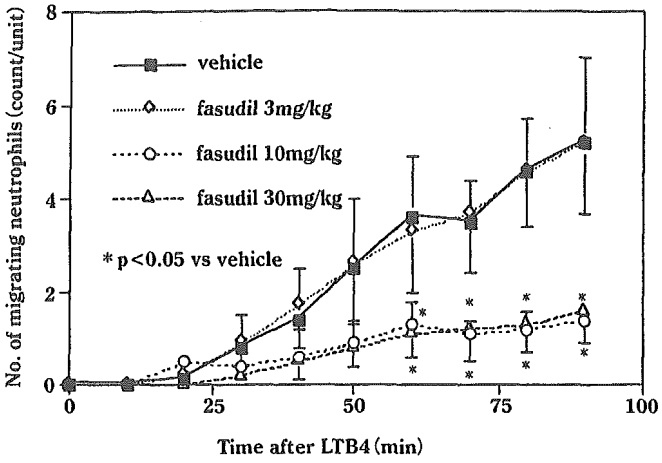

Figure 3

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±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 30μM of fasudil[3]. In rats, the maximum plasma concentration of fasudil after intraperitoneal administration of fasudil at 10mg/kg was approximately 15μM[23], 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 obstruction[5]. It has also been reported that fasudil 10 and 30mg/kg significantly prevented the development of myocardial fibrosis in a chronic myocardial damage model in rats [24]. 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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Abstract

The purpose of this study was to evaluate circulatory and respiratory responses to a breath-holding stress test in surgical patients at the bed-side using continuous and non-invasive monitoring with arterial tonometry and pulse oxymetry. Sixty-one patients were assigned into four groups: normal healthy patients (Cont), elderly patients (Elder), hypertensive patients (HT) and diabetic patients (DM). The breath-holding stress test was conducted in the supine position at the functional residual capacity level and in room air. Breath-holding time, changes in heart rate (HR), mean arterial pressure (MAP), arterial oxyhemoglobin saturation using a pulse oxymeter (SpO_2) and the recovery time of SpO_2 were measured. Breath-holding time was significantly shorter in the HT group (30 ± 2.0sec, $p<0.05$) and tended to be shorter in the Elder group (31 ± 3.0sec, $p=0.08$) compared with the Cont group (41 ± 2.9sec). The maximum mean arterial blood pressure (Max-MAP) was higher in the Elder (105 ± 4.0mmHg) ($p<0.05$) and HT (128 ± 5.6mmHg) ($p<0.05$) groups compared with the Cont group (93 ± 4.0mmHg). However, ΔMAP, ΔHR, Min-SpO_2, and ΔSpO_2 were not significantly different among the four groups. Our results suggest that non-invasive continuous monitoring facilitates evaluation of stress responses to breath-holding in preoperative patients, and that the breath-holding stress test causes sympathetic augmentation, resulting in increases in MAP and HR by approximately 15%, concomitant with a decrease in SpO_2 to 90-94%. The magnitude of the response is similar regardless of age and existence of HT and DM.

Key words; Breath-holding, stress test, homodynamic, preoperative, tonometry

Introduction

Preoperative evaluation of a patient is one of the most important roles of anesthesiologists, especially given the recent clinical tendency for increased proportion of geriatric surgical patients. The breath-holding stress test is a simple stress test available to anesthesiologists and can be conducted during the preoperative visit. However, the magnitude and time-course of stress responses to the breath-holding stress test remain unclear. The purpose of this study was to evaluate circulatory and respiratory responses to the breath-holding stress test in patients using continuous and non-invasive monitoring and to compare the responses among normal healthy, elderly, hypertensive, and diabetic patients.

Materials and Methods

Surgical patients were studied after obtaining ethical approval for the project from our institution and informed consent from patients. Sixty-one patients

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were enrolled in the study. Patients were assigned into four groups: normal healthy patients (Cont) aged <65 years (n=13, mean age: 36 years, range: 22-64 years), elderly patients (Elder) aged >65 years with no cardiac complications or hypertension (n = 19, mean age: 73 years, range: 65-84 years), hypertensive patients (HT) with a history of HT and currently receiving antihypertensive therapy (n=21), and diabetic patients (DM) receiving insulin therapy (n=8). The breath-holding stress test was conducted at the bed-side in the supine position, and at the end of passive exhalation, namely, at the functional residual capacity (FRC) level and while breathing room air without hyperventilation. Stress responses to the breath-holding stress test were assessed by continuous measurement of oxyhemoglobin saturation using a pulse oximeter (SpO_2), recording of electrocardiogram, continuous monitoring of arterial blood pressure using arterial tonometry, and heart rate (BP508, Nihon Colin Electronics, Komaki, Japan). Breath-holding time, changes in heart rate (HR), arterial pressure, SpO_2 and the recovery time of SpO_2 were measured. All data were expressed as mean±SEM (standard error of the mean). Differences between groups were examined for statistical significance using the Student's *t*-test and one-factor ANOVA with Posthoc test (Games Hwell). A p value less than 0.05 denoted the presence of a statistically significant difference.

Results

Patient demographic data are shown in **Table 1**. The mean ages of the Cont, Elder, HT and DM groups were 28, 74, 66, and 67 years, respectively. The mean arterial pressure (MAP) in the HT group was significantly higher than in the Cont group.

Typical recordings of changes in MAP, HR and SpO_2 during the breath holding test are shown in **Fig. 1**. In this 35-year-old female, the breath-holding time was 42 sec. SpO_2 gradually decreased and reached a minimum value of 88% at 15sec, and thereafter recovered to the pre-breath-holding level at approximately 40sec after cessation of breath-holding. Her MAP and HR increased and reached the maximum changes of 20mmHg and 25 beats/min, respectively, at the end of breath-holding.

Table 1 Patient demographics

	Cont	Elder	HT	DM
n	13	19	21	8
Age (yr)	28±1	74±1*	66±2*	67±4*
Height (cm)	166±3	159±3	154±2*	157±4
Weight (kg)	63±3	57±2	56±2	55±2

Data are mean±SEM.
Cont: control group, Elder: elderly group, HT: hypertensive group, DM: diabetic group.
*$p<0.05$ compared with Cont.

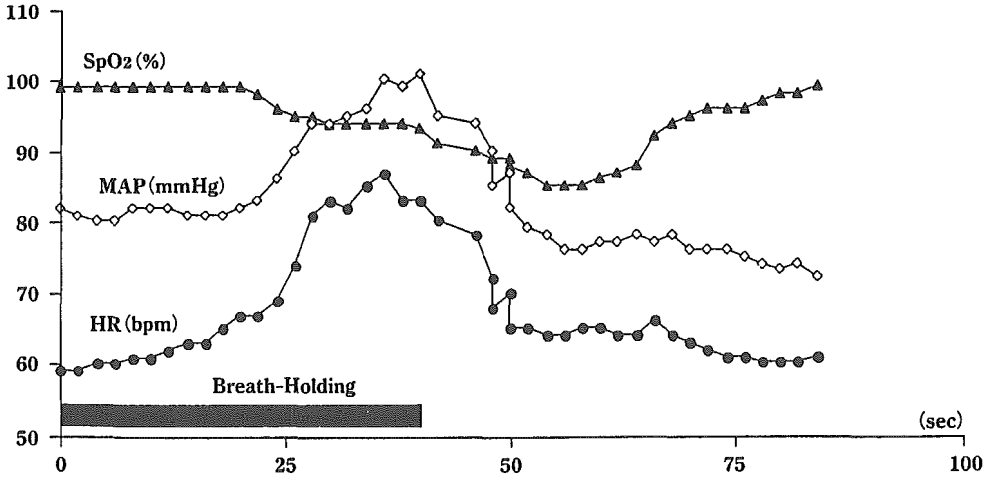

Figure 1 Typical recordings of changes in mean arterial pressure (MAP), heart rate (HR) and SpO_2. See Results.

Table 2 Changes in MAP, HR and SpO_2 during breath-holding test conducted at end expiration during room air breathing

	Cont	Elder	HT	DM
Breath-holding time (sec)	41±3	31±3	30±2*	42±2
Baseline MAP (mmHg)	78±3	89±3	113±5*	87±6
Max-MAP (mmHg)	93±4	105±4*	128±6*	100±7
⊿MAP (mmHg)	16±2	16±2	15±2	13±2
Baseline HR (bpm)	68±4	62±2	74±2	67±5
Max-HR (bpm)	76±3	69±2	81±3	74±7
⊿HR (bpm)	8±11	7±9	8±5	7±9
Baseline SpO_2 (%)	98±0.4	98±0.3	97±0.3	98±0.5
Min SpO_2 (%)	92±3.6	94±4.4	92±4.6	90±5.7
⊿SpO_2 (%)	6.6±4.3	3.8±3.5	4.8±2.7	7.5±4.2
Recovery time (sec)	35±3	33±2	26±2*	37±3

Data are mean±SEM.
MAP: mean arterial blood pressure, HR: heart rate, SpO_2: oxyhemoglobin saturation.
For other abbreviations, see **Table 1**.
*$p < 0.05$ compared with Cont.

