日本財団
The Nippon Foundation

財団法人日中医学協会
2007年度共同研究等助成金—在留中国人研究者—報告書

2008年 3月 13日

財団法人 日中医学協会 御中

貴財団より助成金を受領して行った研究テーマについて報告いたします。

添付資料：研究報告書

中国人研究者名：惠 小剛 惠小剛 印

指導責任者名：林 富 職名：教授

所属機関名：東北大学小児外科

〒980-8574

所在地：仙台市青葉区星陵町1-1

電話：022-717-7237(直通)

1. 助成金額：600,000 円

2. 研究テーマ

子豚を用いてTPN（完全静脈栄養）により肝機能障害におけるアミノ酸の役割についての検討—伊東細胞を介して関与する可能性

3. 成果の概要（100字程度）

本研究では、子豚を用いてTPNを2週間まで持続的に投与した。その結果、肝機能障害の発症にはTPNの成分が関係している事が示唆され、発症メカニズムには活性化を受けた伊東細胞も関与している事が明らかになった。即ち、アミノ酸を加えて投与すると肝細胞の脂肪化が著しくなりアポプトシスも発生する事が判明した。更に、胆汁鬱滞病変を中心とする肝障害はアミノ酸により引き起こされる事実も判明した。一方、伊東細胞の活性化に伴い類洞の正常構造が破壊され肝血流障害が起こり、肝細胞や毛細胆管の構成要素の慢性虚血が引き起こされ最終的に肝機能障害が発症するメカニズムを解明した。将来は、適正なアミノ酸組成の輸液を考案し、伊東細胞の活性化を抑制し、TPNによる肝機能障害を抑えることが可能になると考えられる。

4. 研究業績

(1) 学会における発表 ■無 ・□有（学会名・演題）

(2) 発表した論文 □無 ・■有（雑誌名・題名）

雑誌名 World Journal of Pediatrics

題名 Total parenteral nutrition-induced liver dysfunction: evidence and pathogenesis

—日中医学協会助成事業—

子豚を用いてTPN(完全静脈栄養)により肝機能障害におけるアミノ酸の役割についての検討—伊東細胞を介して関与する可能性

研究者氏名　恵　小剛
中国所属機関　天津市児童病院小児外科
日本研究機関　東北大学病院小児外
指導責任者　教授　林　富
共同研究者名　天江　新太郎，和田　基，西　功太郎，
松浦　俊治，佐々木　英之，風間 理郎

Abstract

Objectives To investigate whether amino acids would cause parenteral nutrition-associated cholestasis-based liver damages, and try to evaluate the possible roles of Ito cells in this pathogenesis.

Materials and methods 15 piglets were randomly assigned to three groups equally. Group 1 piglets were maintained on glucose alone; Group 2 piglets were administrated by amino acid plus glucose mixture; Group 3 represented control piglets receiving standard lab chows ad libitum. All PN (parenteral nutrition) animals were treated for 14 days continuously with isocaloric intravenous regimen. On the postoperative 14th day, all animals were anesthetized and blood samples were collected for liver function test, and liver biopsy specimens were harvested for histological assessment as well as immunohistochemistry for α-SMA, while TUNEL (TdT-mediated dUTP-digoxigenin nick-end labelling) method was employed to detect apoptosis of hepatocytes and TEM (transmission electron microscopy) was also used to observe ultrastructural changes of liver specimens.

Results The concentration of serum total bilirubin and total bile acids were significantly higher in group 2 than the other groups ($p<0.05$); whereas liver enzymes showed no significant differences among three groups except for ALP (Alkaline phosphatase). Morphologically, steatosis was severer with vague sinusoid in group 2 than group 1 with mild cytoplasmic rarefaction in hepatocytes. In addition, immunohistochemistry revealed that the number of activated Ito cells significantly increased in group 2 compared with the other groups. Moreover, apoptotic index of hepatocytes in group 2 was significantly higher than group 1 and control group. TEM observation indicated that dilated bile canaliculi with decreased microvilli and deposition of bile pigments in hepatocytes were demonstrated in group 2. Besides, the sinusoid seemed to become shrank attributed to corresponding expansion of Disse space. In some specimen, we also observed that the slight production of collagen bundles in the Disse space and subendothelial deposition of base membrane-like materials. Apoptotic hepatocytes manifested by the earlier nuclear chromatin condensation characterized by crescent-shaped appearance and late development of apoptotic fragments or bodies.

