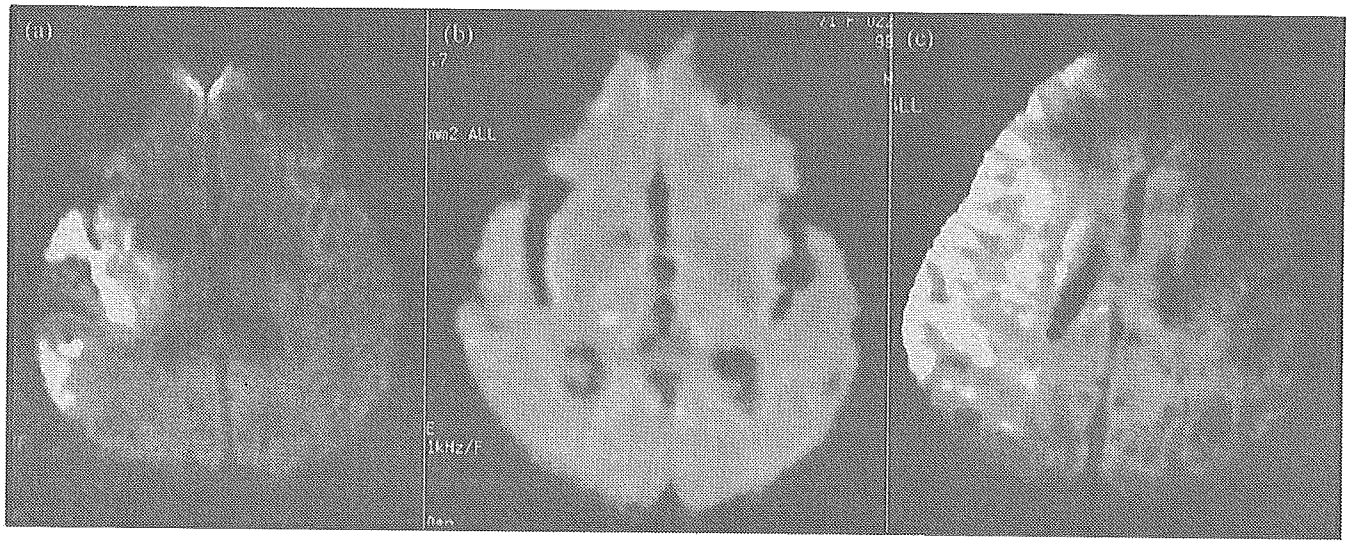

Figure 1: Diffusion-weighted images (DWI) for the three groups classified according to the location of the lesions. (a) Group I: cortical lesions. (b) Group II: lacunar lesions in the basal ganglia. (c) Group III, massive lesions involving one entire hemisphere

Determination of plasma OxLDL levels

To measure plasma OxLDL levels we used the same procedure as in our previous study[8]. Briefly, a sandwich ELISA procedure was performed with monoclonal Ab against oxidative phosphatidylcholine (FOH1a/DLH3; DLH3)[9] and apoB IgG antibody (Boehringer, Germany). The complex was detected by phosphatase-conjugated donkey anti-sheep IgG antibody (Chemicon, USA) and visualized by incubation with a substrate solution containing 1 mg/ml disodium *p*-nitrophenyl-phosphate hexahydrate (Wako, Japan). Absorbance at 405 nm was measured for comparison with a standard curve obtained under the same assay conditions. Simultaneously, we ran a parallel set of ELISA using anti-apoB mAb (OEM, USA) to determine the amounts of apoB in the same lipoprotein fractions. The OxLDL levels were expressed as amount of OxLDL per μg of apoB protein.

Determination of ischemic lesion and size

MRI was performed with a 1.5 Tesla unit (Sigma Horizon; GE Medical System, Milwaukee, WI) with echo-planar capabilities. The acquisition parameters for DWI were as follows: 10,000 ms/95 ms (repetition time/echo time), 128 × 128 matrix, 5 mm-thick sections, 1.5 mm inter-slice gap, 12 axial sections and diffusion gradients of 15 mT/m applied in three orthogonal directions. The *b* value was 0 and 1000 s/mm^2[10]. PWI was performed using the flow-sensitive alternating inversion recovery (FLAIR) method[10]. The FLAIR technique is based on IR-prepared echo-planar imaging sequences. After the collection of slice-selective and non-slice selective IR images, two different images were subtracted to obtain blood-flow imaging. The measurement conditions for FLAIR were as follows: time of repetition (TR)=2 seconds, echo time (TE)=10 ms, inversion time (TI)=1200 ms, slice thickness=8 mm, 5 mm inter-piece gap, field of view (FOV)=24 cm, non-slice selective pulse four times thicker than the selective pulse, matrix=96 × 96 pixels and number of excitations=100. The volumes of hyperintensity on DWI and of hypointensity on PWI were determined by three experienced neuroradiologists (MH, KY, NM) blinded to the clinical status of the patients. Utilizing GE calculating software, the lesion volume on DWI, PWI and the DWI–PWI mismatch were calculated [DWI/PWI mismatch volume (cm^3)=initial lesion volume on PWI minus initial lesion volume on DWI].

Statistics

Sequentially obtained data, expressed as the mean ± SD, were analysed with the Mann–Whitney *U*-test for two-group comparison; ANOVA, followed by Scheffe's test was used for more than three-group comparisons. The correlation between the OxLDL level, and the stroke volume and neuronal deficits was examined by the Spearman rank correlation test. Statistical analyses were performed on a Macintosh computer running statistical software (Stat View 4.0). Statistical significance was considered as $p<0.05$.

RESULTS

Plasma OxLDL level, ischemic volume, neuronal deficits and risk factors in 44 patients with ischemic stroke

The characteristics of our patients are presented in *Table 1* and *Figure 2*. Patients with cortical lesions (GI) had significantly higher OxLDL levels than patients with lesions in the basal ganglia or brain stem (GII), and the controls ($p<0.05$, $p<0.01$, respectively). Admission DWI showed that GI patients tended to manifest a higher ischemic volume than GII patients (18.2 ± 11.0 vs 1.8 ± 2.2 cm^3, mean ± SD). In patients with massive lesions that involved one entire hemisphere (GIII), the infarct volume was significantly larger than in the other two groups (>100 cm^3, $p<0.01$). The abnormal area depicted by PWI and the DWI–PWI mismatch was significantly larger in GI than GII ($p<0.05$ each). Due to their bad clinical condition, only two of six GIII patients

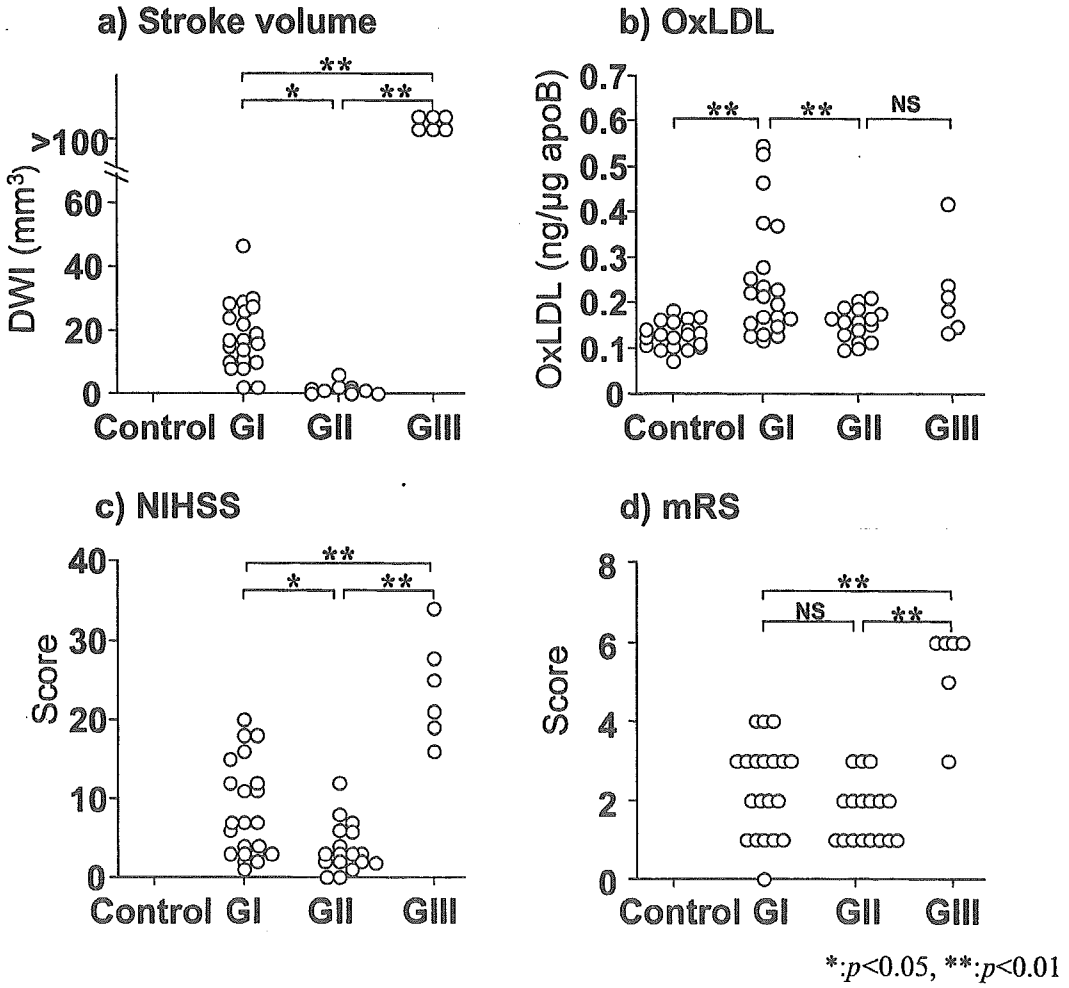

Figure 2: Stroke volume, plasma OxLDL, NIH stroke scale and modified Rankin Scale of each study group. (a) Stroke volume. (b) Plasma OxLDL level. (c) NIH stroke scale. (d) Modified Rankin Scale

were available for PWI and DWI–PWI mismatch study, a number too small for statistical analysis. The admission NIHSS was significantly higher in GI than GII ($p<0.05$) and the mRS score of GIII patients was significantly higher than in the other two groups ($p<0.01$).

Correlation between plasma OxLDL levels, and ischemic volume depicted on DWI and PWI, and DWI/PWI mismatch

As shown in *Figure 3a–c*, in GI, there was a significant correlation between plasma OxLDL and the infarct volume based on DWI ($p=0.01$), PWI ($p<0.01$) and DWI–PWI mismatch findings ($p<0.05$). In GII, plasma OxLDL was not correlated with the ischemic volume assessed by DWI ($r=0.043$, *Figure 3d*) and PWI ($r=0.076$, data not shown). In GIII, plasma OxLDL was not significantly elevated and there was no correlation with the ischemic volume (data not shown).

Correlation between neuronal deficits assessed by NIHSS, plasma OxLDL, and integrated ischemic volume determined by DWI and PWI in GI

As shown in *Figure 4a,b*, in GI the NIHSS score was associated with the initial stroke volume; the association appeared to be stronger with the PWI than the DWI lesion volume. In addition, there was a significant correlation between plasma OxLDL and the initial NIHSS score, but not with the mRS score (*Figure 4c,d*).