Changes in MAP, HR and SpO_2 of the four groups are shown in **Table 2**. Breath-holding time was significantly shorter in the HT group (30±2.0sec) ($p < 0.05$) and tended to be shorter (albeit insignificantly) in the Elder group (31±3.0sec) ($p = 0.08$) than in the Cont group (41±2.9sec). The recovery time was significantly shorter in HT group than in Cont group. Maximum MAP (Max-MAP) was significantly higher in the Elder (105±4.0mmHg) ($p < 0.05$) and HT (128±5.6mmHg) ($p < 0.05$) groups than in the Cont group (93±4.0mmHg). However, ⊿MAP, ⊿HR, Min-SpO_2, and ⊿SpO_2, were not significantly different among the four groups.

Discussion

Preoperative evaluation of surgical patients is mandatory for optimal anesthetic management. Several stress tests have been used in preoperative evaluation. The breath-holding stress test is a simple stress test available to anesthesiologists during their preoperative visit that does not require any invasive monitoring. Current non-invasive continuous monitoring devices, such as arterial tonometry and pulse oximetry, have facilitated evaluation of circulatory and respiratory stress responses to breath-holding. Our present study has demonstrated that breath-holding time was around 30 to 40sec and SpO_2 continued to decrease and reached a nadir at 10 to 20sec after the cessation of breath-holding, in association with maximal increases in blood pressure and heart rate by approximately 15%, which were elicited at the end of breath-holding.

Breath-holding time depends on lung volume and PO_2 of inspired gas[1,2] and it is significantly limited by an increase in $PaCO_2$[2]. It has been shown that breath-holding time is approximately 1min at room air, when PaO_2 decreases to approximately 65-70mmHg and $PaCO_2$ increases by approximately 12mmHg. The rate of the increase in $PaCO_2$ has been shown to be 43mmHg/min during the first 10sec, 13mmHg/min during the next 10sec, and 6mmHg/min thereafter[2]. The endpoint of breath-holding time has been reported to correspond to a $PaCO_2$ of approximately 50mmHg[2,3]. Hyperventilation before breath-holding, therefore, can prolong the breath-holding time to 3-4 min[1].

Sasse, et al[4] has reported, using an invasive arterial blood gas analysis, that breath-holding time at FRC is about 35sec and the arterial $PaCO_2$ increases by 10.2mmHg at the end of breath-holding. Their reported breath-holding time is similar to that of our normal healthy patients (40±2.0sec). Stock, et al[2] also reported that $PaCO_2$ is about 50mmHg after 40sec of apnea at FRC. Therefore, the $PaCO_2$ of our

patients may also be expected to have reached ~ 50mmHg at the end of breath-holding.

The FRC has been shown to increase with aging due to expansion of alveolar spaces and emphysematous lung[5,6], which can prolong the duration of breath-holding. On the other hand, in elderly patients with emphysematous lung, the baseline $PaCO_2$ may be higher than normal. In addition, elderly patients cannot tolerate the dyspneic sensation as compared with normal subjects, resulting in a decrease in breath-holding time. The result of these complex factors is that the breath-holding time of elderly (Elder) patients tended to be shorter ($p<0.08$) than that of normal healthy (Cont) patients in this study.

The breath-holding time in hypertensive patients was significantly shorter than in normal healthy patients, and was similar to that in elderly patients. The age of the hypertensive patients was also similar to that of the elderly patients, which may have contributed to the shorter breath-holding time in hypertensive patients compared with normal healthy patients. The breath-holding stress test has been performed previously in hypertensive patients to evaluate responses of blood pressure to stimuli[7,8], where the systolic blood pressure increased by 12% in normal subjects and by 30-40% in hypertensive subjects after 20sec of apnea. In our study, the increase of mean arterial blood pressure was about 15% in all groups. This only slight increase in blood pressure may be attributed to the use of antihypertensive drugs in our hypertensive patients.

In diabetic patients, the responses to breath-holding were similar to those in normal healthy patients. Since our diabetic patients had received insulin therapy and were not complicated by autonomic neuropathy, the hemodynamic responses appeared to be similar to those in other groups. If diabetic patients also had autonomic neuropathy, the responses obtained may differ. Several cardiovascular tests have been performed to evaluate autonomic neuropathy in diabetic patients, including heart rate variation in response to deep breathing, standing and the Valsalva maneuver, and also the recording of postural change in systolic blood pressure. The breath-holding test may therefore become a suitable alternative to these cardiovascular tests.

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超早期抜管を行う fast track pediatric cardiac surgery における bispectral index (BIS) monitor の有用性

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麻生俊英**　外須美夫*

要　旨

先天性心疾患に対して fast track cardiac surgery を予定された乳幼児21症例で，セボフルランとフェンタニル（10 μg・kg⁻¹ 以下）で麻酔し，bispectral index (BIS) monitor を用いて手術中の鎮静度を評価した。疾患は，ファロー四徴症5症例，心房中隔欠損症4症例，心室中隔欠損症4症例，心内膜床欠損症3症例，肺動脈狭窄症2症例，そのほか3症例であった。フェンタニル使用量は7.3±2.1 μg・kg⁻¹，セボフルラン濃度は0.5-3.0%であった。人工心肺の復温中に一過性にBIS値が70を超える症例があり，セボフルラン濃度を調節して対応し，ほぼ70以下に維持することができた。セボフルランによると考えられる循環抑制は見られず，21症例中19症例は手術室で，2症例はICU入室後3時間以内に抜管することができた。

キーワード：fast track cardiac surgery，超早期抜管，bispectral index (BIS)，低体温，セボフルラン

近年，小児心臓外科手術において fast track cardiac surgery が増加している。fast track cardiac surgery では，術後の早期抜管が重要な要素のひとつで，麻酔方法がポイントとなる[1]。当施設では，先天性心疾患に対する心臓外科手術において fast track cardiac surgery を施行する場合，セボフルランを中心とした麻酔を行い，術中のフェンタニル投与量を10 μg・kg⁻¹ 以下に抑え，手術室での超早期抜管を行っている。fast track cardiac surgery では，できるだけ少ない麻酔薬で，十分な鎮痛と鎮静を得る必要があるが，今回，fast track cardiac surgery を予定された乳幼児心臓外科手術においてBISモニターを用い，手術中に適正な鎮静（麻酔深度）が得られているかを検討した。

1. 対象と方法

北里大学病院で2001年2月から10月までに，先天性心疾患に対し fast track cardiac surgery を施行した乳幼児21症例を対象とした。

前投薬は，ジアゼパム 0.3-0.5 mg・kg⁻¹，ヒドロキシジン 1-2 mg・kg⁻¹ を投与した。導入はチオペンタール 3-5 mg・kg⁻¹，ベクロニウム 0.1 mg・kg⁻¹ を投与し，維持はセボフルランを使用した。人工心肺（CPB）中は人工肺の回路からセボフルランを投与した。術中のフェンタニルの使用量は10 μg・kg⁻¹ 以下とし，臨床所見，BIS値から麻酔深度を判断し，セボフルラン濃度を調節した。また，CPB離脱後の循環動態を安定させるため，CPB離脱の約30分前にオルプリノン 50 μg・kg⁻¹ を投与した。手術中のモニタリングは，ECG，パルスオキシメータ，皮膚温，深部温（膀胱温・咽頭温），観血的動脈圧，中心静脈圧，経食道心エコー，BISモニター（Bispectral Index™ monitor, Aspect Medical System, Ink., USA）を使用した。

手術後，セボフルランの投与を中止し，体動があり，十分な自発呼吸（吸気圧＜−20 cmH_2O，$Paco_2$＜45 mmHg）がある場合に抜管した。

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表 1　手術症例

診断名	症例数
ファロー四徴症	5
心房中隔欠損症	4
心室中隔欠損症	4
心内膜床欠損症	3
肺動脈狭窄	2
単心室	1
両大血管右室起始	1
心室中隔瘤	1
計	21

