

Figure 3

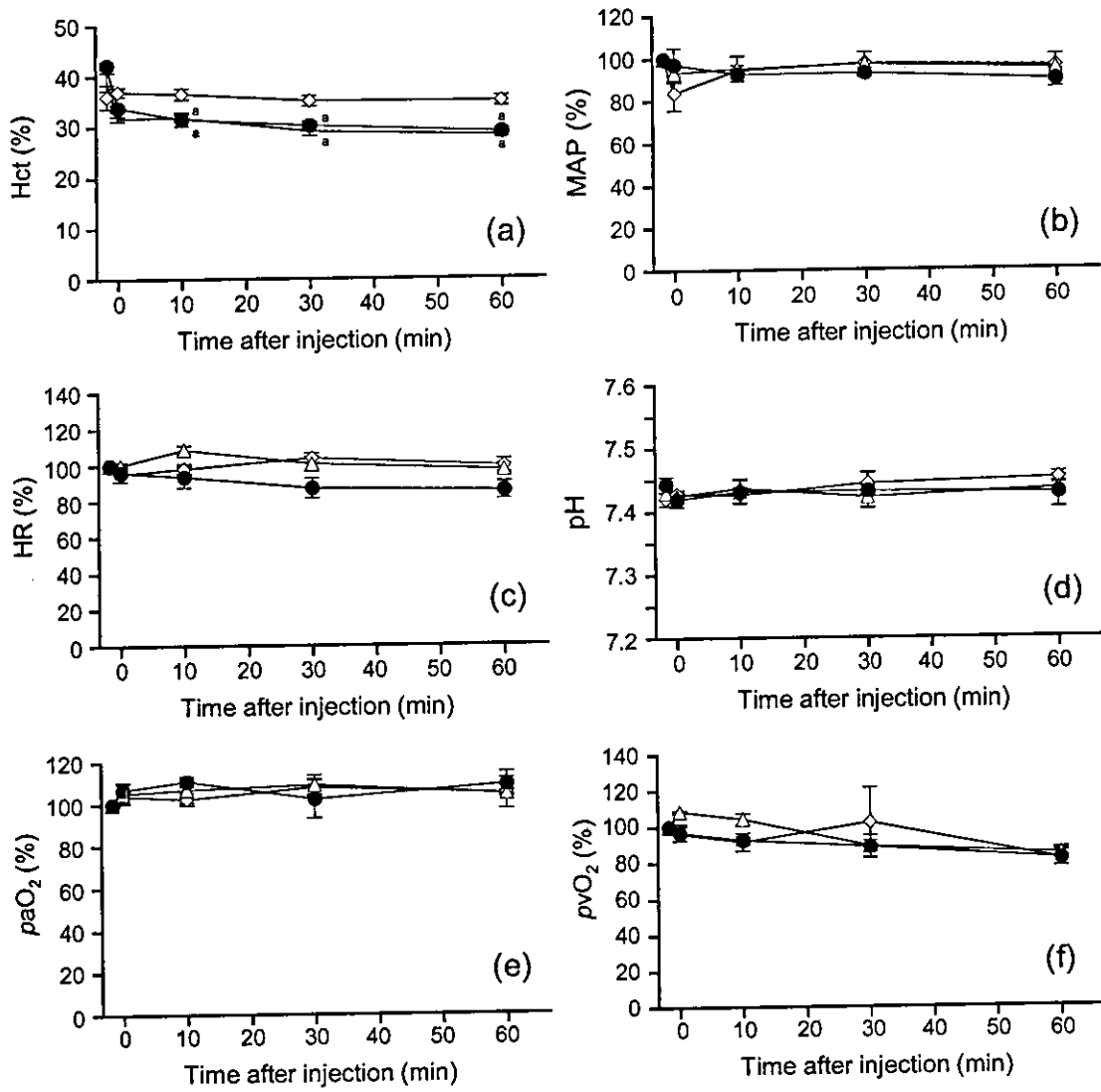


Figure 4

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 \pm 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