Elevation of plasma OxLDL in the early phase is predictive of infarct enlargement

Figure 5 depicts changes in OxLDL and lesion volume determined by DWI that evaluated between stroke onset and 3 days after the insult, in GI and GII patients. In GI, plasma OxLDL remained significantly elevated ($p<0.01$) compared with the control and the elevation was associated with further enlargement of the lesion volume as determined by DWI. However, during the chronic phase after ischemic stroke, it decreased to the control level; this finding coincides with the observations we reported in our previous study[8] (data not shown).

Illustrative case

This 81-year-old woman presented with total aphasia and was admitted to our hospital 3 hours after stroke onset. She was lethargic and her ECG showed atrial

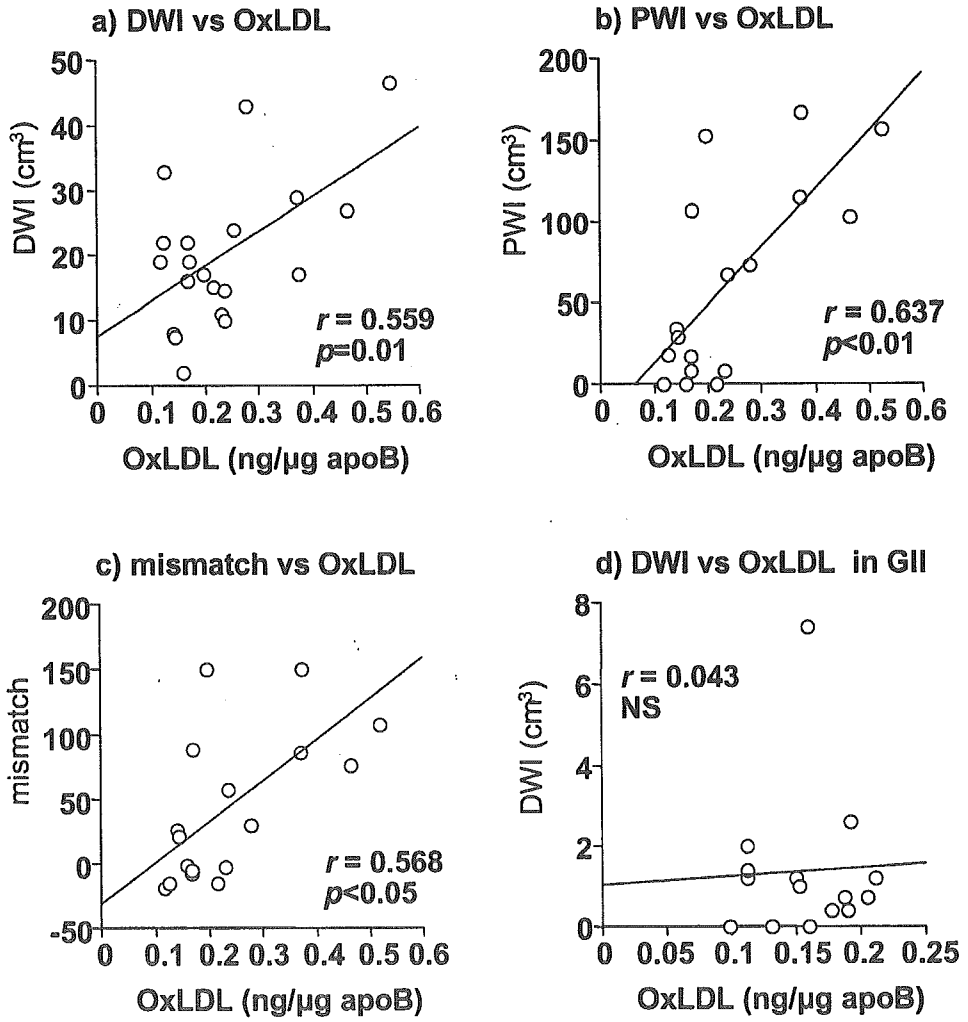

Figure 3: Relationship between plasma OxLDL level and stroke volume in Group I and II patients. (a) Group I, plasma OxLDL and stroke volume (DWI). (b) Group I, plasma OxLDL and stroke volume (PWI). (c) Group I, plasma OxLDL and mismatch volume (DWI and PWI). (d) Group II, plasma OxLDL and stroke volume (DWI)

fibrillation. Her admission NIHSS score was 11. Her initial DWI demonstrated hyperintensity in the left temporal lobe (*Figure 6a,b*); the lesion volume was 27 cm^3. Her plasma OxLDL was 0.298 ng/μg apoB. MRA showed occlusion of the left ICA (*Figure 6c*). We classified this infarction as cardioembolic and assigned her to GI. Serial DWI obtained 3 days after stroke onset showed enlargement of the lesion volume to 42 cm^3 (*Figure 6d,e*) and her plasma OxLDL had increased to 0.418 ng/μg apoB. She regained alertness and her aphasia improved slightly. At discharge, 17 days after the insult, her NIHSS score was 8.

DISCUSSION

The present results of DWI, PWI and DWI–PWI mismatch studies showed that in stroke patients with cortical infarcts (GI), the plasma OxLDL levels were correlated with the initial ischemic volume. Sequential studies over 3 days post-insult showed that a persistent increase in plasma OxLDL was associated with enlargement of the infarct volume. On the other hand, in patients with severe massive infarction involving the entire hemisphere (GIII), plasma OxLDL did not increase significantly. DWI and PWI studies showed that the plasma OxLDL level in patients with small subcortical infarcts remained similar to the control and was not correlated with the infarct volume. In our previous study[8], plasma OxLDL increased immediately after stroke onset especially in patients with cortical infarction and continued to increase for 7 days. It returned to the baseline between 14 and 30 days after the insult. These and our current results suggest that the observed OxLDL elevation reflects progressive oxidative damage in patients with ischemic lesions and may be predictive of enlargement of the lesion during the acute phase. In contrast, in GIII patients plasma OxLDL levels did not increase significantly, although their mRS score at discharge was significantly worse than in the other two groups. Therefore, plasma OxLDL did not reflect irreversible damage to cerebral tissue in patients with massive infarction.

Elevation in free radicals may be an etiological factor in stroke[16] and there is convincing evidence that in

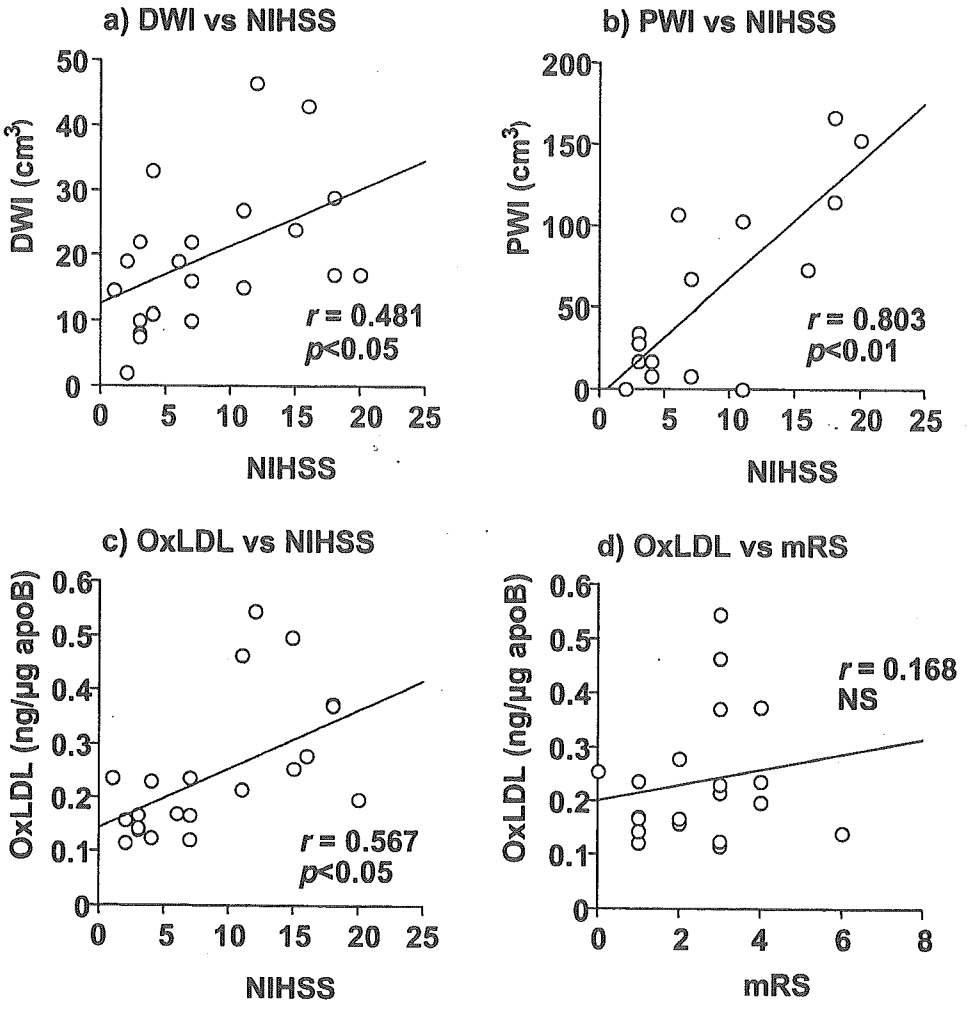

Figure 4: Relationship between NIH stroke scale (NIHSS), and stroke volume and plasma OxLDL in Group I patients. (a) NIHSS and stroke volume (DWI). (b) NIHSS and stroke volume (PWI). (c) NIHSS and plasma OxLDL level. (d) NIHSS and modified Rankin Scale

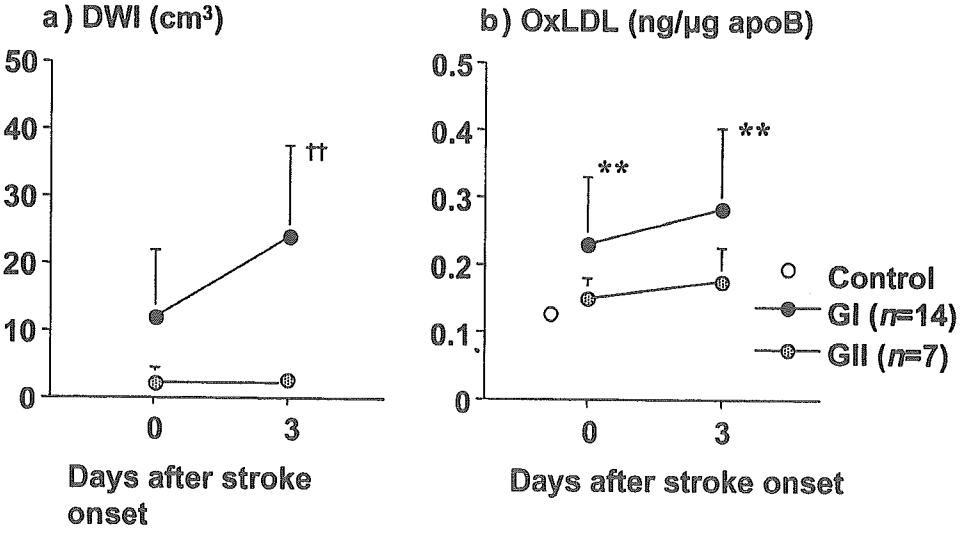

Figure 5: Group I and II patients. Plasma OxLDL level and lesion volume (DWI) at stroke onset and 3 days later. (a) Changes in the lesion volume (DWI). (b) Changes in plasma OxLDL. ††P<0.01 to Day 0. **p<0.01 to control