Conclusions we have demonstrated that not glucose but amino acid was primarily involved in parenteral nutrition-associated cholestasis. Furthermore, aggravated steatosis and apoptosis of hepatocytes were also associated with the supplement of amino acids. Because numbers of Ito cells were activated in group 2, we suggested that the liver toxicity of amino acids would be related to subsequent activation of Ito cells, which would cause compromised microcirculation leading to chronic ischemic injuries to hepatocytes.

Key Words TPN, liver dysfunction, Amino acids, Ito cells, Liver microcirculation

Backgrounds and purposes

The development of progressive liver dysfunction has been a well-recognized complication of

prolonged total parenteral nutrition (TPN). Although more and more investigators have studied the possible mechanisms using animal models or retrospective clinical studies, the authentic pathogenesis was unknown. In recent decades, investigators began to concentrate on the toxic roles of components of TPN solution. Glucose and fat and their absolute or relative concentrations have been incriminated. (1) Fat emulsion, although they may cause fat accumulation in Kuppfer cells, have been essentially excluded as being important in the genesis of cholestasis. (2) Amino acids are most frequently implicated in promoting cholestasis. Vileisis et al treated infants with either 2.5 or 4.0 g/kg of crystalline amino acid in an otherwise equal TPN regimen for at least 2 weeks while routine liver function tests were monitored. Although the frequency of cholestasis was the same in the two groups, the duration of TPN prior to onset of cholestasis was shorter, and the rate of rise of direct bilirubin was greater in the high-amino acids group, suggesting that the amino acid are more toxic when administered in excess. The inference is that amino acids can be toxic when administered at routine rates. (3) Black et al compared several parameters of liver function in essentially well premature neonates receiving amino acid glucose infusion or glucose alone for 1 week and found evidence for hepatic canalicular dysfunction in the group receiving amino acid. (4) These results suggest that amino acids solution has a direct effect on the excretory apparatus of the hepatocyte. Evidences for this view come from observations that amino acid solutions inhibit bile formation, particularly the bile acid-independent fraction in isolated perfused rat liver. (5) Particular amino acids have been also implicated. Methionine at a perfusate concentration of 20 to 30mM obliterated bile salt independent bile flow in perfused rat liver; Alanine inhibited uptake of taurocholate by isolated rat hepatocytes; Taurine supplementation increases bile acid excretion in premature infants and protects from lithocholate cholestasis in young guinea pigs. Of the major constituents of TPN solutions, amino acids are the most likely responsible for cholestasis. Preferential transport of the amino acids across the hepatic sinusoidal membrane may inhibit bile acid uptake. (6) Reduced uptake of bile acids by the hepatocyte results in a decreased transport and secretion of bile acids, the major driving force of bile flow. As opposed to amino acids-induced cholestasis during TPN, few studies reported that the protective role of amino acids in ameliorating calorie overloading-associated cholestasis. In a newborn rabbit TPN model, histological examination of the cholestatic liver showed ballooning and clear cell transformation of the hepatotcytes resembling those observed in the liver with calorie overload. Ikeda et al reported that calorie overload changes in hepatocytes are ameliorated by an increased dose of amino acids. (7)

Therefore, although it has been a fact that TPN could cause intrahepatic cholestasis, the real toxic components and the related mechanisms were poorly understood. Our previous research on TPN-induced liver dysfunction (not yet published) demonstrated that major components including glucose and amino acids must have caused liver damages characterized by steatosis and cholestasis, and we also discussed on the potential implication of Ito cells in this pathogenesis. In the present study, we try to evaluate if glucose or amino acids would be primarily involved in liver dysfunction during TPN administration, further to explore which component would be mostly responsible for the activation of Ito cells in a piglet model.