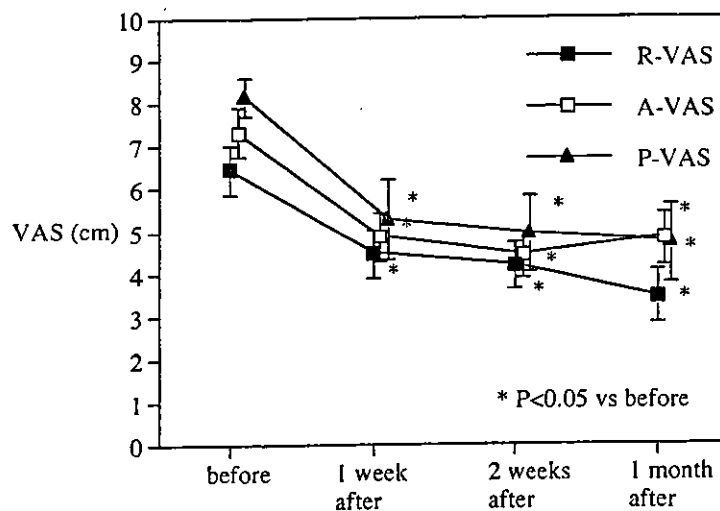


Figure 1. Visual analogue scales (VAS) of rest pain (R-VAS), allodynia (A-VAS), and paroxysmal pain (P-VAS) before and after the oral administration of pranlukast hydrate. All the VAS were significantly decreased after the treatment with pranlukast hydrate.

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 \pm 1.7	4.7 \pm 1.9*	4.6 \pm 3.0*	4.0 \pm 2.9**
	LDG	6.6 \pm 2.2	4.3 \pm 2.3**	3.9 \pm 1.8**	3.0 \pm 2.3**
Allodynia	SDG	6.8 \pm 2.1	5.2 \pm 2.4*	4.9 \pm 2.8*	4.5 \pm 3.1*
	LDG	7.8 \pm 2.6	4.6 \pm 2.5**	4.0 \pm 1.7**	5.1 \pm 2.3**
Paroxysmal pain	SDG	8.4 \pm 1.4	6.5 \pm 3.7	6.5 \pm 3.7	5.1 \pm 4.5
	LDG	8.0 \pm 2.1	4.4 \pm 4.2*	3.8 \pm 3.6*	3.7 \pm 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 relieve 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 antagonist 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 many 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 rash 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

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

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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.

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

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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.

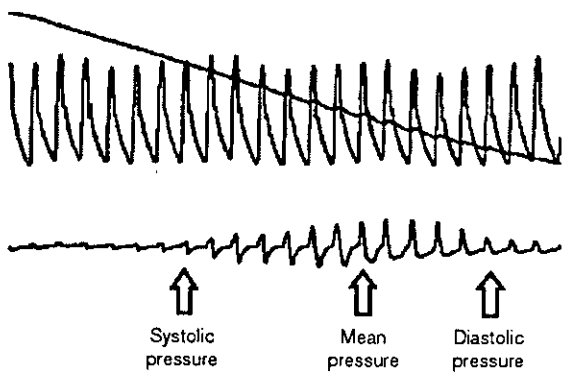
We therefore examined in this study whether faster oscillometry is feasible. We developed a faster oscillometry method by linearizing the deflation rate and by introducing a new pressure determination algorithm based on interpolated data from a small number of beats. We then determined the accuracy of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

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

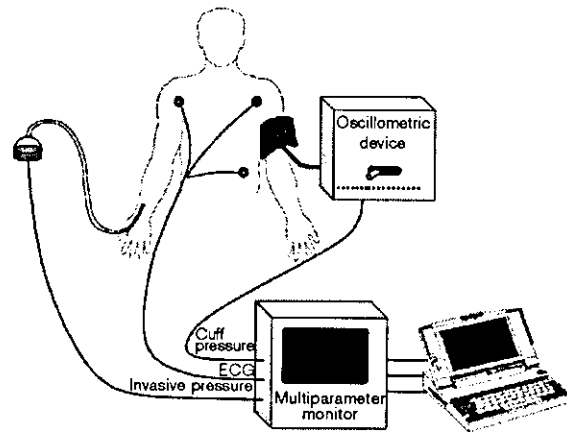


Illustration of an experimental set-up. Blood pressure was measured by a modified oscillometric device every 5 min, with simultaneous invasive pressure recording at the contralateral radial artery. The oscillometric device was switched alternately between the conventional and the faster deflation mode. 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

