SLC, Shizuoka, Japan) were anesthetized with urethane 1.2g/kg given intraperitoneally. A tracheal cannula was inserted to facilitate spontaneous respiration with a mixture of oxygen and room air. A cannula was placed in the femoral vein for drug infusion.

Preparation of hamster cheek pouch:

The hamster cheek pouch preparations were set up as previously described[7,8]. The cheek pouches were pulled out, cut longitudinally, and extended. The connective tissue was elaborately dissected away to expose the microvasculature of the mucous layer. The thin mucous membrane tissue was spread out in a plastic chamber.

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Microscopic observation and recording of leukocyte behavior:

The microvasculature of the hamster cheek pouch (unit: 50μm×100μm) was observed under a transillumination microscope (ECLIPSE, Nikon, Tokyo, Japan), using a W40 water immersion lens (×40, Nikon, Tokyo, Japan) and ×10 eyepieces (Nikon, Tokyo, Japan). Images of the microcirculation were projected onto a color television monitor screen (PVM-20M4J, SONY Co, Tokyo, Japan) via a color TV camera (DXC-5800, SONY Co, Tokyo, Japan) mounted at the top of the microscope. The behavior of the neutrophils in each experiment was recorded with a videotape recorder (SVO-5800, SONY Co, Tokyo, Japan).

Application of fasudil:

Fasudil (Asahi Kasei, Japan) at the dose of 3mg/kg (n=8), 10mg/kg (n=9), and 30mg/kg (n=7) in a total volume of 3ml/kg, or saline of 3ml/kg (n=7) as a vehicle were administrated via the femoral vein for 30 minutes.

Chemotactic agent:

Leukotriene B_4 (Paesel, GMBH, Frankfurt, Germany) was used as a chemoattractant[9]. A stock solution of leukotriene B_4 (30μm in absolute ethanol) was kept at −80℃ and diluted to 300nM with Tyrode's solution immediately before use. Ten minutes after initiating the administration of fasudil or saline, leukotriene B_4 (300nM) was applied to the microvasculature at the observation site with a 50μl micropipette.

Count of neutrophils:

Neutrophils could be individually visualized as bright white cells against the dark background of the blood stream, since they rolled slowly on the endothelial wall. Migration of neutrophils was determined when neutrophils were moving from the venular wall into the interstitial space (unit: 50μm×100μm).

Data analysis

All values are expressed in mean±SD. Data were statistically analyzed with ANOVA and Student's t-test was used for comparisons between the two groups, and paired t-test for comparisons before and after interventions in the same group. $P<0.05$ was considered as a statistically significant difference.

Results

Before the application of leukotriene B_4, several neutrophils were observed to move slowly along the vascular endothelium. The application of leukotriene B_4 caused a transient increase and a subsequent decrease in rolling of neutrophils. There were no significant differences in the alterations in rolling and adhesion of neutrophils among the four groups. Thickened wall was observed both in the vehicle and the fasudil 3mg groups, but not in the fasudil 10mg and fasudil 30mg groups (**Fig. 1**).

Migration of neutrophils started 20min after leukotriene B_4 application in all groups. **Fig. 2** demonstrates typical pictures in which migration of neutrophils occurred at 60min after initiating leukotriene B_4 in the vehicle group. In the vehicle group, 3.6± 1.3 and 5.2±1.8 counts/unit of neutrophils were migrated at 60 and 90min after leukotriene B_4, respectively. In the fasudil-10mg group, 1.3±0.5 and 1.4±0.2 counts/unit, and in the fasudil-30mg group, 1.1±0.5 and 1.6±0.7 counts/unit were migrated at 40 and 60min, respectively. The numbers of migrating neutrophils in the fasudil 10mg and the fasudil 30mg groups were significantly less than those in the vehicle and fasudil 3mg groups (**Fig. 3**).

Discussion

Leukotriene B_4 is a pro-inflammatory mediator synthesized in myeloid cells from arachidonic acid[10]. It induces recruitment and activation of neutrophils, monocytes and eosinophils by stimulating the production of a number of proinflammatory cytokines and mediators[9,10]. In our study, superfusion of leukotriene B_4 could also augment migration of neutrophils in the microvasculature of hamster cheek pouch. Pharmacological inhibition studies support a role for leukotriene B_4 in the pathogenesis of neutrophil mediated tissue damage, and treatments which reduce its production or block its effects may prove beneficial in neutrophil mediated inflammatory diseases[10~12]. Since

