S/D ratio <1	*91 (57.6%)*	*48 (42.1%)*	*0.016*
AR-A duration	*52.3±24.1*	*52.2±21.9*	*NS*
AR-A duration >30 ms	*41 (25.9%)*	*39 (34.2%)*	*NS*
Isovolumetric relaxation time (ms)	*68.5±24.0*	*67.7±22.5*	*NS*
Functional and prognostic indices			
NYHA functional class			
Class I	*71 (45.2%)*	*41 (36.4%)*	*NS*
Class II-III	*85 (53.8%)*	*73 (63.6%)*	
Class IV	*2 (1.3%)*	*0 (0.0%)*	
Charlson co-morbidity index score			
0	*50 (31.6%)*	*47 (41.2%)*	*NS*
1	*49 (31.0%)*	*26 (22.8%)*	
2	*27 (17.1%)*	*18 (15.8%)*	
3	*10 (6.3%)*	*12 (10.5%)*	
≥4	*22 (13.9%)*	*11 (9.6%)*	

The Echo data shown are those obtained on initial recruitment of IDD.
Values are mean ± standard deviation or number of subjects (%).
Normal geometry: RWT ≤0.42 and LVMI ≤115 (95) g/m².*
Eccentric hypertrophy: RWT ≤0.42 and LVMI >115 (95) g/m².*
Concentric hypertrophy: RWT >0.42 and LVMI >115 (95) g/m².*
Concentric remodeling: RWT >0.42 and LVMI ≤115 (95) g/m².*
**Values for females.*
IDD, isolated diastolic dysfunction; NS, non-significant; CHF, congestive heart failure; CKD, chronic kidney disease (serum creatinine ≥2 mg/dl, or regular hemodialysis); ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin-receptor blockers; Echo, echocardiographic; RWT, relative wall thickness; LVMI, left ventricular mass index; NYHA, New York Heart Association.

Discussion

The presence of IDD in asymptomatic individuals is an independent predictor for the development of CHF, so early identification and lifelong treatment of IDD is important for preventing progression of this serious condition? Not a few patients are presumed to be under treatment for IDD, but information is scarse in Japan. Our study design with its long recruitment period in an isolated area of the country was advantageous in delineating the epidemiological and clinical features of patients with IDD. We have made the following observations: (1) the number of outpatients with moderate to severe IDD is 0.5–1.5% of the elderly population; (2) the ratio of IDD to SD patients is larger in females (2.4) than in males (0.7); (3) the prevalence of hypertension and MI of this IDD cohort was similar to the estimates for IDD cohorts in the US, but lower than those of DHF cohorts in the US and Asia, whereas diabetes was more frequently observed in the present IDD cohort than in those from the US; (4) the LV mass index (LVMI) was high, but the LV geometry was diverse (with 31% of patients showing normal geometry; (5) although the majority of IDD patients had a rather low CCI score, their prognosis, even without a history of CHF, was worse than that of general population; and (6) in both genders, higher age at diagnosis and the presence of CKD were independent predictors of mortality, whereas treatment with CCBs had a favorable survival benefit. Additionally, the presence of diabetes (in males) and history of CHF (in females) were indicators of bad prognosis.

In this study, the total numbers of IDD and SD patients increased with age, which is a common observation reported by some previous studies[2,3] SD was dominant in males, whereas IDD prevailed in female patients. We also found that the proportion of IDD patients in the general population was 0.9% for males and 0.5% for females in the age range of 45–84 years, based on the number of survivors in 2003 who were confirmed to have moderate to severe IDD. These estimates differ considerably from the prevalence rates reported by population-based studies in the US and Australia (5.0–6.2% for males and 6.3–7.3% for females)[2,3] In fact, detailed comparison would seem impossible for several reasons, such as ethnic, geodemographic, and methodological differences.

Table 3 Independent Predictors of Mortality in Males and Females With IDD

	β-coefficient	*OR*	*95%CI of OR*	*p value*
Males				
Age (years)	*0.132*	*1.141*	*1.080–1.207*	*<0.001*
CKD	*1.116*	*3.053*	*1.244–7.489*	*0.015*
Diabetes	*1.042*	*2.836*	*1.271–6.326*	*0.011*
CCBs	*–0.169*	*0.184*	*0.068–0.499*	*0.001*
Females				
Age (years)	*0.110*	*1.117*	*1.036–1.204*	*0.004*
CKD	*2.692*	*14.75*	*2.231–97.58*	*0.005*
CHF	*1.884*	*6.578*	*1.284–33.71*	*0.024*
CCBs	*–2.756*	*0.064*	*0.009–0.441*	*0.005*

OR, odds ratio; CI, confidence interval; CCBs, calcium channel blockers. Other abbreviations as in Table 2.

The literature indicates that patients with DHF are more likely to be females than those with SHF. In contrast to these US reports, males were dominant in our IDD cohort (58%) as well in the DHF cohort reported by Tsutsui et al (49–61%)[15] The reasons behind the male predominance remain unknown, but referral bias may explain this finding. Also, the literature indicates gender differences regarding consultation, examination, and treatment in cardiac patients;[16] which were unavoidable in our study owing to its hospital-based design. Thus, for comparing IDD and SD, it would be more appropriate to calculate the IDD/SD ratio after stratification by gender. This ratio was 0.7 for male and 2.4 for female patients, indicating more prevalence of IDD than SD among female patients who underwent Echo in this study. Therefore, we might suppose that a population-based study of IDD that could carefully consider gender matching may demonstrate female predominance of IDD in the general population in Japan.

In a large population-based study conducted in Western

communities, it has been reported that age, hypertension, diabetes, and MI were independent predictors of DD[2,3] In Asian communities, these comorbidities are also associated with DHF. Tsutsui et al reported the clinical characteristics of Japanese patients with DHF (99 patients, average age 69 years) in Fukuoka[15] In their study, the prevalence of hypertension, MI, and diabetes was 60%, 31%, and 28%, respectively. Similarly, Yip et al reported the prevalence of these comorbidities in Chinese patients with DHF (132 patients, average age of 73 years, 56% females) in Hong Kong; they found a prevalence of hypertension, MI, and diabetes of 57%, 14%, and 35%, respectively[17] Compared with those reports, the prevalence of each of these comorbidities (hypertension, MI, and diabetes) was slightly lower in our IDD cohort (46%, 12%, and 24%, respectively). In addition, the majority (90%) of our IDD cohort did not have a history of validated CHF, in contrast with the Asian DHF cohorts in which most patients had such a history[15,17] Therefore, these comorbidities may be significant risk factors for the advancement of IDD to DHF in Asian patients.

Regarding the Western populations, Vasan et al reported the clinical characteristics of 37 white patients admitted with DHF in Framingham (average age of 72 years, 65% females)[18] The prevalence of hypertension, MI, and diabetes was 75%, 24%, and 14%, respectively. In Olmsted County, Senni et al studied 59 white patients with DHF (average age of 78 years, 69% female)[19] and reported a prevalence of hypertension (58%) and MI (15%) nearly similar to ours. In comparison with the estimates reported by studies of Asian populations (described in the previous paragraph), it could be noted that the prevalence of these comorbidities in Caucasian cohorts with DHF is closer to those in Asian communities. A similar prevalence of comorbidities in both DHF and IDD cohorts has been observed in Western communities[2,18,19] Redfield et al conducted a population-based study in Olmsted County, and identified 118 residents with moderate IDD without a previous history of validated CHF[2] The prevalence of hypertension, MI, and diabetes in their cohort was 47%, 10%, and 9%, respectively, notably lower than the values found in some DHF cohorts in the US[18,19] but rather closer to those found in our IDD cohort (except for diabetes).

The Doppler Echo selection criteria used in this study tended to include typically moderate to severe IDD patients; that is, DCT <140 ms, S/D ratio <1, and AR-A duration >30 ms were observed in 45%, 51% and 29%, respectively, of the patients, and the IRT was shortened. When divided by LVMI only, the LV geometry was hypertrophy in 54% of males and 69% of females[5] On the other hand, when divided by LVMI and RWT (relative wall thickness)[5] the proportions of the 4 categories of LV geometry showed little differences, especially in males (Table 2). Thus, identifying IDD may be difficult without using a Doppler Echo technique, owing to the diverse nature of LV geometry.

It is not easy for conventional Doppler Echo techniques to evaluate the diastolic function in patients with chronic AF, so we defined preserved systolic function associated with DCT <140 ms as "restrictive DD", regardless of the presence of AF. There were several reasons for using this definition in our study. First, AF is common in patients with restrictive pathology, even though 12% of patients with normal sinus rhythm in this study had a documented history of AF (ie, paroxysmal AF). Second, an inverse relationship was reported between DCT and pulmonary capillary wedge pressure (PCWP) in patients with AF, so a short DCT highly predicts elevated PCWP[20] Furthermore, patients with and without AF who had a DCT <130 ms showed a similarly poor prognosis[21] Third, this study aimed to evaluate the actual burden of IDD in the community, with its design targeting the high inclusion of clinically significant IDD subgroups (moderate to severe cases only, as the inclusion of mild cases will tend to underestimate the burden of IDD owing to the generally asymptomatic nature of the latter). Thus, we used conventional Echo-Doppler methodology that could be performed in most hospitals, using inclusion criteria that comprised undetermined and suspected IDD individuals (including AF cases) as well.

In comparison with the general population, our IDD patients (either with or without history of validated CHF) had poor survival, which may be related to the impact of IDD itself and/or other coexisting factors (such as diabetes, renal failure, cerebrovascular diseases, and dementia) that would predispose the patients to poor prognosis. The majority of patients (63% of males and 64% of females) had CCI score ≤1, but one-third of them had a score ≥2, which made them a poor prognosis group according to some previous reports[22,23] Additionally, some of the comorbidities (diabetes, CHF, and CKD), which were also scored by the CCI, were shown to be independently associated with death in our cohort. In general, the higher the prevalence of poor prognostic comorbidities, the worse the patient's survival will be. Therefore, aging communities should pay attention to IDD and its associated comorbidities to reduce their future burden.

