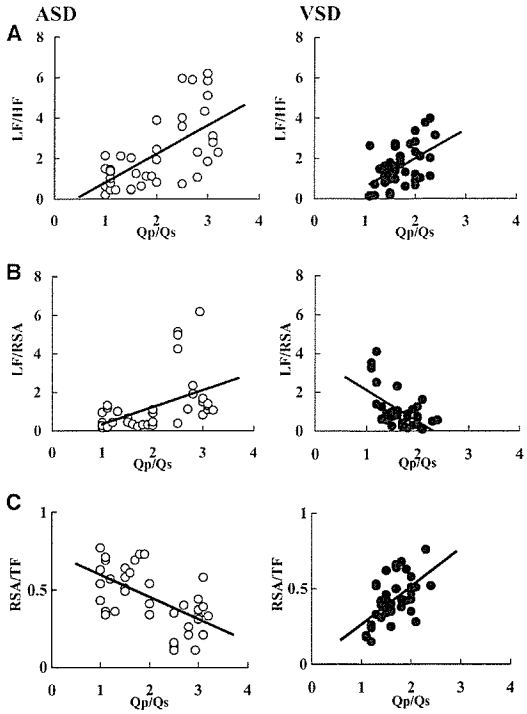

Figure 1. *(A) LF/HF is plotted against Qp/Qs for patients with ASD (open circles, left panel) versus VSD (filled circle, right panel). In both cases, the correlation is positive and significant. LF/HF = normalized ratio of LF power to HF power, Qp/QS = pulmonary to systemic blood flow ratio. (B) LF/RSA is plotted against Qp/Qs for ASD patients (open circles, left panel) and VSD patients (filled circle, right panel). In patients with ASD, the correlation was positive whereas it was negative in patients with VSD. LF/RSA = normalized ratio of LF power to RSA power, Qp/QS = pulmonary to systemic blood flow ratio. (C) RSA/TF is plotted against Qp/Qs for ASD patients (open circles, left panel) at left and VSD patients (filled circle, right panel). In patients with ASD, the correlation was negative while it was positive in patients with VSD. RSA/TF = normalized ratio of RSA power to TF power, Qp/QS = pulmonary to systemic blood flow ratio.*

was increased, with an increase in Qp/Qs, the mechanical influence of breathing movement on the right atrium was attenuated since it was exposed to pressure and volume overload. The comparison of HRV in patients with ASD versus VSD suggests a close participation of the pulmonary stretch receptor reflex pathway, which is associated with breathing movements, and the baroreceptor reflex pathway in the right atrium in the HF component of HRV.

Conclusions

We observed differences in shunt ratio dependence on normalized RSA between patients presenting with VSD and patients with ASD. These observations strongly suggest that volume inflation (mechanical stretch) and low-pressure overload of the right atrium inhibits the respiratory vagal innervation of the sinus node in presence of an ASD.

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シクロデキストリン包接レボブピバカイン硬膜外投与後の血漿レボブピバカイン濃度の推移

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要　旨

マルトシル-β-シクロデキストリン（CyD）で包接したレボブピバカインを硬膜外腔に投与し，レボブピバカイン単独のときに比べて，吸収と消失が遅れるかどうかを調べた。ウサギを1%レボブピバカイン群（単独群，n=6）とCyD包接1%レボブピバカイン群（包接群，n=6）に分け，2重盲検法でいずれかの溶液1 mlを硬膜外腔に注入し，480分まで血漿レボブピバカイン濃度を測定した。包接群の血漿中濃度は，単独群に比べて5，10，15分で有意に高かったが，消失半減期は，78±37分と100±54分で有意差はなかった。レボブピバカインをCyDで包接して硬膜外腔へ投与しても，血中からの消失過程に差はないが，投与後早期に血中濃度が高くなることが分かった。

キーワード：シクロデキストリン，レボブピバカイン，硬膜外麻酔，薬物動態

硬膜外腔へ投与した局所麻酔薬の急激な血中への移行を抑える方法として，局所麻酔薬にエピネフリンを添加する方法[1]，あるいは局所麻酔薬を持続投与する方法[2]などがある。シクロデキストリン（cyclodextrin：CyD）誘導体は，薬物の安定化，溶解性の向上，薬物の局所刺激性の低下，または生体内半減期の調節などを目的に用いられている[3]。2-ヒドロキシプロピル-β-CyDとオピオイドや局所麻酔薬と包接した複合体を，くも膜下腔や硬膜外腔に投与すると，オピオイドや局所麻酔薬の吸収を遅らせ，効果持続時間を延長させることが報告[4][5]されている。

著者らは，レボブピバカインとマルトシル-β-CyDとの包接複合体を用いて，ラットの坐骨神経ブロックを行い，神経障害や副作用もなく，安全に効果持続時間を1.4-2.0倍延長することを確認している[6]。今回は，この包接複合体をウサギの硬膜外腔へ投与して，局所から血中への吸収および全身への分布が遅延しているかどうかを明らかにするために，単味のレボブピバカインを投与したときと比較しながら薬物動態を調べたので報告する。

1. 方　法

本研究は，本学動物実験計画審査委員会の承認を受けた。昼夜12時間サイクルのケージで飼育している自由行動下の雄ウサギ（JW）12匹（1.9-2.2 kg）を用いた。

1%リドカインで浸潤麻酔を行い，ウサギの耳静脈に24ゲージのカテーテルを挿入し，ペントバルビタール（35 mg・kg^{-1}）で麻酔した。10%ポビドンヨードで皮膚消毒後，L5-6より，19ゲージの硬膜外針（ムラコメディカル）を用いて，傍脊椎・抵抗消失法で硬膜外腔を穿刺した。22ゲージのポリウレタンカテーテルを硬膜外腔の頭側に3 cm挿入し，抜けないように皮膚に固定した。右大腿動脈にポリエチレンカテーテル（PE10，ベクトンディッキンソン）を10 cm挿入し固定した。カテーテルは，皮下を通しウサギの背部に固定した。硬膜外カテーテルには生理食塩液を，動

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脈カテーテルにはヘパリン加生理食塩液を満たした。24 時間後，ウサギに神経障害がないことを確認し，次の実験を行った。

レボブピバカインは，丸石製薬（大阪）から提供された。レボブピバカインは，1%の濃度で用い，100 mM のマルトシル-β-CyD（塩水港精糖，横浜）と包接した。ウサギを無作為に 1%レボブピバカイン群（単独群，n=6）とマルトシル-β-CyD 包接 1%レボブピバカイン群（包接群，n=6）の 2 群に分け，2 重盲検法で投与した。いずれかの溶液 1 ml を 30 秒で硬膜外腔に投与し，投与後 0.3 ml の生理食塩液でカテーテル内の薬液を硬膜外腔に押し込んだ。血漿レボブピバカイン濃度を測定するために，投与前（対照），投与後 5，10，15，30，60，120，240，360，480 分に動脈カテーテルから 1 ml ずつ採血した。血液はただちに分離し，血漿を−20℃で保存した。実験終了後は，高用量のペントバルビタール投与下に椎弓切除を行い，硬膜外カテーテルの位置を確かめた。

血漿レボブピバカイン濃度は，質量分析装置を用いて測定した（新日本科学）。検量線の相関係数は 0.99 以上，定量限界は 5 ng・ml^{-1}であった。実測値から最高濃度到達時間（Tmax），最高血漿中濃度（Cmax）を求めた。血漿薬物濃度-時間曲線下面積（area under the curve：AUC）は，台形法を用いて 0-480 分で求めた。血漿中濃度のコンパートメント解析には GraphPad Prism® version 4 (GraphPadSoftware, San Diego) を用いた。測定値は平均値±標準偏差で示した。2 群間の比較には t 検定を用い，$P<0.05$ を有意とした。

2. 結　果

全例で硬膜外カテーテルは硬膜外腔に正しく留置されていた。

両群の経時的な血漿レボブピバカイン濃度および Tmax，Cmax，AUC を表に示した。両群の Tmax，AUC に有意差はなかったが，Cmax は包接群で単独群より有意に高かった（$P=0.046$）。経時的な濃度変化を図に示したが，包接群の血漿濃度は，5 分（$P=0.046$），10 分（$P=0.022$），および 15 分（$P=0.026$）で単独群より有意に高かった。この濃度変化を 2-コンパートメントモデルで解析した結果，単独群と包接群の半減期は，分布相でそれぞれ 5.2±2.1 分と 5.2±4.2 分，消失相でそれぞれ 100.5±53.6 分と 78.1±36.9 分で，両群間に有意差はなかった。

3. 考　察

マルトシル-β-CyD 包接レボブピバカインを硬膜外投与すると，血漿レボブピバカイン濃度は 15 分後まで，レボブピバカインを単独で投与したときより有意に高かった。その後の消失過程に有意差はなかった。

局所麻酔薬に CyD を包接すると，分子量が大きくなり，局所に投与した薬物の吸収を遅らせ，血中濃度の急激な増加を抑制し，全身的な副作用を軽減すると考えられる。2-ヒドロキシプロピル-β-CyD と局所麻酔薬の包接複合体をくも膜下腔や硬膜外腔に投与すると，効果持続時間を延長する[4]。スルフォブチルエーテル-β-CyD とブピバカインまたはリドカインを包接させると，長時間の神経遮断が可能であることが報告[7]されている。