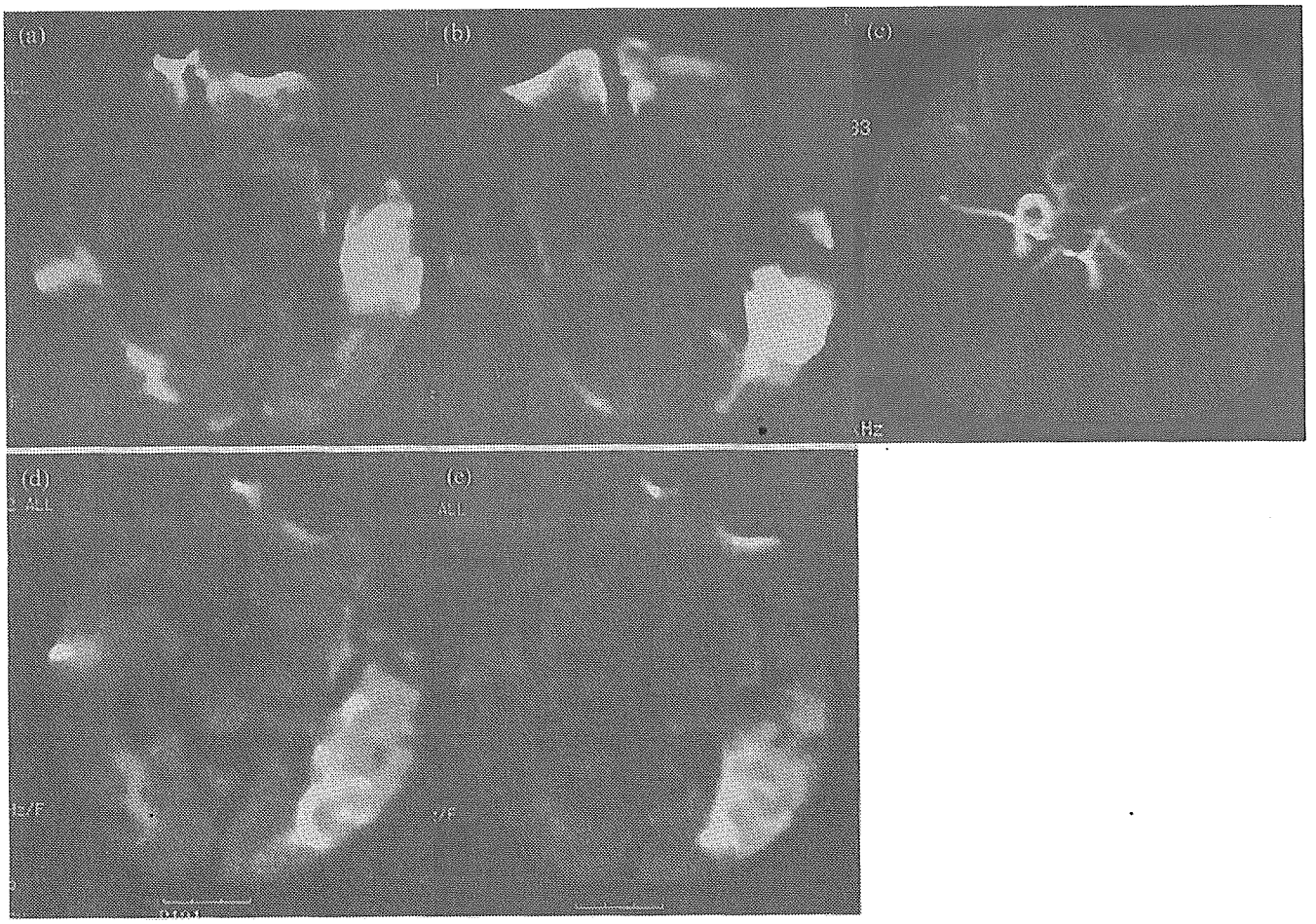

Figure 6: Illustrative case of an 81-year-old woman with a cardioembolic infarction who was assigned to Group I. (a,b) DWI obtained at onset shows a hyperintensity area in the left temporal lobe. (c) MR angiogram demonstrates occlusion of the left internal carotid artery. (d,e) DWI obtained 3 days after onset shows enlargement of the hyperintensity area in the left temporal lobe

ischemic brain injury, reactive oxygen species (ROS) are directly involved in oxidative damage to cellular macromolecules[17,18]. Although brain cells are normally well protected from ROS by antioxidant defences including oxygen radical scavenger enzymes, they are overcome by the over-production of ROS and the consumption of antioxidants in ischemic brain tissue[19]. The excess generation of ROS in damaged cells leads to oxidation of lipoprotein core lipids and of the cell membrane. These modified apolipoproteins and other proteins can induce macrophage activation and the generation of ROS via both the uptake of their scavenger receptors, and the interaction of macrophages and T cells[20], thereby promoting the oxidation of low-density lipoprotein. While, at present, we do not know to what extent extracellular oxidant stress may also directly affect the intracellular redox balance, enhanced extracellular oxidation may also participate in the weakening abrogation of intracellular antioxidant defences. Although increased plasma OxLDL may derive from both systemic and focal oxidative stress, OxLDL elevation shortly after an ischemic insult may be attributable to leakage from brain cells undergoing oxidative damage. This hypothesis is supported by our observation that patients with massive infarct volume in the early phase and patients with cortical infarction in the chronic phase did not manifest high levels of plasma OxLDL.

Chopp *et al.*[21] suggested that nuclear DNA damage following cerebral ischemia involves two distinct mechanisms, oxidative injury and endonuclease-mediated nuclear DNA fragmentation. DNA fragmentation by certain activated endonucleases, including caspase-activated endonuclease, occurs at a relatively late stage of post-ischemic cell death[21,22]. On the other hand, oxidative damage resulting from direct attacks by ROS such as hydroxyl radicals and nitric oxide derivatives in the early phase after the insult may represent an early event that is reversible by DNA repair mechanisms[23–28]

Ren *et al.*[29] documented that in rats, the levels of cytoprotective heatshock protein 70 in the ischemic brain hemisphere were maximally increased at 24 hours post-insult and that the increase persisted for at least 7 days in the ischemic cerebral cortex. This phenomenon may also be reflective of neuroprotection and suggests that, as is the case in humans, oxidative damage in the early stage after stroke insult may be reversible in this

animal model. We posit that in patients with moderately-sized cortical lesions, elevated plasma OxLDL may indicate the survival of salvageable cells under oxidative stress and suggest that these patients should receive aggressive treatment, including the administration of antioxidant drugs.

It is likely that the DWI/PWI mismatch area is indicative of potentially salvageable tissue[10–12]. In animal models, oxidative cerebral damage was present in the penumbra, as well as the ischemic core[30,31]. The data presented here show that increased OxLDL was reflected in DWI and PWI findings, and in the DWI–PWI mismatch ratio. As PWI studies regularly over-estimate the region at risk, efforts are directed at identifying PWI parameters that will more accurately define this region[32]. The findings presented here show that determination of the plasma OxLDL level can be used to strengthen the diagnosis reached by image analysis.

GI and GIII, but not GII patients manifested a high NIHSS score. In GI, the NIHSS score was correlated with plasma OxLDL levels, and with DWI and PWI findings, indicating that in patients with moderate cortical infarction, the plasma OxLDL level can reflect initial neuronal deficits. However, the OxLDL level was not predictive of outcome in GIII because the mRS score in these patients was high despite the absence of significant OxLDL elevation.

CONCLUSION

Our study documents that plasma OxLDL is a useful peripheral biomarker that indicates oxidative damage in patients with moderately-sized infarcts. Studies are underway in our laboratory to evaluate the efficacy of antioxidants, including radical scavengers, to strengthen the antioxidant defences in patients with stroke by evaluating the plasma OxLDL level.

ACKNOWLEDGEMENT

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総　　説

脳卒中診断の最前線

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1999年11月より当院にstroke care unit(SCU)を開設して，24時間体制で急性期脳卒中を受け入れてきた。5年間の急性期脳卒中患者は660名であり，その内訳は脳梗塞370例（55.6%），脳出血141例（21.3%），くも膜下出血97例（14.7%）であった。入院時にくも膜下出血を疑った患者以外はまずstroke MRI（拡散強調画像：DWI，灌流強調画像：PWI，T2強調画像，MRA）を施行した。2004年3月からは臨床機3T-MRIでstroke MRIを施行し，短時間でテンソル画像によるtractographyやMR spectroscopy（MRS）を撮影し，神経繊維の走行や脳代謝についても診断した。その結果1）DWIは大脳病変なら発症後1時間たてば小さな病巣（1 mm^3程度）でも描出できた。2）脳幹病変は発症後3時間以上たてば描出できた。3）DWI/PWI mismatchが50%以上ある主幹動脈閉塞に対して血栓溶解療法が適応となり，術後の評価もstroke MRIで可能であった。4）脳出血急性期でもstroke MRIで診断し得た。5）tractographyやMRSが脳卒中の予後を予測できる可能性がある，ことがわかった。また急性期脳卒中患者の血中酸化LDLを測定すると，脳梗塞患者は発症0－3日にかけて健常者より有意に高く，特に皮質に病巣がある症例で酸化LDLは高かった。これらのことより血中酸化LDLを測定することで脳梗塞の重症度と治療可能域を反映できる可能性を示した。

はじめに

脳卒中は本邦の死亡率の第3位であり，かつ寝たきりの原因の第1位である。脳卒中は特殊疾患であり，その診断と治療は高度の診断機器と専門のスタッフが必要である。それにも関わらず多くの症例は一般の救急施設に搬送され，必ずしも最先端の診断や治療を受けていないのが実状であろう。その一つの原因として大学病院を中心とした医育機関が急性期脳卒中患者を受け入れる体制を構築してこなかったことが考えられる。脳卒中のような特殊な疾患は脳卒中専門医が超急性期から診断治療することで，その予後が大きく改善することがヨーロッパを中心に報告されている[1)]。われわれは国立大学病院としては画期的なシステムとして24時間体制で脳卒中患者を受け入れ診断・治療するstroke care unit（SCU）を1999年11月から開設した[2)]。われわれは近年急速に発達する頭部MRIを利用して脳卒中超急性期にstroke MRIを施行し，正確な診断を心がけてきた[2-5)]。また近年脳梗塞の酸化ストレスのbiomarkerとして，急性期脳卒中症例の血中酸化LDLを測定した[6-9)]。これらの結果を基にして，脳卒中診断の放射線学的，血中生化学的診断の最前線を報告する。

対象と方法

1999年より当院にstroke care unit（SCU）を開設して，24時間体制で急性期脳卒中を受け入れてきた[2-5)]。5年間の急性期脳卒中患者は660名であり，その内訳は脳梗塞370例（55.6%），脳出血141例（21.3%），くも膜下出血97例（14.7%）であった。

1．SCUの体制と診断方法

脳神経外科を中心に，救急診療部，放射線科，循環器内科，神経内科，整形外科，精神神経科，麻酔科，手術部，放射線部の協力を得て，急性期脳卒中患者を24時間体制で受け入れた。超急性期の患者は救急外来受診時にまずstroke MRIを施行した。Stroke MRIは放射線科医が24時間体制でチームを組み，diffusion MRI（DWI），perfusion MRI（PWI），T2-MRI，MRAを緊急で施行した。超急性期脳出血に対してもDWI，T2-MRIで診断でき[10)]，