Study Limitations

First, because this study was hospital-based and not population-based, we may have missed latent IDD patients in the community who did not undergo Echo. Also, individuals with mild DD (impaired relaxation) were not included. Accordingly, estimation of the precise prevalence of the whole spectrum of IDD in the general population was not possible; however, we have shown the proportion of moderate to severe Echo-documented IDD. Moreover, the use of tissue Doppler for identifying IDD was not feasible. Nevertheless, we can suppose that diagnosing most patients with moderate to severe IDD could be easily done using conventional Echo-Doppler, and hence delineating the epidemiological and clinical features of these patients would closely mimic reality. Second, the limiting the study to Sado Island may restrict generalization of the results to other areas in Japan. Third, 72% of patients, especially the asymptomatic ones, did not undergo diagnostic coronary angiography. In this respect, it is known that noninvasive diagnostic processes have limitations in precisely classifying the disease etiology. Fourth, we tried to evaluate diastolic function in all patients; but this was not actually possible, especially in the elderly. Therefore, we might have underestimated the disease burden in the elderly patient category.

Conclusions

We have carried out the first enumeration survey of moderate to severe IDD in a Japanese community. The Sado Heart Failure Study is a highly inclusive hospital-based study that may be able to characterize patients with IDD in the community. Outpatients with moderate to severe IDD are progressively increasing with aging of the popultion. These patients often have multiple coexisting disorders,

making the outcome of IDD poor, regardless of the presence of a CHF history. The disease characteristics and living conditions of patients should be taken into consideration when establishing preventive strategies for HF in the Japanese community.

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治療　ガンマグロブリン無効例への対応

蛋白合成酵素阻害薬ウリナスタチン療法

佐地　勉

Clinical utility of ulinastatin, urinary protease inhibitor in acute Kawasaki disease

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Abstract

Ulinastatin, a trypsin inhibitor, is useful as a first-line or a second-line treatment regimen including alternative therapy for IVIG-resistant or IVIG nonresponder Kawasaki disease(KD) patients. Mechanisms involving protections against tissue organs and endthelial cell and anti-inflammatory effects by ulinastatin, are dependent on the inhibition of PMN-derived elastase, tumor necrosis factor alpha(TNFα), and other proinflammatory cytokines/interleukins(IL-1, IL-6, IL-8). Ulinastatin also suppresses the activation of PMN cells, macrophages, and platelets.

Although almost no statistical data related to the definitive effect in acute stage of KD, ulinastatin have shown possible effects, but not always, in a part of KD patients. The indications of clinical use include shock and pancreatitis. Off-label uses of ulinastatin have been reported in hematological, hepatic, renal, OB/Gy diseases and cardiovascular diseases including vasculitis syndromes. The efficacy of ulinastatin in aKD remained to be investigated.

Key words: ulinastatin, protease inhibitor, Kawasaki disease, TNFα, elastase

はじめに

年間発症数が1万人を超す勢いで増加する川崎病(KD)の最大の治療上の克服すべき問題点は，免疫グロブリンIVIG不応例に対する，二次的治療法の選択である．

2年ごとに行われている第17回全国調査のサーベイランスでは，急性期KD(aKD)に対して初回IVIGへの不応例には，44％の施設で小児科医はIVIGの再投与を選択し，その次には17.6％の頻度でIVIG+ulinastatinが投与されていた[1]．

2003年2月に日本小児循環器学会で制定された，川崎病急性期治療のガイドラインでは，IVIG療法以外の追加治療手段の中に，ulinastatin 5,000単位/kg/日，×3-6回/日，の投与が示されている(日本小児循環器学会学術委員会，川崎病急性期治療のガイドライン．日小循誌 20: 54-62, 2004)．また第18回川崎病全国調査成績では，全体の中での追加治療薬剤はIVIGが15.6％で，次いでulinastatin 7.5％，ステロイド3.1％の順であった(川崎病研究グループ，2005

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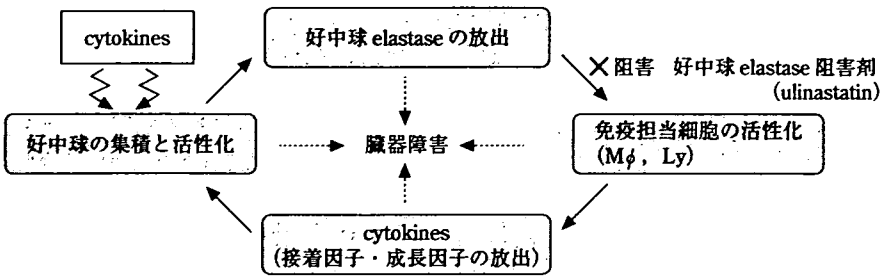

図1 Ulinastatinの作用点
(小川道雄：消化器外科 20: 565-572, 1997より引用)

表1 Ulinastatinの生物学的作用

1. 種々のプロテアーゼ阻害
   trypsin＞α-kimotrypsin＞N-elastase＞N-oathepsinG＞plasmin＞P-elastase＞Hiarulonidase＞lipase＞amylase＞enterokinase
2. サイトカイン産生抑制
   IL-1α, IL-1β, IL-6, IL-8, TNF-α, 接着因子
3. NFκB伝達経路阻止作用
4. 好中球，マクロファージ，血小板の活性化抑制，elastase遊離/活性酸素産生抑制，細胞障害抑制
5. ライソゾーム膜安定化作用
6. 実験的炎症(肺，肝，腎，膵)やショックの軽減
7. 細胞外matrix形成抑制

年度).

1. Ulinastatinの作用機序

a. TNFα抑制作用

冠動脈再灌流障害では，ulinastatinは術後の多核白血球からのelastase，TNFα，IL-6，IL-8の遊離を抑え，また肺の再灌流障害を抑え，肺機能を保護する[2,3].

ulinastatinの単球からのTNFα産生抑制作用は，ショックにおけるその産生か分泌を抑制するのに対し，gabexate mesilateはNFκB活性化を強く抑制するためである[4]．またTNFαにより活性化されて出現する内皮細胞上のICAM-1の発現を抑制して，内皮細胞を保護する機能がある[5].

b. その他の作用

リソゾーム膜の安定化作用により各種の蛋白分解酵素の遊離を抑制する．例えばTNFαを含む心筋抑制因子や毒性因子の遊離阻害作用もある．その他，凝固阻害作用，血管内皮細胞(EC)成長促進などの作用もある．ulinastatinは，特に好中球からのelastaseの放出阻害と，放出後の不活化の両方に作用し，結果的に酸素ラジカルの除去，サイトカイン・接着因子の活性を低下させる(図1)．そのほか，報告されている多彩な生物学的な作用機序をまとめて列記する(表1).

2. 川崎病での作用点

aKDでは，多核白血球(PMN)でPGH_2とTXA_2のmRNAが亢進しているが，ulinastatinはこれを抑制する[6].

さらに好中球により誘導される内皮細胞障害を，顆粒球から分泌された細胞外elastaseの不活化ばかりでなく，好中球に直接作用してelastaseの産生と分泌を抑制し防御すると考えられている[7,8].

aKDに対するulinastatinの最初の使用報告例は，1993年の岡田(山形大)の報告と思われる[9].

その後90年代には症例報告が相次いだ．そ

表2 Ulinastatin 5,000 U/kg×3回/日×1日の併用例と非併用例(IVIG+ASA)の比較

	ulinastatinの前投与11例	ulinastatinの非投与11例	p値
発症病日	34±21	27±29	NS
入院病日	4.7±1.3	4.1±2.4	NS
原田のスコア	3.8±0.9	3.7±0.9	NS
入院時			
WBC(×10³/μl)	15.2±7.0	17.0±5.7	NS
CRP(mg/dl)	7.7±3.5	8.5±5.1	NS
入院後発熱日数	3.9±0.9	4.8±2.8	NS
CRP陽性日数	11.1±3.0	13.3±4.1	NS
γ-glob投与量(g/kg)	1.5±0.7	1.5±0.7	NS
冠動脈病変	1例	1例	NS

の症例研究の結果として①軽症例での単独の効果，②併用によるIVIGの減量効果，③IVIG無効例・不応例・抵抗例，および再燃例への有効性，があげられる[10]．CRP値やWBCの増加が少ない軽症例では，ときにIVIGを使用せずアスピリン(ASA)との併用でも治療が可能である．またIVIG投与や再投与後の再燃例，さらにIVIGに全く反応しない症例や，いわゆる不応例にもときに有効であるという意見が多数を占める．

ulinastatinをfirst lineの治療として用いた場合，57%(5,000単位/kg×6)～64%(5万単位×6)はulinastatin使用後のIVIGを必要としなかった(IVIG回避例)，またIVIGとの併用により，重症例やいわゆるhigh risk例ではIVIGの使用量を減少させているとする報告がある[11,12]．

ほとんどの施設では急性期治療薬の第一選択としてIVIGが用いられているが，約15-20%に存在する不応例に対してulinastatinは追加治療薬の一つとして位置付けられている．

著者らの行った5,000単位/kg×3回/日のpilot studyでは14例中5例に効果があり，軽症例3/3例，再燃例2/5例，重症例1/3例と合わせると，すなわち25例中11例で有効であった[13]．

ulinastatinはelastaseの産生，放出を抑制していることがうかがわれる．しかしIVIG療法でも顆粒球elastaseは低下するため[14]，elastase以外のサイトカイン(IL-1，IL-6，TNFα)の産生抑制やNO_3^-の低下，マクロファージ抑制などの可能性がある．

