TLR2 was identified as a receptor for the peptidoglycan of gram-positive bacteria. Although no endogenous ligand of TLR2 has been found so far, it has been reported that, in endothelial cells (20) and macrophages (21), TLR2 is induced by TLR4 stimulation, thereby sensitizing cells to TLR2 ligands. As TLR4 and TLR2 have a common downstream signaling pathway, which leads to NF-κ B activation, the cross-talk between TLR4 and TLR2 works as a positive feedback loop. Recently, Fan et al. have demonstrated that after intraperitoneal infection of wild type or TLR2 knockout mice by E. coli, the absence of TLR2 induction in the TLR2 knockout mice resulted in reduced expression levels of ICAM-1, and subsequently reduced migration of polymorph nuclear neutrophils into the lung (19). These findings suggest that TLR4 and TLR2 do not merely sense the specific ligands individually but rather act in concert to protect the host from infection (18).

Regarding the role of TLR4 and TLR2 in the pathogenesis of atherosclerosis, Edfeldt et al. reported that in human atherosclerotic plaques, TLR4 and TLR2 were expressed on macrophages invading atherosclerotic plaques and their immunoreactivities were co-localized with the nuclear translocation of NF-κ B, a marker of the activation of this pathway, suggesting that TLR4 and TLR2 could play an important role in macrophage activation in such plaques (11).

In the present study, we demonstrated that expression levels of TLR4 and TLR2 on circulating monocytes are changed in CAD patients. We also found that the cross talk between TLR4 and TLR2 was differentially regulated in SA and UA patients. Thus the major findings of our study are as follows: 1) The expression levels of TLR4 on circulating monocytes were higher in SA and UA patients than in CNT patients and were not significantly different between the SA and UA patients. 2) The expression levels of TLR2 on circulating monocytes were higher in UA patients than in SA and CNT patients, and levels were not significantly different between the SA and CNT patients. 3) The response of TLR2 levels to LPS stimulation was differentially regulated in the SA and UA patients with up-regulation in SA patients and down-regulation in UA patients.

Expression levels of TLR4 in SA and UA patients

Our study showed that TLR4 levels were increased in both SA and UA patients. In human monocytes, increased surface expression of TLR4 by INF-γ has been reported to be associated with enhanced activation of the NF-κ B pathway and subsequent production of inflammatory cytokines in response to LPS stimulation (26). Also, low serum levels of LPS have been detected in healthy subjects and shown to be an independent risk factor for atherosclerosis (27) and at low concentration ranges, LPS has been found to have significant proinflammatory effects on human blood vessels (28).

In subjects with carotid atherosclerosis, it was noted that serum levels of HSP 60, an endogenous ligand for TLR4, were also increased and there was a significant correlation between this and intima-media thickness (25). Another study found that serum levels of fibrinogen, another endogenous ligand for TLR4, were increased in patients with acute coronary syndrome (29). Taken together, these findings suggest that increased levels of TLR4 on circulating monocytes play an important role in the development of coronary plaques in CAD patients.

Expression levels of TLR2 in UA patients

In the present study, TLR2 levels were increased only in UA patients. Matsuguchi et al. (21) reported that in mouse macrophages, TLR4 messenger RNA was constitutively expressed and expression stayed at a constant level after stimulation with LPS or proinflammatory cytokines. On the other hand, TLR2 messenger RNA was expressed at low levels in the absence of stimulation but rose to high levels when cells were stimulated with either LPS or proinflammatory cytokines. Such LPS-mediated TLR2 messenger RNA induction was brought to an end by the blockade from NF-κ B activation, suggesting that the NF-κ B pathway plays an essential role in this regard.

Ritchie *et al.* (30) reported that the activation level of the NF-κ B pathway, as indicated by the DNA binding of NF-κ B in PBMCs, was much higher in UA patients than in SA patients. UA has also been reported to be associated with an inflammatory response, not only at the site of plaque rupture but also in the systemic circulation, as shown by increased plasma levels of CRP and proinflammatory cytokines (31) as well as by the activation of circulating monocytes (32, 33) and CD4-positive T lymphocytes (34). In these studies, the inflammatory marker levels were shown to be higher in UA than in SA patients. In our study, both activation of the NF-κ B pathway in monocytes and increased levels of proinflammatory cytokines could explain the increased level of TLR2 expression on peripheral monocytes observed in the UA patients.