くも膜下出血を疑った症例のみ最初に緊急CTを施行した。

2．Stroke MRIによる治療方針の決定

Stroke MRIにより以下の条件を満たせば緊急の脳血管撮影を行い，血栓溶解療法を行うことにしている（図1）[5,11-14]。①DWIで病巣が小さく，かつPWIで大きな血流低下領域がある。すなわちDWI/PWI mismatchが大きい（50%以上ある），②MRAで主幹動脈（内頸動脈，中大脳動脈水平部，椎骨脳底動脈）に70%以上の狭窄あるいは閉塞がある，③血流再開が発症から6時間以内に可能である。以上の条件を満たす症例はすぐに脳血管撮影を行った。

3．3T-MRIの導入

2004年3月からは臨床機3T-MRIでstroke MRIを施行し，短時間でテンソル画像によるtractographyやMR spectroscopy(MRS)を撮影し，神経繊維の走行や脳代謝についても診断した。

4．血中酸化LDLの測定

急性期脳卒中患者の血清を採取し，血清中のOxLDLを板部らが開発した酸化LDLモノクロール抗体(DLH3)を用い，抗ApoB抗体とのsandwich ELISA法でwell washを利用して半定量的に計測した[7]。またこれらの値とstroke MRIで得られた脳虚血体積の関連性を検討した[9]。

結　果

1）拡散強調画像による脳虚血巣の診断

DWIでは大脳病変なら発症後1時間以上経過した症例では微少な虚血巣（1 mm³程度）でも描出できた（図2）。また脳幹病変でも発症後3時間以上たてば描出可能であったが，延髄病変では3時間以内の小梗塞では描出されない症例があり，症状が脳幹病変を疑わせる症例ではfollow-upのDWIが必要であった[15]（図3）。

2）Stroke MRIによる治療方針の決定

DWI/PWI mismatchと入院時のNIH stroke score (NIHSS)は逆相関した[11]。またDWI/PWI mismatchが50%以上ある主幹動脈閉塞に対して動脈内血栓溶解療法を行ったところ，術後出血は以前の症例と比較して激減した。再開通した症例の梗塞を免れた領域をrescued volume

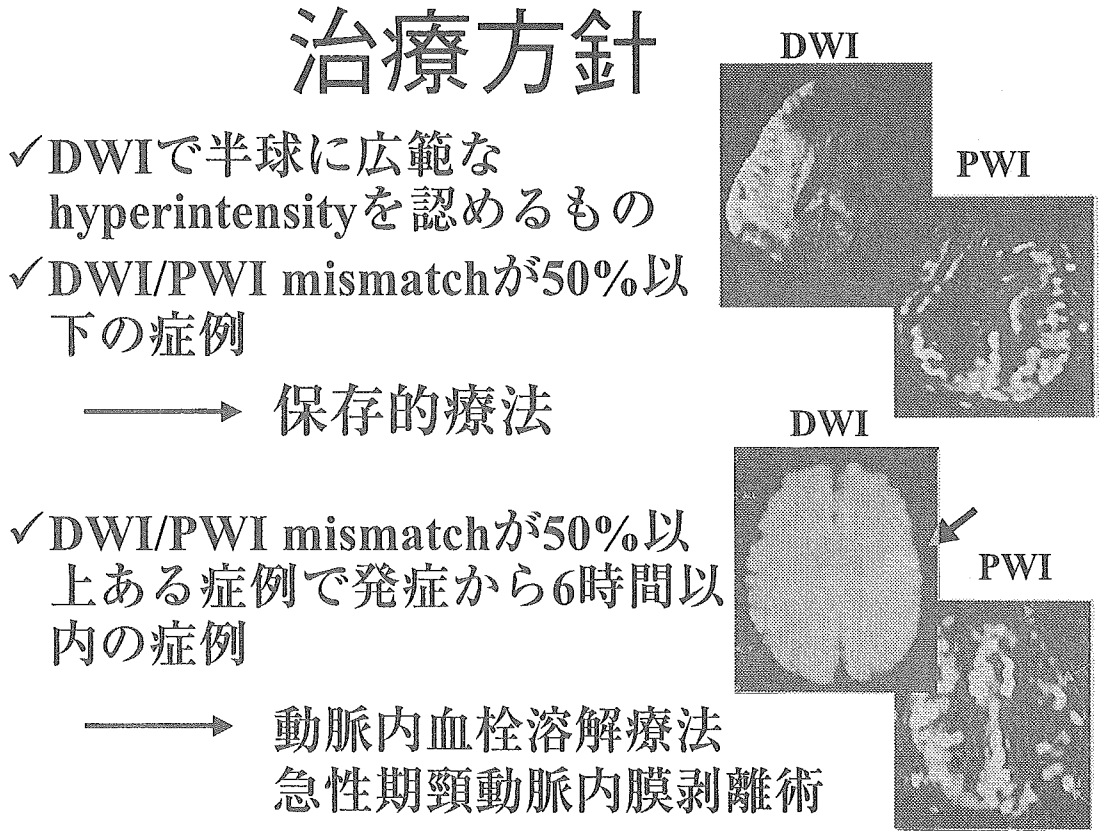

図1　stroke MRIによる治療方針の決定

として術後の評価を行ったところ，final NIHSS と rescued ratio が逆相関した（助かった領域が多いほど NIHSS は低いスコアー）。ゆえに術後の評価も stroke MRI で可能であった[11]。

3）Stroke MRI による急性期脳出血の診断

患者が片麻痺や意識障害で受診した場合，神経兆候だけでは出血と梗塞との鑑別は不可能である。従来は脳卒中患者が受診した場合，まず頭部 CT を施行し，脳出血があるかどうかを診断した。しかし，われわれは上記の様な症状で脳卒中が疑われる症例に対して，まず stroke MRI を施行した。9例の発症後40分から13時間までの脳出血患者に対してまず stroke MRI を施行した。この段階で脳出血患者の DWI は脳虚血と比較して病巣は heterogeneous で血腫周囲には DWI では hypointensity

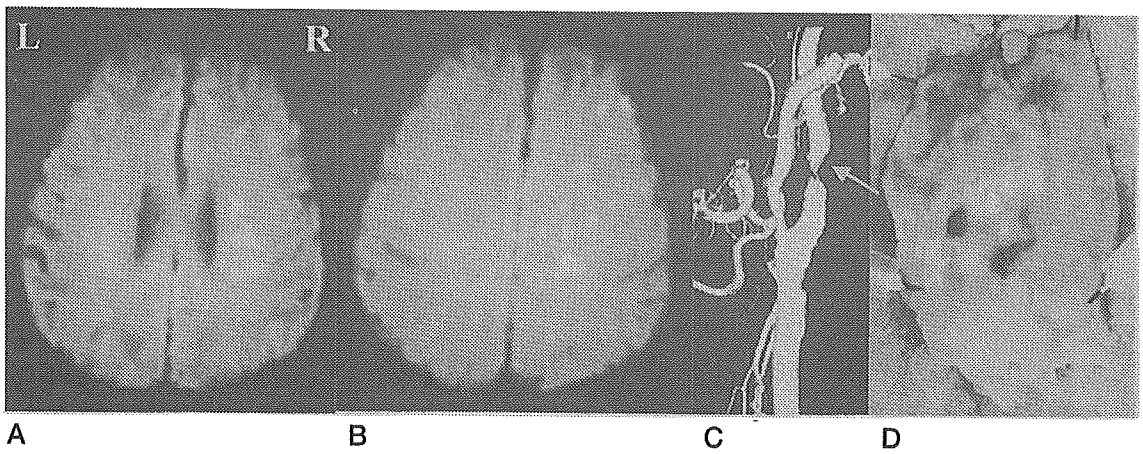

図2　67歳　男性の入院時 stroke MRI

A，B：入院時の DWI で左大脳白質に小さな脳梗塞が散在して存在している。この像から artery to artery による脳梗塞が考えられた。
C：脳血管撮影（3D-angiography）で頸部頸動脈に重度の狭窄があることが確認できた。
D：頸動脈内膜剥離術で頸動脈に潰瘍を伴うアテロームプラークが認められ，それを摘出した。

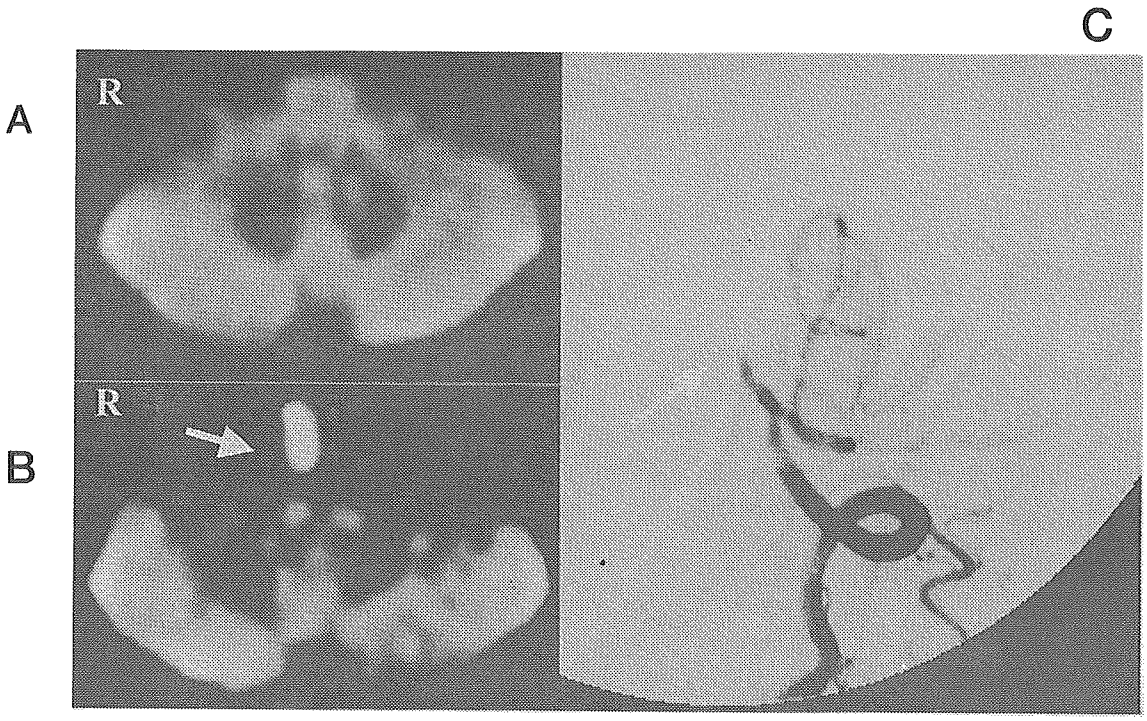

図3　51歳男性，延髄梗塞の入院時の DWI と follow-up DWI

A：発症2時間目の initial DWI. 脳幹の梗塞巣ははっきりしない。
B：発症19時間目の follow-up DWI では右延髄内側に明らかな虚血巣を示す。
C：脳血管撮影では右椎骨動脈の閉塞を認めた。

rimが認められた（図4）。これらの症例は確認のため頭部CTを施行したところ全例が脳出血であった。この結果からその後すべての症例がstroke MRIで脳出血と診断され，確認の意味での頭部CTは省略している。