3. Ulinastatinの併用効果

著者らは，ulinastatin(5,000単位/kg×3回/日)+IVIG(1g/kg/1日)+ASA(50mg/kg)の3者併用と，IVIG(1g/kg/1日)+ASAを比較しulinastatin投与24時間後でIVIG投与前の臨床検査を比較した[10](**表2**)．その結果，ulinastatinの前投与ありのIVIG+ASAでは，若干入院後発熱期間が短く，CRPも低下したが，冠動脈拡大率も有意差は得られなかった．その結果，投与前年齢，入院病日，原田スコア，WBC，CRPに有意差なく，また治療後のCRP陽性日数，IVIG投与量，冠動脈病変合併率にも有意な差がみられなかった．つまり，IVIG+ulinastatin併用(IVIGの前投与)がIVIG単独療法に勝るという結論は得られなかった．他の報告でも，冠動脈病変合併率は差がない[15]．

しかし，ulinastatin使用前後で，サイトカイン，superoxide dismutase，NO_3^-，neopterineを測定し変動を観察してみると，NOx(NO_3^-)とIL-1βの低下作用が強い傾向がみられた(**図2**)．NO_3^-は30.9±17.3から使用後，18.4±9.4(μmol/l)(p<0.01)へと有意に低下した．

4. Ulinastatinの対象疾患

承認されている適応症は2つの疾患群である．

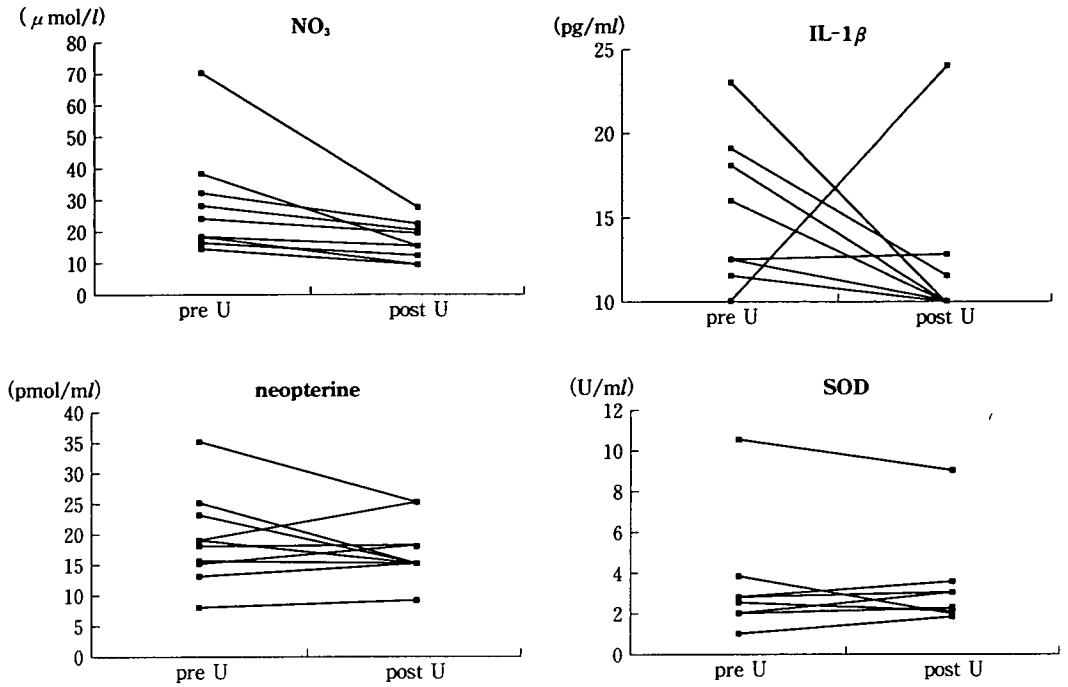

図2 Ulinastatin使用前後のNO_3, IL-1β, neopterine, SODの変化

表3 Ulinastatin(ミラクリット)の適応疾患とoff-labelとして有効な疾患

適応疾患：
1. 急性循環不全(出血性，細菌性，外傷性，熱傷性ショック)における循環動態の改善
2. 急性膵炎(術後，外傷性，ERCP後)，慢性再発性膵炎の急性増悪期

適応外off-label使用の報告：

炎症性疾患：
川崎病，血管性紫斑病，Stevens-Johnson症候群，関節リウマチ，肺アスペルギルス症

消化器疾患：
潰瘍性大腸炎，肝切除後肝障害

腎疾患：
腎機能障害，シスプラチン腎障害，溶血性尿毒症症候群

婦人科疾患：
切迫早産(頸管中コラーゲン分解抑制)，子宮収縮抑制，絨毛羊膜炎，子宮頸管炎，膣炎，双胎間輸血症候群，羊水塞栓

ショック：
体外循環後循環不全，熱傷，癌転移抑制，外科手術周術期管理，出血性ショック

血液疾患：
DIC，AML，造血器腫瘍性疾患，ペルオキシダーゼ陽性白血病

その他：
糖尿病性末梢神経傷害，ラットの実験的関節炎

適応外のoff-label使用での有用性の報告もある(**表3**).

成人での使用量は，急性膵炎：25,000-50,000単位/500 ml輸液/1-2時間×1-3回/日で，急性循環不全時：10万単位/500 ml輸液/1-2時間×1-3回/日である．もう一つの抗elastase薬

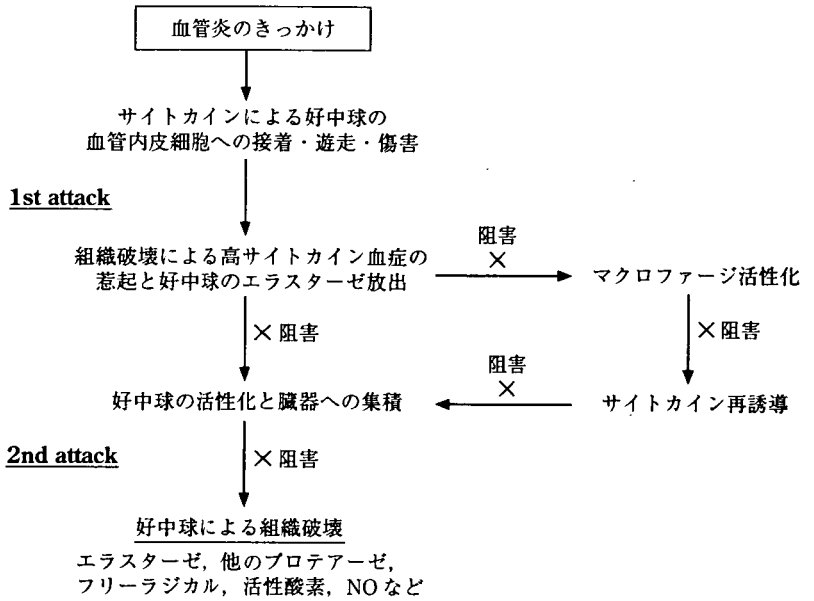

図3 組織障害における高サイトカイン血症と好中球エラスターゼ
(×阻害：ulinastatinによる阻害)(文献[16]より引用)

sivelestat(エラスポール)は好中球のelastaseを選択的に阻害して血管内皮細胞傷害を抑制し，蛋白漏出や出血を軽減する．ARDS/ALIでは呼吸器管理日数の短縮や呼吸状態の改善が認められる．

最近では，組織障害によって，サイトカインが放出されてfirst attackが起こり，それに続いて好中球が活性化されるsecond attackが生じるという2段階の障害仮説が提唱されている(図3)．

おわりに

aKDにおけるIVIG不応例の中には冠動脈後遺症を来す頻度が極めて高い．その治療薬剤の選択には臨床医にも迷いがあり，ごく一部の症例にみられる巨大冠動脈瘤という最悪のシナリオが回避できないことがある．その中で我が国でのみ使用できるulinastatinの可能性は，諸外国の研究者にも期待されているところが多く，このまま永遠のmisteryになってほしくないと感じている．

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Circ J 2007; **71**: 357–362

Abnormal Tissue Doppler Images are Associated With Elevated Plasma Brain Natriuretic Peptide and Increased Oxidative Stress in Acute Kawasaki Disease

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Background The aims of this study were to evaluate myocardial mechanics using pulsed tissue Doppler imaging (TDI), and to determine the relationship between abnormal myocardial performance and plasma brain natriuretic peptide (BNP) levels and oxidative stress in acute Kawasaki disease (KD).

Methods and Results Consecutive TDI parameters, including peak systolic velocity (Sw) and early (Ew) and late diastolic excursion of the mitral annuli were obtained in 42 patients with KD (mean age: 2.4±0.4 years) in weeks 1, 2, and 3, and during convalescence. Plasma BNP level and urinary 8-isoprostane were also examined during the acute phase. These data were then compared with TDI profiles from 62 healthy children, plasma BNP levels in 38 controls with other febrile illnesses, and urinary 8-isoprostane levels in 13 healthy children. Ew in week 1 was significantly lower than in controls, subsequently normalizing in the convalescent stage. Plasma BNP level in acute KD patients was significantly higher (65±9 pg/ml) than in controls (13±2 pg/ml). Urinary 8-isoprostane level in acute KD patients was significantly higher as compared with control (596±37 vs 379±26 pg/ml Cr, $p<0.05$). There was a significant negative correlation between week 1 Sw and plasma BNP level ($r=-0.55$, $p=0.0001$). Change in Sw velocity in the BNP ≥51 group was significantly greater than in the BNP <51 group. There was a significant negative correlation between week 1 Sw and urinary 8-isoprostane level ($r=-0.48$, $p=0.001$).