表 2　患者背景

年齢 (months)	29.5±24.3
体重 (kg)	12±4.9
手術時間 (min)	238±114
麻酔時間 (min)	335±119
フェンタニル ($\mu g \cdot kg^{-1}$)	7.3±2.1
セボフルラン濃度 (%)	
手術開始-CPB 開始	0.5-3.0
CPB	1-1.5
CPB 離脱-手術終了	0.5-2.0

(平均値±標準偏差)

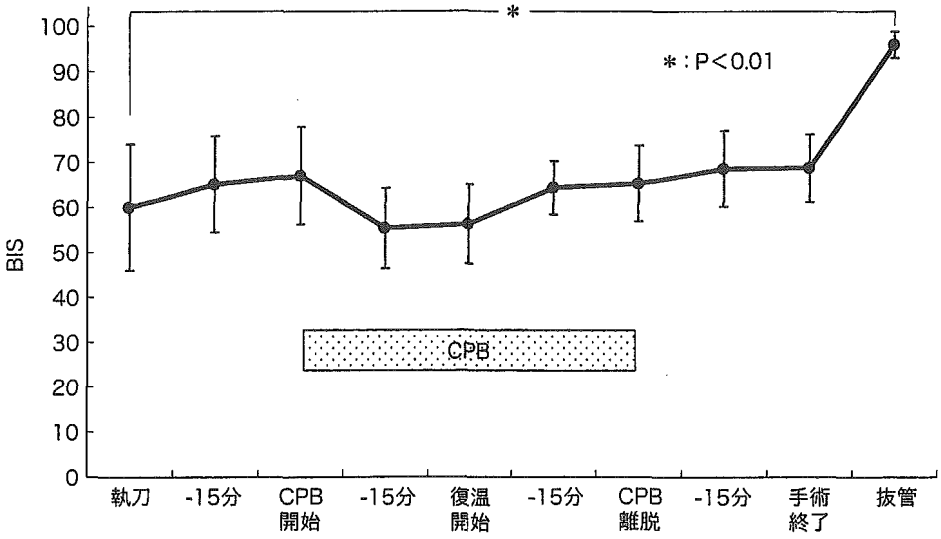

図　BIS の推移

手術中の BIS 値の変化を示す。執刀，執刀後 15 分，CPB 開始，CPB 開始後 15 分，復温開始，復温開始後 15 分，CPB 離脱，離脱後 15 分，手術終了，抜管における BIS 値を平均した。執刀時の BIS 値に比べ，抜管時は有意に上昇した（$P < 0.01$）。そのほかの時点では，執刀時と有意差はなかった。

統計はすべて平均値±標準偏差で表した。BIS 値の手術開始前との比較は，分散分析後，Bonferroni の補正を行った paired t 検定を用い，$P < 0.05$ を有意とした。

2. 結　果

表 1，表 2 に症例を示す。手術中にセボフルラン投与によると考えられる循環抑制は認められなかった。手術室で抜管した症例は 21 症例中 19 症例で，2 症例は肺高血圧のため手術室で抜管せず，術後 3 時間以内に ICU で抜管した。再挿管された症例はなかった。

手術開始時，手術開始 15 分，CPB 開始時，CPB 15 分，CPB 離脱時，離脱 15 分，終了時，抜管時のそれぞれの BIS 値を平均して，手術中の推移として図にまとめた。人工心肺中の中等度低体温から復温する際に BIS 値が一過性に 70 を超えたのが 11 症例（52%）あり，セボフルラン濃度を上げることによって調節した。CPB 中を含め，手術中の循環動態を変動させずに，BIS 値をほぼ 70 以下に保つことができ，手術開始時の BIS 値と有意差はなかった。抜管時の BIS 値

は90以上（96±2.9）に有意に上昇した。

3. 考　察

fast track cardiac surgeryでは，術後の早期抜管が重要な要素のひとつである。Michaelら[2]は，心房中隔欠損症根治術の経費削減のひとつとして，手術室での抜管を推奨している。また，心臓外科手術後の手術室での抜管は，患者にとって不利益にならないと報告[1]されている。われわれの施設では，竹内ら[3]の報告を参考に，早期抜管の対象をすべてのbidirectional Glenn，Fontan手術，乳幼児中期以降で体重5 kg以上の根治術，体重約5 kg以上のBT shunt，PA bandingとし，肺高血圧症と中等度以上の弁逆流の残存を適応外とした。術中のフェンタニル使用量を10 $\mu g \cdot kg^{-1}$以下とし，心収縮力増強作用と血管拡張作用を有するオルプリノンを使用し，経食道エコーによって残存病変や心機能を評価し，CPB離脱前に心腔内の空気をエコーで確認しながら除去して空気塞栓による痙攣を予防することで，術後早期抜管が可能であった[4][5]。

早期抜管には，短時間作用型の麻酔薬を使用し，オピオイドの使用量を減らす必要があり，手術中，特にCPB中の覚醒のリスクが増える可能性がある。心臓外科手術での術中覚醒の頻度は他の手術より高いが，報告[6]~[8]によってばらつき（0.3-23%）があり，麻酔方法が影響していると考えられている。揮発性麻酔薬や静脈麻酔薬は，中潜時聴性誘発電位を抑制する作用がオピオイドより強く[9][10]，術中の覚醒頻度が少ないと報告[11]されている。fast track cardiac surgeryでは揮発性麻酔薬や静脈麻酔薬を用いて麻酔するため，オピオイドを中心とした従来の心臓外科麻酔法より術中覚醒が少ないと考えられる[6]。

この研究では，鎮静度の指標のひとつとしてBIS値をモニターした。BIS値といくつかの臨床的な鎮静スコアとの関連から，BIS値が50-70に維持されていれる場合，適切な鎮静が得られていると考えられている[12]。また，亜酸化窒素・セボフルランで麻酔を維持した場合，BIS値が50となる呼気中セボフルラン濃度は0-2歳児で平均1.55%，2-12歳児で平均1.25%と報告[13]されている。この研究では，セボフルラン濃度0.5-3.0%で，BIS値を50-70に維持することができた。

CPB中のBIS値の変動について，いくつか報告があり，成人ではCPB中の低体温によってBIS値が低下すると報告[14]されている。小児の中等度低体温でのCPBで，亜酸化窒素-イソフルラン-フェンタニルで麻酔しBIS値を測定したところ，最低体温でのBIS値の低下がみられたが有意差はなく，低体温からの復温中に体動や自律神経反射，代謝亢進のサインがなくてもBIS値の有意な上昇がみられ，この時期に鎮静レベルが浅くなっている可能性があると報告[15]されている。また，人工肺から揮発性麻酔薬を併用している場合，CPB離脱時の心筋抑制を避けるために揮発性麻酔薬の濃度を下げることがあり，これと復温が重なるため，術中覚醒のリスクが高くなる可能性がある[6]。この研究でも復温中のBIS値の上昇がみられたが，セボフルランの濃度を上げることで対処でき，セボフルランによる循環抑制はみられなかった。復温中は，BIS値を指標として適切なレベルまでセボフルラン濃度を上げ，鎮静度を保つ必要があることが示唆された。

術後の早期抜管が重要なポイントとなるfast track cardiac surgeryにおいては，必要最小限の麻酔薬を使用することが望ましく，この点からBIS値は有用で，必要なモニターのひとつであると考えられる。

小児心臓外科手術で，BIS値をモニターしてセボフルランを使用し，ほぼ良好な鎮静度を維持することができた。また，フェンタニルの使用量を10 $\mu g \cdot kg^{-1}$以下に抑えることができ，術後早期抜管が可能であった。

本稿の要旨の一部は，日本麻酔科学会第49回大会（2002年，福岡市）で発表した。

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ABSTRACT

Bispectral Index Monitoring in Fast Track Pediatric Cardiac Surgery

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Background : We evaluated the changes in the bispectral index (BIS) as a potential indicator of level of consciousness in infants and children undergoing fast track cardiac surgery.

Methods : Twenty-one children undergoing fast

track cardiac surgery were recruited into this study. Anesthesia was maintained with inhaled sevoflurane and intravenous fentanyl $10\mu g \cdot kg^{-1}$. Cardiopulmonary bypass (CPB) with mild hypothermia and an immediate tracheal extubation protocol were used. BIS was recorded throughout the operation.

Results: In average, BIS was kept almost under 70 with 0.5-3.0% of sevoflurane. During rewarming from mild hypothermia, BIS increased temporarily over 70 in about a half of children. We, therefore, treated them by increasing sevoflurane concentration. Nineteen children were extubated in the operating room, and two patients were extubated in ICU within three hours after surgery.