Materials and methods

1 Animals and experimental design

Fifteen piglets with body weight ranging from 10 to 15kg were randomly assigned to three groups equally. Group 1 was glucose alone (50g/kg/d); Group 2 was glucose plus amino acids (glucose 50g/kg/d; amino acid 7.5g/kg/d); Group 3 was control receiving standard lab chows ad libitum. All

animals underwent the same catheter cannulation through external jugular vein in the aseptic manner. For control group, animals received physiologic saline infusion (20ml/h) for maintaining patency of catheter. For TPN groups, infusion lasted for 14 days continuously by fasting and deprivation of tap water. Besides, the infusion volumes were adjusted to keep same and isocaloric intravenous infusion regimen was also considered. Besides glucose or amino acids, electrolytes, vitamins and trace elements were given, but lipid was not included.

2 Blood samples and liver specimens

Blood samples for liver function tests were collected on the 14th postoperative day. The parameters include albumin (Alb), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TB), alkaline phosphatise (ALP), γ-glutamyl transpeptitdase (γ-GT) and total bile acid (TBA). Liver biopsy specimens were also harvested on the terminal day of this study. One part of light microscopy was fixed in 10% neutral formalin solution and embedded in paraffines; the residual part for TEM was prefixed with 2.5% glutaraldehyde in 0.1M phosphate buffer at 4°C.

3 Immunohistotchemistry

All liver samples were immunostained with a monoclonal antibody to α-SMA (**dilute 1:800; DAKO**) to identify activated Ito cells with streptavidin-biotin complex immunostaining. As a negative control, normal rabbit IgG was used instead of the primary antibody. No specific immunoreactivity was detected in these sections. The positive expression of activated Ito cells was recognized by brown luminescence. The intensity of immunostaining was scored in light of specific scoring system.

4 Detection of apoptosis of hepatocytes by TUNEL

TUNEL was performed with peroxide *In Situ* apoptosis detection kit according to manufacture's instructions. (**CHEMICON S7101, USA**) The apoptotic hepatocytes exhibited brown stained nuclei. Twenty random fields were counted for each slide. The numbers of apoptotic hepatocytes were expressed as the percentages of the total number of the counted hepatocytes. (Apoptotic index)

5 Transmission electron microscopy (HITACHI 7100, JAPAN)

After primary fixation for 48 hours, specimens were washed with PBS, followed by secondary fixation in 1% osmium tetroxide in 0.1M PBS at 4°C for 2 hours. After washing with PBS, they were dehydrated and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate, and then examined with a transmission electron microscope.

Results

All 15 piglets were survival without events in this study. Group 2 and control piglets obtained the equivalent body weight gain, but Group 1 piglets showed almost stagnant body weight.

1 Liver function tests

After two weeks, the levels of serum TB and TBA in Group 2 were significantly elevated compared with that in group 1; however, serum enzymes level showed no significant changes except for the elevated ALP level in group 2. (**See Table 1**)

Table 1 Results for liver function tests

Group	Alb (g/dl)	TB (mg/dl)	AST(IU/L)	ALT(IU/L)	ALP(IU/L)	γ-GT(IU/L)	TBA (μmol/L)
1(n=5)	2.13±0.32	0.06±0.01	44.00±19.52	29.67±10.59	199.00±52.85	30.67±9.02	3.33±0.58
2 (n=5)	2.63±0.21	0.18±0.07*	46.67±35.53	37.00±13.28	654.67±112.61$^{\Delta}$	29.00±14.93	15.00±2.65 *
3 (n=5)	2.97±0.50	0.02±0.0	35.33±10.12	29.33±17.04	433.00±175.12	20.67±6.43	2.93±0.12

$^{\Delta}$Significant difference was determined between Group 2and 1(p=0.01);* significant difference was seen between

Group 2 and 1, or between Group 2 and control group. (p<0.05) Duncan's test was used following ANOVA.