Conclusions Latent abnormal tissue Doppler profiles, possibly reflecting long-axis systolic and diastolic dysfunction have been noted in KD patients. Abnormal myocardial mechanics may contribute to the increased plasma BNP level and enhanced oxidative stress may contribute to cardiac dysfunction in KD. (*Circ J* 2007; **71**: 357–362)

Key Words: Brain natriuretic peptide; Kawasaki disease; Oxidative stress; Tissue Doppler echocardiography

Kawasaki disease (KD) is a systemic vasculitis that primarily affects small and medium-sized arteries[1] Transient myocardial dysfunction in children with acute KD has been reported[2,3] and moreover, myocardial inflammation, including myocarditis and microvascular damage in the myocardium, has been shown to cause aberrations in cardiac function[4] Several recent studies have discussed the usefulness of tissue Doppler imaging (TDI) to evaluate left ventricular (LV) function[5–11] Tissue Doppler measurement of myocardial Doppler velocity can be used to measure long-axis functions, which seem to be both more sensitive to minor disturbances in LV function and relatively preload-independent[11,13–11] In the present study we used TDI to evaluate disturbance of myocardial function in acute KD patients.

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The plasma brain natriuretic peptide (BNP) level is associated with cardiac function in adult patients with congestive heart failure[15,16] Although elevation of BNP has been reported in acute KD patients[17] no relation between BNP and cardiac function has been found.

Reports have suggested that increased systemic oxidative stress is associated with progression of cardiovascular disease, including ischemia–reperfusion injury, atherosclerosis, and heart failure[18–24] The isoprostanes are a complex family of compounds produced from arachidonic acid via a free radical-catalyzed mechanism[18,19] The level of 8-isoprostane is used as a pathophysiological marker of lipid peroxidation. We previously reported elevation of urinary 8-isoprostane levels in acute KD patients[25] and although the exact pathological role of increased oxidative stress in acute KD is uncertain, we believe that it is associated with oxidative injury to systemic vessels[25] We hypothesize that this combined with disturbance of myocardial microcirculation may contribute to myocardial dysfunction in acute KD. Therefore, the purpose of the present study was to investigate the relationship between abnormal myocardial performance and plasma BNP levels, and to investigate the association between abnormal myocardial performance and enhanced oxidative stress.

Methods

Forty-two children with acute KD (boys/girls: 24/18, mean age: 2.4±0.4 years) were enrolled. All patients satisfied the diagnostic criteria; those with atypical KD were excluded. The children were admitted to hospital between April 2002 and July 2003. All patients were treated initially with a single dose of intravenous immune globulin (IVIG), 1–2 g/kg, in a single infusion over 12h and were given aspirin orally within 7 days of onset. IVIG was begun 5.0± 0.7 days (range 4–7 days) after onset. Twenty-eight children received a single dose of IVIG (total dose 1–2 g/kg), 8 required additional IVIG following the initial IVIG treatment (total dose 3–4 g/kg), and 6 underwent steroid pulse therapy (20–30 mg/kg of methylprednisolone after IVIG) because of IVIG-resistant KD. Mean plasma C-reactive protein (CRP) level before initial IVIG therapy was 8.0± 0.6 mg/dl. Limited pericardial effusion and mitral valve regurgitation were observed in 7 and 6 patients, respectively. Gallop rhythms were detected in 4 patients and 2 patients underwent short-term dobutamine therapy because of transient cardiac hypokinesis. Transient coronary artery dilatation was observed in 2 patients after that treatment regimen and in 2 convalescent-phase patients.

Echocardiography

Echocardiographic measurements were obtained with a commercial ultrasound system (Acuson SEQUOIA C256; Siemens, Mountain View, CA, USA). Complete 2-dimensional and conventional color flow imaging and Doppler echocardiography were performed initially. Heart rate (HR), LV shortening fraction (LVSF), LV end-systolic dimension (LVDs), LV end-diastolic dimension (LVDd), and mitral valve inflow Doppler pattern analysis of peak E-wave and A-wave velocities were calculated in the standard manner. End-systolic wall stress (ESWS) and HR-corrected velocity of circumferential fiber shortening (VCFc) were also calculated using established techniques[26] After these echocardiographic evaluations, apical 4-chamber perspective tissue Doppler echocardiography was performed to measure longitudinal annular velocities at the lateral mitral wall. The first systolic (Sw), early diastolic (Ew), and late diastolic (Aw) tissue Doppler velocities were measured at the lateral mitral wall and averaged over 3 cycles in accordance with previous reports[5–14] These echocardiographic evaluations were done in week 1 (before starting IVIG therapy), week 2 (≈7 days after starting IVIG therapy), week 3 (≈14 days after starting IVIG therapy), and in the convalescent stage (a mean of 5, 12, 19, and 36 days after onset). All patients agreed to initial echocardiographic evaluations before starting IVIG therapy in week 1. Changes from baseline (%) in Ew, Aw, and Sw were calculated as: {(the velocities at each phase−the velocities on week 1)/the velocities on week 1}× 100. The transmitral E/Ew ratio was calculated for each patient. Echocardiographic measurements in 62 age-matched healthy children without cardiac disease (mean age: 2.4± 2.0 years) were also obtained as the control (TDI control group). All examinations were done by the same physician. Intraobserver variability was estimated as 4%.

Measurement of Plasma BNP and Urinary 8-Isoprostane Concentrations

Blood samples were obtained to measure the plasma concentration of BNP. Acute phase concentrations were measured before IVIG therapy (mean 5.0±0.7 days after onset) and during the convalescent phase (mean 36±1 days after onset), using an immunoradiometric assay from a commercially available kit (Shionogi Co Ltd, Osaka, Japan). Plasma BNP levels in 38 age-matched and CRP-matched (age: 2.8± 0.8 years, CRP: 9.2±0.8 mg/dl) children with other acute febrile illnesses were measured as a control (BNP control group) for comparison with the acute KD group.

Urinary 8-isoprostane levels were measured during the acute phase before IVIG therapy (mean 5.0±0.7 days after onset). Levels in 13 healthy children (mean age 3.0±1.0 years) were obtained as a control (8-isoprostane control group). The concentration of free 8-isoprostane was analyzed using a commercially available enzyme immunoassay kit (Cayman Chemical, MI, USA)[27] To eliminate contaminants, urine samples were purified before analysis with ODS gel (Silica Gel ODS-Q3, Fiji Gel, Tokyo, Japan) followed by a NH_2 Sep-Pak column (Sep-Pak, Vac NH_2, Waters, MA, USA) as previously reported[28] The detection limits of the assay were between 7.8 and 200 pg/ml. Microtiter assay plates were scanned with a computer-controlled adjustable wavelength microtiter plate reader. Results are expressed as pg/mg urinary creatinine (Cr).

The study protocol was approved by the ethical committees of the medical centers involved, and informed consent was given by all subjects.

Statistical Analysis

Results are expressed as means±SEM. The statistical significance of differences between groups was determined by 1-way ANOVA followed by Tukey-Kramer's test. The unpaired T-test was used to analyze the difference between the control group and KD group in urinary 8-isoprostane levels. Differences in consecutive changes of echocardiographic parameters among each group were analyzed by repeated measures ANOVA. Pearson's correlation coefficient analysis was used to assess the association among echocardiographic parameters, plasma BNP and urinary 8-isoprostane level. All data analyses were performed with a commercially available statistical analysis software package (Statview 5.0, Abacus Concepts Inc, Calabasus, CA, USA). $P<0.05$ was considered significant.

Results

TDI in Acute KD Patients (Table 1)

HR was significantly lower after IVIG treatment. Blood pressure (BP), ESWS, VCFc, LVDd, LVDs, and LVSF did not significantly change. Ew velocity in week 1 was significantly lower than in the controls and subsequently normalized during the convalescent stage. Mitral inflow E/A and Ew/Aw at week 1 were both significantly lower than in controls and normalized in the convalescent phase. This finding was observed for Ew/Aw compared with E/A. E/Ew at week 1 was significantly higher as compared with control, but subsequently normalized during the convalescent phase. Sw, VCFc, ESWS, and LVSF showed no significant change at any stage. Sw velocity did not significantly correlate with LVSF at any stage ($r=0.1$, $p=0.19$). There were no significant differences between children with or without pericardial effusion, mitral valve regurgitation, or coronary artery lesion. There were no significant differences in TDI parameters between the responders to initial IVIG therapy (n=28) and non-responders (n=14).

Table 1 Conventional Echocardiographic and Tissue Doppler Parameters in Acute KD Patients

		KD			
	Control	*Week 1*	*Week 2*	*Week 3*	*Convalescent phase*
HR (beats/min)	102 (3)	136 (4)*	108 (3)†	107 (2)†	108 (2)†
Systolic BP (mmHg)	98 (1)	100 (1)	93 (2)	94 (1)	97 (1)
UCG					
LVDd (cm)	2.9 (0.1)	2.9 (0.1)	2.9 (0.1)	3.0 (0.1)	3.0 (0.1)
LVDs (cm)	1.9 (0.1)	1.8 (0.1)	1.9 (0.1)	1.9 (0.1)	1.9 (0.1)
LVSF (%)	35 (0.5)	34 (0.8)	35 (0.6)	36 (0.5)	36 (0.6)
ESWS	54 (2)	55 (2)	52 (2)	54 (2)	56 (2)
VCFc	1.14 (0.02)	1.15 (0.02)	1.16 (0.02)	1.15 (0.01)	1.15 (0.1)
Mitral inflow E/A	2.0 (0.1)	1.4 (0.1)*	1.6 (0.1)*	1.6 (0.1)*	1.8 (0.1)
TDI					
Sw	9.7 (0.2)	9.7 (0.2)	9.0 (0.2)	9.3 (0.2)	9.5 (0.2)
Ew	20.3 (0.5)	16.2 (0.6)*	17.1 (0.7)*	18.9 (0.6)†‡	20.1 (0.6)†‡
Aw	8.5 (0.3)	11.4 (0.6)*	8.2 (0.3)†	8.5 (0.3)†	8.5 (0.3)†
Ew/Aw	2.5 (0.1)	1.6 (0.1)*	2.2 (0.1)†	2.3 (0.1)†	2.5 (0.1)†
E/Ew	5.6 (0.2)	7.3 (0.2)*	6.3 (0.3)	5.7 (0.2)	5.4 (0.2)
Change of Sw (%)		0	−4 (3)	−1 (3)	2 (3)
Change of Ew (%)		0	6 (2)	18 (3)	27 (3)†‡