Conclusions: BIS was kept within the level of adequate sedation during surgery. However, since the increase in BIS during the rewarming phase could reflect light anesthesia, caution should be taken around this phase.

key words: bispectral index (BIS), fast track pediatric cardiac surgery, early extubation, sevoflurane

特　集

外傷性大量出血による周術期心停止患者の生存率と男女差

小澤章子*，外須美夫*

はじめに

「女性は男性に比べ大量出血に強い」と古くから言われている．臨床的にしばしば実感するが，臨床における大量出血と性差についての報告はみられない．われわれは，外傷による大量出血患者の手術中に心停止を来たした症例を検索し，心停止からの蘇生率，生存率を男女で比較検討した．

対　象

平成6年から15年までの10年間に，北里大学病院中央手術室に搬入された外傷症例で，手術室入室から手術中に心停止を来たした症例のうち，心停止の主原因が大量出血と判断されたものを対象とした．外傷の原因は交通事故，転落，刺創で，受傷部位は胸部，腹部あるいは両方の臓器損傷がある症例とした．頭蓋内出血を有するものと四肢の損傷のみのものは除外した．また，対象年齢は15から60歳までとした．検索は，北里大学病院情報処理センターのデータベースを用い，過去の麻酔記録を調査した．

結　果

10年間の麻酔科管理症例は50,979例で，男性は24,918例(49%)，女性26,061例(51%)であった．そのうち，緊急外傷手術症例で手術時に大量出血で心停止を来たした症例は19例で全体の0.037%で，男女比は12：7(15～60歳)で，平均年齢は，男性が38歳，女性が33歳であった．受傷部位は，男性が胸部6例，腹部9例，骨盤1例で，女性は胸部2例，腹部6例，骨盤1例(重複あり)であった．男女とも腹部の受傷が多かった．頭部外傷は，生命予後に出血以外の要素が関与するため，四肢のみの外傷は救命救急センターでの止血が行い易いため，今回の調査から除外した．また，家族からの聞き取り調査で，この19例中のうち，受傷前までに中等度以上の合併症(高血圧，虚血性心疾患など)を有していた症例はなかった．

心停止19例は全例心臓マッサージを行ったが，男性12例中，入室時に心臓マッサージを行ったものは1例で，その他は術中の施行であった．女性7例では入室時には2例，術中に5例が心臓マッサージを行った．蘇生に成功し手術室から生存して退室できたのは，男性が12例中6例(50%)，女性は7例中6例(85.7%)で女性が男性より高率であった(**図1**)．入室時または術中に開胸心マッサージを行った症例は男女ともに2例ずつあったが，男性は2例とも術中に死亡したにも拘らず，女性は2例とも蘇生に成功し，十分な血圧を得て手術室を退室した．

術中の平均輸血量は，MAPが男性で28単位，女性で25単位，FFPが男性で28単位，女性で24単位であった．

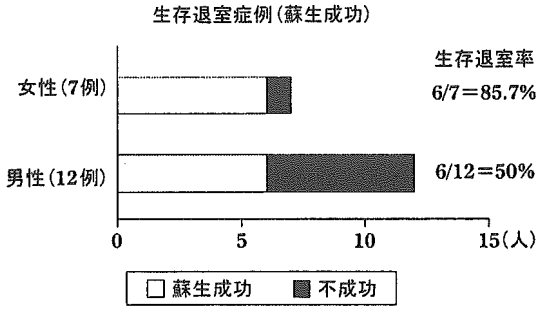

図1　生存退室症例（蘇生成功）

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入室時のヘモグロビン値の平均は，男性7.0，女性7.3g/dl，術中のヘモグロビン最低値は男性7.9，女性4.8g/dlであった．手術室退室時，男性は8.1と上昇したが，女性は5.0と改善しなかった(図2)．男性は輸血を行うことで全例が貧血の改善をみたが，女性は大量の輸血にも拘らず貧血が進行した．ヘマトクリットも同様の傾向がみられた．入室時の血小板値の平均は，男性8.0，女性4.2×10000/mm^{3}で，血小板輸血を行い，退出時には男性6.6，女性6.0であった．術中の動脈血BEは入室時に男性が−12.5，女性が−21.0，退室時男性が−9.8，女性で−13.0mEq/lで，術中のどの時点をとっても女性の方が低かった(図3)．

生存退室症例12例(男女とも6例ずつ)について男女別に調べた．平均血圧は，男性は入室時，退室時で変化なく55mmHgであったが，女性は25から50と著明に改善した．また，ヘモグロビン値は，男性が入室時・術中・退室時と6.4・8・10.2と上昇したのに比べ，女性は8・5・4.7とむしろ低下した．BE値に関しては，男性が−9.5・−13.3・−5，女性は−20・−18・−11と両者とも改善しているが，女性の方が，どの時点においても男性より低値であった．

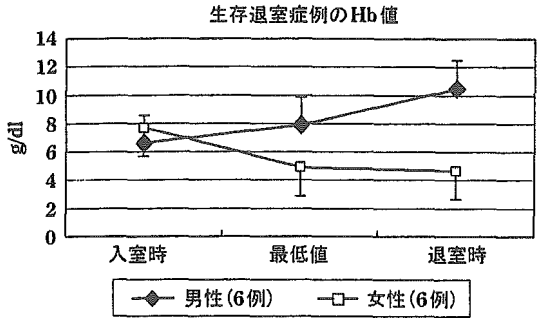

図2 生存退室症例のHb値

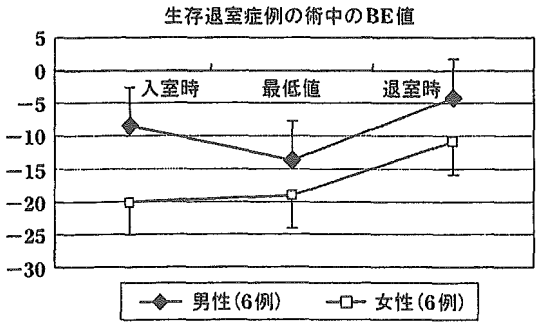

図3 生存退室症例のBE値

女性の生存退室症例は，入室時平均血圧，術中のヘモグロビン値，動脈値BEのいずれも男性に比べて低値であるにも拘らず，蘇生率は高かった．

考　察

最低ヘモグロビン値は女性の方が低く，動脈血BEの最低値も女性に低かったことから，女性が男性より出血が軽度で組織酸素化が維持されていたわけではない．それにも拘らず，大量出血による心停止からの生存率が女性に高かった．今回は高齢者は対象から除外しており，両群とも元々健康成人と思われることから，基礎疾患や動脈硬化の違いというより，大量出血に対する生体の耐性に男女差がある可能性が示唆される．

出血性ショックモデルにおける性差について，いくつかの報告がある．まず，出血性ショック-蘇生モデルにおいて，Kueblerらは性成熟期の雌のラットは同じ体重の雄に比べて循環血液量が多かった[1]と述べており，性成熟期には非妊時から妊娠，出産時の大量出血に対応するための準備が行われている可能性がある．出血性ショックによる低酸素性組織障害についても雌が優位であるという報告が多くみられる．外傷による出血性ショック-蘇生後に，雌は雄に比べて，内皮細胞機能，組織灌流が保たれる[2]，マクロファージや多核白血球の活性化が抑制されるため細胞，組織障害が抑制される[3,4]など，組織障害が軽度であることが示されている．また，雌では腸粘膜損傷，肺障害が少なく，雄の血漿中の一酸化窒素濃度の上昇が組織障害と正の相関関係にあり，性周期と組織障害の関連性を示唆する報告も見受けられる[5]．

出血性ショック後，雌ではインターロイキン(以下IL)-1，2，3，6や副腎皮質ホルモンの分泌が増強すること[6]や，エストラジオール治療をした群ではIL-6やマクロファージ機能が増強すること[3]，IL-10で治療後，雄では生存率の上昇がみられたが雌では変化がないこと[7]などにより，出血性ショック後の内分泌・免疫応答にも性差があることが知られている．また，女性ホルモンは，出血後の免疫応答の増強に重要なだけでなく，その後に生じる敗血症や感染を起こしにくくして生存率が上がるという報告もある[8]．いずれも，出血に際して雌が雄よりも生命維持に対する耐性があり，性ホル