2 Histological changes

Compared with mild steatosis in Group 1, the control specimens showed normal liver histological micrographs, but the Group 2 specimens displayed the significantly aggravated histological lesion manifested by vacuolation of hepatocytes. **(See Fig 1)**

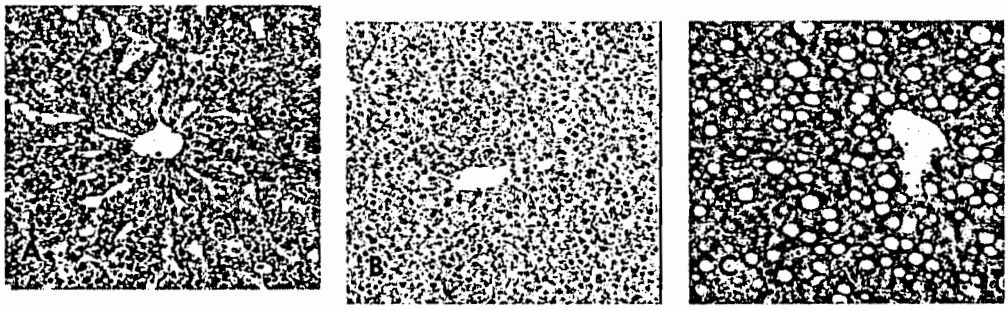

Fig1 Micrographs of liver specimens after treatment for two weeks (HE, x200) A, normal histological picture in control group; B, not severe changes except for mild cytoplasmic rarefaction in Group 1; C, dramatic vacuolation of hepatocytes with obscure sinusoid in Group 2.

3 Immunohistochemistry

In Group 2, considerable activated Ito cells immunoreactive to α-SMA antibody were demonstrated, whereas they were scarcely or sporadically found in Group 1 or control group. The scoring result indicated that there was significant difference between Group 2 and Group 1 or control group. **(See Fig 2 and Table 2)**

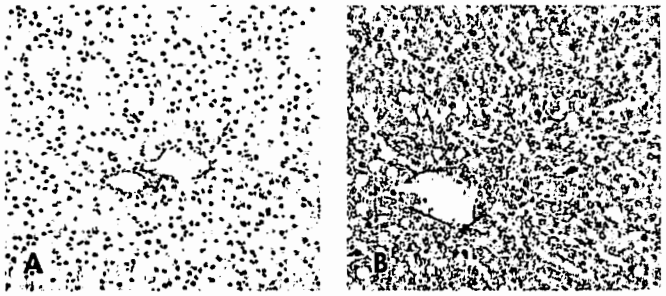

Table 2 scoring results of liver Ito cells

Group	Scoring				
	0	1	2	3	4
1 (n=5)	4	1	0	0	0
2 (n=5)*	0	0	0	1	4
3 (n=5)	5	0	0	0	0

Fig2 Immunohistochemistry for Ito cell in liver specimen. (x200) **A**, negatively *p<.05 significantly higher expression of

Ito cells in detected in control or Group 1; **B**, massively detected in Group 2. (Brown stained) Group2

4 TUNEL assay

Apoptotic index indicated that there was significant increase in apoptotic hepatocytes in Group 2. (**Group 1, 0.85±0.46; Group 2, 28.59±5.25; control group, 0.94±0.73 p<0.05**). Besides, the apoptotic hepatocytes were more conspicuous in centrilobular region than peripheral area in hepatic lobule. (**See Fig 3**)

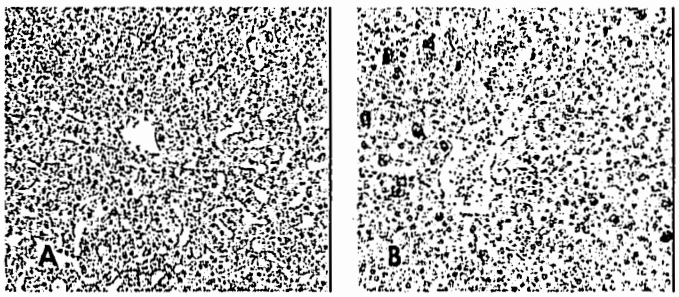

Fig3 Hepatocyte apoptosis by use of TUNEL method (Original magnification x200). A, apoptotic hepatocytes were seldom

found in Group 1 and 3; B, significantly increased apoptotic hepatocytes converging on central part of lobule in Group 2.