*KD, Kawasaki disease; HR, heart rate; BP, blood pressure; UCG, ultrasonic cardiography; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; LVSF, left ventricular shortening fraction; ESWS, end-systolic wall stress; VCFc, heart rate-corrected velocity of circumferential fiber shortening; Mitral inflow E/A, early transmitral flow velocity to late transmitral flow velocity ratio; TDI, tissue Doppler imaging; Sw, systolic tissue Doppler velocity; Ew, early diastolic tissue Doppler velocity; Aw, late diastolic tissue Doppler velocity; E/Ew, early transmitral flow velocity to early diastolic tissue Doppler velocity ratio. *p<0.05 vs control, †p<0.05 vs week 1, ‡p<0.05 vs week 2.*

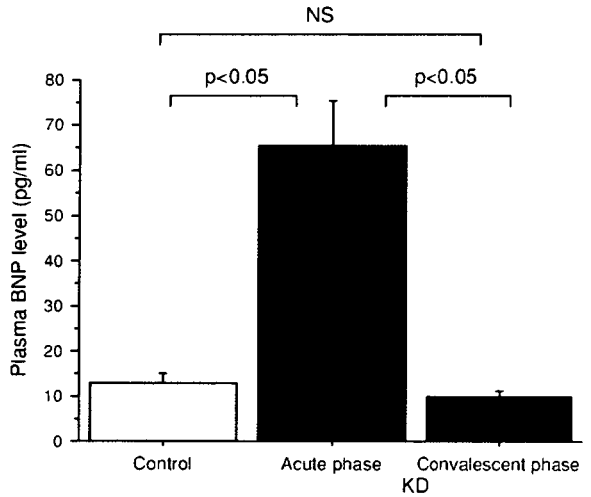

Fig 1. Plasma brain natriuretic peptide (BNP) level in patients with acute Kawasaki disease (KD) was significantly higher than in the control group.

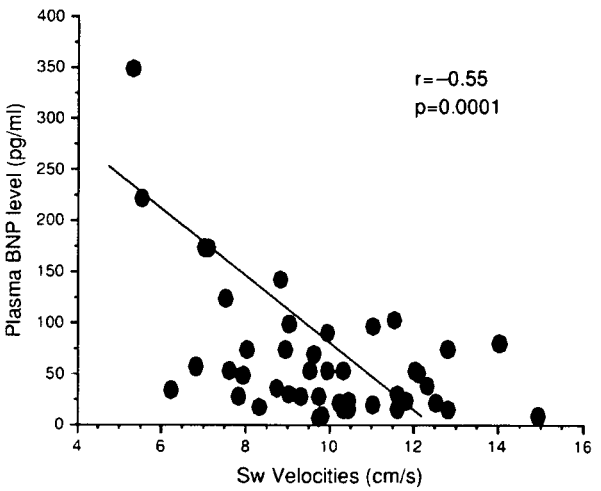

Fig 2. Correlation between plasma brain natriuretic peptide (BNP) level and week 1 systolic tissue Doppler velocity (Sw velocity) was a significant negative correlation.

Plasma BNP Level in Acute KD

Plasma BNP level in acute KD was significantly higher (65±9 pg/ml) than in the control group (13±2 pg/ml), but normalized in the convalescent phase (11±1 pg/ml). No patient had a plasma BNP level above 50 pg/ml in the convalescent phase (Fig 1).

Correlation Between Plasma BNP Level and TDI Profile

There was a significant negative correlation between VCFc in week 1 and acute phase plasma BNP level (r=−0.37, p=0.018). There was a significant negative correlation between LVSF in week 1 and acute phase plasma BNP level (r=−0.42, p=0.003). There was a significant and stronger negative correlation between Sw velocity in week 1 and acute phase plasma BNP level than for VCFc and LVSF (r=−0.55, p=0.001) (Fig 2). We subdivided the KD group into 2 groups using median BNP (51 pg/ml): BNP ≥51 (n=21) and BNP <51 (n=21). The change in Sw velocity in the BNP ≥51 group was significantly greater than in the BNP <51 group (p<0.05) (Fig 3). Although the increase in LVSF in the BNP ≥51 group was also significantly greater than in the BNP <50 group (p<0.05) (Fig 4), Ew, Ew/Aw, E/Ew, and E/A did not significantly differ between groups.

Urinary 8-Isoprostane Level in Acute KD

The pre-IVIG therapy urinary 8-isoprostane level was significantly higher than in the control group (596±37 vs 379±26 pg/ml Cr, p<0.05)

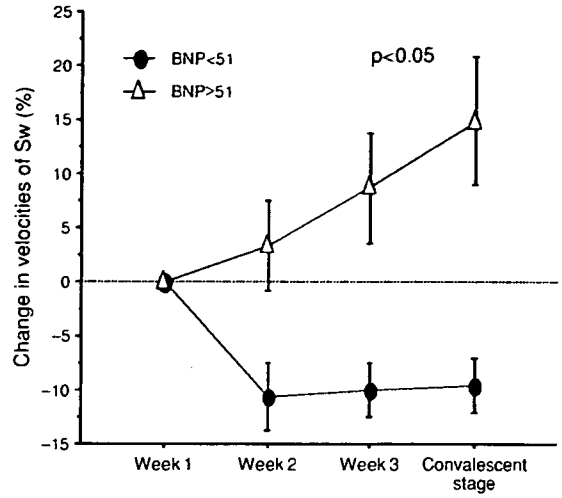

Fig 3. Time course of changes in the systolic tissue Doppler velocity (Sw velocity). Increases in Sw velocity in the brain natriuretic peptide (BNP) ≥51 pg/ml group were significantly greater than those in the BNP <50 pg/ml group.

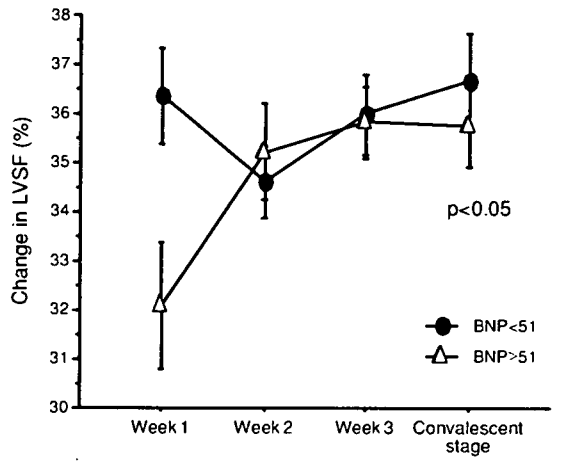

Fig 4. Time course of changes in left ventricular shortening fraction (LVSF). The increase in the LVSF of the brain natriuretic peptide (BNP) ≥51 pg/ml group was significantly greater than in the BNP <50 pg/ml group.

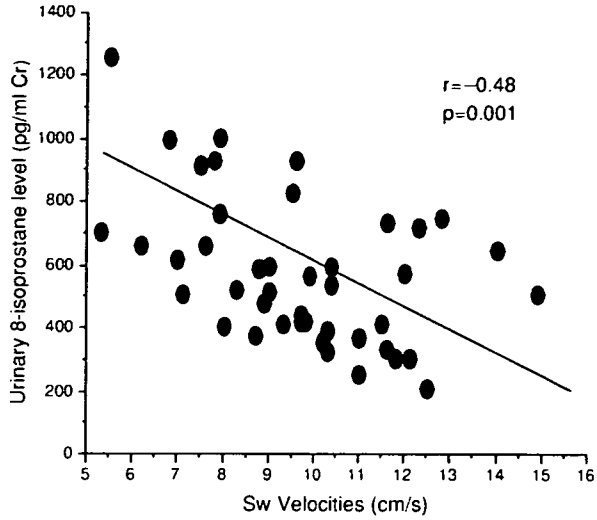

Fig 5. Correlation between systolic tissue Doppler velocity (Sw velocity) in week 1 and urinary 8-isoprostane level was a significant negative correlation. Cr, creatinine.

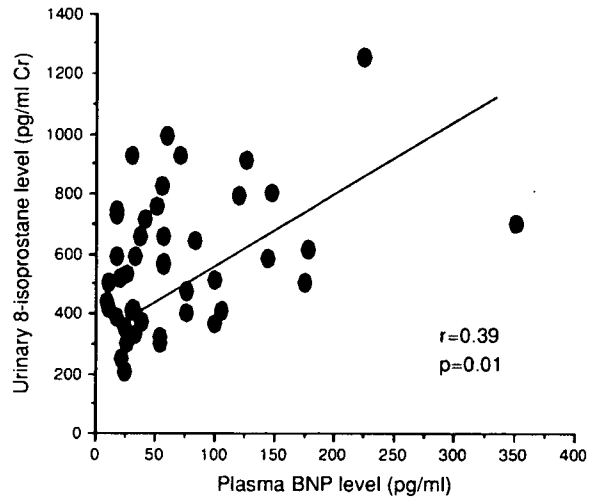

Fig 6. Correlation between plasma brain natriuretic peptide (BNP) level and urinary 8-isoprostane level was a significant positive correlation. Cr, creatinine.

Correlation Between Urinary 8-Isoprostane Level and TDI Profile

LVSF in week 1 was significantly negatively correlated with urinary 8-isoprostane level (r=–0.34, p=0.02). There was a significant negative correlation between Sw velocity at week 1 and urinary 8-isoprostane level (r=–0.48, p=0.001) (Fig 5). VCFc showed no significant correlation with urinary 8-isoprostane level (r=–0.25, p=0.13). E/Ew in week 1 showed a weak, but significant, positive correlation with urinary 8-isoprostane level (r=0.31, p=0.04).

Correlation Between Plasma BNP Level and Urinary 8-Isoprostane Level

The plasma BNP level was weakly but significantly positively correlated with the urinary 8-isoprostane level (r=0.39, p=0.01) (Fig 6).