モンが関与していることを示唆している．

また，外傷と性差についても，女性が優位であるという報告が多い．Offnerらは，level 1の外傷センターでの5年間のprospective studyで，外傷術後の重症感染症は有意に男性に多いと報告している[9]．Monafaらは，North CarolinaのLevel 1のtrauma centerにおける4年間の重症外傷患者を性別と年齢別(15～45歳(若年者)と45歳以上)に分けてretrospectiveに検討した．若年者では，男性において，多臓器不全，長期ICU，長期入院が多く，死亡率が高かったが，45歳以上では，性差を認めなかった[10]．McKinleyらは，Houstonのlevel 1 trauma centerでの1年間のprospective studyを行った．重症外傷後の多臓器不全患者58名(男性38名，女性20名)において，血行動態安定化のために必要とした輸液量，肺動脈カテーテル挿入率，酸素化が維持されたときの肺動脈楔入圧を測定したところ，いずれも女性は低値であった．蘇生に対する反応も，女性の方が良好であった[11]．これらは，若年層つまり性成熟期の女性は，男性や性ホルモン減少期の女性よりも外傷後の生存率が高いことを意味している．

さらに，蘇生に関しては，院外心停止者の心拍再開率は女性が男性に比べて有意に高かったという報告がある．Swedenの10年間のretrospective studyで，院外心停止症例23,797例(そのうち女性は27.9%)で，来院後の蘇生成功率は女性が16.4%と男性の13.2%よりも有意に高かった．平均年齢が高い，VFが少ない，心原性心停止が少ない，心停止時の目撃者が少ない，bystander CPRを受ける機会が少ない，など，女性の方が男性に比べて条件が悪いにも拘らず蘇生率が高いことは，性ホルモンの影響が大きいとしている[12]．この理由の1つとして，自律神経系の反応に性差がある可能性があると述べている．Airaksinenら[13]は，冠動脈の血流低下が起きた際，女性の方が迷走神経優位となり，その結果，徐脈という有益な抗不整脈作用が発現しVTやVFなどの致死的な頻脈を避けられる，としている．

ストレス時，特に大量出血時に雌に生体の耐性がある理由は，出産時の大量出血に対する生体の防衛機構の1つと思われる．性ホルモン分泌期間(性成熟期)は，妊娠，出産時の出血に備えて凝固系，免疫系，自律神経系などが準備段階にあり，大量出血時にこれらが賦活化され，凝固の亢進や低酸素状態に耐えられるような組織循環，頻脈を避ける血行動態を作り出す可能性がありうる．同じ雌でも，性成熟期か否かで生体内環境が異なるとの報告があり[5]，妊娠が可能かどうかが，生体内バランスを決定している可能性は大きい．動物界を含め生命体の成り立ちを振り返ると，雌に対しては妊娠可能な数(個数)が多く存続すること，雄に対しては，様々な試練を乗り越えて生き残った強い遺伝子が必要とされることが，種の保存のためには合理的であるように思える．

今回の調査では，対象とした心停止症例が少なく男女差を明らかにするのは難しい．また，15歳から60歳までという年齢制限を行ったが，性成熟期という意味では女性に関しては15歳から40歳まで，という範囲での処理を行った方が，より詳細な情報が得られたかもしれない．今回，母集団とした全手術症例数は多く(50,979例/10年間)，そこから患者背景を絞ったため対象症例が少なくなったが，関連因子となる前提条件はある程度揃えることができた．臨床における出血性ショックは，対象年齢，疾患，疾患の進行度，術式，合併症，術者，施設など多くの因子が関与し同一条件で検討することは困難な中で，今回の検討で大量出血時の男女の反応の違いについて，臨床的に1つの傾向を示すことができたと思われる．

結　　語

外傷患者の大量出血による周術期心停止症例の手術室での生存率は，女性の方が男性より高率であった．外傷症例では元来健康成人と思われるので，基礎疾患や動脈硬化の違いということより，大量出血に対する生体の耐性に男女差がある可能性が示唆される．

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Lack of Interleukin-1 Receptor Antagonist Modulates Plaque Composition in Apolipoprotein E–Deficient Mice

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Objective—Interleukin (IL)-1 plays an important role in atherosclerosis. IL-1 receptor antagonist (IL-1Ra) is an endogenous inhibitor of IL-1. However, the role of IL-1Ra in the development of atherosclerosis is poorly understood.

Methods and Results—Mice that lacked IL-1Ra (IL-1Ra−/−) were crossed with apolipoprotein E-deficient (E−/−) mice and formation of atherosclerotic lesions was analyzed after 16 weeks or 32 weeks consumption of a normal chow diet. This study focused on the comparison of atherosclerotic lesion between IL-1Ra+/+/apoE−/− (n=12) and IL-1Ra$^{+/-}$/apoE−/− mice (n=12), because of the significantly leaner phenotype in IL-1Ra−/−/apoE−/− mice compared with the others. Interestingly, atherosclerotic lesion size in IL-1Ra+/−/apoE−/− mice at age 16 weeks was significantly increased (30%) compared with IL-1Ra+/+/apoE−/− mice ($P<0.05$). At 32 weeks, the differences of lesion size between these mice failed to achieve statistical significance. However, immunostaining demonstrated an 86% ($P<0.0001$) increase in the MOMA-2–stained lesion area of IL-1Ra+/−/apoE−/− mice. In addition, α-actin staining in these lesions was significantly decreased (−15%) compared with those in IL-1Ra+/+/apoE−/− mice ($P<0.05$).

Conclusions—These results suggest an important role of IL-1Ra in the suppression of lesion development during early atherogenesis and furthermore indicate its role in the modulation of plaque composition. (***Arterioscler Thromb Vasc Biol.* 2004;24:1068-1073.)**

Key Words: atherosclerosis ■ immune system ■ inflammation ■ interleukins ■ macrophage

Interleukin (IL)-1 plays an important role in immunity, cell damage, and cell proliferation, and is produced and secreted by a variety of cells including those responsible for controlling immunity.[1] Cytokines, including IL-1, characteristically form a network in which the production of a specific cytokine leads to serial production of others. In addition to immune reactions, IL-1 has numerous systemic functions, such as promoting fever, stress response, and modulating insulin and lipid metabolism.[2–3]

Atherogenesis is a complex process in which endothelial cell (EC) and smooth muscle cell (SMC) activation appears to be a central theme.[4] IL-1 is produced by ECs and SMCs as well as macrophages/monocytes and hepatocytes.[5,6] Furthermore, stimulation and activation of ECs and SMCs by IL-1 causes a wide range of inflammatory processes within the atheroma, such as the enhanced expression of leukocyte adhesion molecules,[5,7] clotting factors and inhibitors of fibrinolysis,[8] and chemokines,[9] as well as increased proliferation of SMCs,[4] suggesting a central role for IL-1 in the development of atherosclerosis.

The IL-1 receptor antagonist (IL-1Ra) is a structural homologue of IL-1[10] that occupies the type I IL-1 receptor with higher affinity and an association rate constant similar to that of IL-1[11] but is unable to recruit the IL-1 receptor accessory protein, required to mediate intracellular signaling.[12,13] Thus activity of IL-1 is counter-regulated by its endogenous inhibitor IL-1Ra.[9,14] A previous report showed that IL-1Ra is expressed in ECs and atherosclerotic lesions.[15] Recently, we investigated the contribution of IL-1Ra to neointimal formation after injury by comparing IL-1Ra–deficient (IL-1Ra−/−) mice with wild-type (IL-1Ra+/+) mice.[16] Intimal thickness and the intima to media ratio were significantly elevated in the IL-1Ra−/− mice compared with the IL-1Ra+/+ mice. Immunostaining for IL-1Ra revealed that IL-1Ra protein was indeed expressed in the endothelium as well as inflammatory cells of the adventitia in IL-1Ra+/+ mice, but was absent in IL-1Ra−/− mice. These results suggested that the IL-1Ra plays an important role in the suppression of neointimal formation after injury. Furthermore, treatment with recombinant IL-1Ra proved an effective therapy for atherosclerosis in apoE-deficient C57BL/6J (apoE−/−) mice.[17] Moreover, IL-1Ra gene polymorphism is significantly associated with coronary artery disease.[18] These findings suggest that endogenous IL-1Ra may also suppress other occlusive vascular response to injury, such as atherosclerosis.

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To directly address the question of whether deficiency of IL-1Ra promotes the development of atherosclerotic lesion and/or can modulate the phenotype of atheroma, we took advantage of IL-1Ra−/− mice generated recently.[2] Using hypercholesterolemic apoE−/− mice as an animal model of atherosclerosis, we established three genotypes (IL-1Ra+/+/apoE−/−, IL-1Ra+/−/apoE−/−, and IL-1Ra−/−/apoE−/− mice) by cross-breeding.