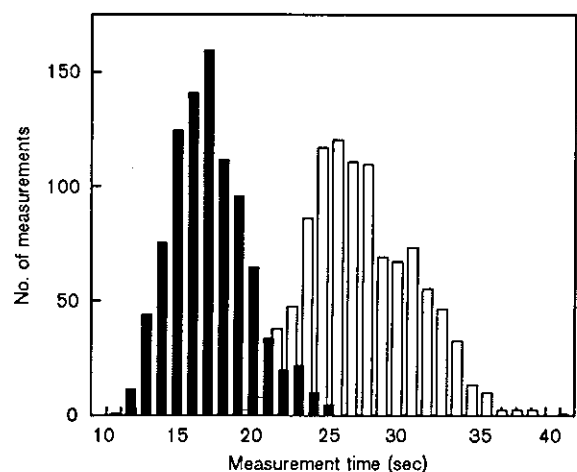
Data were expressed as mean \pm standard deviation. Correlations between variables were obtained by linear regression analysis. Coefficients of determination (r^2) were reported.

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.

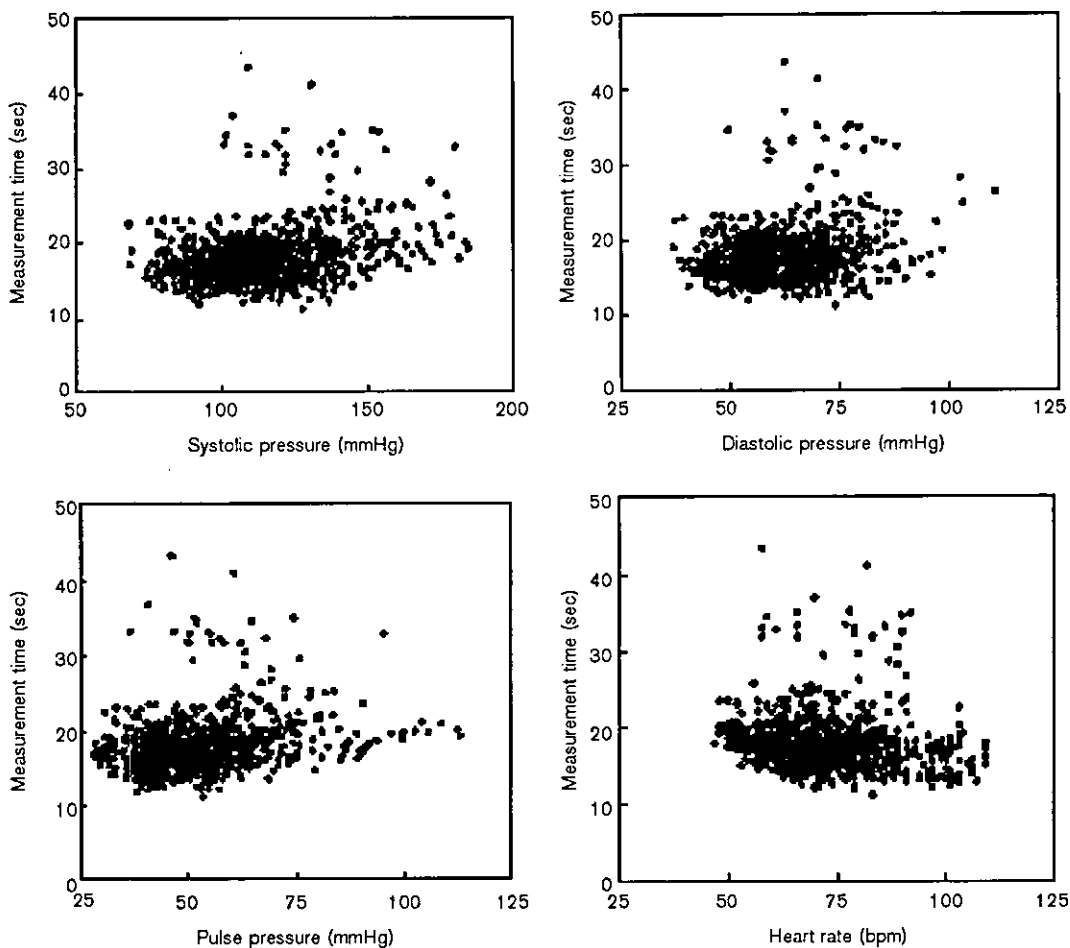
In Figure 5 we compared systolic (left panel) and diastolic (right panel) pressure distribution obtained from pooled measurements by conventional (open bars)