Discussion

Diastolic Function in Acute KD

The mean week-1 Ew velocity was significantly lower in the patients than in the controls, but normalized during the convalescent phase. It has been reported that decreased Ew velocity in conjunction with a decreased Em/Am ratio is strongly correlated with the isovolumic LV relaxation constant (τ)[29] and reflects early diastolic recoil dysfunction[30,31] Decreased preload index and increased ESWS in acute KD have been reported[3] Although we also used ESWS as an after-load marker for the LV in this study, ESWS did not significantly change at any stage. HR was significantly higher in week 1 as compared with controls, but significantly decreased after week 2 because almost all patients become afebrile after IVIG or steroid pulse therapy. Although HR significantly changed between weeks 1 and 2, Ew

velocity increased by only 5%, with no significant increase between week 1 and week 2 and significant accentuation after week 3. This suggests that the change in Ew velocity in acute KD is relatively independent of both ESWS and HR. It has been reported that HR and BP do not have a significant effect on Ew velocity[10,32] On the other hand, some studies report that increasing HR influences the diastolic parameters, especially Aw[33,34] In contrast to the Ew velocity, the Aw velocity showed a significant reduction from week 1 to week 2, which resulted in a significantly increased Ew/Aw from week 1 to week 2. The influence of a change in HR on Ew/Aw needs to be taken into account.

Systolic Function in KD

It has been reported that Sw velocity sensitively reflects regional long-axis ventricular systolic function[6,9] and we used Sw as a marker of longitudinal systolic function. We also used LVSF as an approximate indicator. In the present study, VCFc and LVSF did not significantly change at any phase. Newburger et al reported that both LVSF and VCFc were commonly suppressed within 10 days of onset[2] contrary to the results of the present study. In their study, only 58% of patients received IVIG within 10 days of onset; in the present study, all patients received IVIG within 7 days of onset. This difference in initiating IVIG therapy may explain the differing results. Moran et al reported rapid improvement of abnormal mechanics after IVIG[3] a finding consistent with ours. Takahashi suggested that relatively renewable structures such as intramyocardial microvessels explain the functional reversibility of abnormal myocardial mechanics in acute KD[4]

Plasma BNP Level in Acute KD

In this study, significantly increased plasma BNP levels were observed in acute KD patients, but these quickly normalized in the convalescent stage, which is consistent with an earlier report[17] The mechanism responsible for the increased plasma BNP level in acute KD is not yet known. Kawamura et al maintain that pro-inflammatory cytokines, such as interleukin-1β and TNF-α, cause myocarditis and stimulate secretion of BNP in acute KD[17] They also reported no significant correlation between plasma BNP level and LV ejection fraction (LVEF) in acute KD. In contrast, in the present study, LVSF, VCFc, and Sw in week 1 significantly correlated with plasma BNP level. Moreover, the change in Sw velocity from baseline significantly differed between the BNP ≥51 pg/ml (high BNP) and BNP <51 pg/ml (low BNP) groups. These results suggest the Sw velocities obtained by TDI are more closely associated with increased plasma BNP level than with either LVSF or VCFc. Suppressed myocardial mechanics may partly contribute to BNP production from the ventricle in acute KD. Agricola et al assumed that TDI velocities are a more sensitive marker than the LVEF because TDI is relatively preload-independent and because of the architecture of myocardial fibers[35]

Oxidative Stress in Acute KD

Urinary 8-isoprostane levels were significantly higher as compared with controls, suggesting that enhanced systemic oxidative stress may contribute to acute KD pathogenesis. Further study is necessary to clarify the mechanism responsible for this.

Week 1 LVSF and Sw velocity were significantly negatively correlated with urinary 8-isoprostane level. Wolfman et al reported a significant negative correlation between 8-isoprostane level and LVEF in patients with chronic heart failure[36] and Mobert et al[37] reported that 8-isoprostane induced a concentration-dependent decrease in coronary flow; alterations in coronary flow were associated with a parallel reduction of LV pressure and dP/dt (max). 8-isoprostane is a potent vasoconstrictor[38-40] an effect that may contribute to enhanced peripheral vascular tone and have a negative influence on coronary arterial and intracardiac microvascular blood flow, thus leading to depressed cardiac functional capacity in acute KD patients. Interestingly, we observed a significant correlation between plasma BNP level and urinary 8-isoprostane level. Nonaka-Sarukawa et al reported a significant correlation between urinary F2-isoprostane concentration and plasma BNP level in patients with congestive heart failure[23] The mechanisms responsible for these correlations are not yet known. In the present study, both BNP and 8-isoprostane levels correlated with week 1 Sw velocity. Therefore, we assume that ventricular dysfunction associated with enhanced oxidative stress resulting in suppression of cardiac capacity may contribute to increased production of plasma BNP from the ventricle.

Study Limitations

First, high-dose IVIG therapy for acute KD might affect the TDI profile because it has high osmolarity[41] and is associated with volume overload. Second, because urinary 8-isoprostane may be influenced by many factors, it is difficult and premature to conclude that this substance has a clear effect on oxidative stress. Thirdl although an increased urinary 8-isoprostane level may reflect systemic oxidative stress, it is difficult to evaluate its local production and effects in the heart. Fourth, the influence of the administration of aspirin, a cyclooxygenase inhibitor, cannot be excluded, because small amounts of 8-isoprostane can be formed by human platelets and monocytes through a cyclooxygenase-dependent mechanism. Fifth, the 8-isoprostane levels in KD were only compared with healthy subjects; its levels in febrile conditions other than KD have not been revealed. Sixth, the number of patients enrolled in this study was quite low. Finally, the observation period in this study was very short; further long-term observation is necessary.

Conclusion

Latent abnormal tissue Doppler profiles, possibly reflecting long-axis systolic and diastolic dysfunction, have been noted in acute KD patients. Increased plasma BNP level is associated with abnormal tissue Doppler profiles, suggesting that abnormal myocardial mechanics may partly contribute to the increased plasma BNP level observed in acute KD. Increased urinary 8-isoprostane level indicates the presence of enhanced oxidative stress in acute KD and the urinary 8-isoprostane level correlates with transient myocardial dysfunction, suggesting that enhanced oxidative stress may contribute to cardiac dysfunction in acute KD.

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CARDIOVASCULAR PATHOLOGY

Original Article

Isolated necrotizing arteritis (localized polyarteritis nodosa): examination of the histological process and disease entity based on the histological classification of stage and histological differences from polyarteritis nodosa

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Abstract

Introduction: Although isolated necrotizing arteritis (INA) has been thought to be an isolated form of polyarteritis nodosa (PAN), a detailed histological comparison between INA and PAN has not been performed. Therefore, we examined the disease entity of INA based on the histological comparison of both diseases. In addition, a histological classification of INA, in which the histological process of INA is included, was described. **Methods:** A histological study, including CD3, CD20, and CD68 immunostains, was performed in seven operated patients with INA. Five untreated patients with PAN were also examined. **Results:** In INA, arteritis with fibrinoid necrosis occurred in small and medium-sized arteries in a single organ. INA was divided histologically into acute (five cases) and healed stage (two cases). Endothelial injury and medial degeneration, followed by fibrinoid necrosis, occurred in the acute stage, and regression of fibrinoid necrosis and fibrosis were present in the healed stage. Infiltration of predominant T lymphocytes and macrophages was also observed in the affected arteries. Histological comparison between INA and PAN led to the finding that the extension of fibrinoid necrosis in the entire arterial wall, which indicates severe wall destruction, intense proliferation of fibroblasts and aneurysm formation occurred in PAN alone. **Conclusions:** We demonstrated some histological differences between INA and PAN. Based on the histological similarities and differences between INA and PAN, it was concluded that INA shall be classified as a mildly wall destructive form of PAN-type arteritis located in a single organ.
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Keywords: Isolated necrotizing arteritis; Polyarteritis nodosa; Histological comparison; Aneurysm

1. Introduction

Vasculitis located in a single organ is defined as isolated or localized vasculitis [1]. Among the isolated vasculitides, isolated necrotizing arteritis (INA), also called localized polyarteritis nodosa (PAN), is the most common disorder [1]. INA is characterized by fibrinoid necrosis in small and medium-sized arteries and the histological appearance resembles that of PAN [1]. In INA, the occurrence of fibrinoid necrosis is well recognized, but the histological process of the disease has not been determined. Therefore, in the present study, we made a histological classification of

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the stages of INA in which the histological process of INA is included.

INA has been considered to be a single-organ manifestation of PAN [1]. However, aneurysm formation is relatively frequently observed in cases with PAN [2], but none of the cases with INA had aneurysm [3–10]. These findings indicated the possibility of histological differences of the affected arteries between INA and PAN. Therefore, in the present study, we performed a histological comparison of the affected arteries between INA and PAN and examined the disease entity of INA based on the comparison.

INA is incidentally found in an operated organ because of the lack of clinical data indicating vasculitis. INA is generally a self-limiting disorder, and medication is not necessary [1]. On the other hand, in the case with PAN, treatment with steroids or immunosuppressive drugs is usually performed [11]. Therefore, the histological diagnosis of INA or PAN is important for the routine pathological diagnosis in operated organs, and so we describe the histological diagnostic points of INA or PAN based on the data obtained from the present study.