4）MRIによる機能的神経診断

3T-MRIが導入されたのち，stroke MRIの測定時間が大幅に短縮され，かつ拡散強調画像を利用し，神経繊維の走行を描出できるようになった（tractography）。こ

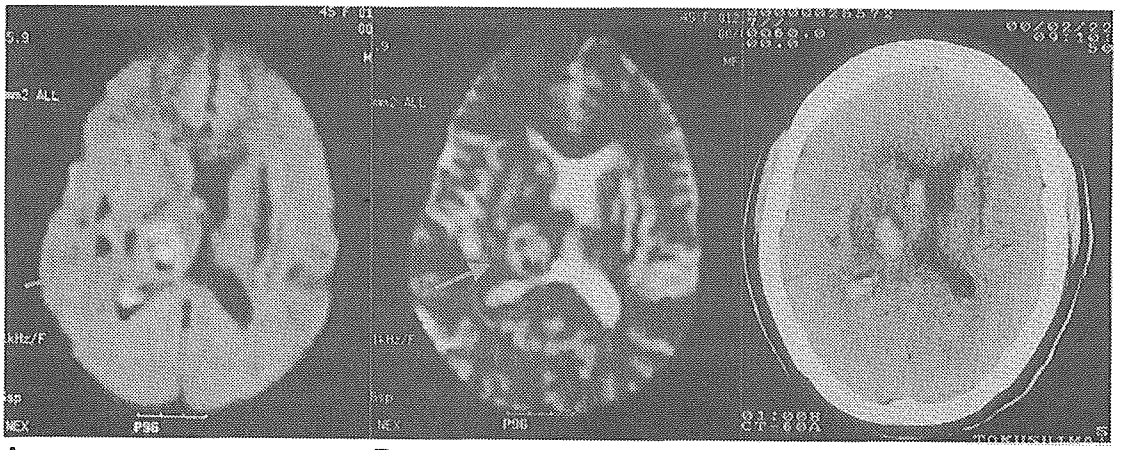

図4　49歳　女性　視床出血のDWI

A，B：
発症1時間20分後のinitial DWIで右視床にheterogenous massを認める。
C：引き続き行われた頭部CTで右視床出血を確認した。

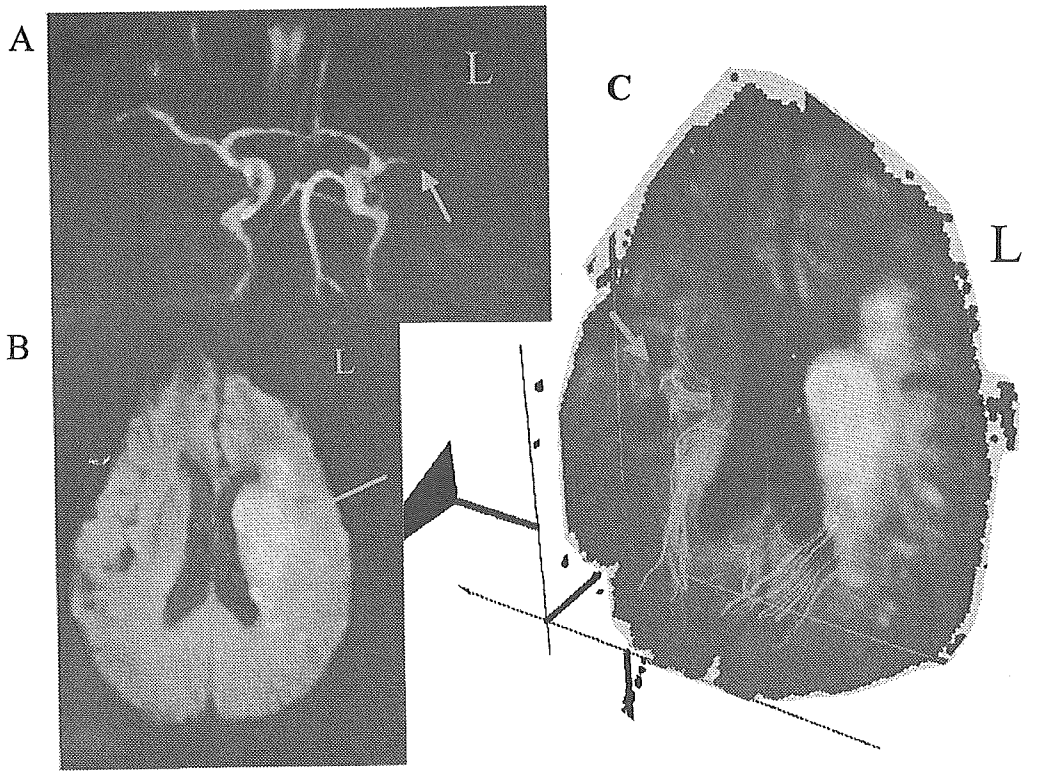

図5　72歳　男性　左中大脳動脈閉塞症例のDWIとtractography

A：発症12時間目のinitial MRAで左中大脳動脈水平部の閉塞を認める。
B：発症12時間目のinitial DWIでは左放線冠に脳虚血巣を認めるが，特に前方部分のintensityが著明である。
C：同時に施行したテルソン画像によるtractographyでは正常側で認められる前頭葉からの神経繊維（赤矢印）は病巣側では断裂しているが，放線冠後方部の虚血巣ではtractは病巣を貫いている。

れにより脳出血や脳梗塞による神経繊維の断裂が描出でき，予後を推測できる可能性を示した（図5）。また脳の代謝をMRSで短時間で評価でき，未だ脳梗塞に陥っていない領域でも代謝が退化している領域を描出できるようになった（図6）。

4）血中酸化LDLの測定

急性期脳卒中患者の血中酸化LDLを測定すると，脳梗塞患者は健常者より有意に高く，また脳出血より高い値を示した[8]。特にラクナ梗塞より皮質に病巣を持つ患者の血中酸化LDLが高かった[8]（図7）。この血中酸化LDLのピークは発症3日目にかけて認められ，2週間をすぎるとbase lineに復した（図8）。またstroke MRIでDWI/PWI mismatchが大きく，ペナンブラ領域がある症例で血中酸化LDLは高く，逆にmismatchのない大

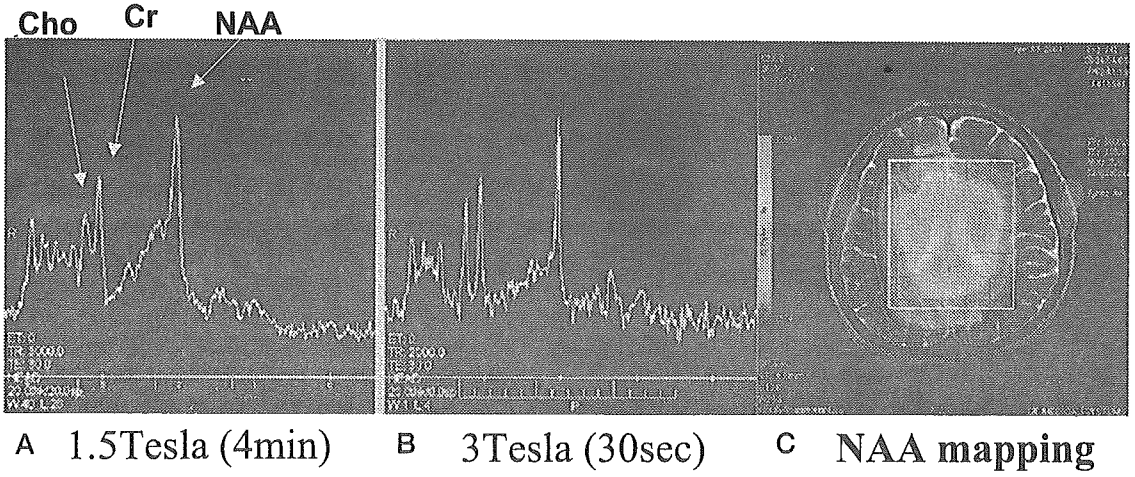

図6　MRSとNAA mapping

A：1.5T-MRIによるMRSでは4分の測定時間がかかる。
B：3T-MRIでは40秒の測定時間でかつS/N比がよいMRSが測定できる。
C：3T-MRIでspectroscopic imaging(CSI)によりNAAをMRI画像上にmappingができる。

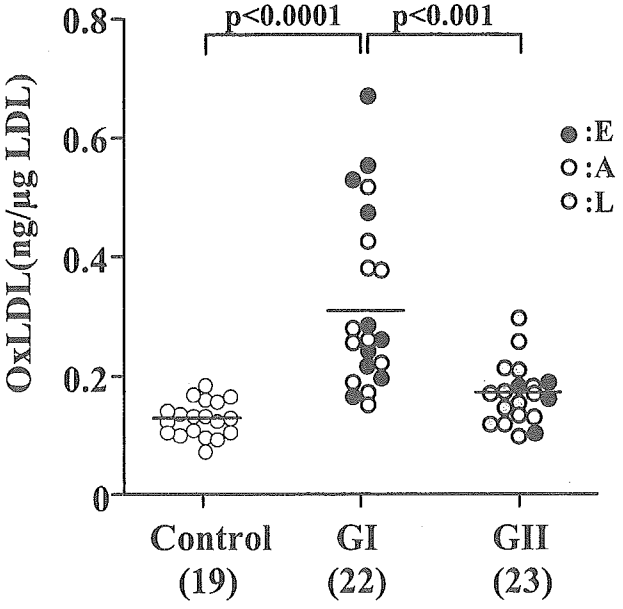

図7　急性期脳梗塞患者の血中LDL

皮質に梗塞巣を持つ群(GI)はそれ以外の小梗塞群(GII)およびコントロール群の酸化LDLに比較して有意に高値を示した。
E：embolic stroke, A：atherosclerotic stroke, L：lacunar stroke

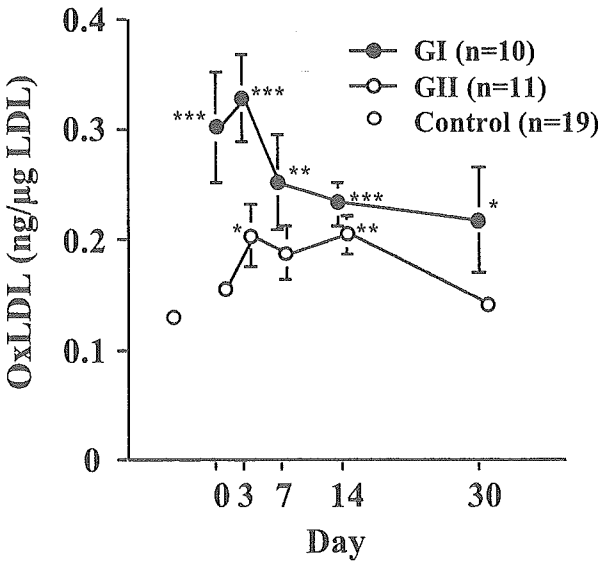

図8　血中酸化LDLの脳梗塞患者での経時変化

皮質梗塞群(GI)は発症3日目にかけて高値を示し，2週間を過ぎてbase lineに復した。

梗塞例やラクナ梗塞では低くかった[9]。これらのことより血中酸化LDLを測定することで脳梗塞の重症度と治療可能域を反映できる可能性を示した。

考　察

MRIで拡散強調画像がとれるようになりそれが極短時間で施行できるようになってから，脳卒中の診断は飛躍的に進歩した。当院でも1.5TMRIが導入され，かつecho planar法が導入された1997年からは超急性期の脳卒中にstroke MRIで診断してきた。1999年11月からは国立大学では全国に先駆けてSCUを開設し，放射線科の協力ものと24時間体制でstroke MRIが施行できるようになった[2-5,12]。この結果，当院の脳卒中に対する診断はさらに向上し，これに伴い，適切な治療ができるようになったと確信している。すなわち，それまでは6時間以内の超急性期の脳虚血では症状があってもどの部位に虚血巣があり，またどの血管が閉塞しているかもわからず，画一的な治療法をとらざるを得なかった。また重症の患者には頭部CTやT_2MRIで虚血巣がなく，脳血管撮影で主要血管の閉塞を確認した後，血行再建術の適応を決定していた[16]。Stroke MRIが導入されてからは大脳皮質の病巣は発症から1時間が経過すれば1 mm^3程度の小さな梗塞巣でも描出され，また主幹血管の狭窄，閉塞が瞬時にわかるようになった。また脳血流画像も同時に撮影でき，それによるDWI/PWI mismatchが血行再建術の適応基準として使えるようになった[11,13,14]。これにより適切な血行再建術が施行できるようになり，術後の出血が激減し，予後が良くなっている。3T-MRIが導入されてからは，撮影時間が短縮され，tractographyやMRSが追加して施行できるようになった。今後はこれらを解析して，症例の予後がinitial MRIで予測できる可能性があり，症例を重ねて検討したい。