Freville ら[8]は，2-ヒドロキシプロピル-β-CyD とブピバカインとの包接複合体を硬膜外に投与して，血中ブピバカイン濃度を包接していないときと比較している。この報告でも，包接複合体で消失相に変化はないが，吸収相は遅延している。包接複合体では，局所麻酔薬が硬膜外腔に長くとどまり，血中濃度の上昇を抑え，作用時間を延長させると考えられる。

今回，硬膜外投与した CyD 包接レボブピバカインでは，投与早期の 15 分まで，血中濃度が高いことが示された。このことは，CyD で包接すると，硬膜外腔から血中への移行が逆に速くなったためと考えることもできる。硬膜外局所麻酔薬の薬物動態は，経口投与される薬物と同様に，一次吸収過程と一次消失過程を含むコンパートメントモデルで表され，2 分画・一次吸収モデルで解析される[9][10]。今回の測定では，両群とも 5 分の時点で，Cmax を示し，ピークはさらに前にあると考えられ，この計算式をそのまま当てはめることはできないと思われた。ヒトの硬膜外腔投与後の血中濃度の変動[1][2]，CyD で包接することによって吸収や消失が遅れることを考慮して，5 分から 480 分と

表　硬膜外腔投与したレボブピバカインの血漿濃度

		時間（分）								
	前	5	10	15	30	60	120	240	360	480
単独群	0	1,692	1,105	785	592	375	208	59	22	18
	0	842	635	496	377	325	230	73	29	9
	0	923	658	563	426	309	195	72	31	17
	0	701	570	480	391	327	236	159	45	23
	0	957	699	698	441	287	193	54	17	6
	0	1,082	717	607	426	316	161	37	12	0
平均		1,033	731	605	442	323	204	76	26	12
標準偏差		347	191	118	77	29	28	43	12	9
包接群	0	1,772	1,376	1,331	940	590	282	117	33	10
	0	1,049	805	634	479	369	212	75	47	8
	0	1,126	809	611	458	330	193	52	19	7
	0	1,652	1,154	831	610	377	222	60	21	25
	0	1,467	1,001	1,247	511	346	272	53	20	8
	0	1,725	1,262	1,096	844	630	340	119	33	15
平均		1,465*	1,068*	958*	640	440	253	79	29	12
標準偏差		311	237	311	204	133	55	31	11	7

単独群は1%レボブピバカインを1 ml，包接群は100 mM マルトシル-β-シクロデキストリン包接1%レ
*：両群間で $P<0.05$ であることを示す。

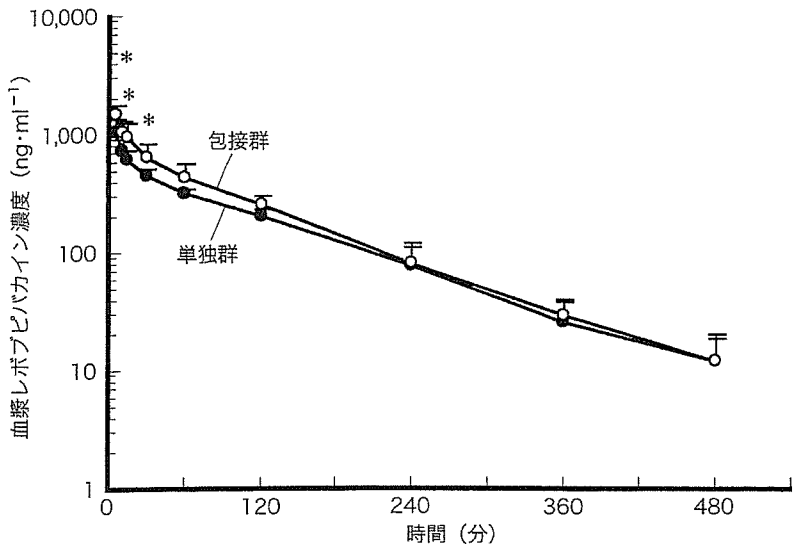

図　血漿レボブピバカイン濃度の推移
マルトシル-β-CyD 包接レボブピバカイン群（包接群）とレボブピバカイン群（単独群）の血漿濃度の変化
平均値±標準偏差と＊は両群間で $P<0.05$ であることを示す。

長時間の測定を試みたため，1，2分の早期の血漿レボブピバカイン濃度を測定しなかった。しかし，ウサギの硬膜外腔へブピバカインを投与した報告[8]では，単味のブピバカインで，投与後0.5分ですでにCmaxに達し，5分後にはかなり減少している。CyD包接ブピバカインでも投与後0.5分

Tmax (分)	Cmax ($ng \cdot ml^{-1}$)	AUC_{0-8h} ($ng \cdot hr \cdot ml^{-1}$)
5	1,692	1,359
5	842	1,147
5	923	1,133
5	701	1,362
5	957	1,070
5	1,082	986
5	1,033	1,176
0	347	154
5	1,772	2,011
5	1,049	1,286
5	1,126	1,120
5	1,652	1,402
5	1,467	1,440
5	1,725	2,048
5	1,465*	1,551
0	311	387

ボブピバカインを1 ml，硬膜外腔に投与した。

でCmaxに達するが，吸収が速い単味のブピバカインに比較し変化は緩やかである。これらの結果などを総合して，今回の著者らの研究では，単独群と包接群ともTmaxは1分前後にあったと考えられた。ただし，単独群では5-15分で，すでに血中レボブピバカイン濃度が低下したため，CyDとの包接によって局所からの吸収が遅れ，消失の速い単味のレボブピバカインとの間にずれが生じ，包接群で血中レボブピバカイン濃度が高くなったと考えられた。

硬膜外投与後，時間が経過すると薬物濃度は指数関数的に減少し，片対数で表した消失相は直線になる。このことから，2-コンパートメントモデルを適用して半減期を求めたが，群間に有意差はなかった。レボブピバカインは，マルトシル-β-CyDで包接しても，血中からの排泄は，単味のときと同じであると考えられた。レボブピバカインは複合体から速やかに解離するためと考えられた[3]。

CyDは，親水性，疎水性，およびイオン性誘導体に大別され，置換基の種類を変えることで，種類も用途も多様である[3)11)]。このため局所麻酔薬とCyD誘導体の種々の組み合わせが考えられる。局所麻酔薬のレボブピバカインは，ブピバカインの光学異性体のS（−）体のみからなる。R（+）体と比べて麻酔効力は変わらないが，中枢神経毒性や心毒性が少ない利点がある[12)13)]。また，マルトシル-β-CyDは，CyDの中で安全性が高い[3)14)]。CyDによる包接効果は，CyDの種類や量，濃度などでも変化するし，置換基の種類，導入の位置，平均置換度とその分布も関係する。また，今回のウサギの硬膜外投与による急激な血漿レボブピバカイン濃度の変動は，ヒトで10-15分後にCmaxに達するのと大きく異なっている[1)2)]。種による違いも考慮しなければならない。硬膜外投与によるCyD複合体の薬物の移行については，0.5-1分の早期の時間での測定なども含め，さらなる研究が必要と考えられた。

マルトシル-β-CyDで包接したレボブピバカインをウサギの硬膜外腔に投与すると，レボブピバカインを単独で投与したときに比べて，血中からの消失過程に差はないが，血漿レボブピバカイン濃度は投与15分後まで，単独で投与したときより高くなることが分かった。

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ABSTRACT

Plasma Levobupivacaine Concentrations following Epidural Administration of Levobupivacaine Conjugated with or without Maltosyl-beta-cyclodextrin

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Background：To investigate the pharmacokinetics of complexation of levobupivacaine with maltosyl-beta-cyclodextrin, the plasma concentrations of levobupivacaine were measured following epidural administration of levobupivacaine conjugated with maltosyl-beta-cyclodextrin or levobupivacaine alone in a rabbit model.

Methods：Rabbits were randomly divided into two groups, levobupivacaine (1%) group (n=6) and levobupivacaine (1%) conjugated with maltosyl-beta-cyclodextrin (100 mM) group (n=6). One ml of each solution was randomly injected through an epidural catheter placed at L5-6. The plasma levobupivacaine concentrations were measured before and 5, 10, 15, 30, 60, 120, 240, 360, and 480 min after injection.

Results：The plasma levobupivacaine concentrations were significantly higher in the levobupivacaine conjugated with maltosyl-beta-cyclodextrin group than in levobupivacaine group at 5 min (1,465±311, 1,033±347 ng・ml^{-1}), 10 min (1,068±237, 731±191), and 15 min (958±311, 605±118). There were no differences in area under the curve (1,551±387, 1,176±154 ng・hr・ml^{-1}) and elimination half life (100±54, 78±37 min) between the two groups.