2. Methods

Among the patients operated in Juntendo Hospital during 1991 to 2003, seven patients (four females and three males with ages ranging from 16 to 56 years) showed INA (Table 1). Among them, INA was incidentally found in an operated organ, including the uterus (four cases), gallbladder (one case), pancreas (one case), and connective tissue around the spermatic cord (one case), and none of them had clinical data indicating systemic vasculitis before and after the operation (Table 1). The follow-up period after the operation in the patients ranged from 3 to 10 years (mean, 5 years). The incidence of INA in the uterus was 2.3% (4 per 1746 cases) in uteruses subjected to hysterectomy. The operated organs were fixed with formalin solution, and histological sections from the tissues were stained with H&E, elastic van Gieson, Azan, and Giemsa stains in the usual manner. Immunohistochemical studies were performed with the avidin–biotin peroxidase complex technique using the following antibodies: CD3 (F7.2.38, Dakopatts, Glostrup, Denmark; 1:25), CD20 (L26, Dakopatts; 1:200), CD 31 (JC/70A, Dakopatts; 1:50), CD34 (My10 and 8G12, Becton Dickinson, San Jose, CA, USA; 1:50), CD68 (KP1, Dakopatts; 1:50), smooth muscle actin (1A4, Dakopatts; 1:50), Desmin (D33, Dakopatts; 1:100), and factor VIII related antigen (polyclonal, Dakopatts; 1:700). Heat-induced epitope retrieval was performed in citrate buffer (pH 6.0 or 7.0) before the immunostaining for CD3, CD20, CD31, CD34, and CD68. Enzyme (proteinase-K, 10 min) antigen retrieval was used for factor VIII related antigen.

For the pathological comparison between INA and PAN, we selected five untreated cases (four autopsied cases and one operated case) with PAN from the case list of PAN in our group (Table 2). The diagnosis of PAN of the accumulated cases was made on the basis of the American College of Rheumatology criteria [12], and pathological findings of pulmonary and hepatic lesions in the accumulated autopsy cases were already reported in our previous studies [11,13]. In an operated case, histological and

Table 1
Clinical findings in seven operated cases with INA

Case no.	Age (y)/sex	Underlying disorders	Resected tissues
INA1	41/F	Leiomyoma of uterus	Uterus
INA2	43/F	Leiomyoma of uterus	Uterus
INA3	43/F	Leiomyoma of uterus	Uterus
INA4	46/F	Leiomyoma of uterus	Uterus, left ovary and Fallopian tube
INA5	29/M	Testicular torsion	Spermatic cord
INA6	16/M	Chronic pancreatitis	Pancreas (body and tail) and spleen
INA7	56/M	Cholecystolithiasis	Gallbladder

Table 2
Clinical and pathological findings in five cases with PAN

Case no.	Autopsy or operation	Age (y)/sex	Affected organs	Stage of arteritis[a]	Aneurysm
PAN1	Autopsy	10/F	Whole body[b]	AI	+
PAN2	Autopsy	39/M	Whole body[b]	AI, GT, and HGT	+
PAN3	Autopsy	67/F	Whole body[b]	AI, GI, and HGT	+
PAN4	Autopsy	70/M	Whole body[b]	AI, GI, and HGT	+
PAN5	Operation[c]	61/M	Stomach and spleen	AI and GI	+[d]

[a] Classification of stages according to Arkin [2]: acute inflammatory (AI), granulation tissue (GT), healed granulation tissue (GHT).

[b] Affected organs were spleen, liver, gastrointestinal tract, gallbladder, and genital system in all cases, and additional involvement of kidney and lung in three cases.

[c] Subtotal gastrectomy and splenectomy were performed for gastric carcinoma.

[d] Aneurysm formation in the branch of mesenteric artery was confirmed by angiography.

Table 3
Pathological findings of arteritis in seven operated cases with INA

Case no.	Affected organ	No. of affected arteries	Size of affected arteries (μm)	Stage of arteries	Aneurysm
INA1	Uterus (cervix)	Nine	150–500	Acute	–
INA2	Uterus (cervix and body)	Ten	120–350	Acute	–
INA3	Uterus (cervix)	Two	250, 330	Acute	–
INA4	Uterus (cervix)	Two	540, 740	Acute	–
INA5	Connective tissue around spermatic cord	One	140	Acute	–
INA6	Pancreas (tail)	One	520	Healed	–
INA7	Gallbladder (fundus)	One	250	Healed	–

immunohistochemical studies from the tissue sections were performed in the same manner as for the cases with INA. In the autopsy cases, the histological findings focusing on the comparison between INA and PAN were examined using conventional staining (H&E, elastic van Gieson, and Azan) glass slides.

3. Results

3.1. Pathological features of INA

In all cases, necrotizing arteritis was found in a single organ, and the number of affected arteries ranged from 1 to 10 (Table 3). The size of the affected arteries ranged from 120 to 740 μm in diameter (Table 3). The arteritis was divided into two stages, acute and healed.

Acute stage (five cases, INA1–5). Initially, the endothelium showed degeneration, necrosis, and desquamation, and in the subendothelial space, edema, hemorrhage, and fibrinous exudation occurred (Fig. 1A, B). There were degenerative changes in some parts of the media, and mild infiltration of the inflammatory cells, admixed with histiocytes, neutrophils, and lymphocytes, was noted in the intima and media (Fig. 1A, B). Thereafter, fibrinoid necrosis, which was located in the intima and inner media, occurred (Figs. 1C–E and 2A). In the media, the degenerative

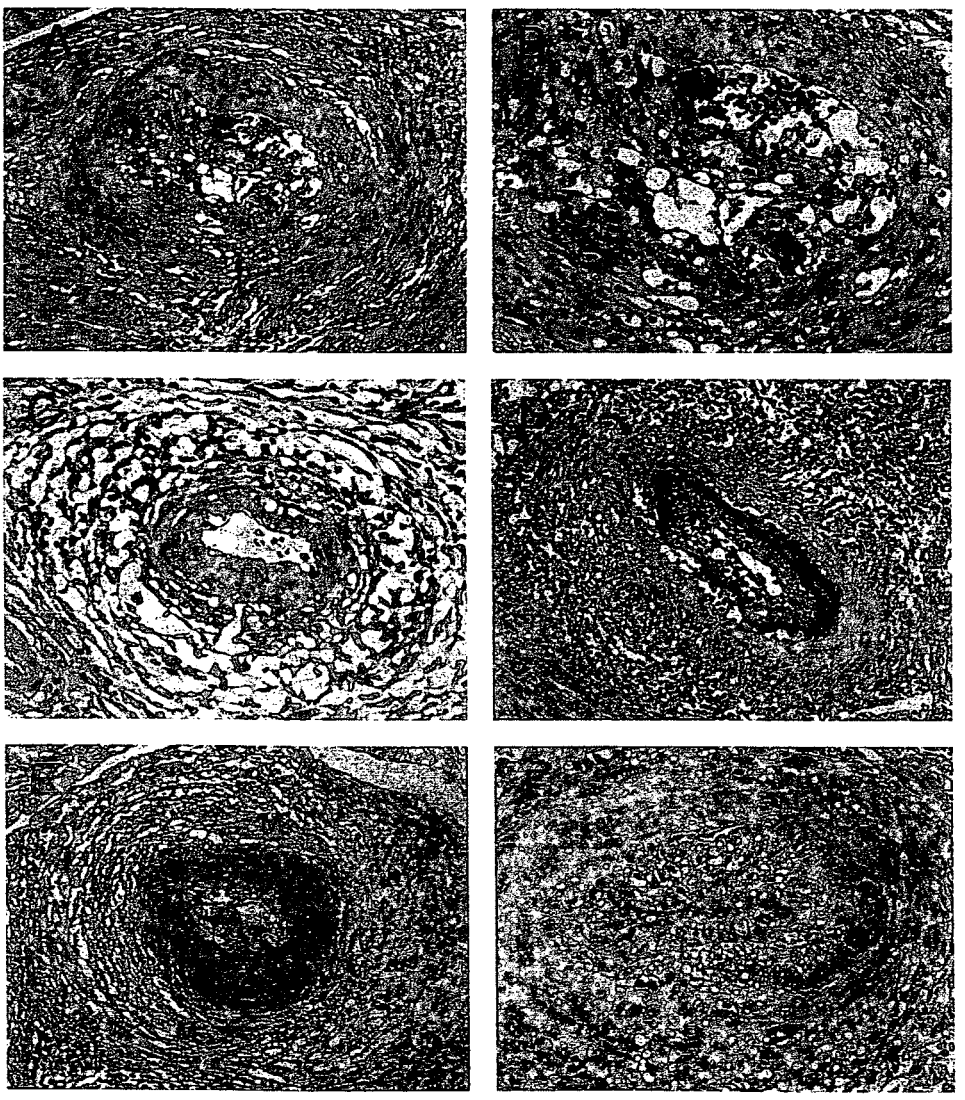

Fig. 1. Histological appearance of acute (A–E) and healed stages (F) in INA. (A) In a medium-sized artery, the endothelium shows desquamation, and edema and hemorrhage are present in the subendothelial space. In a part of the media (indicated by an arrow), degeneration is present (H&E, original magnification ×200). (B) High-power view of A. The endothelium shows degeneration and necrosis, and fibrinous exudation and infiltration of neutrophils, lymphocytes, and histiocytes are present in the intima and inner media (H&E, original magnification ×400). (C) Fibrinoid necrosis (FN) occurs in the intima and inner media in a small artery (H&E, original magnification ×400). (D, E) FN in the intima and inner media, degeneration of media (M), and intense infiltration of inflammatory cells are present in a medium-sized artery. The proliferation of fibroblasts is mild and the medial rim can be traced (H&E, original magnification ×200). (F) There are edematofibrous thickening in the intima, regression of fibrinoid necrosis (indicated by an arrow) in the intima and inner media, and slight fibrosis in the media and adventitia (H&E, original magnification ×400).