画像診断は飛躍的に向上したが，脳卒中には心筋虚血の診断に使用している血中CPK，WBCなどの血中バイオマーカーがないのが実状である。もし，入院時の採血で脳卒中の重症度や病型が診断できれば，症例に対する治療法の効果判定や，予後予測に役立つと思われる。酸化LDLは動脈硬化に関与する重要な物質であるが，最近までは血中では測定できないものと考えられていた。板部らが開発した方法で血中にも血管壁の1／1000の濃度で存在することがわかり，心筋虚血例では健常人と比較して有意に上昇していることが報告されていた[7]。われわれは急性期脳梗塞では健常人と比較して有意に血中酸化LDLが上昇していることを初めて報告し，発症3日目までにそのピークがあることを報告した[8]。またこの上昇は小さなラクナ梗塞より皮質梗塞で高く，DWI/PWI mismatchが高い症例ほど血中酸化LDLが高いことを示した[8,9]。以上より，放射線学的診断に加えて血中バイオマーカーとしての酸化LDLが今後脳梗塞の病型診断やその重症度，治療効果の判定に役立つ可能性を示した。

結　語

脳卒中の診断は日々進歩しており，症状が重症度や病型が瞬時に判断ができるようになってきた。Stroke MRIや血中バイオマーカーを駆使して，できるだけ迅速かつ正確な治療ができれば，脳卒中が原因で寝たきりになる率を下げ得ると考えている。

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Original Contribution

Inhibition of brain damage by edaravone, a free radical scavenger, can be monitored by plasma biomarkers that detect oxidative and astrocyte damage in patients with acute cerebral infarction☆

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Abstract

We assess the availability of plasma biomarkers to monitor the brain damage and the therapeutic efficacy of edaravone. The study consisted of 51 patients with ischemic cerebral infarcts. They were divided into 2 groups: GI (n = 24) had cortical lesions, and GII (n = 27) had lesions in the basal ganglia or brain stem. Edaravone was administered to 27 randomly selected patients (GIa, n = 13; GIIa, n = 14) and its efficacy was studied by comparing their plasma OxLDL, S-100B, and MnSOD levels to those in patients without edaravone (GIb, n = 11, GIIb, n = 13). Three days after the start of edaravone, plasma OxLDL was significantly lower in GIa than GIb patients (0.177 ± 0.024 ng/μg apoB vs 0.219 ± 0.026, $P < 0.05$). In GIIa patients, pre- and posttreatment plasma OxLDL was not significantly different (0.156 ± 0.013 vs 0.152 ± 0.020). In GIa patients, S-100B and MnSOD were significantly lower than in GIb patients ($P < 0.05$). The neurological condition at the time of discharge had recovered in GIa but not GIb patients. Ours is the first evidence to confirm the efficacy of edaravone by plasma biomarkers. In patients with cortical infarcts, edaravone reduced oxidative damage, thereby limiting the degree of brain damage.

Keywords: Acute cerebral infarction; Free radical scavenger; Plasma biomarker; Free radicals

Introduction

Upon energy failure during brain ischemia, polyunsaturated fatty acids are released from membrane phospholipids. This is followed by activation of the arachidonate cascade that includes the lipoxygenase pathway, the production of oxygen radicals, and lipid peroxidation, which is thought to contribute to neuronal injury [1–3].

Edaravone exerts antioxidant action that suppresses free radicals including hydroxy, peroxy, and alcoxy radicals; it has been used to treat patients with acute stroke [4]. Clinical and experimental studies [5–8] have shown that it exerts protective effects against oxidative actions. The neuroprotective efficacy of edaravone and its beneficial effect on functional outcomes have been demonstrated in patients with acute ischemic stroke [9]. However, the role of its antioxidant actions in its therapeutic effects remains to be elucidated. We previously reported that patients with atherothrombotic and cortical infarcts manifested significantly higher plasma oxidized low-density lipoprotein (OxLDL) levels than did patients with lacunar infarcts and

Abbreviations: OxLDL, oxidized low-density lipoprotein; NIHSS, NIH Stroke Scale; mAb, monoclonal antibody; SOD, superoxide dismutase; DWI, diffusion-weighted imaging; PWI, perfusion-weighted imaging; FLAIR, flow-sensitive alternating inversion recovery; IR, inversion-recovery; 8-OHdG, 8-hydroxydeoxyguanosine; MCAOR, middle cerebral artery occlusion/reperfusion.

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age-matched controls [10]. Plasma OxLDL levels were correlated with the infarct volume and the admission NIH Stroke Scale (NIHSS) score but not with the modified Rankin scale on discharge [11]. On the other hand, not all patients with severe large infarcts over the hemisphere had high plasma OxLDL levels and to some degree, the increased plasma OxLDL level at stroke onset was associated with expansion of the infarct size 3 days after the insult. Our results suggested that since increased plasma OxLDL reflects brain oxidative damage in patients with moderate cortical infarction and predicts the presence of salvageable regions, it is helpful to monitor OxLDL in stroke patients.

Protein S-100B, an acidic Ca^{2+}-binding protein (20 kDa) that comprises a major component of cytosol, exists predominantly in astrocytes and Schwarzman cells [12–14]. It is produced and released by astrocytes and can stimulate the activation of microglia and astrocytes, and it plays a pivotal role in the occurrence of delayed infarct expansion and prolonged suppression of neuronal functions in the peri-infarct area [15–17]. S-100B levels in cerebrospinal fluid or plasma are now used as a biomarker for evaluating the presence and severity of brain damage and to predict the prognosis after acute ischemic or traumatic brain injury [18]. In patients with acute ischemic stroke, serum S-100B levels correlate with infarct size and neurological and functional outcomes [19,20]. Therefore, S-100B levels may be useful for the evaluation of neuroprotective therapies [21].

To investigate whether its efficacy is reflected in the level of plasma biomarkers of brain damage, we compared plasma OxLDL and S-100B levels in ischemic stroke patients who did, or did not, receive edaravone. Here we first demonstrate that the therapeutic efficacy of edaravone can be monitored by plasma biomarkers that detect oxidative and astrocyte damage in acute cerebral infarction.

Methods

Subjects

Our study population consisted of 51 patients with ischemic cerebral infarcts. They were 31 men and 20 women, ranging in age from 40 to 82 years (67.1 ± 12.5, mean ± SD). The controls were 19 age-matched healthy volunteers who had no history of cerebrovascular accidents (9 men and 10 women, aged 61.2 ± 9.6 years, range 35–78). The patients had been admitted consecutively between February 2002 and July 2003 to the Stroke Care Unit at the University of Tokushima Hospital. Prior informed consent was obtained from all study participants or their relatives. Our study was approved by the ethics committee of the University of Tokushima.

All patients underwent magnetic resonance imaging (MRI) examination immediately at admission; echocardiography and extracranial duplex ultrasound were also performed in all patients. A diagnosis of stroke was based on clinical findings. An NIHSS [22,23] score was assigned at admission and discharge to determine neurological deficits. Baseline data (age, sex), conventional vascular risk factors (hypertension, diabetes mellitus, hyperlipidemia), and previous atrial fibrillation were recorded. Patients whose pertinent data could not be evaluated at the time of stroke onset and those with hemorrhagic infarction were excluded from this study. Based on the location of the ischemic lesions [11], the patients were divided into two groups (Table 1 and Fig. 1A). In GI patients (n = 24), the infarct was located in cortical regions in the cerebral hemisphere and involved the frontal, parietal, and temporal lobe or the occipital lobe and cerebellum (Fig. 1A,a). In GII patients (n = 27), the infarct involved basal ganglia regions in the anterior circulation (putamen, caudate head), corona radiata, or brain stem and thalamus (Fig. 1A,b). The stroke subtypes were defined according to the TOAST classification system [23]. Of the 51 patients, 16 (31.4%) had cardioembolic, 13 (25.5%) had atherothrombotic, and 22 (43.1%) had lacunar infarcts. The atherothrombotic infarct group included patients with clinical and imaging findings of either significant stenosis or occlusion of a major artery or a branch of the cortical artery, presumably due to atherosclerosis. The cardioembolic infarct group included patients with arterial occlusion presumably due to an embolus arising in the heart. The lacunar infarction group included patients with one of the traditional clinical lacunar syndromes and no evidence of cerebral cortical dysfunction, and patients whose MRI did not show lesions exceeding 1.5 cm in diameter (Fig. 1A,b). Randomization was based on a computer-generated random number table; from this, a

Table 1
Characteristics of patients recruited in this study at admission

	Group I		Group II	
	GIa	GIb	GIIa	GIIb
Age	71.8 ± 6.9	60.1 ± 11.2	70.5 ± 13.2	67.2 ± 17.6
Male/female (n)	8/5	6/5	10/4	7/6
E/A/L	7/6/0	6/5/0	2/1/11	1/1/11
BP (mm Hg)				
Systolic	158.1 ± 10.3	171.0 ± 8.6	181.6 ± 8.7	182.2 ± 8.7
Diastolic	78.0 ± 3.6	90.2 ± 6.0	96.9 ± 5.8	90.8 ± 6.0
NIHSS	10.9 ± 2.5	12.5 ± 2.8	5.4 ± 1.3	6.7 ± 3.7
Clinical biochemistry				
CRP (mg/dl)	2.0 ± 1.0	2.5 ± 1.3	0.5 ± 0.3	1.2 ± 0.5
LDH (mg/dl)	231 ± 15.8	261.3 ± 25.2	228.9 ± 23.6	280.3 ± 41.9
TG (mg/dl)	98.4 ± 15.7	100.6 ± 13.2	120.8 ± 16.3	86.2 ± 20.3
TC (mg/dl)	151.7 ± 7.6	151.1 ± 11.3	196.5 ± 13.4	188.8 ± 16.7
LDL (mg/dl)	125.4 ± 6.4	131.0 ± 10.8	175.1 ± 46.5	171.6 ± 18.8
HDL (mg/dl)	42.3 ± 3.4	44.6 ± 3.1	46.5 ± 3.0	42.2 ± 2.9
BG (mg/dl)	116.2 ± 8.1	125.0 ± 3.7	127.7 ± 7.2	154.2 ± 35.3
BUN (mg/dl)	15.6 ± 1.1	18.6 ± 3.4	13.2 ± 0.9	15.7 ± 1.1
CRN (mg/dl)	0.8 ± 0.1	1.0 ± 0.1	0.7 ± 0.1	0.8 ± 0.1

E, cardioembolic; A, atherothrombotic; L, lacunar infarction; BP, blood pressure; TC, total cholesterol; BG; blood glucose; CRN, creatine.