5 Transmission electron microscopy

Under TEM, following meaningful changes have been observed. Firstly, the dilation of bile canaliculi accompanied by the loss of microvilli was usually seen only in group 2. Besides, some hepatocytes stored dark matter represented bile salts or pigments. Secondly, different phases of apoptotic hepatocytes could be easily found. Thirdly, base membrane-like materials produced by activated Ito cells brought about endothelial fenestrae disappear replaced by subendothelial deposition. Furthermore, activated Ito cells became "plump" in volume with long cytoplasmic processes, and slight collagen bundles produced around them. Lastly, the loss of lipid droplets in some activated Ito cells could be found. (**See Fig 4**)

Discussion

It is unknown whether steatosis and cholestasis are separate entities or represent a continuum of one disorder in TPN-induced liver dysfunction. Although different TPN regimens have been devised to explore harmful ingredients or clarify the mechanisms, it is controversial or undecided. In this study, the elevated serum TB and TBA level, together with bile canaliculi and accumulation of bile components in hepatocytes indicated that cholestasis developed in Group 2. Likely, the obvious fatty degeneration and hepatocytes apoptosis were shown in Group 2. These evidences indicated that amino acids are toxic to liver and could concomitantly induce steatosis and cholestasis. Before, it is hard to elucidate this composite liver damage. However, we proved that Ito cells were extensively activated, and these pathologic changes predominately located in the centre part of lobules. In addition, some studies reported that the activation of Ito cells has implicated in regulating sinusoidal blood and liver microcirculation. (8) We suggested that decreased sinusoidal blood supply maybe contribute to liver damages. Firstly, the replace of normal fenestrae by subendothelial base membrane could hamper the two-way communication between hepatocytes and sinusoid; Secondly, the proliferative activated Ito cells and deposition of collagen fibres could inflate Disse space through "pushing or squeezing"sinusoid resulting in increased blood resistance. Lastly, as activated Ito cells were transformed into myofibroblasts and obtained contractility, the tonic contraction could participate in increasing the resistance of blood

flow in sinusoid. Consequently, the compromised microcirculation not only amplified steatosis, but also induced hepatocyte apoptosis. Considering the direct effects of amino acids, we also suggested the accelerating roles in the form of physical pressure or ischemic attack on apparatus of bile system induced by Ito cells activation.

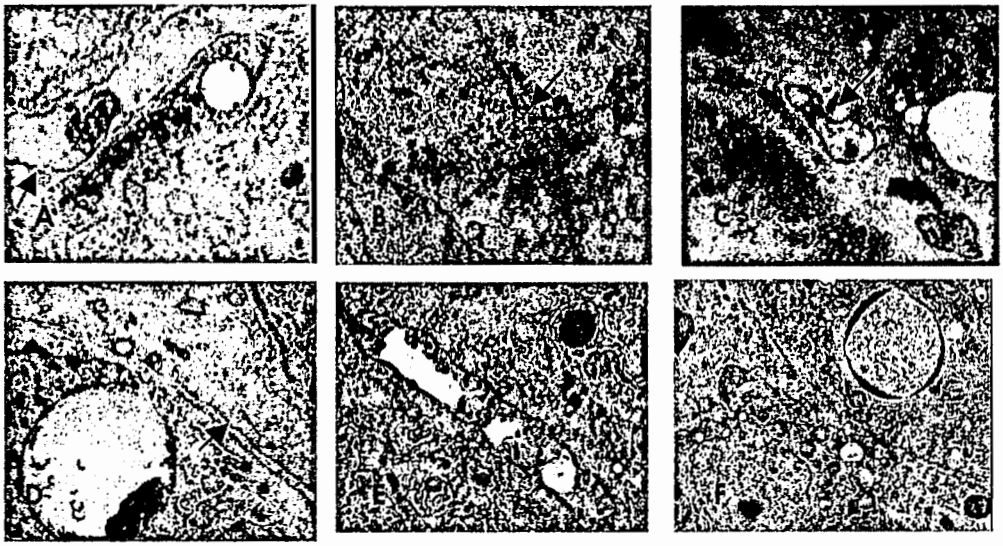

Fig 4 TEM micrographs (Original magnification x1000) A, normal sinusoid with subendothelial fenestrae; D, fenestrae structure was disappeared and replaced by base membrane like materials in Group 2. B, normal bile canaliculus in Group 1 and control; E, dilated bile canaliculus with decreased microvilli in Group 2; C, activated Ito cells with indentation attributed to loss of lipid droplet "push"sinusoid in Group 2; F, earlier apoptotic hepatocyte characterized by the chromatin condensation.