Methods

Animals

The generation of IL-1Ra−/− mice used in this study has been described previously.[2,16] These mutant mice lacked all 4 isoforms of the IL-1Ra. These mice were backcrossed to the C57BL/6J strain for 8 generations. The apoE−/− mice were obtained from the Jackson Laboratory (Bar Harbor, Me). IL-1Ra−/− mice were crossed with apoE−/− mice and IL-1Ra+/−/apoE+/− mice were backcrossed into the apoE−/− background to produce IL-1Ra+/−/apoE−/− mice. These mice were then intercrossed to generate homozygous apoE−/− mice bearing the IL-1Ra allele combination of either +/+, +/−, or −/−. Screening for apoE was performed by phenotypic assays. Blood specimens were obtained, and apoE deficiency in these mice was detected based on elevation of serum cholesterol as described previously.[19] IL-1Ra genotyping was performed by polymerase chain reaction analysis of tail DNA as described previously.[2] Throughout the experiment, the mice were fed a normal diet containing 4.6% crude fat with <0.02% cholesterol (CLEA Japan, Inc) to avoid the induction of severe hypercholesterolemia, which has its own consequences on the immune system.[20] In this study, we used only male mice to rule out gender differences. The studies were performed according to the protocols approved by the National Defense Medical College Board for Studies in Experimental Animals.

Plasma Lipid Measurements

After fasting for 7 hours, plasma total cholesterol, high-density lipoprotein cholesterol, and triglyceride levels were measured by enzymatic assays as previously described by Hedrick et al.[21]

Tissue Preparation and Histology

After tail-cuff systolic blood pressure was measured in the mice, male mice at either 16 or 32 weeks of age were euthanized with pentobarbital and perfused with 0.9% NaCl, followed by 4% paraformaldehyde. After perfusion, the aorta was harvested, fixed overnight in 4% paraformaldehyde, embedded in OCT compounds (Tissue-Tek; Sakura Finetechnical Co, Tokyo, Japan) and sectioned (10-μm thickness). All samples were routinely stained with hematoxylin-eosin, Masson trichrome, and oil red O. Immunohistochemistry was performed on each section. Smooth muscle cells were visualized with α-smooth muscle cell actin (SMA) staining (Roche), and mouse macrophages/monocytes were visualized with clone MOMA-2 (BioSource International). The sections were visualized using a Vectastain ABC kit (Vector Laboratories) with DAB as the substrate.

Quantification of Atherosclerotic Lesions

Aortic sinus sections were prepared as previously reported.[22,23] The area of the lesion was measured using National Institutes of Health (NIH) image 1.55 (public domain software). The values reported represent the mean lesion area from 5 sections for each animal. The quantification of the macrophage and SMCs accumulation in the lesion was determined by calculating the percentage of the MOMA-2 or α-SMA, respectively, positive area to the total cross-sectional vessel wall area. The extent of atheroselerosis in the mouse aorta was also determined using an "en face" method.[24]

Enzyme-Linked Immunosorbent Assay

The serum levels of IL-1β and IL-1Ra were determined by enzyme-linked immunosorbent assay as described previously.[25,26]

Analysis of Gene Expression by Real-Time Quantitative Polymerase Chain Reaction

The aortas of 32-week-old mice were dissected and kept in liquid nitrogen. Total RNA was extracted from the aortas using TriReagent (Sigma) and quantity was determined by measuring the absorbance at 260 nm. Reverse-transcription was performed with AMV Reverse Transcriptase XL (Takara Biochemicals, Japan). Quantitative gene expression analysis was performed on an ABI PRISM 7700 machine (Applied Biosystems) using SYBR Green technology. The following oligonucleotide primer pairs were used: IL-1β sense, 5′- TGG TGT GTG ACG TTC CCA TT-3′; antisense, 5′-CAG CAC GAG GCT TTT TTG TTG-3′; VCAM-1 sense, 5′- TTT GCC GAG CTA AAT TAC AC-3′; antisense, 5′-ATT CTC CCA TAT TGA ACA ACT A-3′; ICAM-1 sense, 5′- TGC GTT TTG GAG CTA GCG GAC CA-3′; antisense, 5′-CGA GGA CCA TAC AGC ACG TGC AG-3′; MCP-1 sense, 5′- GCC CAG CAC CAG CAC CAG-3′; antisense, 5′-GGC ATC ACA GTC CGA GTC ACA C-3′. The optimum number of cycles was set for each gene product with uniform amplification. Each mRNA level was expressed as the ratio to 18S RNA expression.

Statistical Analysis

The results are shown as the mean±SE. Differences between groups were determined using 1-way ANOVA and a multiple comparison test. Two groups were compared using Student t test. A value of $P<0.05$ was regarded as a significant difference.

Results

The systolic blood pressures were similar among the 3 genotypes at 16 weeks of age (IL-1Ra+/+/apoE−/− mice [n=12]: 87.2±1.2 mm Hg, IL-1Ra+/−/apoE−/− mice [n=12]: 86.6±1.1 mm Hg, IL-1Ra−/−/apoE−/− mice [n=10]: 85.2±0.9 mm Hg; P=NS). However, the body weight of IL-1Ra−/−/apoE−/− mice was significantly less compared with that of either IL-1Ra+/+/apoE−/− or IL-1Ra+/−/apoE−/− mice (Figure I, available online at http://atvb.ahajournals.org). Furthermore, plasma lipid analysis revealed that total cholesterol levels of IL-1Ra−/−/apoE−/− mice were significantly elevated compared with those of the IL-1Ra+/+/apoE−/− mice. Moreover, high-density lipoprotein cholesterol levels of IL-1Ra−/−/apoE−/− mice were lower than those of either IL-1Ra+/+/apoE−/− or IL-1Ra+/−/apoE−/− mice. In contrast, no significant differences in body weights or plasma lipid levels were observed between IL-1Ra+/+/apoE−/− and IL-1Ra+/− /apoE−/− mice. This study therefore compared atherosclerotic lesions between IL-1Ra+/+/apoE−/− and IL-1Ra+/−/apoE−/− mice to exclude differences in body weight or lipid levels as confounding factors. Notably, IL-1Ra serum levels in IL-1Ra+/−/apoE−/− mice (169.4 pg/mL) were approximately half of those in IL-1Ra+/+/apoE−/− mice (332.9 pg/mL) and furthermore the levels of IL-1β in IL-1Ra+/−/apoE−/− mice tended to be higher compared with those in IL-1Ra+/+/apoE−/− mice (data not shown).

Early Atherogenesis

Aortic root atherosclerotic lesions of IL-1Ra+/−/apoE−/− mice at 16 weeks of age were significantly larger than those in IL-1Ra+/+/apoE−/− mice (Figure II, available online at

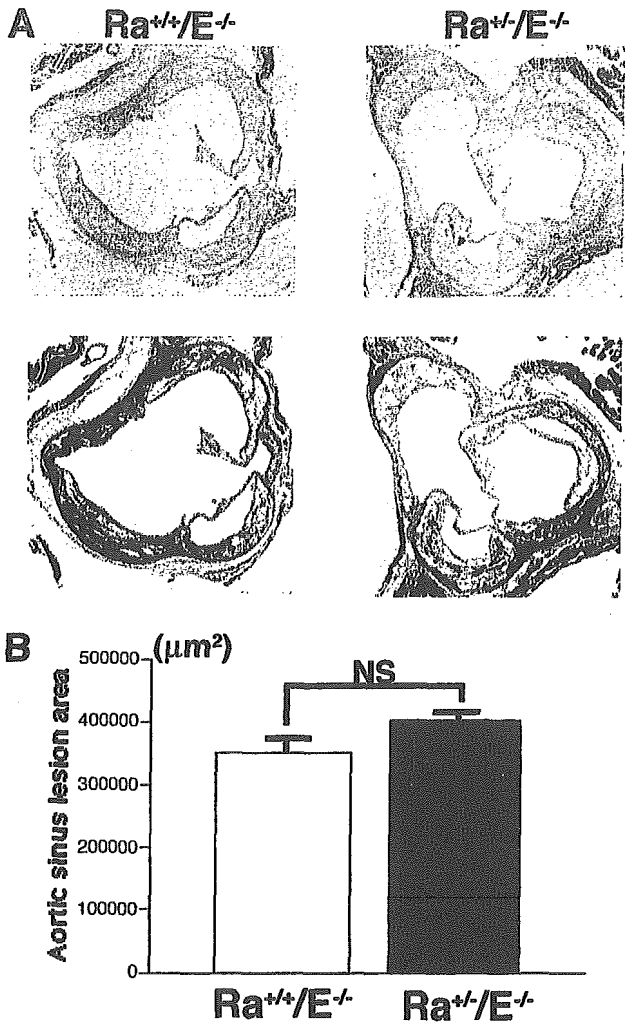

Figure 1. A, Representative photomicrographs of sections of aortic sinus plaque from IL-1Ra+/+/apoE−/− (Ra+/+/E−/−) (left) and IL-1Ra+/−/apoE−/− (Ra+/−/E−/−) mouse (right) 32 weeks old. Adjacent sections were processed for hematoxylin and eosin (upper panels), and elastin staining (bottom panels). Original magnification ×50. B, Quantitative comparison of the atherosclerotic lesion sizes in the aortic sinus between the Ra+/+/E−/− (n=12) and the Ra+/−/E−/− (n=12) mice at 32 weeks old.

http://atvb.ahajournals.org). Atherosclerotic lesions were also examined throughout the aorta. The percent of lipid deposits within total aorta was also significantly elevated in IL-1Ra+/−/apoE−/− mice (25.2%±2.1%, n=12) in comparison to IL-1Ra+/+/apoE−/− mice (14.0%±1.2%, n=12; $P<0.0001$). Neither percent MOMA-2–positive nor α-SMA–positive area showed significant difference between these 2 groups (data not shown).