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.

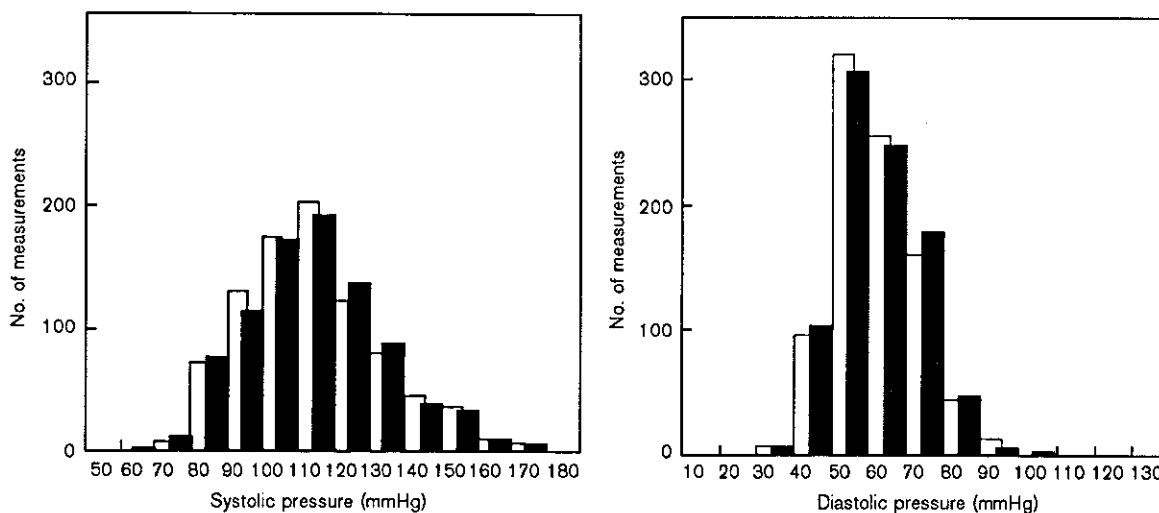
and faster (solid bars) oscillometry. These histograms show the similarity of pressure values obtained by the two different oscillometric methods as a whole, though the corresponding two measurements were 5 min apart.

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 \pm 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). Correla-