Conclusions

In the present study, we established the amino acids-induced liver toxicity biochemically and morphologically, indicating that severer fatty degeneration and cholestasis as well as hepatocyte apoptosis could simultaneously occur. Amino acids-dependent Ito cells activation maybe initiate or deteriorate these liver lesions by influencing on sinusoidal microcirculation. However, the present short-term TPN study fails us to draw a definite conclusion, and long-term experiment about relationship between amino acids and Ito cells would be further expected. It would be better if the evaluation of sinusoidal microcirculation secondary to the introduction of amino acids could be conducted in the future.

References

1 Rager R, Finegold MJ. Cholestasis in immature infants: Is parenteral alimentation responsible? J Pediatr 1975;86:264-269

2Beale EF, Nelson RM, et al. Intrahepatic cholestasis associated with parenteral nutrition in infants. Pediatrics 1979;64:342-7

3 Vilersis RA. Prospective controlled study of parenteral nutrition-associated cholestatic jaundice. J Pediatr 1980;96(5):893-7

4 Black DD. The effect of short-term parenteral nutrition on hepatic function in human neonate. J Pediatr 1981;99:445-9

5 Graham MF, Tavill AS. Inhibition of bile flow in isolated perfused rat liver by amino acid mixture. Hepatology 1984;(4)69-73

6 Blitzer BL. Amino acid inhibition of bile acid uptake by basolateral membrane vesicles. Gastroenterology 1983;(84):1364A

7 Ikeda Y, Soda S. Are hepatomegaly and jaundice attributable to "glucose overload"? Acta Chir Scand 1979;494;170-172
8 Housset C, Rockey DC, Bissell DM. Endothelin receptors in rat liver: lipocytes as a contractile target for endothelin-1. Proc Natl Acad Sci USA 1993;90:9266-70

2008/3/1

財団法人日中医学協会

2007 度共同研究等助成金－在留中国人研究者－報告書

2008 年 2 月 20 日

財団法人　　日中医学協会　御中

貴財団より助成金を受領して行った研究テーマについて報告いたします.

添付資料：　研究報告書

中国人研究者名：　孟　翔峰
指導責任者：　熱田　充　　職名：　教授
所属機関名：　長崎大学医歯薬総合研究科
所在地：〒852－8588　長崎市坂本 1－7－1
電話：95－819－7688　内線：4352

1. 助成金額：600,000 円

2. 研究テーマ

シランカップリング剤の成分とｐＨ値と湿潤湿気がデユアルキュア型レジン接着剤のシリカベースマシナブルセラミックスとの接着耐久性及ぼす効果

3. 成果の概要

シランカップリング剤とレジン接着剤を適切に組み合わせることで，シランカップリング剤の劣化を防止できることがわかりました．さらに，シランカップリング剤のシロキサンの量は接着耐久性には影響を及ぼさず，シランカップリング剤の pH が一部，レジン接着剤のセラミックスに対する濡れ性が最も大であることが明らかになりました.

4. 研究業績

(1) 学会における発表　　有

Meng X, Yoshida K, Atsuta M: Micro-shear bond strength of self-adhesive resin cement to silica-based machinable ceramic, The 2nd joint meeting of the Japan prosthodontic society and the greater New York academy of prosthodontics, Tokyo, Japan, 2007.

(2) 発表した論文　有

Meng X, Yoshida K, Atsuta M (2007) Influence of light irradiation condition on micro-shear bond strength of dual-cured resin luting agents. Dent Mater J, 26 (4): 575-581.

－日中医学協会助成事業－

シランカップリング剤の成分とｐＨ値と湿潤湿気がデユアルキュア型レジン接着剤のシリカベースマシナブルセラミックスとの接着耐久性及ぼす効果

研究者氏名　孟　翔峰
日本研究機関　長崎大学医歯薬総合研究科
指導責任者　教授　熱田　充
共同研究者　吉田　圭一

要旨

Objective. To evaluate the correlation between siloxane quantity, pH value and wettability of five silane coupling agents with resin bond durability of ceramic.