Cross talk between TLR4 and TLR2 in SA and UA patients

The cross talk between TLR4 and TLR2 was differentially regulated in the SA and UA patients. In the SA patients, TLR2 levels were increased by TLR4 stimulation with LPS, as previously reported for mouse macrophages and human endothelial cells. In contrast, in the UA patients, in which basal TLR2 levels were elevated, TLR2 levels were decreased by LPS stimulation. Given that the increased TLR2 levels on monocytes due to TLR4 stimulation works as a positive feedback loop, the decrease in TLR2 levels due to TLR4 stimulation in the UA patients

could have a protective role against excess stimuli. This response involving the TLR2 expression level is similar to that seen in activation levels of monocytes derived from UA patients due to LPS stimulation.

In this regard, Zalai *et al.* (33) measured the migration capacity and membrane fluidity of circulating monocytes as indices of their activation levels and showed that at baseline, monocyte activation levels were higher in UA than in SA patients. After LPS stimulation, the migration capacity of monocytes was increased in normal donors, but this was not the case for monocytes isolated from UA patients. The monocytes from the UA patients, however, could have already been activated and resistant to further activation. If the down-regulation of TLR2 levels after LPS stimulation has a protective role against excess stimuli, the elucidation of the mechanism of this regulation could lead to the identification of a new therapeutic option for modulating immunity activation levels in patients with CAD.

Study limitations

Our results suggested that the systemic inflammatory response in SA patients was associated with increased TLR4 expression levels on circulating monocytes, while the more prominent systemic inflammatory response in UA patients was associated with increased TLR2 expression levels. However, to confirm these observations, it would be necessary to investigate any changes in the expression pattern of these TLRs in the same patients. It would also be important to determine whether the induction of TLR2 expression is an event preceding plaque rupture or just a consequence of it. These issues should be clarified in future studies.

Conclusions

In the present study on surface expression levels of TLR2 and TLR4 on circulation monocytes in SA and UA patients, we showed that TLR4 levels were increased in both SA and UA patients, while TLR2 levels were increased only in UA patients. With LPS stimulation, the TLR2 levels were up-regulated in the SA patients but down-regulated in the UA patients.

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Associations of plasma C-reactive protein levels with the presence and extent of coronary stenosis in patients with stable coronary artery disease

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Abstract

Prospective studies showed plasma high sensitivity C-reactive protein (hsCRP) levels to be a powerful predictor of cardiac events. However, the association between hsCRP levels and the extent of coronary stenosis in patients with coronary artery disease (CAD) remains controversial. We investigated the association between hsCRP levels and the extent of coronary stenosis in 273 patients undergoing elective coronary angiography. Plasma hsCRP levels were higher in patients with CAD than in those without CAD (0.70 mg/l versus 0.56 mg/l, $P < 0.02$), but hsCRP levels did not correlate with the number of >50% stenotic vessels and were not a significant factor for CAD. However, after the exclusion of 76 patients taking statins, a step-wise increase in hsCRP levels was found depending on the number of >50% stenotic vessels: 0.50 in CAD(−), 0.68 in 1-vessel, 0.77 in 2-vessel, and 0.88 mg/l in 3-vessel disease ($P < 0.01$). The hsCRP levels also correlated with the numbers of >50% and >25% stenotic segments ($r = 0.30$ and 0.32, $P < 0.001$). Multivariate analysis revealed the hsCRP levels to be a significant factor for CAD. Thus, after the exclusion of patients with statins, plasma hsCRP levels were found to be associated with the presence and extent of coronary stenosis in patients with stable CAD.

Keywords: Coronary artery disease; C-reactive protein; Statin; Inflammation

1. Introduction

Inflammation has been recognized to play an important role in both the initiation and progression of coronary artery disease (CAD) [1,2]. Several prospective studies [3–5] recently showed that plasma high sensitivity C-reactive protein (hsCRP) levels, which are one of the markers of systemic inflammation, are a powerful predictor of future myocardial infarction (MI) and cardiac death among apparently healthy individuals. The high hsCRP levels have also been reported to be associated with an increased risk of further coronary events in patients with CAD [6–8]. However, the association between the plasma hsCRP levels and the extent of coronary stenosis in patients with CAD remains controversial. Some studies previously demonstrated such associations [8–10], whereas others could not find [11–15]. We recently reported the median value of the hsCRP levels in 3515 Japanese healthy people to be 0.2 mg/l [16], which seems to be much lower than those reported in other ethnic groups: 1.6 mg/l in Americans [17] and 1.9 mg/l in Turks [18]. However, there has been no report showing the

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hsCRP levels in Japanese patients with stable CAD. Our study was done to elucidate the association between the plasma hsCRP levels and the extent of coronary stenosis and to also clarify the hsCRP levels of Japanese patients with stable CAD.