tion 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

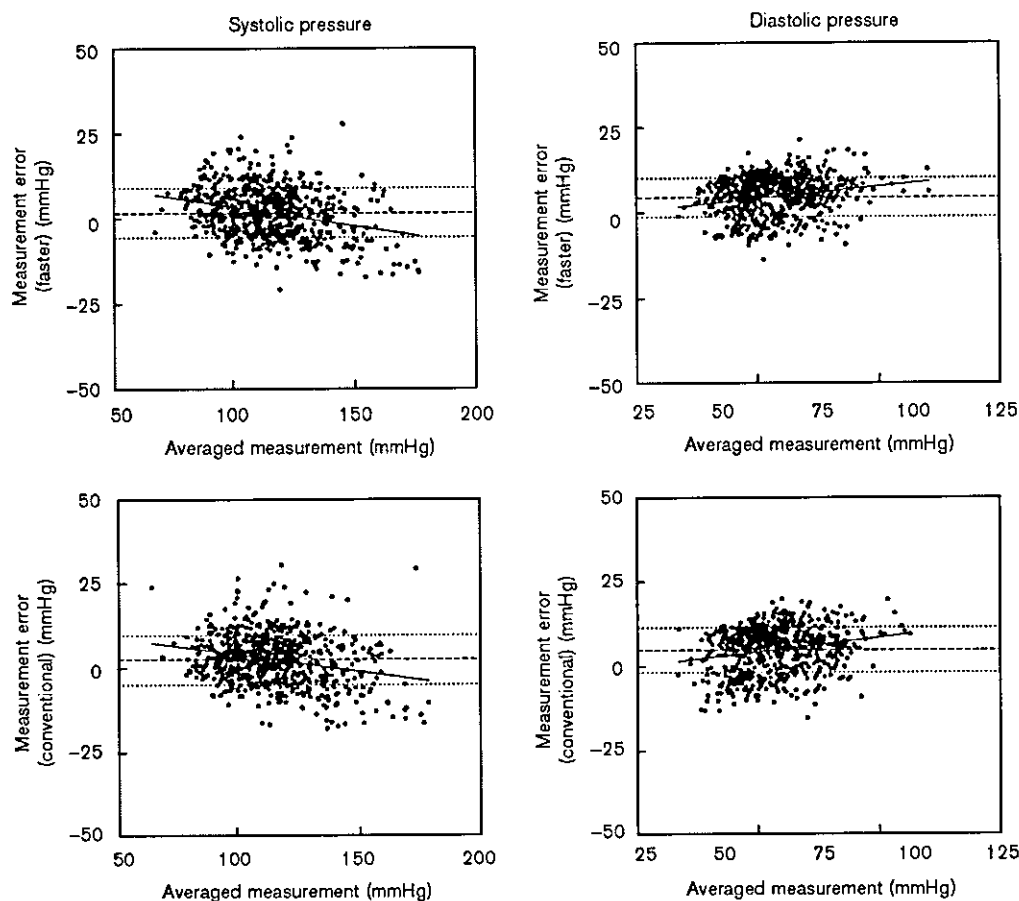
might contribute to reduce any correlation, this contribution seems small judging by the poor relation between the invasive-oscillometric pressure differences and the right-left oscillometric pressure differences (data not shown).

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 \pm 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

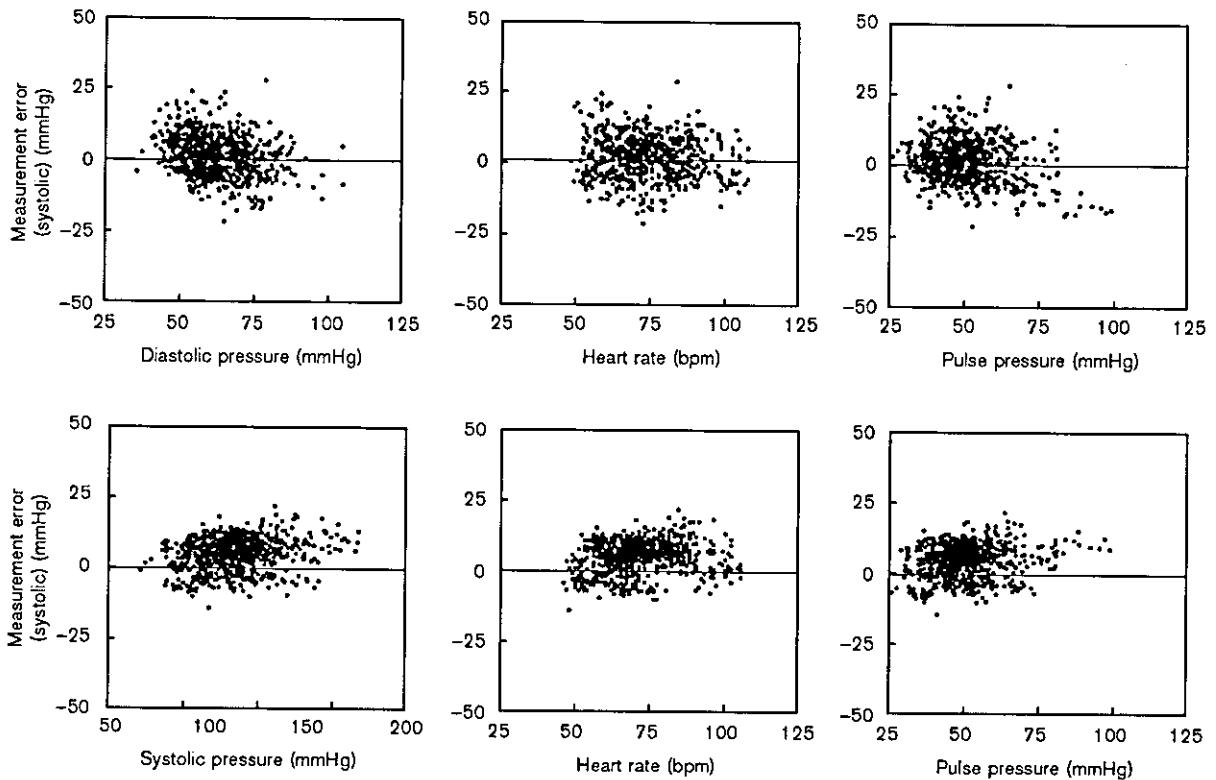
did not increase cuff inflation speed because this is likely to increase pain.