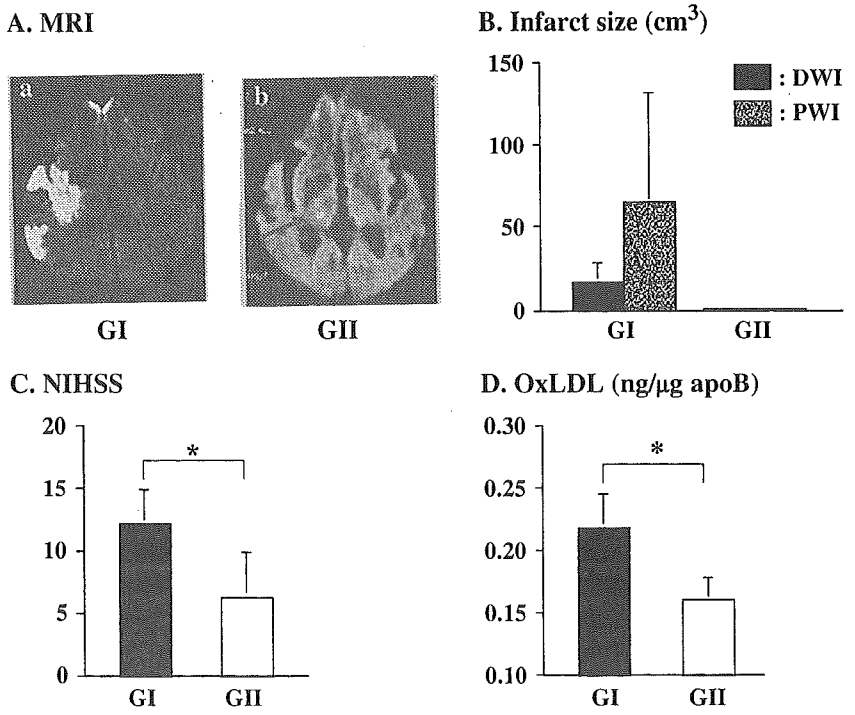

Fig. 1. MRI, infarct size, NIHSS, and plasma OxLDL levels at onset of acute cerebral infarction. In group I (GI, n = 24) the infarct was located in cortical regions in the cerebral hemisphere and involved the frontal, parietal, and temporal lobe or the occipital lobe and cerebellum (A,a). In group 2 (GII, n = 27), the infarcts (arrow) involved basal ganglia regions in the anterior circulation (putamen, caudate head), corona radiata, or brain stem and thalamus (A,b). The infarct size, determined by diffusion- and perfusion-weighted imaging (B), the NIHSS score (C), and plasma OxLDL levels (D) were significantly greater in GI than GII patients. Data are the means ± SD. *P < 0.05 by the Mann-Whitney U test.

study coordinator who was not involved in patient care created the randomization list assigning treatment group. The 51 patients were randomly divided into those who did (n = 27), and did not (n = 24), receive edaravone; 13 GI and 14 GII patients received the drug. Hereafter they are designated GIa and GIIa, respectively. Edaravone administration started within 24 h of stroke onset; it was infused at a dose of 30 mg, twice a day, for 14 days. Patients who did not receive edaravone are designated GIb (n = 11) and GIIb (n = 13). As other medicines, all patients with cardioembolic infarction of the GI group received glycerol (400 ml/day for 3–7 days), and all patients with atherothrombotic infarct of GI and GII groups and all with lacunar infarction of GII group received thromboxane A2 synthetase inhibitors (160 mg/day for 7 to 14 days).

No patients with massive infarcts (ischemic volume > 100 cm^3) that involved the cortex and basal ganglia were included in this study. MR angiography (MRA) or cerebral angiography revealed occlusion/stenosis of the internal carotid artery (ICA) or horizontal portion of the middle cerebral artery (MCA) in 16 of the 24 G1 patients (66.7%).

Blood sampling

Venous blood samples for the OxLDL assay and other biochemical analyses were obtained immediately after MRI examination at admission, and on Days 3, 7, and 14 after stroke onset. To measure plasma OxLDL, S-100B, and MnSOD levels, blood was drawn into tubes containing EDTA-2Na and separated by centrifugation at 4°C, 3000 rpm. Other routine chemical laboratory assays were performed according to protocols established by our Clinical Laboratory Department.

Isolation of LDL

LDL isolation was by potassium bromide stepwise density-gradient ultracentrifugation as described previously [24]. Standard OxLDL was prepared by incubating LDL with 5 μM $CuSO_4$ at 37°C for 3 h; anti-OxLDL (mAb) was prepared as described previously [24].

Determination of plasma levels of OxLDL, S-100B, and MnSOD

To measure the plasma OxLDL level we used the same procedure as in our previous study [10,11]. This procedure has been found acceptable by others [25,26]. Briefly, sandwich ELISA was performed with mAb against oxidative phosphatidylcholine (FOH1a/DLH3; DLH3) [27] and apoB IgG antibody (Boehringer, Germany). The complex was detected by phosphatase-conjugated donkey anti-sheep IgG antibody (Chemicon, USA) and visualized by incubation with a substrate solution containing 1 mg/ml disodium p-nitrophenylphosphate hexahydrate (Wako, Japan).

Absorbance at 405 nm was measured for comparison with a standard curve obtained under the same assay conditions. Simultaneously, we ran a parallel set of ELISA using anti-apoB mAb (OEM, USA) to determine the amounts of apoB in the same lipoprotein fractions. The OxLDL level was expressed as the amount of OxLDL per microgram of apoB protein.

S-100B levels were measured in triplicate by curopium-based ELISA according to the manufacturer's instructions. In other clinical and experimental studies [28,29], plasma S-100B increased a little within 12 h after ischemia and peaked at 3 days postinsult. Therefore, we determined the level at 3 days after the insult. MnSOD was measured at the same time as S-100B using commercial kits (Amersham Bioscience, NJ).

Determination of ischemic lesion and size

MRI was performed with a 1.5 T unit (Sigma Horizon; GE Medical System, Milwaukee, WI) with echo-planar capabilities. The acquisition parameters for diffusion-weighted imaging (DWI) were 10,000 ms/95 ms (repetition time/echo time), 128 × 128 matrix, 5-mm-thick sections, 1.5-mm interslice gap, 12 axial sections, and diffusion gradients of 15 mT/m applied in three orthogonal directions. The *b* value was 0 and 1000 s/mm [11].

Perfusion-weighted imaging (PWI) was performed using the flow-sensitive alternating inversion-recovery (FLAIR) method [30]. The FLAIR technique is based on inversion-recovery (IR)-prepared echo-planar imaging sequences. After the collection of slice-selective and non-slice-selective IR images, two different images were subtracted to obtain blood-flow images. The measurement conditions for FLAIR were time of repetition (TR) = 2 s, echo time (TE) = 10 ms, inversion time (TI) = 1200 ms, slice thickness = 8 mm, 5-mm interpiece gap, field of view (FOV) = 24 cm, non- slice-selective pulse = 4× the selective pulse, matrix = 96 × 96 pixels, and number of excitations = 100.

The volumes of hyperintensity on DWI and hypointensity on PWI were determined by a radiologist with 20 years of experience who was blinded to the clinical status of the patients. Utilizing GE calculating software, lesion-volume mismatches on DWI, PWI, and DWI-PWI were calculated. (DWI/PWI mismatch volume (cm^3) = initial lesion volume on PWI minus initial lesion volume on DWI).

Statistics

Sequentially obtained data, expressed as the mean ± SD, were analyzed with the Mann Whitney *U* test for two-group comparisons; ANOVA followed by Scheffe's test was used for more than three-group comparisons. Statistical analyses were performed on a Macintosh computer running statistical software (Stat View 5.0). Statistical significance was considered as $P < 0.05$.

Results

Characteristics of patients with ischemic stroke

As shown in Table 1, there was no significant difference with respect to age, blood pressure at admission, and general biochemical data among the 51 patients in the four groups. The NIHSS score was significantly higher in GI than GII patients ($P < 0.05$, Table 1, Fig. 1C) and the infarct size at admission was larger in GI than GII patients ($P < 0.05$, Fig. 1B). There were DWI/PWI mismatches in most of GI patients (Fig. 1B).

Edaravone inhibits the increase of plasma OxLDL in the acute phase after cerebral infarction

As shown in Fig. 1D, plasma OxLDL at admission was significantly higher in GI than GII patients (0.219 ± 0.016 and 0.221 ± 0.028 ng/μg apoB for GIa and GIb vs 0.156 ± 0.013 and 0.157 ± 0.028 for GIIa and GIIb; $P < 0.05$). Of the 24 GI patients, 13 received edaravone; compared to pretreatment levels, their plasma OxLDL was significantly decreased 1 day after the start of treatment ($P < 0.05$); the difference from GI patients who did not receive edaravone was also significant ($P < 0.01$, Fig. 2A).

Plasma OxLDL in GIa patients continued to decrease gradually in the course of edaravone treatment and reached the baseline on Day 14 (Fig. 2A). In GIb patients, it increased until the third day; at that time it was significantly higher than in GIa patients (0.219 ± 0.026 vs 0.177 ± 0.024, $P < 0.05$; Fig. 3E). In the 19 normal age-matched controls, plasma OxLDL was 0.13 ± 0.009. At 7 days, OxLDL was significantly lower in GII patients who did (GIIa) than those who did not (GIIb) receive edaravone ($P < 0.05$, Fig. 2B). These results indicate that edaravone is efficacious against oxidative brain damage especially in patients with cortical infarcts (GI) and that OxLDL is an appropriate plasma biomarker for monitoring its effectiveness.

Decreased OxLDL levels after edaravone treatment are correlated with changes in plasma S-100B and MnSOD

On the third day after the insult, the S-100B level was significantly higher in GIb than GIIb patients ($P < 0.05$, Fig. 3A), suggesting that damage to astrocytes was more severe in patients with cortical than lacunar infarcts. Like plasma OxLDL, plasma S100-B levels were significantly lower in GIa than GIb patients (377 ± 98 ng/L vs 633 ± 179 ng/L, $P < 0.05$; Figs. 2A and 3A), there was no significant difference between GIIa and GIIb patients with respect to plasma S100-B (Fig. 3B). On the third day after the start of edaravone administration, plasma OxLDL levels were similar in GIIa and GIIb patients (0.152 ± 0.020 vs 0.161 ± 0.017, Fig. 3F) and not different from the levels obtained just after the insult (0.156 ± 0.013 vs 0.157 ± 0.028, Fig. 2B).