2. Methods

2.1. Study patients

We measured the plasma hsCRP levels in 273 consecutive patients (198 male; mean age: 64 ± 8 years, range: 40–85 years) who underwent elective coronary angiography (CAG) for suspected CAD at the National Defense Medical College Hospital from July 1999 to July 2003. The results were compared with the findings of coronary angiograms. Any patients with MI within 6 months, those with unstable angina who had anginal pain at rest within 1 month, or those with a history of percutaneous coronary intervention (PCI) or coronary artery bypass surgery were excluded. Of the 273 patients, 149 (55%) had hypertension (blood pressures of ≥160/95 mmHg or on drugs), and 118 (43%) had hyperlipidemia (total cholesterol level of >240 mg/dl or on drugs), of whom 76 were taking lipid-lowering drugs (statins). Diabetes mellitus (fasting glucose level of ≥126 mg/dl or on hypoglycemic drugs or insulin) was present in 77 (28%) patients, and 54 (20%) were smokers (≥5 cigarettes/day). Our study was approved by the ethics committee of the hospital. After written informed consent was obtained, blood samples were taken in a fasting state on the morning of the day when angiography was performed. The plasma hsCRP levels were measured using the BNII nephelometer (Dade Behring, Japan). The serum total and HDL cholesterol levels were measured on a Hitachi 7600 analyzer using standard enzymatic methods (Kyowa Medics, Japan). The LDL cholesterol levels were also measured by a direct enzymatic method with a commercially available kit (Cholestest LDL, Daiichi Pure Chemicals, Japan).

2.2. Coronary angiography

Coronary angiograms were recorded by a femoral approach with the Judkins technique using a cineangiogram system (Toshiba, Japan). Coronary angiograms and clinical histories were all evaluated by Y. Momiyama, blinded to the hsCRP data. CAD was defined as at least one coronary artery having >50% luminar diameter stenosis. The extent of coronary stenosis was represented as the numbers of >50% stenotic vessels and >50% and >25% stenotic segments. Moreover, the degree of stenosis in each segment was scored from 0 to 4 points (0, ≤25%; 1, 26–50%; 2, 51–75%; 3, 76–90%; 4, >90% stenosis), and the extent score of coronary stenosis was defined as the sum of the scores of all segments. Coronary artery segments were defined according to the Coronary Artery Surgery Study classification.

2.3. Statistics

Any differences between the two groups were evaluated by the unpaired t-test for parametric variables, by the Mann–Whitney U-test for nonparametric variables, and by the χ^2-test for categorical variables. Any differences among the three or more groups were evaluated by the analysis of variance with Scheffe's test for parametric variables, by the Kruskal–Wallis test for nonparametric variables, and by the χ^2-test for categorical variables. Since the distributions of the measured hsCRP levels and the measured extent of coronary stenosis were highly skewed, the correlations between the plasma hsCRP levels and the extent of coronary stenosis were evaluated by Spearman's rank correlation test. A forward step-wise multiple logistic regression analysis was used to elucidate the association between CAD and the hsCRP levels. A P value of <0.05 was considered to be statistically significant. The results are presented as the mean value ± S.D. or the median value.

3. Results

Of the 273 patients, CAD was found in 175 patients (64%), of whom 72 had 1-vessel disease, 61 had 2-vessel disease, and 42 had 3-vessel disease. Of the 175 patients with CAD, 12 (7%) had a history of MI >6 months ago. Compared with the 98 patients without CAD, the 175 with CAD were predominantly male, had higher rates of hypertension and diabetes and also had lower HDL-cholesterol levels (Table 1). The plasma hsCRP levels were higher in patients with CAD than in those without CAD (median value 0.70 mg/l versus 0.56 mg/l, $P < 0.02$). The hsCRP level of >2.0 mg/l, which we previously reported as the upper limit in healthy Japanese [16], was found in 24% of patients with CAD versus 12% without CAD ($P < 0.05$). The hsCRP levels tended to increase depending on the number of >50% stenotic vessels: 0.56 mg/l in CAD(−), 0.70 mg/l in 1-vessel, 0.70 mg/l in