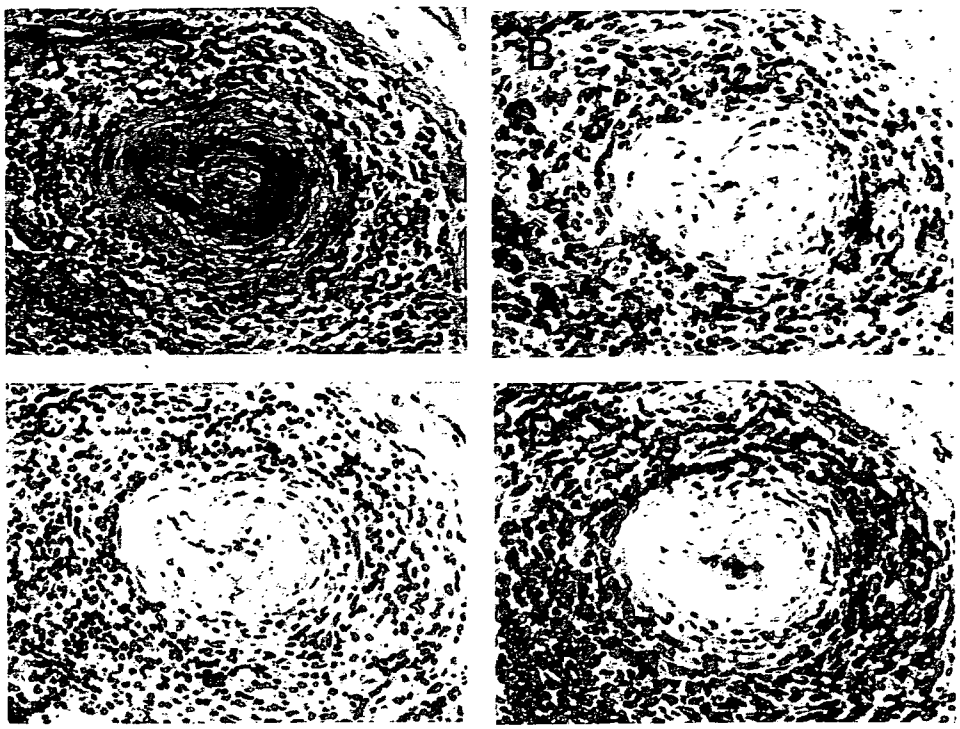

Fig. 2. Histological appearance and subpopulation of lymphocytes in a small artery with INA. (A) There are fibrinoid necrosis (FN) in the intima and inner media and intense infiltration of inflammatory cells in the media and adventitia (H&E, original magnification ×400). (B) Many T lymphocytes infiltrate in the arterial wall (CD3 stain, original magnification ×400). (C) There is sporadical infiltration of B lymphocytes in the arterial wall (CD20 stain, original magnification ×400). (D) Many histiocytes infiltrate in the arterial wall (CD68 stain, original magnification ×400).

changes associated with the disappearance of fibromuscular elements became more severe, but the medial rim could be traced. (Figs. 1D, E and 2A). Associated with the medial injury, mild proliferation of fibroblasts was present in the injured media and adventitia (Fig. 1D, E). Inflammatory cell infiltration occurred in the entire arterial wall, and intense

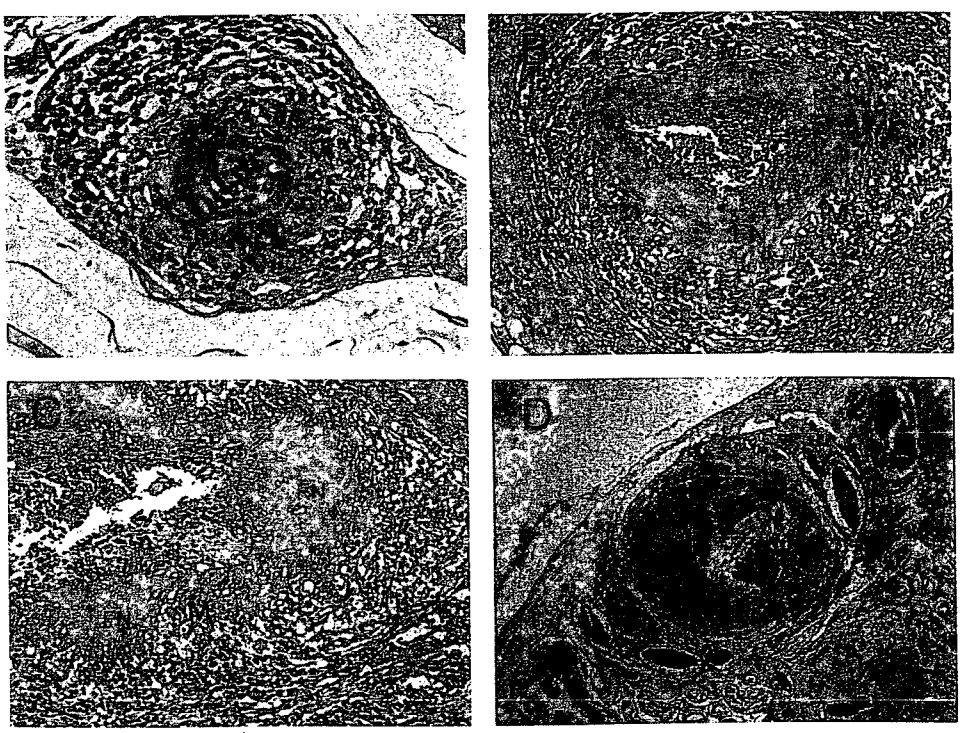

Fig. 3. Characteristic histological findings in PAN are shown: fibrinoid necrosis (FN) from intima to adventitia (A, C), intense proliferation of fibroblasts in media (M) and adventitia (A, B, C), and aneurysm formation (indicated by an arrow in D) [H&E, original magnification ×400 (A); ×200 (B); ×200 (C); ×40 (D)].

infiltration of inflammatory cells was frequently seen in the adventitia (Figs. 1D, E and 2A). Macrophages and T lymphocytes were the most predominant cell type, and B lymphocytes were scarce and did not exceed 10% of the inflammatory cells (Fig. 2B–D). The infiltration of neutrophils occurred in varying degrees (Figs. 1D, E and 2A).

Healed stage (two cases, INA6 and 7). Edematofibrous thickening in the intima, regressive fibrinoid necrosis in the intima and inner media, and slight fibrosis in the media and adventitia were present (Fig. 1F). Mild inflammatory cell infiltrate was noted in the arterial wall, especially in the adventitia (Fig. 1F).

In the examined cases, the histological stages consisted of the acute or healed stage, and an admixture of both stages was not found (Table 3). The segmental occurrence of the arteritis was confirmed by serial and deep-cut section examination. None of the affected arteries showed aneurysm formation.

3.2. Pathological comparison between INA and PAN

Most of the affected arteries in the patients with PAN presented the acute stage of PAN histologically. Thus, histological comparison was performed in the acute stage of both INA and PAN. The affected arteries in PAN frequently showed histological appearance similar to that of INA. However, extension of fibrinoid necrosis from the intima and inner media to the outer media and adventitia (Fig. 3A, C), intense proliferation of fibroblasts (Fig. 3A–C), and aneurysm formation (Fig. 3D) were found in PAN alone. In addition, inmmunohistochemical stains demonstrated the predominant infiltration of T lymphocytes and macrophages in the affected arteries in an operated case of PAN, which is the same result as with INA.

4. Discussion

After the first report on INA by Plaut [3], only the study by Abu-Farsakh et al. performed a pathological comparison between INA and PAN [7]. Although they reported that the proportion of the inflammatory cell infiltrate was similar in both diseases, they did not perform a comparison of the histological appearance in both diseases [7]. Therefore, the present study is the first examination of the comparison of the histological appearance between both diseases. Subsequently, we found some differences in the affected arteries in both diseases. The extension of fibrinoid necrosis in the entire arterial wall, which produces severe wall destruction, was found in PAN alone. On the other hand, in the cases with INA, the media rim can be traced in the affected arteries. Based on these findings, we concluded that severe destruction of the arterial wall occurs in PAN, but the destruction is mild in INA, and these differences led to the presence of aneurysm in PAN and the absence of aneurysm in INA.

In the present study, we made the classification of the histological stages of INA, in which the disease process is included. In INA, endothelial injury and medial degeneration occur initially. Thereafter, fibrinoid necrosis occurs. The infiltration of inflammatory cells is also observed in the acute stage, and severe infiltration of inflammatory cells was frequently observed in the phase with fibrinoid necrosis. The acute stage in PAN showed a histological process similar to that of INA. The inflammatory cells consisted mainly of T lymphocytes and macrophages in both INA and PAN. In PAN, a T-cell-mediated immune mechanism for the development and perpetuation of the disease has been considered [14]. Therefore, the same mechanism as that of PAN may occur in the development and perpetuation of INA. The degree of wall destruction in the affected arteries in both diseases (as described and discussed above) was mild in INA and severe in PAN. Based on the similar histological process and different wall destruction between INA and PAN, we think that INA is a mildly wall destructive form of PAN-type arteritis that is located in a single organ.

Necrotizing arteritis with fibrinoid necrosis in medium-sized arteries consisted of PAN, Kawasaki disease, and INA [1,15]. In the collagen vascular disease, PAN-type arteritis was present and the histological appearance of the arteritis was indistinguishable from PAN [1,13,16]. In addition, in Kawasaki disease, necrotizing arteritis, which is indistinguishable from PAN, occurs [1,16]. Collagen vascular disease and Kawasaki disease had peculiar clinical findings, and, thus, the diagnosis of such diseases was usually made before the operation. Therefore, the pathological diagnosis of INA or PAN becomes a subject of discussion in operated organs. For the pathological diagnosis of INA or PAN, the important findings were that the extension of fibrinoid necrosis in the entire arterial wall, intense proliferation of fibroblasts, and aneurysm formation are present in PAN alone. If such findings are found in the affected artery in an operated organ, the pathological diagnosis of PAN is desirable. However, the affected arteries frequently had histological appearances that are difficult to classify as PAN or INA. In the cases having such arteritis, only clinical data are useful for the differentiation of both diseases [1,8].

In conclusion, we demonstrated some histological differences between INA and PAN. Based on the histological similarities and differences between INA and PAN, INA shall be classified as a mildly wall destructive form of PAN-type arteritis that is located in a single organ.

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