Advanced Atherosclerosis

Lesion size and morphology were also analyzed at 32 weeks of age to determine the effect of IL-1Ra on advanced atherosclerosis. Although lesion size in IL-1Ra+/−/apoE−/− mice tended to be elevated compared with that in IL-1Ra+/+/apoE−/− mice (Figure 1A), the differences did not achieve statistical significance (Figure 1B). En face analysis of the extent of atherosclerosis in the aortas also showed the decrease of difference between IL-1Ra+/−/apoE−/− (38.2%±1.9%, n=12) and IL-1Ra+/+/apoE−/− mice (30.7%±1.8%, n=12; $P<0.05$). However, immunohistochemical analysis revealed a significant decrease in α-SMA stained lesion area IL-1Ra+/−/apoE−/− compared with IL-1Ra+/+/apoE−/− mice (Figure 2A). The percent α-SMA positive area was 49.0%±3.7% in IL-1Ra+/+/apoE−/− mice (n=12) versus 41.9%±3.3% in IL-1Ra+/−/apoE−/− mice (n=12) ($P<0.05$; Figure 2B). These data demonstrate that diminished IL-1Ra expression modulates the lesional α-SMA content.

Notably, IL-1Ra+/+/apoE−/− mice demonstrated features of fibrous plaques, containing necrotic cores and foam cells that were covered by a fibrous cap (Figure 3A). IL-1Ra+/−/apoE−/− mice showed markedly increased lesional macrophage content compared with that of IL-1Ra+/+/apoE−/− mice (Figure 3A). Quantitative analysis of immuno-staining of lesions in the IL-1Ra+/−/apoE−/− mice showed a 1.9-fold increase in MOMA-2 staining compared with that within IL-1Ra+/+/apoE−/− mice (45.6%±3.7% versus 24.4%±2.0%; $P<0.0001$) (Figure 3B).

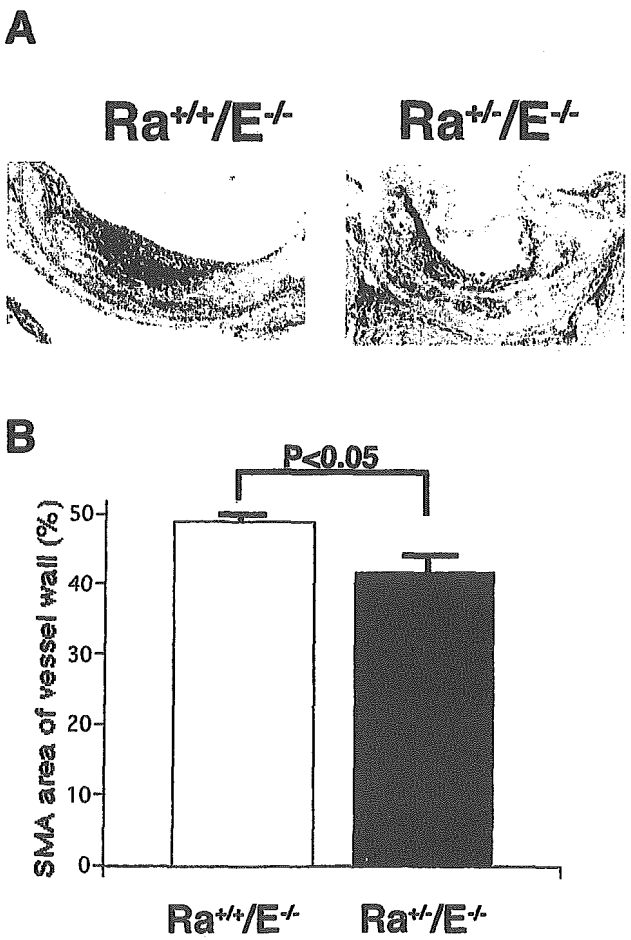

Figure 2. A, Representative photomicrographs of sections of advanced atherosclerotic plaques (immunohistochemical staining for α-smooth muscle cell actin) from the aortic sinus of the IL-1Ra+/+/apoE−/− (Ra+/+/E−/−) (left) and IL-1Ra+/−/apoE−/− (Ra+/−/E−/−) mouse (right) 32 weeks old. Original magnification ×100. B, Quantitative analysis of α-SMA staining in sections from Ra+/+/E−/− (n=12) and Ra+/−/E−/− (n=12) mice at 32 weeks old.

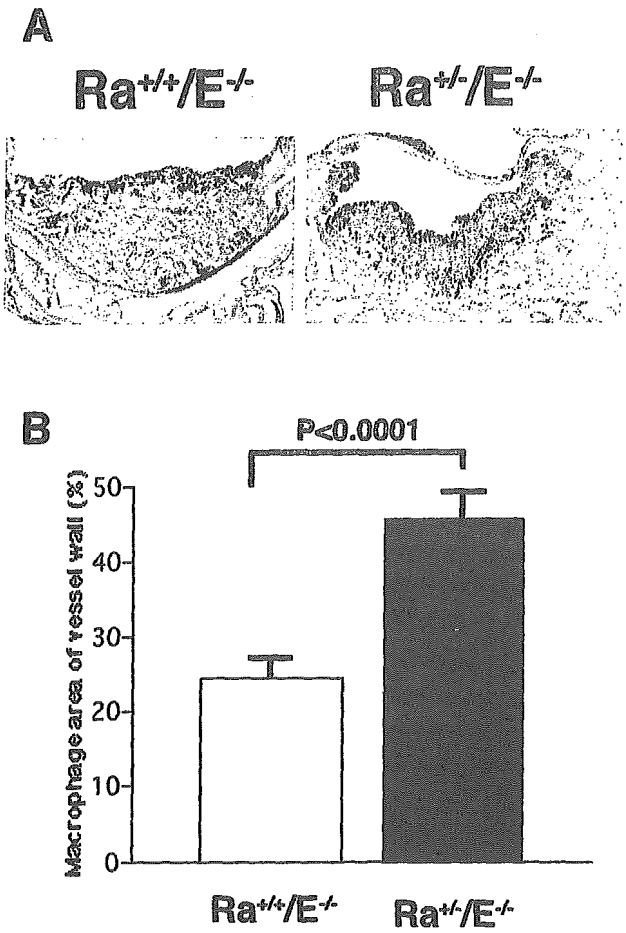

Figure 3. A, Representative photomicrographs of sections of advanced atherosclerotic plaques (immunohistochemical staining for MOMA-2) from the aortic sinus of IL-1Ra+/+/apoE−/− (Ra+/+/E−/−) (left) and IL-1Ra+/−/apoE−/− (Ra+/−/E−/−) mouse (right) 32 weeks old. Original magnification ×100. B, Quantitative analysis of MOMA-2 staining in sections from Ra+/+/E−/− (n=12) and Ra+/−/E−/− (n=12) mice at 32 weeks old.

mRNA Levels of Cytokine, Chemokine, and Adhesion Molecules in the Aorta

To investigate the effect of IL-1Ra on the modulation of plaque composition, we next investigated the mRNA expression levels of cytokine, chemokine, and adhesion molecules in the aorta. The mRNA was extracted from the aorta of each mouse at 32 weeks. The results of real-time polymerase chain reaction revealed that the level of IL-1β mRNA in IL-1Ra+/−/apoE−/− mice was significantly increased by 268% compared with IL-1Ra+/+/apoE−/− mice. Furthermore, the level of MCP-1 mRNA in IL-1Ra+/−/apoE−/− mice was also significantly increased by 442% compared with IL-1Ra+/+/apoE−/− mice. Regarding adhesion molecules, mRNA levels of both ICAM-1 (238%) and VCAM-1 (904%) in IL-1Ra+/−/apoE−/− mice were significantly higher than those in IL-1Ra+/+/apoE−/− mice. These observations suggest that deficiency of IL-1Ra may induce the development of atherosclerosis and accumulation of many macrophages/monocytes in the lesion, possibly by enhancing mRNA expression of IL-1β, MCP-1, and adhesion molecules.