Table 1
Clinical characteristics of patients with and without CAD

	CAD (n = 175)	CAD(−) (n = 98)	P value
Age (years)	65 ± 8	63 ± 8	NS
Gender (male)	135 (77%)	63 (64%)	<0.05
Body mass index (kg/m^2)	25 ± 4	24 ± 4	NS
Hypertension	107 (61%)	42 (43%)	<0.01
Systolic BP (mmHg)	138 ± 18	132 ± 17	<0.02
Hyperlipidemia	82 (47%)	36 (37%)	NS
Statins (+)	53 (30%)	23 (23%)	NS
Total cholesterol (mg/dl)	206 ± 35	207 ± 32	NS
LDL-cholesterol (mg/dl)	128 ± 31	124 ± 26	NS
HDL-cholesterol (mg/dl)	49 ± 14	58 ± 17	<0.001
Diabetes mellitus	62 (35%)	15 (15%)	<0.001
Current smoking	37 (21%)	17 (17%)	NS
Plasma hsCRP (mg/l)	0.70	0.56	<0.02
hsCRP >2.0 mg/l	42 (24%)	12 (12%)	<0.05

Data are presented as the mean value ± S.D. or the number (%) of patients. Plasma hsCRP levels are presented as the median value. BP: blood pressure.

Table 2
Correlations between plasma hsCRP levels and the extent of coronary artery stenosis

	r	P value
All study patients (n = 273)		
The number of >50% stenosis	0.20	<0.002
The number of >25% stenosis	0.23	<0.001
The extent score of stenosis	0.21	<0.002
Patients without statins (n = 197)		
The number of >50% stenosis	0.30	<0.001
The number of >25% stenosis	0.32	<0.001
The extent score of stenosis	0.31	<0.001

2-vessel, and 0.82 mg/l in 3-vessel disease, but the differences among the four groups did not reach statistical significance. The hsCRP levels correlated with the numbers of >50% and >25% stenotic segments (r = 0.20 and 0.23) and the extent score of coronary stenosis (r = 0.21) (P < 0.002) (Table 2). However, in a multivariate analysis, the hsCRP levels were not a significant factor for CAD (Table 3).

Since lipid-lowering drugs (statins) can affect the plasma hsCRP levels [19,20] or may cause the regression of coronary stenosis [21,22], the 76 patients who were taking statins were excluded. After the exclusion of such patients, the hsCRP levels were much higher in patients with CAD than in those without CAD (median 0.79 mg/l versus 0.50 mg/l, P < 0.001). The hsCRP level of >2.0 mg/l was found in 24% of patients with CAD versus 5% without CAD (P < 0.005). A step-wise increase in the hsCRP levels was found depending on the number of >50% stenotic vessels: 0.50 mg/l in CAD(−), 0.68 mg/l in 1-vessel, 0.77 mg/l in 2-vessel, and 0.88 mg/l in 3-vessel disease (P < 0.01) (Fig. 1). The hsCRP levels also correlated better with the numbers of >50% and >25% stenotic segments (r = 0.30 and 0.32) and the extent score of coronary stenosis (r = 0.31) (P < 0.001) (Table 2 and Fig. 2). The multivariate analysis revealed the hsCRP levels to be a significant factor associated with CAD independent of conventional risk factors (Table 3). The odds ratio for the presence of CAD was 4.4 (95% CI: 1.4–13.9) for the hsCRP level of >2.0 mg/l.

Table 3
Associations of the presence of CAD with risk factors and plasma hsCRP levels (multiple logistic regression analysis in the 273 study patients and in the 197 patients without statins)

	Odds ratio	95% CI	P value
All study patients (n = 273)			
Age (per 10 years increase)	1.5	1.1–2.1	<0.02
Hypertension	1.9	1.1–3.3	<0.025
Diabetes	2.6	1.3–5.0	<0.01
HDL-cholesterol (per 10 mg/dl increase)	0.7	0.6–0.8	<0.001
Patients without statins (n = 197)			
Gender (male)	3.4	1.5–7.4	<0.005
Hypertension	2.8	1.4–5.4	<0.005
HDL-cholesterol (per 10 mg/dl increase)	0.8	0.6–0.9	<0.01
hsCRP >2.0 (mg/l)	4.4	1.4–13.9	<0.02

The dependent variable was the presence of CAD. The analysis included age (per 10 years increase), gender, hypertension, hyperlipidemia, diabetes, smoking, and HDL-cholesterol (per 10 mg/dl increase), and hsCRP (>2.0 mg/l) levels.