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

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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.

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原 著

Migration of neutrophils elicited by leukotriene B₄ is inhibited by fasudil, a Rho-kinase inhibitor, in the microvasculature of hamster cheek pouch

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原著

Migration of neutrophils elicited by leukotriene B₄ is inhibited by fasudil, a Rho-kinase inhibitor, in the microvasculature of hamster cheek pouch

Akiko Ozawa*, Yoshihiro Nara*, Kimotsuki Hiroshi*
Eri Nakahara*, Hirotsugu Okamoto*, Sumio Hoka*

Abstract

This study was designed to investigate possible effects of a Rho-kinase inhibitor, fasudil, on migration of neutrophils induced by leukotriene B₄. The neutrophil behavior was observed in the microvasculature of hamster cheek pouch using a trans-illumination microscope. Superfusion of leukotriene B₄ caused an increase in the number of neutrophils adhering the endothelium and migrating through the endothelium outside the venules. The migration induced by leukotriene B₄ was significantly attenuated in hamsters receiving intravenous infusion of 10 and 30mg/kg of fasudil prior to the leukotriene B₄ 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 hemorrhage¹⁾ 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 migration²⁾. It has been shown that neutrophil chemotaxis as well as neutrophil and macrophage infiltration is inhibited by fasudil and its active metabolite, hydroxyfasudil³⁻⁶⁾. 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 10–16 weeks 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: $50\mu\text{m} \times 100\mu\text{m}$) was observed under a transillumination microscope (ECLIPSE, Nikon, Tokyo, Japan), using a W40 water immersion lens ($\times 40$, Nikon, Tokyo, Japan) and $\times 10$ 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 administered via the femoral vein for 30 minutes.

Chemotactic agent:

Leukotriene B_4 (Paesel, GMBH, Frankfurt, Germany) was used as a chemoattractant⁹. A stock solution of leukotriene B_4 ($30\mu\text{M}$ in absolute ethanol) was kept at -80°C 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 $50\mu\text{l}$ 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: $50\mu\text{m} \times 100\mu\text{m}$).

Data analysis

All values are expressed in mean \pm 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 acid¹⁰. 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 diseases¹⁰⁻¹². Since



Figure 1

Photomicrograph of microcirculation in hamster cheek pouch. Intact venular wall was observed before superfusion of leukotriene B₄ (upper). Sixty min after superfusion of leukotriene B₄, 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.