Conclusions：The results of this study indicated that the absorption of levobupivacaine conjugated with maltosyl-beta-cyclodextrin from the epidural space and the elimination from the blood were similar to plain levobupivacaine.

key words：cyclodextrin, levobupivacaine, epidural anesthesia, pharmacokinetics

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Effect of 2,6-di-O-methyl-α-cyclodextrin on hemolysis and morphological change in rabbit's red blood cells

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ABSTRACT

The effects of 2,6-di-O-methyl-α-cyclodextrin (DM-α-CyD) on hemolysis and morphological changes in rabbit's red blood cells (RBC) were examined, compared with those of α-cyclodextrin (CyD) and 2-hydoxypropyl-α-cyclodextrin (HP-α-CyD). The hemolytic activity of α-CyDs increased in the order of HP-α-CyD < α-CyD < DM-α-CyD. The three α-CyDs induced morphological changes of RBC from discocyte to stomatocyte. At the same concentration (3 mM) of α-CyDs, DM-α-CyD and α-CyD released phospholipids, rather than cholesterol, and DM-α-CyD markedly released proteins from RBC membranes, compared to α-CyD and HP-α-CyD. The treatment of RBC with DM-α-CyD lowered the extent of a fluorescent sphingomyelin analogue from lipid rafts of RBC membranes in a concentration-dependent manner. These results suggest that DM-α-CyD has higher hemolytic and morphological change activity than α-CyD and HP-α-CyD through more extraction of phospholipids including sphingomyelin and proteins, not cholesterol, from RBC membranes than α-CyD and HP-α-CyD.

1. Introduction

One of the most substantial requirements for drug and drug carriers is to have either no or low levels of intrinsic cytotoxicity. To evaluate cytotoxicity, the cell culture system has been frequently used, e.g. a dye exclusion assay, ^{51}Cr release assay, MTT assay, LDH assay and ^{3}H-thymidine uptake assay. Studies with isolated red blood cells (RBC), which have no nucleus, mitochondria, endoplasmic reticulum, or other organelles, may provide simple and reliable information to classify the drug and drug carriers according to their cytotoxicity because the interaction of drugs and drug carrier's with plasma membranes must be the initial step of cell damage (Sheppard et al., 1969).

Cyclodextrins (CyDs) and their hydrophilic derivatives form inclusion complexes with hydrophobic molecules. CyDs can improve the solubility, dissolution rate and bioavailability of the drugs, and so the widespread use of CyDs is well known in the pharmaceutical field (Uekama and Otagiri, 1987; Szente and Szejtli, 1999). CyDs have been reported to interact with cell membrane constituents such as cholesterol, phospholipids and phosphatidylinositols, resulting in the induction of hemolysis of RBC (Ohtani et al., 1989; Fauvelle et al., 1997; Debouzy et al., 1998). Additionally, randomly methylated β-cyclodextrin (M-β-CyD) disrupted the structures of lipid rafts and caveolae (Galbiati et al., 2001; Simons and Ehehalt, 2002), which are lipid microdomains formed by lateral assemblies of cholesterol and sphingolipids in the cell membrane, through extraction of cholesterol from the microdomains (Anderson and Jacobson, 2002). We previously reported that CyDs induce shape changes in erythrocytes and induce hemolysis at higher concentrations (Irie et al., 1982). The magnitude of the

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hemolytic activity of the parent CyDs is reported to increase in the order of γ-CyD < α-CyD < β-CyD (Irie et al., 1982; Ohtani et al., 1989). Recently, various hydrophilic CyD derivatives have been developed to improve aqueous solubility and complexation ability of parent CyDs. Of various hydrophilic CyD derivatives, hydroxypropylated CyDs, sulfobutyl ether CyDs and branched CyDs have been demonstrated to be able to lower the hemolytic activity of each parent CyD (Irie and Uekama, 1997). Conversely, the methylation of the hydroxyl group at the 2 and 6 positions of a glucopyranose unit of β-CyD, is known to severely augment the hemolytic activity in high concentrations (Uekama and Otagiri, 1987). However, the effects of 2,6-di-O-methyl-α-CyD (DM-α-CyD) on morphological changes and hemolysis of RBC are still unknown. Recently, we reported that DM-α-CyD is predominantly likely to release CD14, a glycosylphosphatidylinositol (GPI)-anchored protein, from lipid rafts through the extractions of phospholipids from murine macrophages without cytotoxicity compared to α-CyD and 2-hydroxypropyl-α-cyclodextrin (HP-α-CyD) (Arima et al., 2001; Motoyama et al., 2005). Therefore, we hypothesized that DM-α-CyD interacts with RBC more strongly than α-CyD and HP-α-CyD. In the present study, we compared the effects of DM-α-CyD on morphological changes and hemolysis of RBC with those of α-CyD and HP-α-CyD, and examined the involvement of lipid rafts of RBC membranes in the morphological changes and hemolysis of RBC induced by α-CyDs.

2. Materials and methods

2.1. Materials

α-Cyd and HP-α-CyD (average degree of substitution = 4.1) were donated from Nihon Shokuhin Kako (Tokyo, Japan). α-CyD and DM-α-CyD were used after recrystallization with water and methanol, respectively. N-6-((N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-hexanoil)sphingosylphosphocholine (NBD-sphingomyelin) was purchased from Invitrogen (Tokyo, Japan). All other chemicals and solvents were of analytical reagent grade.

2.2. Hemolytic activity

RBC were isolated from Japanese white male rabbits (Kyudo, Tosu, Japan) as described previously (Uekama et al., 1981; Irie et al., 1982). Isolated RBC were centrifuged at 1000 × *g* for 5 min and washed three times with 10 mM of phosphate buffered-saline (PBS, pH 7.4). Five percent of RBC suspension in PBS was incubated with 2 ml of PBS (pH 7.4) containing α-CyDs for 30 min at 37 °C. After centrifugation (1000 × *g* for 10 min) the optical density of the supernatant was measured at 543 nm. Results were expressed as percent of total hemolysis, which was obtained when RBC were incubated in water only. All hemolytic assays were carried out on the same day of blood collection.

2.3. Scanning electron microscopy (SEM)

The observation of RBC with SEM was carried out as reported previously (Irie et al., 1982). RBC were treated with α-CyDs using the same methods as used for hemolytic assay. After fixation of samples with 2% (w/v) glutaraldehyde at 25 °C for 30 min, RBC were resuspended with distilled water. After samples were dried at 25 °C for overnight, they were sputtered with gold (5 min, 6–8 mA) using an Ion Coater IB-3 (EIKO Engineering, Ibaraki, Japan) and observed with a Hitachi S-510 SEM (Hitachi, Tokyo, Japan) at an accelerating voltage of 8–15 kV.

2.4. Assay of various membrane components released from RBC to supernatant

After RBC were incubated with α-CyDs at 3 mM for 30 min as described above, the RBC suspension was centrifuged (1000 × *g* for 10 min). The resulting supernatant (1.5 ml) was mixed with 3 ml of chloroform/methanol (15:2, v/v). After 10 min shaking, the phase of chloroform was recovered and evaporated to concentrate membrane components. The concentrations of phospholipids, cholesterol and total proteins in supernatants were determined using a Phospholipids-test Wako®, Cholesterol-test Wako® (Wako Pure Chemical Industries, Osaka, Japan) and Bradford protein assay kit (Bio-Rad Laboratories, Tokyo, Japan), respectively. Total phospholipids and cholesterol levels in RBC were determined according to the method reported by Bligh and Dyer (1959).

2.5. Purification of low-density membrane domain of RBC

Low-density lipid raft-enriched domains were isolated as described previously (Lisanti et al., 1995). RBC were labeled with NBD-sphingomyelin according to the method reported by Dekkers et al. (2000). In brief, 2 ml of rabbit blood was incubated with 12.5 μg/ml of NBD-sphingomyelin at 4 °C for 30 min. The blood was centrifuged at 1000 × *g* for 5 min and the pellet was washed three times with cold PBS. Two millilitres of the α-CyDs solutions was added to 1 ml of the RBC suspension. After further incubation at 4 °C for 30 min, RBC were lysed with 1% (w/v) Triton X at 4 °C for 10 min. After centrifugation at 10,000 × *g* for 10 min, pellet was resuspended with 2 ml of 0.5 M of sodium carbonate (pH 11) and homogenized extensively using a Potter-elvehjem type homogenizer (10 strokes), a Polytron tissue grinder and a sonicator. The resulting homogenate was brought to 45% (w/v) sucrose solution by the addition of 2 ml of 90% (w/v) sucrose in MES-buffered saline (MBS; 25 mM MES, pH 6.5, 150 mM NaCl) and overlaid with two layers of 35% (w/v) and 5% (w/v) sucrose solution in MBS containing 0.25 M carbonate (4 ml each). The gradient was then centrifuged at 200,000 × *g* for 18 h using a Beckman Optima™ L-70 K with a Beckman SW41Ti rotor (Palo Alto, CA). For the analysis of the resulting gradient, 12 fractions (each 1 ml) were collected from the top to the bottom of the gradient. The 4–6 fractions and 9–11 fractions were pooled as low-density and high-density membrane fractions, respectively. Fluorescent intensity of NBD-sphingomyelin was determined by a Hitachi F-4500 spectrofluorometer at 25 °C. The excitation and emission wavelengths were 466 nm and 536 nm, respectively. The concentration of cholesterol in low-density fractions was determined by Cholesterol-test Wako® (Wako Pure Chemical Industries, Osaka, Japan).

2.6. Flow cytometry

A hundred microlitre of rabbit blood was added to 900 μl of PBS and then incubated with 5 μg/ml of NBD-sphingomyelin at 4 °C for 30 min. The blood was centrifuged (1000 × g for 5 min) and the pellet was washed with PBS. The α-CyDs solutions were added to the resulting NBD-labeled RBC suspension at various concentrations. After further incubation at 4 °C for 30 min, RBC were resuspended in HBSS and quantified using a FACSCalibur flow cytometer with CellQuest software (Becton Dickinson, Mountain View, CA).