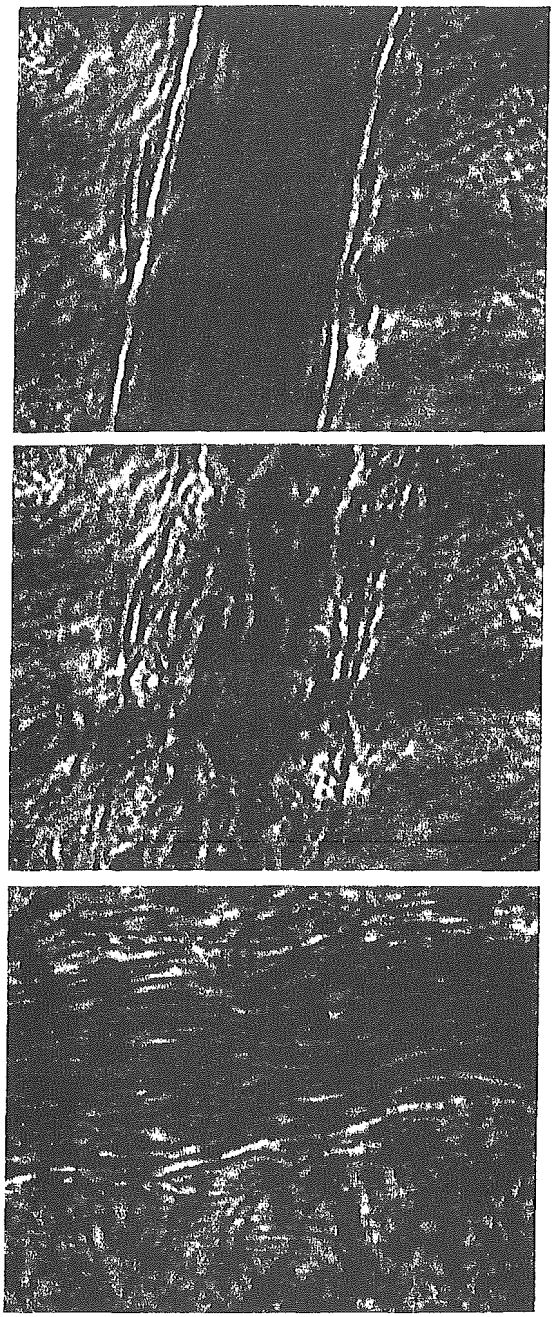

Figure 1

Photomicrograph of microcirculation in hamster cheek pouch. Intact venular wall was observed before superfusion of leukotriene B_4 (upper). Sixty min after superfusion of leukotriene B_4, the venular wall was thickened in a hamster receiving a vehicle (middle), whereas the venular wall was not thickened in a hamster treated with fasudil 30mg (lower). The thickened venular wall comprised of infiltrated neutrophils.

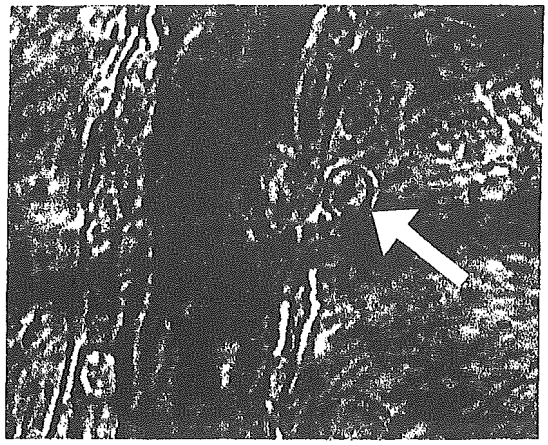

Figure 2

Photomicrograph of microcirculation in hamster cheek pouch. Migration of neutrophils was observed at 60 min after initiating leukotriene B_4 in a hamster receiving a vehicle. An arrow indicates a migrated neutrophil.

neutrophil migration elicited by superfusion of leukotriene B_4 was inhibited by pretreatment of fasudil in this study, it is suggested that fasudil can be beneficial in prevention of neutrophil mediated tissue damage.

Studies in animal models show fasudil to be promising in the treatment of stroke, angina, and renal fibrosis[5,13~15]. Fasudil and its active metabolite, hydroxyfasudil, inhibit Rho-kinase more effectively than they inhibit other protein kinases; e.g., protein kinase C, or myosin light chain kinase[16,17~19]. There is accumulating evidence that Rho is important regulators of endothelial barrier properties by influencing both the endothelial actin-based cytoskeleton and the integrity of interendothelial junctions[20]. The Rho family of small GTPases regulates many facets of cytoskeletal dynamics that underlie changes in cell shape and adhesion during migration[21,22]. It has also been shown that Rho-kinase is involved in controlling the development of polarity and migration of neutrophils[2]. All these together suggest that the inhibitory action of fasudil on neutrophil migration occurs through the inhibition of the Rho-kinase pathway. However, we could not exclude the possibility that fasudil causes the inhibitory effect through inhibition of other protein kinases because fasudil has a wide spectrum of action against