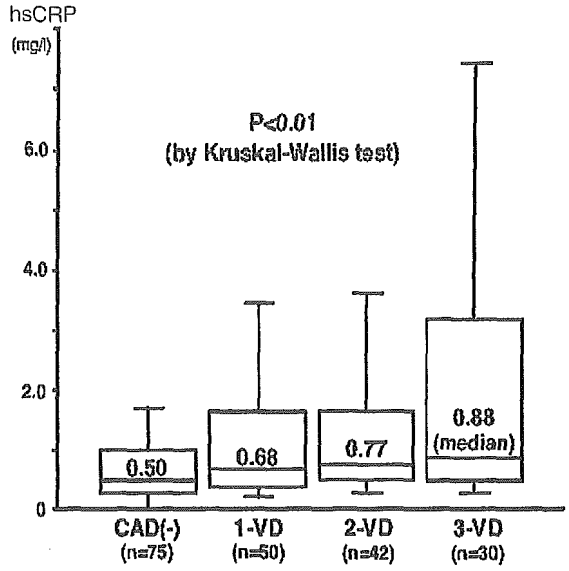

Fig. 1. The plasma hsCRP levels in patients with and without CAD. After the exclusion of patients with statins, a step-wise increase in the hsCRP levels was found depending on the number of >50% stenotic vessels. The central line represents the median, the boxes span from the 25th to 75th percentiles, and the error bars extend from the 10th to 90th percentiles. 1-VD, 1-vessel disease; 2-VD, 2-vessel disease; and 3-VD, 3-vessel disease.

4. Discussion

We investigated the plasma hsCRP levels in 273 Japanese patients undergoing elective CAG: 175 with CAD and 98 without it. The hsCRP levels were higher in patients with CAD than in those without CAD. However, after the

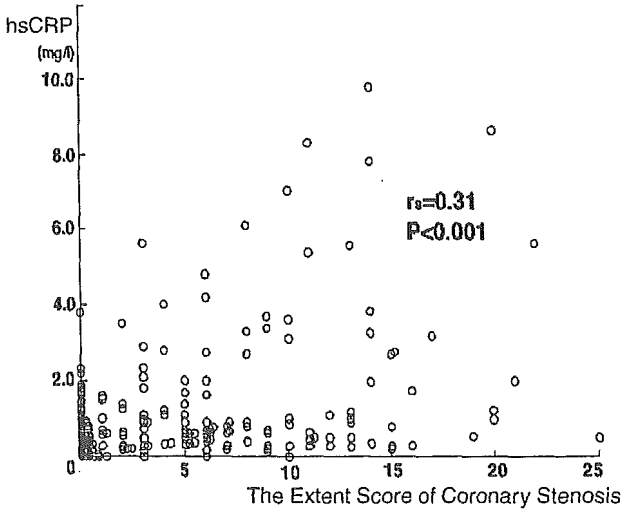

Fig. 2. The correlation between the plasma hsCRP levels and the extent score of coronary stenosis in 197 patients without statins. After the exclusion of patients with statins, the hsCRP levels correlated better with the extent score of coronary stenosis (r = 0.31).

Table 4
Reported hsCRP levels of patients with CAD

Study	Reference no.	Country	hsCRP (mg/l)	Method	Age (years)	BMI (kg/m^2)
Haverkate et al.	[8]	UK	1.7*	Abbott	56	N/A
Veselka et al.	[12]	Czech	2.8*	BNII	61	N/A
Rifai et al.	[11]	Syria	3.4	BNII	51	N/A
Erren et al.	[10]	Germany	4.0	BNII	61	26
Erbagci et al.	[13]	Turkey	11.3*	BNII	60	27
Zebrack et al.	[9]	USA	12.3	Abbott	N/A	N/A
Taniguchi et al.	Present	Japan	0.7	BNII	65	25

The hsCRP levels are presented as the median value (*geometric mean). Age and BMI are presented as the mean value. BMI, body mass index; N/A, not available.

exclusion of patients with statins, the hsCRP levels were found to be associated with the presence of CAD and the extent of coronary stenosis.

The association between the plasma hsCRP levels and the extent of coronary stenosis in patients with CAD remains controversial. Three studies reported no association between the hsCRP levels and the number of >50% stenotic vessels in 100–200 patients undergoing CAG [11–13]. However, these studies did not evaluate the numbers of stenotic segments or the extent score. Azar et al. [14] reported no correlations of the hsCRP levels with the extent score and the number of stenotic vessels in 98 patients. However, the correlations were assessed by simple linear correlations but not by Spearman's rank correlations. Moreover, 20% of patients had MI >2 weeks ago, and some had PCI >2 weeks ago. Hoffmeister et al. [15] also reported no correlation with the extent score. However, some patients were taking statins, and 62% of patients had MI within 2 years.