2.7. Statistical analysis

Data are given as the mean ± S.E.M. Statistical significance of means for the studies was determined by analysis of variance followed by Scheffe's test. p-Values for significance were set at 0.05.

3. Results

3.1. Hemolytic activity of α-CyDs

Fig. 1 shows the hemolytic activity of CyDs for RBC in PBS. The hemolytic activity of α-CyDs increased in the order of HP-α-CyD < α-CyD < DM-α-CyD: DM-α-CyD, α-CyD and HP-α-CyD induced hemolysis at approximately 3 mM, 6 mM and 120 mM, respectively (Fig. 1). These results indicate that hemolytic activity of DM-α-CyD is stronger than α-CyD and HP-α-CyD.

3.2. Effect of DM-α-CyD on morphological changes of RBC

Sheetz and Singer (1974) proposed that two types of morphological changes of erythrocytes, echinocyte (or crenation) and stomatocyte (or invagination), were caused by amphiphilic drugs. We previously reported that natural CyDs induce stomatocyte in human and rabbit erythrocytes (Irie et al., 1982; Ohtani et al., 1989). Hence, we investigated whether DM-α-CyD causes some morphological changes of RBC. Five percent of RBC suspensions with PBS were incubated with CyDs for 30 min at 37 °C. After fixation of RBC by glutaraldehyde, samples were observed with a SEM. RBC treated with 3 mM and 5 mM DM-α-CyD showed stomatocytes and spherostomatocytes, respectively (Fig. 2D). Similar results were observed in RBC treated with α-CyD at the concentrations of 6 mM and 12.5 mM (Fig. 2B). Meanwhile, RBC treated with HP-α-CyD at the concentrations of 120 mM and 200 mM showed morphological changes in spherostomatocytes and spherocytes, respectively (Fig. 2C). These results indicate that DM-α-CyD induced a morphological change in stomatocytes, but not echinocytes, as α-CyD did, although the onset concentrations of α-CyDs to induce morphological changes and hemolysis were totally different.

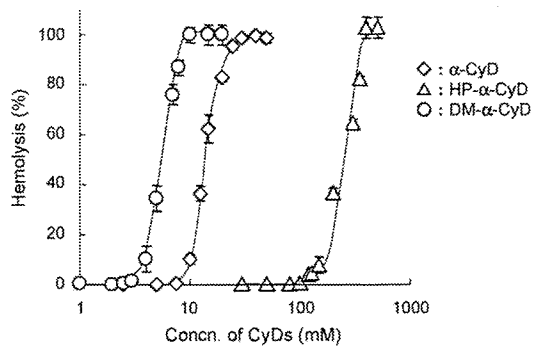

Fig. 1 – Hemolytic activity of α-CyDs on RBC in isotonic PBS. Five percent of isolated RBC suspensions with PBS were incubated with 2 ml of α-CyDs for 30 min at 37 °C. Results were expressed as percent of total hemolysis, which was obtained when RBC were incubated in water only. Each point represents the mean ± S.E.M. of 3–7 experiments.

3.3. Effect of DM-α-CyD to release membrane components of RBC

The magnitude of hemolytic activity of CyDs is affected by the solubilizing effects of CyDs on membrane components such as phospholipids, cholesterol and proteins (Irie and Uekama, 1997; Ohtani et al., 1989). In general, the acyl chain of phospholipids fits tightly into the hydrophobic cavity of the smallest α-CyD and more loosely into the larger inner space of β- and γ-CyDs, whereas the side chain of cholesterol is preferably included in the β-CyD cavity. Of the three parent CyDs, γ-CyD has the least lipid selectivity (Irie and Uekama, 1999). To gain insight into the mechanism for hemolysis and morphological changes induced by DM-α-CyD, the effect of DM-α-CyD to release membrane components from RBC was investigated. After treatment of RBC with α-CyDs, the concentrations of phospholipids, cholesterol and total proteins released in the supernatants were determined using the assay kits, and then the fractions of the levels of phospholipids and cholesterol released by α-CyDs into supernatants to their total levels in RBC were calculated. At the concentration of 3 mM, DM-α-CyD and α-CyD significantly released phospholipids from RBC, compared to HP-α-CyD (Fig. 3A), and the extent of phospholipids released by the treatment with DM-α-CyD was approximately 15% of total phospholipids in RBC. However, DM-α-CyD or α-CyD did not markedly release cholesterol from RBC to supernatant (Fig. 3B). The effects of DM-α-CyD to release total proteins were stronger than α-CyD and HP-α-CyD at the concentration of 3 mM (Fig. 3C). These results suggest that DM-α-CyD induces hemolysis and morphological changes of BRC through the extractions of phospholipids and proteins, not cholesterol, from RBC membranes.

3.4. Effect of DM-α-CyD on the extent of sphingomyelin and cholesterol in lipid rafts of RBC

Lipid rafts are dynamic assemblies of proteins and lipids that float freely within the liquid-disordered bilayer of cellular membranes but also can cluster to form larger, ordered platforms (Lisanti et al., 1995; London and Brown, 2000; Simons and Toomre, 2000). Lipid rafts are mainly composed of sphingolipids such as sphingomyelin and cholesterol in the cell membranes (Ahmed et al., 1997; Barenholz, 2004). Singh and

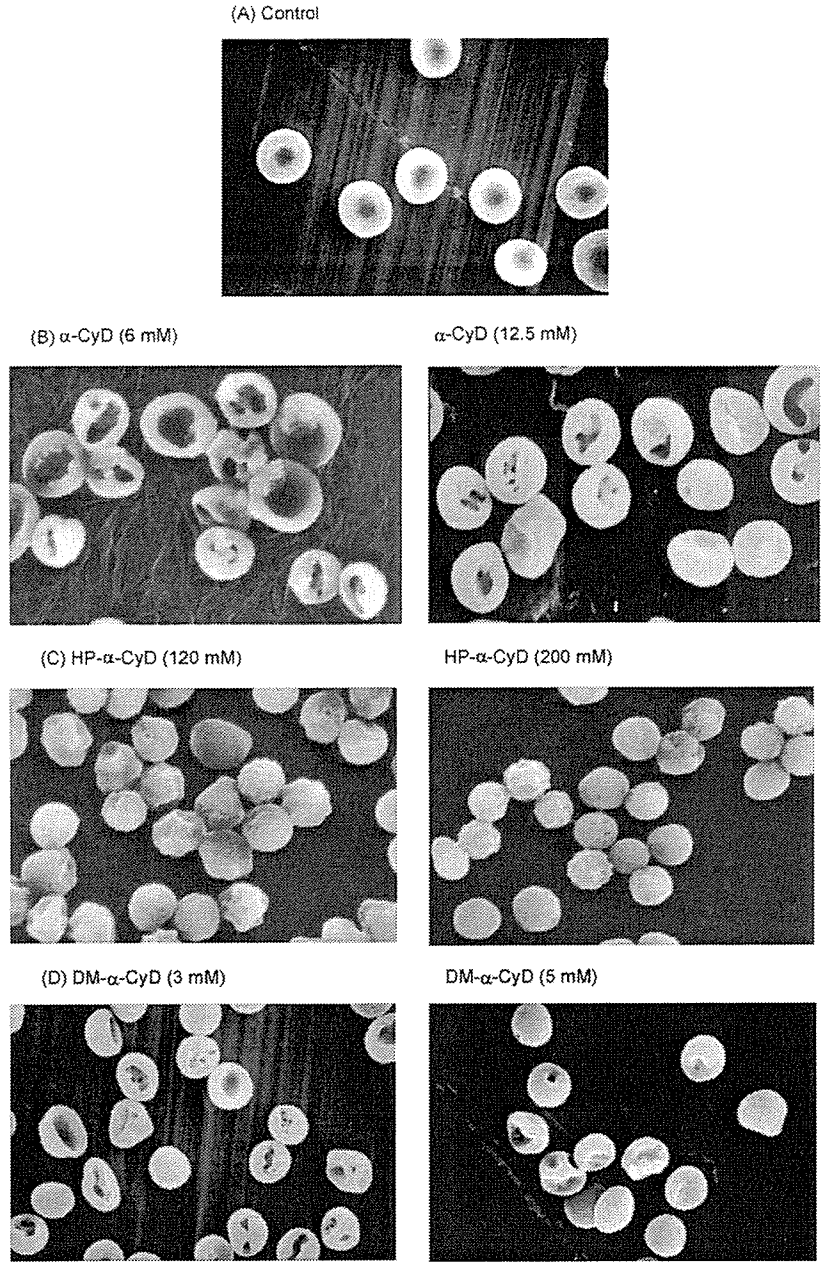

Fig. 2 – (A–D) Scanning electron microscopic analysis of morphological changes in RBC induced by α-CyDs. RBCs were treated with α-CyDs using the same methods as used for hemolytic assay. After fixation of samples with 2% (v/v) glutaraldehyde, RBC were resuspended with distilled water. The samples were dried, and then were sputtered with gold using an Ion Coater IB-3, followed by observation using a Hitachi S-510 SEM. These pictures show representative data for three experiments.