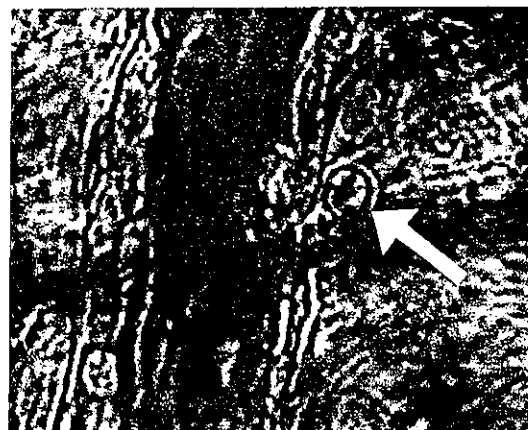


Figure 2

Photomicrograph of microcirculation in hamster cheek pouch. Migration of neutrophils was observed at 60 min after initiating leukotriene B₄ in a hamster receiving a vehicle. An arrow indicates a migrated neutrophil.

neutrophil migration elicited by superfusion of leukotriene B₄ 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 junctions²⁰. 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 neutrophils². 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

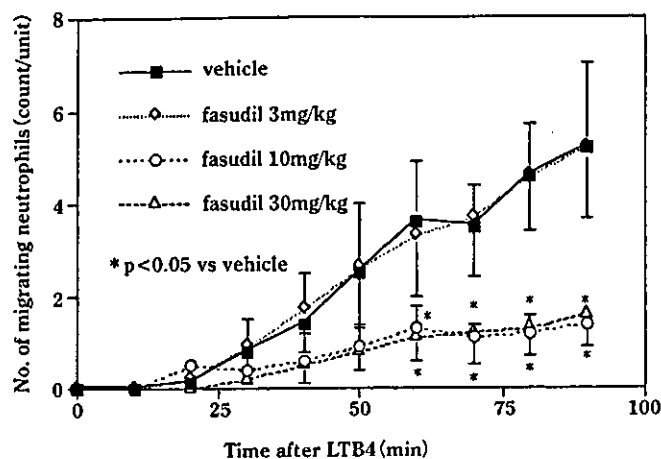


Figure 3

Changes in the numbers of migrating neutrophils after superfusion of leukotriene B_4 . The abscissa indicates the time lapse after the application of leukotriene B_4 . The ordinate indicates the numbers of migrating neutrophils in the detection window. Values are shown as mean \pm SD. Fasudil 10mg and 30mg significantly inhibited the migration of neutrophils elicited by leukotriene B_4 superfusion ($p < 0.05$).

several kinds of protein kinase. Thus, the detailed mechanisms underlying the inhibition of neutrophil migration by fasudil have not been identified in this study.

In the present study, a significant inhibition in the migration of neutrophils induced by leukotriene B_4 was observed in hamsters receiving 10mg/kg and 30 mg/kg of fasudil. It has been reported that a significant inhibition of neutrophil chemotaxis induced by various chemoattractants, including N-formyl-methionyl-leucyl-phenylalanine, was observed at 3 to 30 μ M of fasudil³⁾. In rats, the maximum plasma concentration of fasudil after intraperitoneal administration of fasudil at 10mg/kg was approximately 15 μ M²³⁾, which is equivalent to the effective concentration for inhibition of neutrophil chemotaxis. Plasma concentrations of fasudil were not measured in this study, but intravenous infusion of fasudil 10mg/kg for 30min in hamsters could be suspected to reach a similar level as intraperitoneal administration of fasudil at the same dose.

It has been reported that fasudil 10mg/kg, which was administered daily for 2 weeks also attenuated interstitial fibrosis and macrophage infiltration in rat kidneys with unilateral ureteral obstruction⁵⁾. It has also been reported that fasudil 10 and 30mg/kg sig-

nificantly prevented the development of myocardial fibrosis in a chronic myocardial damage model in rats²⁴⁾. These previous reports are in agreement with our results in hamsters in terms of the doses of fasudil inhibiting neutrophil or macrophage infiltrations and the accompanied possible tissue damage.

In conclusion, this study demonstrated that fasudil, a Rho-kinase inhibitor, reduced neutrophil migration elicited by leukotriene B_4 in the microvasculature of hamster cheek pouch, representing a new therapeutic strategy for neutrophil-mediated tissue damage.

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原著

Preoperative non-invasive assessment of
stress response to breath-holding test

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