Haverkate et al. [8] showed that the hsCRP levels weakly correlated with the number of stenotic vessels in 2121 patients undergoing CAG, although 1030 patients were those with unstable angina. Zebrack et al. [9] also reported the correlations with the numbers of stenotic vessels and segments and the extent score in 2554 patients, but the correlation coefficients were very low (0.02–0.08). However, the correlations were evaluated by simple linear correlations, and 35% of their patients consisted of those with unstable angina. Erren et al. [10] reported the hsCRP levels to correlate with the extent score using Spearman's rank correlation test. The correlation coefficient was 0.29. Even in their study, 32% of patients had statins, and 5% were those with unstable angina. The hsCRP levels are affected by statins [19,20] and are elevated in unstable angina and acute MI [6]. After MI, CRP levels dramatically increase, returning to baseline levels in 3–4 weeks [23]. In the present study, we excluded any patients with MI within 6 months or those with unstable angina within 1 month. However, the hsCRP levels did not correlate with the number of stenotic vessels. After the exclusion of patients with statins, the hsCRP levels were found to correlate with the number of stenotic vessels and better with the numbers of stenotic segments and the extent score (r = 0.30–0.32). The hsCRP levels were an independent factor for CAD in patients without statins. Our results suggest that the inclusions of patients with statins as well as those with MI or unstable angina mask the associations between the hsCRP levels and CAD. In patients with stable CAD who are not taking statins, the hsCRP levels would reflect the extent of coronary atherosclerosis.

Although some ethnic differences in the hsCRP levels have been reported between Indo-Asians and Caucasians [24], we recently reported the median value of the hsCRP levels in Japanese healthy people to be 0.2 mg/l [16], which seems to be much lower than those reported in other ethnic groups: 1.6 mg/l in Americans [17] and 1.9 mg/l in Turks [18]. In the present study, we showed the median value of the hsCRP levels in Japanese patients with CAD to be 0.70 mg/l, which was also much lower than those reported in other ethnic groups [8–13] (Table 4). Since obesity is known to increase the hsCRP levels [25], the lower hsCRP levels in Japanese patients with CAD may be due to smaller body mass index. A twin study showed the hsCRP levels to have a moderate degree of heritability [26], and some CRP gene polymorphisms were reported to influence the hsCRP levels [27,28]. Some genetic factors may also contribute to the low hsCRP levels in Japanese. However, further study is needed to elucidate the ethnic differences in the hsCRP levels between Japanese and other ethnic groups.

In conclusion, after the exclusion of patients who were taking statins, the plasma hsCRP levels were found to be associated with the presence and extent of coronary artery stenosis in patients with stable CAD. In such patients, the hsCRP levels would reflect the extent of coronary atherosclerosis. However, Japanese patients with CAD appear to have lower hsCRP levels than those of other ethnic groups, thus suggesting some ethnic differences in the hsCRP levels between Japanese and other ethnic groups.

Acknowledgment

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Human plasma protein modified to bind oxygen reversibly

Albumin engineered for artificial blood

A modified version of human serum albumin (HSA) that binds oxygen has been created by British and Japanese researchers. The work marks a first step towards a new form of artificial blood.

HSA is the most abundant plasma protein in human blood. It naturally binds with haem, an iron-containing porphyrin group that is a central component of haemoglobin, to produce a complex that can be oxidised. Chemists and structural biologists from Waseda University, Tokyo, and Imperial College London have developed a version of this HSA–haem complex that can reversibly bind oxygen, rather than react with it.

After studying the crystal structure of the complex, the researchers experimented with different versions produced by introducing modified plasmids into the yeast *Pichia pastoris*. Replacing a specific tyrosine residue in the HSA–haem complex with a hydrophobic amino acid such as leucine or phenylalanine and introducing histidine as a proximal base led to effective oxygen binding. This modified complex could reversibly bind oxygen with an affinity only one order of magnitude less than that of haemoglobin.

Artificial blood based on HSA-haem will not induce high blood pressure

Previous candidates for artificial blood are based on haemoglobin and liable to induce high blood pressure. This would not happen with an artificial blood based on the HSA–haem complex, say the researchers, because of an electrostatic repulsion between HSA and blood vessel walls.

Much work remains, admits Stephen Curry, reader in structural biology at Imperial. 'The lifetime of oxygen binding at the HSA–haem complex is still too short for practical use,' he told *Chemistry World*. 'At present we are trying to engineer additional mutations in the protein in order to enhance the oxygen binding properties still further.'