Kishimoto (1983) previously reported that α-CyD solubilizes complex lipids such as ceramide, a lipid moiety of glycosphingolipids. We therefore hypothesized that DM-α-CyD can extract sphingolipids such as sphingomyelin from lipid rafts. To address this hypothesis, we determined the extent of NBD-sphingomyelin in low-density membrane domains purified as lipid rafts fraction by a sucrose gradient centrifugation method using a fluorescent spectrometer. Here we used a

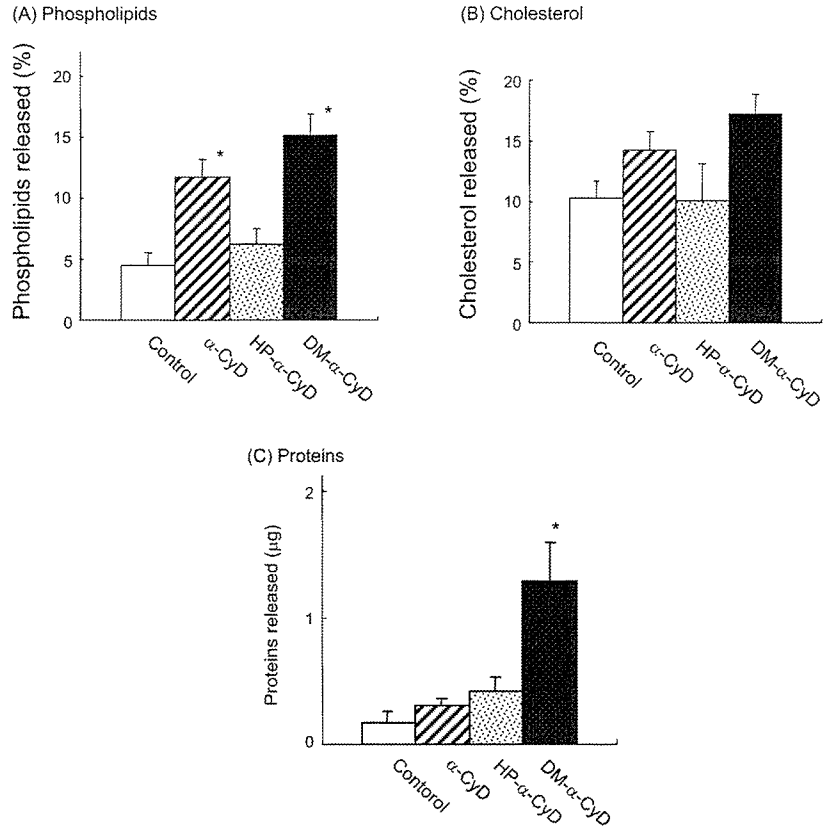

Fig. 3 – (A–C) Effects of α-CyDs on the release of membrane components from RBC. After incubation with α-CyDs at 3 mM for 30 min, the supernatant was mixed with a mixed solution of chloroform/methanol (15:2, v/v). After shaking, the organic phase was recovered and then was evaporated to concentrate membrane components. The concentrations of phospholipids and cholesterol released from the RBC were determined using a Phospholipids-test Wako® and Cholesterol-test Wako®, respectively. The concentration of total proteins released from RBC to the supernatants was determined with the Bradford protein assay method. Each value represents the mean ± S.E.M. of 4–8 experiments. *$p < 0.05$, compared to control.

NBD-sphingomyelin due to the ease with which the extent of the sphingolipid can be determined. The NBD-shingomyelin labeled in RBC membranes was predominantly localized in low-density membrane fractions (4–6 fractions), but not in high-density membrane fractions (Fig. 4A). The treatment of RBC with DM-α-CyD and α-CyD significantly lowered the extent of NBD-sphingomyelin in low-density membrane fractions at 3 mM and 6 mM, which were their onset concentrations to induce hemolysis, respectively (Fig. 4A).

Next, we measured the extent of NBD-sphingomyelin of cell surface after treatment with DM-α-CyD with a flow cytometer. DM-α-CyD and α-CyD shifted the curve corresponding to NBD-sphingomyelin to the left-hand side at 3 mM and 6 mM, respectively (Fig. 4B). In addition, the effect of DM-α-CyD to release NBD-sphingomyelin from lipid rafts was in a concentration-dependent manner (Fig. 4C). Moreover, we examined the effects of CyDs on the extent of cholesterol in lipid rafts (Fig. 5). Treatment of RBC with DM-α-CyD or α-CyD did not show a significant change of the cholesterol level in lipid rafts, compared with control. Taken together, these results strongly suggest that DM-α-CyD releases sphingomyelin, rather than cholesterol, from lipid rafts of RBC.

4. Discussion

In the present study, we revealed that hemolytic activity of DM-α-CyD was stronger than α-CyD and HP-α-CyD. In addition, DM-α-CyD induced morphological change of RBC from discocyte to stomatocyte, but not echinocyte, and then induces hemolysis through extractions of phospholipids including sphingomyelin and proteins rather than cholesterol from RBC membranes.

It is well known that RBC shows various shape changes depending on the set of agents including amphiphilic drugs, salt, pH, ATP and cholesterol. The first explanation of these shape changes was provided by Sheetz and Singer (1974), who proposed that the mechanism of shape changes induced by amphiphilic drugs is based on the different environment between the outer and inner leaflets of lipid bilayers. Yin

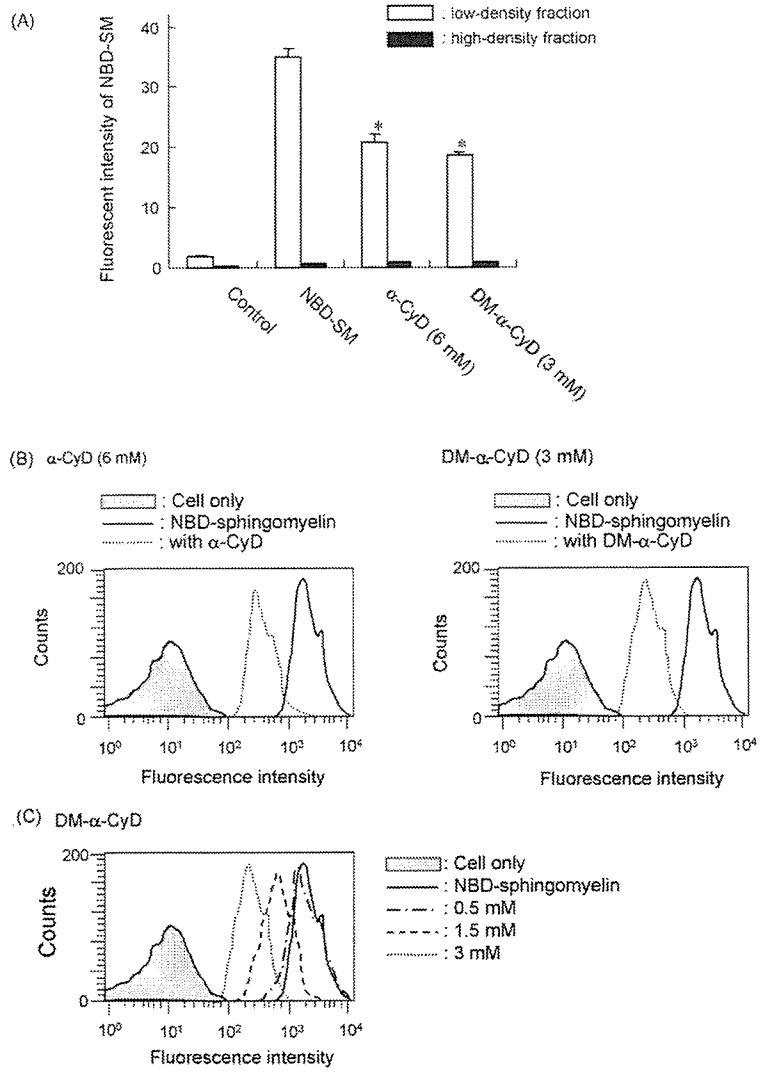

Fig. 4 – Effects of α-CyDs on the extent of NBD-sphingomyelin in lipid rafts of RBC membranes. (A) RBCs were treated with α-CyDs for 30 min at 4 °C. Low-density membrane fractions of RBC membranes were prepared by a sucrose gradient centrifugation. The amount of NBD-sphingomyelin in lipid rafts was determined with a fluorescent spectrometer. The concentrations of α-CyDs were onset of hemolysis. Each value represents the mean ± S.E.M. of 3–4 experiments. *$p < 0.05$, compare to low-density fraction treated with NBD-sphingomyelin alone. (B and C) Flow cytometric analysis of NBD-sphingomyelin in RBC. Ten percent of RBC suspension were incubated with NBD-sphingomyelin at 4 °C for 30 min. After washing with PBS, α-CyDs solutions were added to the suspension at the various concentrations. Further incubation at 4 °C for 30 min, the cells were resuspended in HBSS and were quantified using a FACSCalibur flow cytometer with CellQuest software. (B), the onset concentrations of α-CyDs to induce hemolysis. (C), a concentration-dependency for DM-α-CyD. These figures show representative data for three experiments.

et al. (2005) recently proposed that regulation of inhomogeneous rigidities in cell membrane is involved in morphological change of RBC. The invagination of RBC is known to be caused by the intercalation of cationic amphiphilic compounds into the inner leaflet of RBC membranes (Sheetz and Singer, 1974). However, it is acknowledged that CyDs cannot penetrate into cell membrane without modification because of high molecular weight and hydrophilicity (Uekama and Otagiri, 1987), which are different from surfactants which first incorporate into the membranes and then extract membrane components into micelles (Irie and Uekama, 1999).