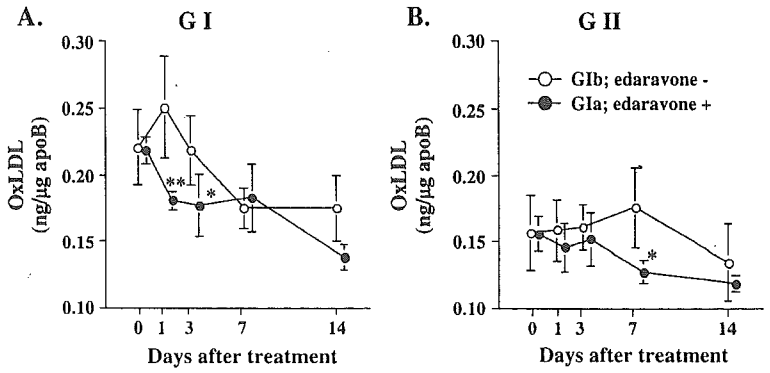

Fig. 2. Changes in plasma OxLDL levels in patients with cerebral infarcts receiving edaravone treatment. Plasma OxLDL levels in GIa patients decreased starting with the first day of treatment compared to untreated (GIb) patients (A). In the group of patients whose infarcts were primarily lacunar (GII), 7-day edaravone treatment resulted in a moderate decrease in the OxLDL level compared to untreated patients (B). Data are the means ± SD. $*P < 0.05$, $**P < 0.01$ by the Mann-Whitney U test.

MnSOD is up-regulated in response to oxidative stress. As shown in Figs. 3C and 3D, the MnSOD level was significantly higher in GIb than GIIb patients at 3 days after the insult ($P < 0.05$). Like plasma OxLDL, plasma MnSOD was significantly lower in GIa than GIb patients on Day 3 (288 ± 27 mg/dl vs 419 ± 67 mg/dl, $P < 0.05$, Fig. 3C).

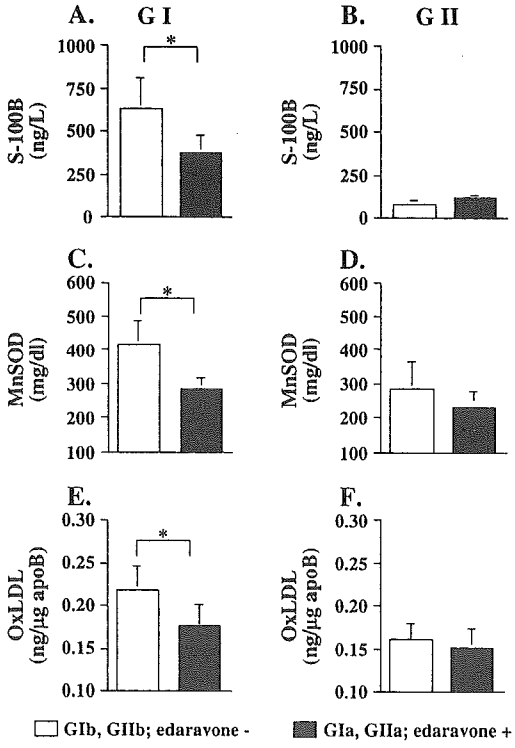

Fig. 3. Plasma levels of S-100B, MnSOD, and OxLDL on the third day after the start of edaravone treatment. The S100-B level was higher in GIb than GIIb patients ($P < 0.05$; A and B). The S100-B level in GIa patients was significantly lower than in GIb patients (A) and mirrored the decrease in plasma OxLDL (E). The S-100B level in GIIa and GIIb patients was not significantly different (B). The MnSOD levels were significantly higher in GIb than GIIb patients ($P < 0.05$; C and D). In GI patients, edaravone treatment significantly decreased the plasma MnSOD level (C) while in GII patients, edaravone had no significant effect (D). Data are the means ± SD. $*P < 0.05$ by the Mann-Whitney U test.

There was no significant difference between GIIa and GIIb patients with respect to plasma MnSOD (Fig. 3D).

Recovery of neurological deficits by patients treated with edaravone

The admission NIHSS score was significantly higher in GI than GII patients ($P < 0.05$, Fig. 1C). As shown in Fig. 4, edaravone treatment resulted in a significant lowering of the score in GI patients (from 10.9 ± 2.5 to 7.0 ± 1.2, $P < 0.05$, Fig. 4A) and correlated with the decrease in plasma OxLDL (Fig. 4C). The NIHSS score of GIb patients did not change between admission (12.5 ± 2.8) and discharge (13.7 ± 4.2) (Fig. 4A); it decreased somewhat in GIIa patients (from 6.4 ± 2.3 to 3.6 ± 1.2) but not in GIIb patients (6.7 ± 3.7 (admission), 6.8 ± 3.6 (discharge)) (Fig. 4B). Based on our observations we conclude that edaravone helps to limit oxidative brain damage in patients with moderate cortical infarcts and thereby limits the degree of focal neurological deficits.

Discussion

While edaravone has gained acceptance as a treatment for patients with acute cerebral infarction [31], the assessment of its therapeutic efficacy based on its antioxidant actions remains to be studied in detail. We compared plasma OxLDL, S-100B, and MnSOD levels in infarct patients who did, or did not, receive edaravone to determine whether its efficacy can be assessed by plasma biomarkers. In patients with acute cerebral infarction who received edaravone treatment, plasma OxLDL was decreased. In contrast, patients who were not treated manifested persistently high OxLDL levels. In particular, edaravone-treated patients with cortical infarcts exhibited a decrease in plasma S-100B and MnSOD levels at 3 dayd after the insult that paralleled a decrease in their OxLDL levels. Edaravone treatment led to the recovery of neurological deficits. Ours is the first

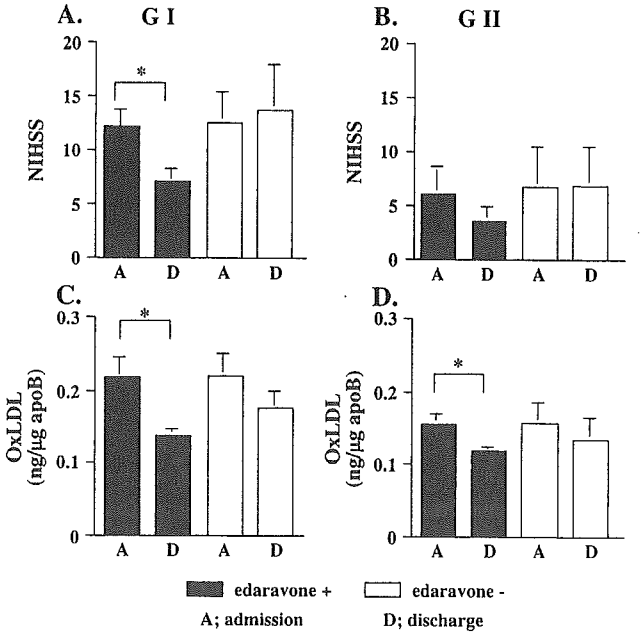

Fig. 4. Effect of edaravone on neurological deficits. The admission NIHSS score was significantly higher in GI than GII patients ($P < 0.05$) as shown in Fig. 1C (A and B). At the time of discharge, GIa patients had a significantly lower score than at admission (7.0 ± 1.2 vs 10.9 ± 2.5, $P < 0.05$; A); this decrease was correlated with the decrease in their plasma OxLDL level (C). In GIb patients, the discharge score (13.7 ± 4.2) was not significantly different from the admission score (12.5 ± 2.8) (A). In GIIa patients there was a moderate difference between the admission (6.4 ± 2.3) and discharge score (3.6 ± 1.2) and in GIIb patients there was no change (6.7 ± 3.7 vs 6.8 ± 3.6) (B). Data are the means ± SD. *$P < 0.05$ by the Mann-Whitney U test.

demonstration that edaravone effectively inhibits oxidative brain damage especially in patients with cortical infarcts, and that its efficacy is reflected by the level of the plasma biomarkers we studied.

Using 8-hydroxydeoxyguanosine (8-OHdG) as a marker of DNA oxidative damage in a middle cerebral artery occlusion/reperfusion (MCAOR) model, we previously demonstrated by immunohistochemical analysis that damage was mainly present in the neuronal cells in the cortical region but not the caudate putamen and that it is augmented outside the infarct border, the so-called ischemic penumbra [32]. Cortical oxidative damage peaked at 24 h after MCAOR and then gradually decreased to the baseline at 7 days postischemia despite the presence of the infarct region. Moreover, the 8-OHdG level in the brain was reflected by the plasma level. These findings support our current observations that plasma oxidative biomarkers reflect cortical oxidative damage and that the antioxidative effects of edaravone result in the protection of neuronal cells in the peri-infarct area.

In our previous clinical studies [10,11] we showed that plasma OxLDL as an oxidative marker was correlated with the infarct size calculated from DWI and PWI studies and their mismatch in patients with moderate cortical infarcts. On the other hand, not all patients with severe hemispheric infarcts manifested high OxLDL levels. Therefore, we postulated that patients with moderate cortical infarcts and increased OxLDL levels might benefit from treatment with edaravone.

Arachidonate itself and lipoxygenase products from the arachidonate cascade induce brain edema, partly due to disruption of microvascular integrity [33,34]. Edaravone prevents peroxidative cell damage due to hydroperoxyeicosatetraenoic acids from the arachidonate cascade and attenuates arachidonate-induced edema in a rat model [3]. Therefore, its anti-edema mechanism(s) may in part be related to the inhibition of ischemic neuronal and cerebrovascular cell injury by products of the arachidonate cascade [33,34]. Propyl galalate (PG), an in vivo and in vitro antioxidant, did not exhibit significant effects in a stroke model despite its strong activity in restraining superoxide radicals [35]. Studies are underway in our laboratory to examine what mechanism(s) other than free radical scavenging can explain the ability of edaravone to rescue the brain from cerebral ischemia.

The overproduction of S-100B might contribute to pathology via the participation in a glial activation cycle that leads to neuroinflammation and neuronal dysfunction. S-100B stimulates iNOS activity and mRNA levels in rat cortical astrocytes; therefore, it induces neuronal cell death through NO release from astrocytes [36]. Enhanced synthesis of S-100B by peri-infarct reactive astrocytes may play a role in the occurrence of delayed infarct expansion. The appearance of activated or reactive astrocytes, characterized by an increase in cell volume and elongated cytoplasmic processes, has been observed in several pathogenic conditions related to ischemic brain injury [12]. We found that among edaravone-untreated patients, those with cortical infarcts had significantly higher S-100B levels on the third postinsult day than patients with lacunar infarcts. Both necrotic cell damage in the penumbral zone of focal infarction and a breakdown in membrane integrity due to cytotoxic and vasogenic edema may result in the leakage of S-100B from the cytosol into extracellular compartments [37]. The parallel decrease in the plasma level of OxLDL and S-100B in edaravone-treated patients indicates that these biomarkers mirror the extent of substantial brain damage. Besides inhibiting oxidative damage in cortical neurons, edaravone may prevent microglia acivation that leads to inflammation and dysfunction of neuronal cells. Using S-100B transgenic and knockout mice, Wainwright et al. [38] demonstrated that glial fibrillary acidic protein and S-100B levels were significantly increased in S-100B transgenic mice. This is consistent with heightened glial activation and neuroinflammation in response to injury. The decrease in the S-100B level by edaravone may shield astrocytes and result in limiting the extent of brain damage.

MnSOD is up-regulated in response to oxidative stress because its promoter region contains response elements for the redox-sensitive transcription factors of many inflammatory-related genes [39] and its up-regulation may be a key