Jon Evans

Reference
T Komatsu *et al*, *J. Am. Chem. Soc.*, 2005, **127**, 15933

Chemistry World, December 2005, p. 18 (Royal Society of Chemistry)

見えてきた!! ナノテク □24□

ニプロ

人工酸素運搬体

救急救命用

血液型照合なしで大量使用

酸素輸液の用途に

ニプロはヘモグロビン小胞体と呼ばれるヘモグロビンナノカプセル型人工酸素運搬体を開発している。早稲田大学や慶応義塾大学、オキシジェニクス（東京都港区、高木智史社長）との共同研究だ。手術時に血液型の照合なしで大量に使用できる救急救命用酸素輸液としての用途が期待されている。安全性の確保と量産化のめどがつき、07年度にも臨床試験に入る。

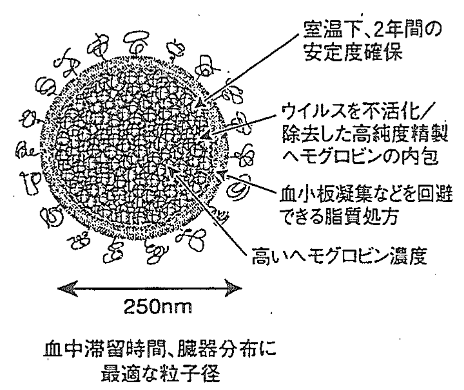
ヘモグロビン小胞体の粒径は約250ナノメートル。1枚膜リポソーム（リン脂質の2分子膜）を形成し、ヒトの赤血球とほぼ同等量（100cc中に10グラム）のヘモグロビンを内包する。

リポソーム処方で懸念されるのが血小板の活性化で血液が凝集すること。新しい脂質をリポソーム素材に含むことで血液との親和性を良くし、血液適合性を高めることに成功した。ポリエチレングリコールをリポソーム表面につけている点も、手術中に使用する血小板製剤の凝集発生を防ぎ、生体適合性を高めるのに有効だ。

ウイルスの不活化・除去には加熱殺菌とナノフィルターの2段階で行い、安全性を高めている。熱に弱いヘモグロビンに「一酸化炭素を付加して精製することで、60度Cの高温で10時間加熱できるよう工夫した。技術的に難しかったが、それが一番の特徴」（甲斐俊哉ニプロ医薬品研究所製剤研究室室長）だ。

デオキシ体（脱酸素化）にして瓶に詰めるため、常温で2年間保存できる。大きさが赤血球の約30分の1なので将来は心筋梗塞など血管が詰まる虚血性疾患の酸素供給治療法としても活用する。がんの放射線治療の時に併用すれば治療効果を上げることも期待できる。

ヘモグロビン小胞体の特徴



日本化薬

高分子ミセル化製剤

ナノテク利用の薬物送達システム

安全・利便性優れ高い効果

日本化薬はナノテクノロジーを用いた高分子ミセル化製剤の実用化を目指している。現在、抗がん剤として「NK911」（高分子ミセル化塩酸ドキソルビシン）が第2相臨床試験の段階にある。「NK105」（高分子ミセル化パクリタキセル）も第1相臨床試験が行われている。

開発が順調に進めば、副作用の強い抗がん剤であっても「安全性が高く、利便性に優れ、効果も高い」（才野哲之執行役員創薬本部長）薬へとチェンジできるものと期待されている。

新しい薬物送達システム（DDS）として期待が集まる高分子ミセルのアイデアは、東京女子医大の岡野光夫教授、東大大学院の片岡一則教授らが提唱した。ミセルとは水になじみやすい分子を外側に、油になじみやすい分子を内側に並べた高分子球体で、親油性に富む抗がん剤などの薬効成分を内部に収める。

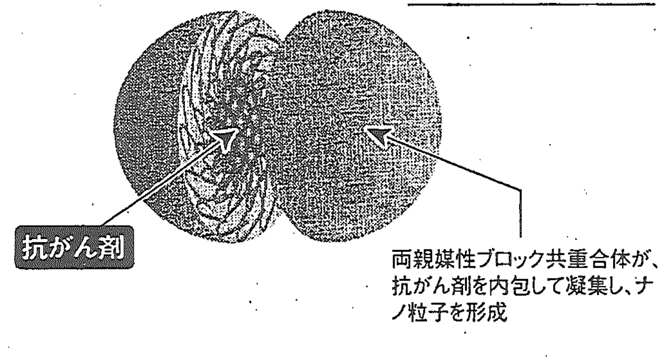
一般に高分子物質は腫瘍部位に集積・滞留しやすい性質がある。高分子ミセル化製剤を体内に送り込めば、がんの周囲に選択的に集まるため、そこでミセルを自壊させれば抗がん剤を集中的に放つことが可能となる。