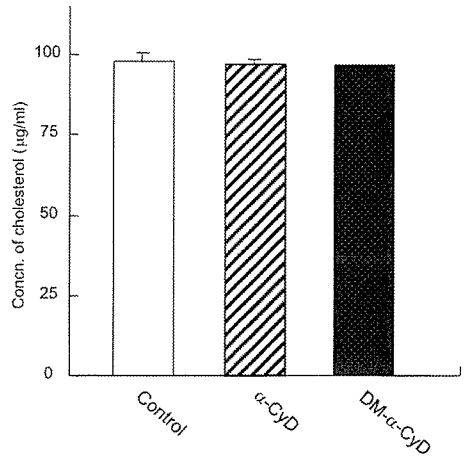

Fig. 5 – Effects of α-CyDs on the extent of cholesterol in lipid rafts of RBC membranes. RBCs were treated with α-CyDs for 30 min at 4 °C. Low-density membrane fractions were prepared by a sucrose gradient centrifugation. The amount of cholesterol in low-density fractions was determined using a Cholesterol-test Wako®. The concentrations of α-CyDs were onset concentrations to induce hemolysis. Each value represents the mean ± S.E.M. of four experiments.

The magnitude of hemolytic activity of α-CyDs could be attributed to the ability to solubilize phospholipids. In regard to the interaction between CyDs and membrane components, α-CyD and β-CyD are known to solubilize phospholipids and cholesterol, respectively, and γ-CyD has the least lipid selectively (Irie and Uekama, 1999). Of β-CyD and its hydrophilic derivatives, the hemolytic activity increased in the order of HP-β-CyD < β-CyD < DM-β-CyD, consistent with the solubilizing ability of β-CyDs for cholesterol (Irie and Uekama, 1997). These lines of evidence suggest that the solubilizing effect of α-CyDs used here on phospholipids increase in the order of HP-α-CyD < α-CyD < DM-α-CyD.

DM-α-CyD may induce morphological changes of RBC through altering the ratio of cholesterol/phospholipids in lipid bilayers of RBC membrane. It is well known that the lipid bilayer of the membrane contains nearly equal amounts of unesterified cholesterol and phospholipids, and any changes in the ratio of cholesterol/phospholipids or distribution of membrane components may affect membrane deformability (Lange et al., 1982; Mohandas and Chasis, 1993). In the present study, we revealed that DM-α-CyD induced morphological changes of stomatocyte of RBC (Fig. 2). With respect to membrane lipids released from the RBC membranes, DM-α-CyD extracted phospholipids, rather than cholesterol, from RBC membranes to supernatant (Fig. 3B). Therefore, these results suggest that the effect of DM-α-CyD on RBC membranes is similar to those of α-CyD, although the magnitude of the interaction between α-CyDs and RBC membranes may be different.

RBC treated with HP-α-CyD showed morphological changes in spherostomatocytes and spherocytes, respectively (Fig. 2C). These slight different morphological changes induced by HP-α-CyD from those by α-CyD and DM-α-CyD may be, at least in part, due to high osmotic pressure because the osmotic pressure values determined using an osmometer were 427 ± 2 mOsm and 542 ± 2 mOsm at the concentrations of 100 mM and 200 mM HP-α-CyD, respectively. Therefore, the high osmotic pressures as well as the membrane component extraction from RBC membranes induced by HP-α-CyD may be involved in the induction of the somewhat unique morphological changes of erythrocytes.

Lipid rafts are known to be cholesterol- and glycosphingolipid-rich membrane microdomains and to connect membrane skeleton in erythrocytes (Brown and London, 2000; Ciana et al., 2005). It is noted that sphingomyelin is mainly localized in an outer leaflet of lipid rafts and plays a key role in the formation of lipid rafts enriched in cholesterol (Jacobson et al., 1995; Barenholz, 2004). Based on the information, we examined the effects of α-CyDs on the extent of NBD-sphingomyelin in lipid rafts of RBC membranes. Consequently, α-CyD and DM-α-CyD were found to lower the extent of NBD-sphingomyelin in lipid rafts of RBC (Fig. 4). Hence, the morphological change toward stomatocyte of RBC induced by DM-α-CyD could be ascribed to the lowering of phospholipids including sphingomyelin in the outer leaflet of lipid rafts of RBC membranes, which may contribute in part to formation of stomatocytes via an inward bending of the RBC membranes.

The release of proteins from RBC membranes may be involved in the morphological changes and hemolysis of RBC induced by the treatment with DM-α-CyD. It is well known that spectrin and spectrin-interacting proteins such as ankyrin, band 3, band 4 and NCAM120 maintain the shape and elasticity of RBC (Bialkowska et al., 1994; Birchmeier and Singer, 1977; Discher and Carl, 2001; Leshchyns'ka et al., 2003; Pant et al., 1983; Platt et al., 1993; Tsuji and Ohnishi, 1986; Vanderpuye et al., 1988). In addition, stomatin, flotillins and GPI-anchored proteins are known to be major integral proteins of lipid rafts of erythrocyte (Salzer and Prohaska, 2001; Samuel et al., 2001). Meanwhile, Irie and Uekama (1999) demonstrated that CyD extracts membrane proteins such as bands 3, 4, 5 and 6 from erythrocyte. In the present study, the effects of DM-α-CyD to release total proteins from RBC membranes were strikingly higher than those of α-CyD and HP-α-CyD (Fig. 3C). Taken together, it is possible that DM-α-CyD releases spectrin, spectrin-interacting proteins and lipid rafts-enriched proteins from the RBC membranes. This presumption may be supported by our finding that DM-α-CyD releases CD14 from lipid rafts of murine macrophages (Motoyama et al., 2005). In fact, our preliminary study indicate that DM-α-CyD released β-actin and flottilin from RBC membranes under the present experimental conditions. Therefore, one possibility may be proposed that DM-α-CyD induce the morphological change toward stomatocyte through the change in lipid-protein interaction from RBC membranes.

Apart from the safety viewpoint of CyDs, it is well known that lipid rafts of RBC membranes are involved in an infection of a number of vacuolar pathogens, including the malaria parasite *Plasmodium falciparum*, although erythrocytes are non-endocytotic (Samuel et al., 2001). Recently, Ziegler et al. (2004) reported that antiplasmodial activity of triterpene betulinic

acid and its analogues may be caused by modifications of lipid rafts through the incorporation of the compounds into the lipid bilayers of erythrocytes. Therefore, it is possible to assume that DM-α-CyD has antiplasmodial activity as well.

In conclusion, we revealed that DM-α-CyD has hemolytic activity stronger than α-CyD and HP-α-CyD. DM-α-CyD was found to induce the morphological change to stomatocyte through the selective extraction of phospholipids including sphingomyelin and proteins, rather than cholesterol, from RBC membranes. These results will provide useful information for the pharmaceutical and cell biological applications of methylated CyD derivatives.

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●原　　著●

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新生児集中治療室（NICU）におけるMRSA感染撲滅対策

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Key Words：NICU，MRSA，nosocomial infection，infection control

要　　旨

NICU病棟において基本的な接触感染に対する標準予防策の厳密な実施の下に，保菌者の除菌を追加することにより，1998年11月から5年間の年月を要して病棟開設以来23年でMRSAを初めて撲滅できた。まず対策開始初期の6回の保菌者除菌の結果を基礎にして，有効な除菌方法を見出した。保菌患児にはムピロシン軟膏による鼻腔・外耳道消毒・皮膚の強酸性水による消毒・気管内挿管児は挿管チューブの周囲へのムピロシン軟膏の塗布を同時に行うことにより児の50～80％以上が除菌できた。そして保菌職員には同軟膏を1日2回7日間鼻腔への塗布でほぼ除菌できた（重症アトピー性皮膚炎1例だけ例外）。さらに除菌対策を行っていく上で，初めて勤務交代の保菌職員によるMRSA導入が容易に起こることが判明した。このことはNICUにおけるMRSA感染症予防対策は，単にNICU内部の問題ではなく，病院全体ひいては地域，そして大きくは国全体における成人のMRSA保菌を減少に向かわせる以外に根本的な予防策はないことを意味する。

緒　　言

1961年に英国で初めてMRSAが報告され，1970年代に世界各国でMRSA感染の増加がみられ深刻な問題となった。しかし1980年代に入ると，各国で抗生剤使用の厳密な対策が行われた結果減少したが，日本においてのみ急激な増加を示した。その原因は，グラム陰性桿菌対策のために新しいセフェム系薬剤の開発が進められ，予防的投与も含めて多くの新しい抗生剤が成人領域で使用され更に耐性化を促したためである[1]。1980年代後半から，一部のNICUにおいてMRSA感染症が報告され始め，1990年代半ばから後半にかけて全国のNICUで問題になってきた。志村ら[2]の報告から解析すると，1994年には北海道・関東・九州の一部のNICUからMRSA感染症が見られていたが，1996年には中部・関西・四国そして全国への拡がりをみせ，発症数増加も多くの施設で見られている。特に新生児TSS様発疹症neonatal TSS-like exanthematous disease（NTED）について，高橋らは本症の原因がMRSAの産生するTSST-1毒素に由来し，この毒素がスーパー抗原として作用することを証明した[3)4)]。さらに本症は1995年には19/74（25.7％），1998年には91/152（59.9％）の高率でNICUを持つ施設で見られており，全国のNICU・新生児病室で問題になっている[5)6)]。