Effect of Complete IL-1Ra Deficiency on Atherosclerotic Lesion

We also analyzed the extent of atherosclerosis in the aortas of 10 IL-1Ra−/−/apoE−/− mice at 32 weeks of age. The lesion area of IL-1Ra−/−/apoE−/− mice was larger than that of IL-1Ra+/+/apoE−/− mice (Figure 4A and 4B). The percent of lipid deposits within the total aorta was also significantly elevated in IL-1Ra−/−/apoE−/− mice (36.2%±1.6%, n=10) in comparison to IL-1Ra+/+/apoE−/− mice (30.7%±1.8%, n=12; $P<0.05$). Interestingly, numerous inflammatory cells were observed in the adventitia of IL-1Ra−/−/apoE−/− mice but not of IL-1Ra+/+/apoE−/− mice (Figure 4A through 4D). Masson trichrome-stained section showed stronger destruction of the elastic lamina within the media in IL-1Ra−/−/apoE−/− mice compared with IL-1Ra+/+/apoE−/− mice (Figure 4C and 4D). Immunostaining revealed that the number of α-SMA-positive cells in the medial layers of the aorta from IL-1Ra−/−/apoE−/− mice decreased (Figure 4E) but not

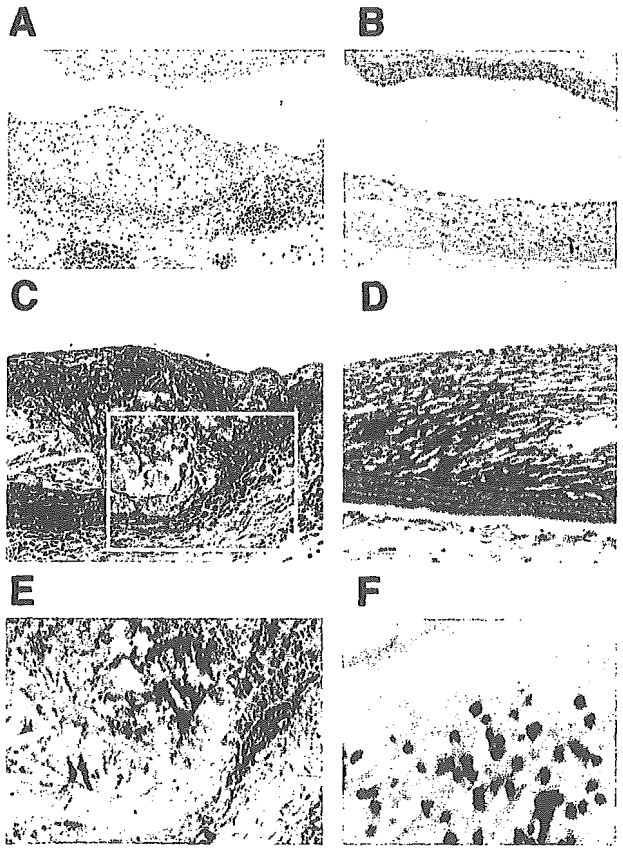

Figure 4. Representative photomicrographs of descending aorta of IL-1Ra−/−/apoE−/− and IL-1Ra+/+/E−/− mice at 32 weeks old. Histology stained by hematoxylin and eosin of descending aortas of IL-1Ra−/−/apoE−/− (A) and IL-1Ra+/+/E−/− mice (B). Original magnification ×100. Histology stained by elastin staining of descending aortas of IL-1Ra−/−/apoE−/− (C) and IL-1Ra+/+/E−/− mice (D). Original magnification ×150. Boxed area is shown (E). E, Section was stained by immunohistochemical staining for α-SMA. Original magnification ×150. F, The panel shows MOMA-2 staining of adventitia of the IL-1Ra−/−/apoE−/− mice 32 weeks old. Most inflammatory cells in the adventitia stained positively for MOMA-2. Original magnification ×200.

IL-1Ra+/+/apoE−/− mice (data not shown). Most inflammatory cells in the adventitia of IL-1Ra−/−/apoE−/− mice stained positive for MOMA-2 (Figure 4F). These results suggest that a complete IL-1Ra deficiency may cause not only atherosclerosis but also severe aortitis.

Discussion

In the present study, lowering serum levels of IL-1Ra in IL-1Ra+/−/apoE−/− mice to approximately half of those in IL-1Ra+/+/apoE−/− mice resulted in the significant increase of atherosclerotic lesions formed. These results are supported by a previous study, demonstrating that IL-1Ra−/− mice showed an increase in fatty streak lesion size in the diet-induced atherosclerosis model.[27] On the contrary, IL-1Ra–overexpressing apoE−/− mice were protected from aortic lesion formation without changing plasma lipid levels.[27] Recently, we demonstrated decreased severity of atherosclerosis in apoE−/− mice deficient for IL-1β.[28] These reports suggest that IL-1 signaling promotes inflammation in the vascular wall, thus contributing to the development of atherosclerosis.

Although the sizes of aortic sinus lesions in IL-1Ra+/−/apoE−/− mice at 32 weeks tended to be larger compared with those in IL-1Ra+/−/apoE−/− mice, the differences failed to achieve statistical significance. However, immunohistochemical analysis revealed a marked increase in the MOMA-2 stained lesion area in IL-1Ra+/−/apoE−/− mice compared with those in IL-1Ra+/+/apoE−/− mice. Furthermore, lesional α-SMA staining was significantly decreased. These results suggest that endogenous IL-1Ra has little implications on the suppression of atherosclerotic lesion size in advanced atheroma but plays an important role in early atherogenesis and modulates plaque composition during lesion progression. The present study is the first to demonstrate that IL-1Ra plays an important role in the modulation of advanced plaque composition, because Devlin et al reported about the only early fatty streak lesions in IL-1Ra−/− mice.[27] Furthermore, our present findings might have clinical implications. Unstable atherosclerotic plaques are characterized by increased accumulation of macrophages and decreased SMC content, rendering lesions more prone to rupture and subsequent vessel thrombosis than stable plaques with less macrophages and increased accumulation of SMCs.[29] Although the murine model of atherosclerosis used here does not allow a direct evaluation of plaque vulnerability to rupture, our results suggest that IL-1Ra deficiency is likely to alter plaque stability.

Our real-time polymerase chain reaction analysis revealed that the lack of IL-1Ra caused the upregulation of IL-1β, MCP-1, and adhesion molecules at the mRNA levels in the aorta. These changes may contribute to the enhanced accumulation of macrophages/monocytes in the advanced plaque. MCP-1 belongs to the group of CC chemokines that are involved in the recruitment of leukocytes to inflammatory sites and might be critically involved in monocyte/macrophage recruitment to atherosclerotic lesions.[30] Furthermore, previous studies have shown that antibody blockade of VCAM-1 significantly reduced monocyte rolling and adhesion in perfused carotid arteries isolated from apoE−/− mice[31,32] and that local overexpression of MCP-1 at the vessel wall induces the infiltration of macrophages and formation of atherosclerotic lesions.[33] These reports suggest that MCP-1 and adhesion molecules play an important role in the recruitment of monocytes to the arterial intima.

Finally, in the present study atheroma in IL-1Ra−/−/apoE−/− mice displayed inflammation of the adventitia. These results are supported by a previous report. Nicklin et al showed that IL-1Ra−/− mice (on the 129/O1a × MF1 background) had aortic inflammation and that provided evidence for the formation of some aneurysm.[34] This group suggested that IL-1Ra plays an important role in the suppression of aortic inflammation. Although our IL-1Ra−/− mice on the C57BL/6J background did not show aortic inflammation, IL-1Ra−/−/apoE−/− mice at 32 weeks old had severe aortitis. These results suggest that chronic inflammation caused by atherosclerosis and/or hypercholesterolemia might trigger inflammation in the adventitia in mice deficient for IL-1Ra. Thus, the present findings suggest a novel pathway implicated in the development of aneurysm caused by atherosclerosis and/or hypercholesterolemia, because previous reports demonstrated that adventitial inflammation, induced by hypercholesterolemia and irritants ($CaCl_2$ or thioglycollate), induced the development of aortic aneurysm in rabbits.[35] However, further studies are needed to clarify these mechanisms using our IL-1Ra−/−/apoE−/− mice.

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