日本化薬は89年からミセルの安定生産技術の習得と、臨床に向けた知見の集積とを開始。「高分子の開発に低分子の化学者を充てる」（西川政孝ナノテクノロジー推進室長）逆転の発想で、ミセルを安定的に量産する上での阻害要因を、一つずつ見つけ出してはつぶしていった。

今では内部に収める抗がん剤に応じてミセルをオーダーメードする技術も確立。日本オリジンのナノテクDDSの完成を急いでいる。

高分子ミセル化抗がん剤

当社のナノテクDDS製剤



日刊工業新聞

年月日	05	10	05	ページ	01	NO.	004	合マーク				

人工酸素運搬体
実証プラント建設
ニプロが20ℓ規模

ニプロはナノカプセル型人工酸素運搬体「ヘモグロビン小胞体」の実証プラントを12月中に建設する。共同研究を行うオキシジェニクス（東京都港区、高木智史社長、03・5733・1683）の京都研究所（京都市下京区）内に20ℓ規模の設備を建設する。投資額は3億円。血液型照合が不要で大量使用できる救急救命用酸素輸液として臨床試験を行い、段階的に量産化を進める。

ニプロは早稲田大学、慶応義塾大学による「ヘモグロビン小胞体」共同開発プロジェクトに02年から加わり、事業化を進めてきた。これまでに動物実験で安全性を確認、生産技術にめどがついたことから実証プラントを設けることにした。07年7月には同設備で生産した製品を使い、第1相臨床試験に入る。

さらに08年夏には第2相臨床試験に向け、60―70ℓ規模のプラントを子会社のニプロファーマ大館工場（秋田県大館市）内に建設する計画。投資額は約20億円。

ヘモグロビン小胞体は、粒径約250ナノ（1ナノは10億分の1）と人の赤血球の約30分の1の大きさで、100cc中10㌘の濃度でヘモグロビンを内包する。救急用の酸素輸液や心筋梗塞など、血管が詰まる虚血性疾患の酸素供給治療剤としても活用が期待されている。常温で2年間保存できることから、備蓄用としても使える。

原料には献血後、使われなかった血液の提供を受けて使用する。加熱殺菌とナノフィルターの2段階で血中のウイルスの不活化と除去を行う。熱に弱いヘモグロビンに一酸化炭素を付加することで、60度Cの高温で10時間加熱できるように工夫し、安全性を高めた。

2005,12,27 日刊工業新聞

大学発ベンチャーの挑戦 75

献血血液で酸素輸液
早慶のシーズ事業化
オキシジェニクス

オキシジェニクスは早稲田大学理工学部と慶応義塾大学医学部の研究技術シーズを事業化したバイオ・ベンチャー。外科手術など輸血時に投与して血中のガス交換を行うセル型酸素輸液「Oxygen Carrier（オキシジェンキャリア）」を開発中だ。

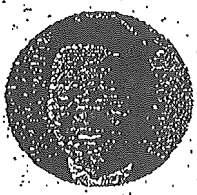
救命救急時や外科手術時の酸素輸液として、実用化できると見た高木智史社長が、両大学の共同研究成果に基づき設立。

土田英俊早稲田大学名誉教授ら4人が技術顧問兼創業者に名を連ねる。

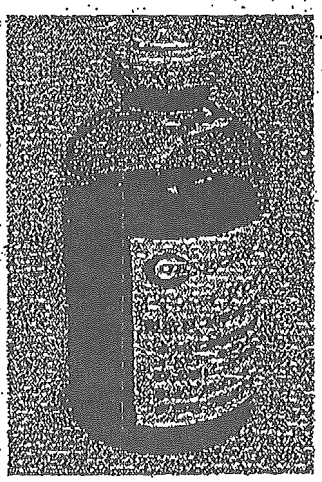
生材料のヘモグロビンだ。ナノフィルター処理を行うことでウイルス不活化・除去を行い、安全性をより高めている。

主要拠点は3カ所。京都研究所（京都市下京区）はキャリア製造と製剤技術開発を担当。横浜研究所（横浜市鶴見区）は小動物や細胞を使って生物学的評価を行う。筑波研究センター（東京都新宿区）は筑波研究からのパイプライン構築を目指している。

早ければ来年中にも株式上場を予定する。海外での治験も視野に入れており、脂質ベースのバイオ・ベンチャーとして世界一を目指している。（大阪・大塚久美）



高木智史社長



開発中の酸素輸液「オキシジェンキャリア」

▽本社＝東京都港区、03・5733・1683▽社長＝高木智史氏▽設立＝02年12月▽資本金＝17億9629万9000円▽従業員＝45人