われわれの施設では，1998年11月からNICU病棟において基本的な接触感染に対する標準予防策の厳密な実施の下に，保菌者の除菌を追加することにより病棟開設以来23年間でMRSAを初めて撲滅できた。基本的なMRSA感染予防対策に則り，行ってきた実際の対策項目の内容を示しながらその経緯を述べる。

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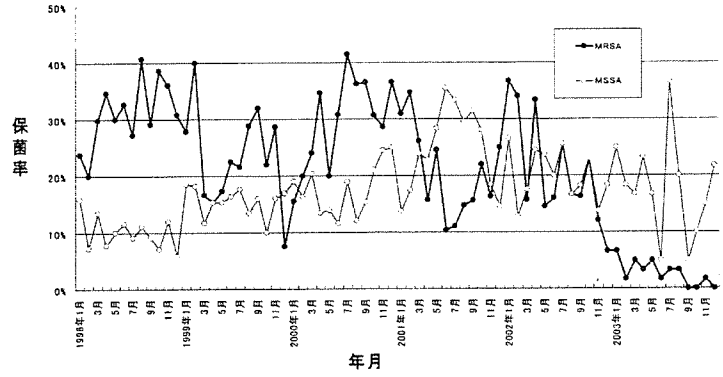


図1　MRSAとMSSAの月別保菌率の推移

対象と方法

対象は1998年11月から2003年12月までの大阪府立母子保健総合医療センターNICU入院児全例（1,090名）とした。週1回の入院患児の保菌状態を検査する定期的細菌モニタリングデータ（定期の培養として気管吸引物：週1回，鼻腔：MRSAが陽性例は週1回，便：対象菌として緑膿菌・セラチア・セパチア・サルモネラが陽性例は週1回）および職員の監視培養データ（鼻腔培養で頻度は2～4ヶ月に1回）とそれに基づくムピロシンによる保菌職員治療結果を集積することで，今回の解析と検討を行った。職員の鼻腔モニタリング検査はマーサチェック（鼻腔用：KK日研生物医学研究所製）を用いた。これは，職員自身が添付綿棒で鼻前庭を拭い，清潔に培地へ入れることができる。MRSAが選択的に増殖され培地が赤変するのでスクリーニングに適している。MRSAの確定はPBP'2の検出キットMRSA-LA「生研」，コアグラーゼ型はぶどう球菌コアグラーゼ型別用免疫血清「生研」（共にKKデンカ生研製）を用いて判定した。職員毎に番号を振り分け，鼻腔拭き取り検体にはその番号だけを付け，検査部へ送付した。検体番号付職員リストと，番号別検査結果報告は当院産業医に連絡された。保菌陽性者へは産業医から個別に封筒にて告知され，職員個人の責任において除菌治療された。保菌患児の皮膚消毒に使用した強酸性水は，三浦電子株式会社製の「オキシライザOXM-01A」を使用して作成したアクア酸化水と呼ばれ，pH2.7以下，+1,000～1,200mVの高い酸化還元電位，20～60ppmの溶存塩素を含んでいる。

結　果

1. MRSAとMSSAの保菌率の変化

NICUにおけるMRSAとMSSAの保菌率の変化を**図1**に示す。1998年11月以降にMRSA感染予防対策を開始して，初期の6回の保菌者除菌の結果を基礎にして，それ以降も対策を持続して，約4年の経過の中で，NICU病棟におけるMRSA保菌者の撲滅までに至った。この経過の中で，**図1**からMSSAの保菌率が，MRSAの保菌率の減少と相反する形で増加してきていることがわかる。これは大きな意味での菌交代現象とも言える。

2. MRSA感染予防対策の詳細

1998年11月から，NICU病棟において基本的な接触感染に対する標準予防策の厳密な実施のもとに，保菌者の除菌を追加した。

1）感染予防の基本原則[7]

①分娩後早期からの母親によるだっこ・カンガルーケア（皮膚）と早期授乳（口腔内・腸管内）。

②母乳哺育でビフィズス菌の腸内での定着。

③児に触れる前後の消毒剤による手洗いと沐浴の個別化と保菌児へのガウン使用。

④計測器具の個別化（体温計・聴診器などを個人別にする）と保育器の終末消毒。終末消毒とは器具全体が滅菌できていることを意味する。

⑤職員が保菌しないように注意し，保菌した場合には，保菌児増加が治まった時期を見計らって，保菌児と共に除菌。

2）MRSA保菌者の除菌対策

1998年11月から**表1**のように除菌対策を開始した（**図4**ではその時期を下向きの小矢印6本で示した）。

表1　MRSA感染予防対策：保菌者の除菌対策

			患者			職員		対策内容
回数	月日	期間	対象	MRSA保菌	対策内容	細菌検査対象	MRSA保菌	
1	98.11.02～11.08	7日間	保菌者全員	13人	・鼻腔内ムピロシン軟膏塗布（3日間） ・挿管児ハベカシントイレッティング（7日間）	74人	1人	・1日2回鼻腔内ムピロシン軟膏塗布（7日間）
2	98.12.14～12.20	7日間		17人	・鼻腔内ムピロシン軟膏塗布（3日間） ・挿管児ハベカシントイレッティング（7日間） ・ハイアミン浴（7日間）	74人	0人	
3	99.02.22～03.01	7日間		18人	・鼻腔内ムピロシン軟膏塗布（7日間） ・挿管児ハベカシントイレッティング（7日間） ・ハイアミン浴（7日間） ・1人のみ酸性水噴霧	81人	2人	・1日2回鼻腔内ムピロシン軟膏塗布（7日間）
4	99.04.08～04.14	7日間	1～3回までの保菌者とルチンでの培養検査でMRSAが検出されている児（合わせて7人）	7人	・鼻腔内ムピロシン軟膏塗布（7日間） ・挿管児ハベカシントイレッティング（7日間） ・ハイアミン浴（7日間） ・1人のみ酸性水噴霧			
5	99.05.21～05.27	7日間	1～4回までの保菌者とルチンでの培養検査でMRSAが検出されている児（合わせて9人）	6人	・鼻腔内ムピロシン軟膏塗布（7日間） ・挿管児ハベカシントイレッティング（7日間） ・酸性水噴霧 ・2人のみ外耳道内ムピロシン塗布			
6	99.06.23～06.30	7日間	1～5回までの保菌者とルチンでの培養検査でMRSAが検出されている児（合わせて10人）	10人	・鼻腔内ムピロシン軟膏塗布（7日間） ・挿管児ハベカシントイレッティング（7日間） あるいはバンコマイシントイレッティング（7日間） ・酸性水噴霧 ・2人のみ外耳道内ムピロシン塗布 ・挿管チューブ周囲ムピロシン軟膏塗布	19人	2人	・1日2回鼻腔内ムピロシン軟膏塗布（7日間）

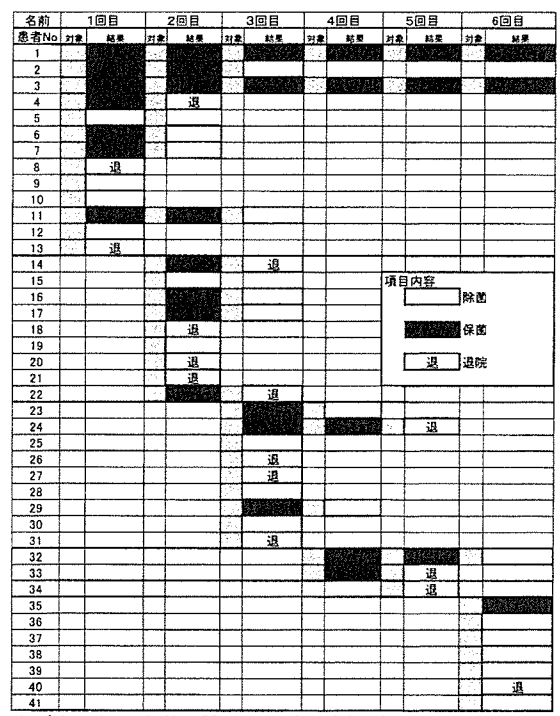
MRSA保菌患児の除菌と共に，職員の保菌状態を把握し，保菌職員も同時に治療することとした。ただし，本人への告知は産業医により行われ，他者は関知しなかった。MRSA保菌職員は，個人の責任においてムピロシン軟膏を朝夕2回7日間鼻腔に塗布した。

この除菌対策は，除菌効果の評価を行うためと，ムピロシン耐性菌を作らないために，1週間の保菌患児・職員の同時除菌後，3週間から2ヶ月の間隔をおいて実施され，その都度除菌効果を評価して次の対策手順変更に役立てた。

3）MRSA除菌対策開始後の変化

①MRSA除菌について

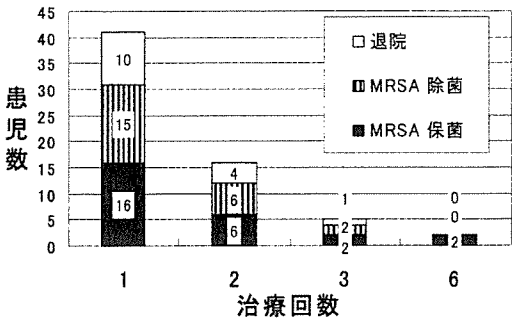
1998年11月から除菌対策を感染予防基本原則の下に開始したが，**表1**に示すように，簡単には保菌児の減少には繋がらなかった。**図2**から保菌状況の詳細をみると，除菌率は第1回（5/13：38%），第2回（4/17：24%），第3回（6/18：33%）であった。初回は鼻腔消毒・気管内消毒のみで，皮膚・外耳道消毒を行っていなかったために，除菌率が低いと考えられた。しか



注：症例1と3は気管切開児でこのあと除菌された。

図2　MRSA除菌対策時の患児の保菌状況

し2回以降も皮膚をハイアミン浴で行ったが，第4回（2/7：29％），第5回（0/6：0％）は外耳道消毒も加えたが改善を認めなかった。そこで，第6回は菌のハイアミンへの消毒剤耐性を考え全員に強酸性水（沐浴後全身に噴霧した後に乾いたリネンで拭う）を使用し（6/10：60％）という効果を得た。**図3**では除菌治療回数による効果を示した。この対策方法では，治療3回までにほぼ除菌できるデータとなった。さらに，後述する気管チューブ周囲へのムピロシン軟膏塗布の追加により，挿管児も含めて，50～80％の除菌率となった。一方，**表2**でみられるように，職員での保菌者は80名中数名しかなく，ムピロシンによる鼻腔塗布で再度保菌する職員は，ほとんどいなかった。この4年間に，再度保菌した職員は，重症のアトピー性皮膚炎のためにステロイド軟膏を手に使用されていた1名のみであった。



消毒6回の2名（症例1と3）は気管切開の気管チューブから菌検出

図3　ムピロシンによる治療回数と除菌効果
（Pediatrics International（2003）45，p242　Figure5より改変）

②職員の配置換えによる変化

図4で2000年4月と7月（一つ星印）には保菌患児が急増した。原因の詳細を検討すると，この時期に一致して職員のモニタリングで新規採用のレジデント

表2　保菌職員への対策

	1983	1984	1991	1996	1997	1998.11	1999.2	1999.8	2000.4	2000.8	2001.1	2002.6	2003.4～6
検査職員数	48	67	75	75	75	74	81	90	81	33	92	97	102
S. aureus	13	19	31	20	ND	30	22	37	34	14	ND	ND	ND
保菌率（%）	27%	28%	41%	27%		41%	27%	41%	42%	42%			
MRSA	2	3	18	0	3	1	2	5	3	2	5	1	3
保菌率（%）	4.2%	4.5%	24.0%	0.0%	4.0%	1.3%	2.5%	5.6%	3.7%	6.1%	5.4%	1.0%	2.9%
職員治療	なし	なし	イソジンゲル鼻腔塗布		ムピロシン鼻腔塗布	ムピロシン鼻腔塗布	ムピロシン鼻腔塗布	ムピロシン鼻腔塗布	ムピロシン鼻腔塗布	ムピロシン鼻腔塗布	ムピロシン鼻腔塗布	ムピロシン鼻腔塗布	ムピロシン鼻腔塗布
変更点			コット児に接触時はマスク着用			保菌患児治療開始				新職員の鼻腔検査陽性者は事前治療			

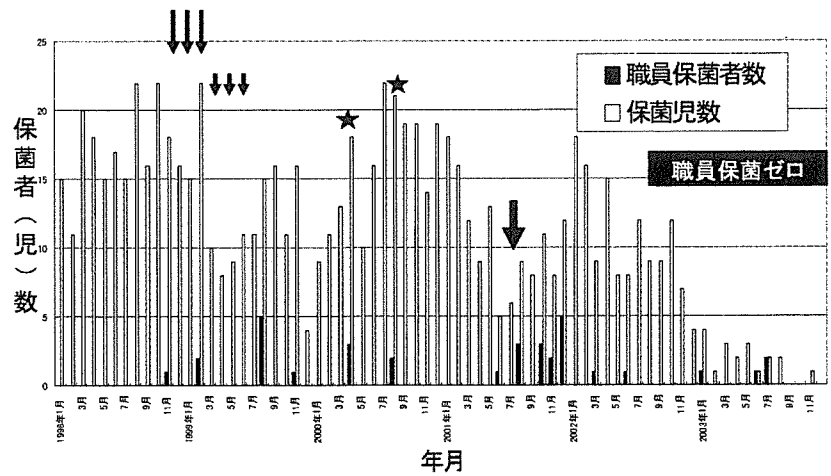


図4　MRSAの職員保菌者数と保菌児数

が前の施設で保菌していたMRSAをNICU入院児に感染させたことが判明した。また同時期に採用した新人看護師1名が，病棟に入る前には陰性であったが，入って2ヵ月後には陽性になった。これらの事実は，手洗いや自己の癖に慣れていない新人の職員が，他へ菌を移しやすい，あるいは自身で保菌しやすいことを示していると考えられた。2000年8月以降（実際には**図4**において大きな矢印の部分から）は，新たにNICU病棟に勤務する新生児科の職員は，すべて入棟前に鼻腔検査を行った。それ以降は入棟前に保菌職員は除菌されるため「保菌職員ゼロ」となって病棟でのアウトブレイクは消失した。これらの事実は他病棟（あるいは他施設）で保菌した人が，職員として勤務される場合には，病棟へ入る前に検査をして除菌することが重要であることを示している。

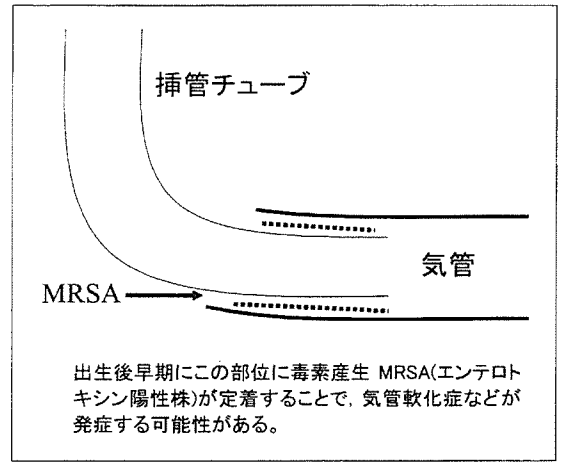


図5　気管内挿管におけるMRSAの残存部位（点線図）
（Pediatrics International（2003）45，p242　Figure6より改変）

4）気管チューブ周囲へのMRSA定着について

図5に示したように，気管内チューブを使用している場合には，その周囲にMRSAが定着することが多い。抗生物質を静脈内で全身投与しても，気管上皮細胞からの分泌については全くデータがない。**図2**における症例1と3（気管切開が行われているNICUの長期入院児）では，6回の気管内投与を繰り返したが無効であった。そこでチューブの周囲にムピロシン軟膏を薄く塗った気管チューブに入れ替えた。同時に鼻腔・外耳道・皮膚消毒を予定通り施行して，両名ともに1回で除菌ができた。一般の挿管チューブでも行いえるので，その他の部位の消毒と同時並行すると除菌が可能である。以後そのようにして，この期間中に4名以上の超早産MRSA保菌児を挿管中に除菌に成功した。挿管チューブ周囲の軟膏塗布に関しては，気道粘膜上の除菌効果として非常に大きいことが判明した。またこの方法による粘膜の変化やその他副作用的なものは全く生じなかった。

5）除菌対策後のMRSAコアグラーゼ型の変化について

図6に除菌対策後のMRSAコアグラーゼ型の変化について示した。1998年までは，開院当初からの優勢株としてコアグラーゼⅦ型が主であったが，除菌対策を始めると，全国の成人領域そしてNICUに多いⅡ型（エンテロトキシン産生・TSST-1毒素産生のNTED〈新生児TSST様発疹症〉の原因株）に取って代わられた。除菌対策をするほど，この株が残ってくるのは，この株自身のヒトへの親和性の強さを物語っている。

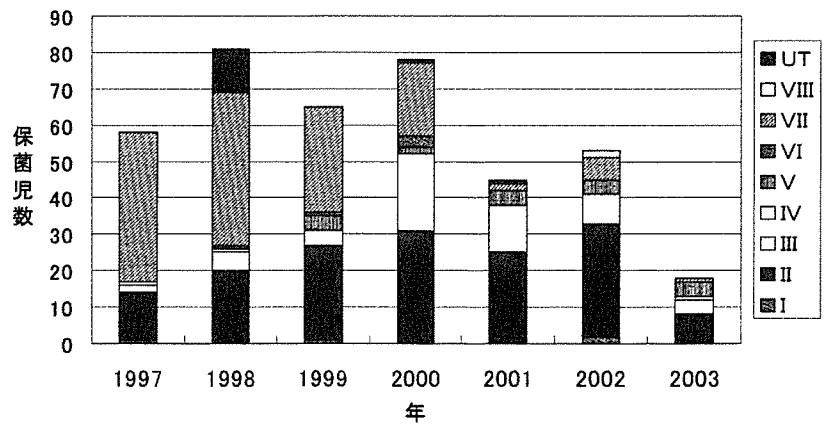


図6　年別・MRSAコアグラーゼ型別の保菌児数推移