Methods. Five silane coupling agents { Monobond S (Ivoclar-Vivadent), Rely XTM Ceramic Primer (3M), Clearfil Ceramic Primer (Kuraray), GC Ceramic Primer (GC), Porcelain Liner M (Sun Medical)} were used. Their siloxane quantity, pH value and contact angle to Heliobond (Ivoclar-Vivadent) was measured irrespectively by a FTIR spectrophotometer, pH-indicator strips and a contact-angle meter. 1.5mm thick ceramic plates (ProCAD, A3) were polished and cleaned, and treated by ten combinations between five silane coupling agents and two dual-cured resin luting agents {Variolink (VLII Ivoclar-Vivadent), Linkmax HV(LMHV, GC)}. Their micro-shear bond strength were measured by after 0, 10,000, and 30,000 time thermal cycling. Data was analyzed by three-way ANOVA, four measure parameter of silane coupling agents were subjected to correlation analysis.

Results. Bond strength was significantly affected by silane coupling agents, thermal cycling, and not by resin luting agents. Significant correlation was established between contact angle and bond strength after TC30,000 of silane coupling agents in VLII and LMHV; and between pH value and bond strength of silane coupling agents in VLII.

Conclusion. The hydrolytic degradation of current dental silane for ceramic bonding might be inevitable under endurance test. The improvement of combination between silane coupling agent with resin luitng agent could affect delay this hydrolytic degradation process, which is depended by the wettability to resin luting agent, partly by pH value, not by siloxane quantity of silane coupling agents.

Key Words　silane coupling agent, pH, siloxane, resin luting agent, machianable ceramic, micro-shear bond strength

緒言：

Silane as a coupling agent is used popularly in resin bond of silica-based ceramic restorative.[1,2] Activated silane can provide a physical and chemical bond between resin luting agent and silica-based ceramic: its silanols could form a direct siloxane bridge with hydroxyls of ceramic surface, meanwhile produce crosslinked siloxane polymolecular layer which could form an interpenetrating polymer network (IPN) with resin luting agent; its organic part could take part in the polymerization with resin matrix to provide a direct covalent link with resin luting agent.[3]

Short-term resin bond strength to silaned ceramic could be obtained, which even could caused cohesive failure of silica-based ceramic in bond test, however this adhesive interfaces were not stable under endurance tests such as thermal cycling or long-term water storage, and different degree reduction of bond strength occurred in various dental silane coupling agents.[4-9] Bond durability degradation is contributed to the hydrolysis of siloxane (-Si-O-Si-) in ceramic surface and IPN under water effect.[10,11] Other field research suggested that the rate of hydrolytic damage of silane could be related to the proportion of Si_{silane}-O-Si_{silane} because the siloxane (Si_{silane}-O-Si_{silane}), siloxane is easier to break than the siloxane (Si_{silica}-O-Si_{silane}), and OH- groups could catalyze the hydrolytic reaction.[12] Miyata et al also suggestd that a weaker silane coupling agent/resin bond

could result in a waker filler/resin in composite with silaned filler.[13]

Current dental silane coupling agents have different silane concentration, activation modes, and supplemental compositions, which produces their different siloxane quantity[14], pH value[14,15], wettability to resin luting agents. These factors could form different adhesive interface structures with resin luting agents, which might decide their water resistance. The fact that many dental silane coupling agents are used in different studies, make a correlation of data from various researchers rather difficult. A further understanding of the role of different properties of silane coupling agent can solve this amibiguity.

In this study, we attempted to explore the relationship siloxane bond quality, pH value and wettability of five commercial dental silane coupling agents with their resin bond durability to ceramic.

対象と方法：

Table 1 Materials tested in this study (information provided by manufactuers)

Material (Abbreviation)	Chemical composition	Lot No.	Manufacturer
Silane coupler:			
Monobond S (MBS)	Ethanol 52%, water 47%, silane 1%, acetic acid	H26975	Ivoclar-Vivadent, Schaan, Liechtenstein
Rely X™ Ceramic Primer (RCP)	Ethanol 70-80%, water20-30%, silane<1%	3UK	3M, ESPE, Seefeld, Germany
Clearfil Ceramic Primer (CCP)	Ethanol, MDP, silane (γ -MPTS) < 5%	00001B	Kuraray co., Ltd, Kurashiki, Japan
GC Ceramic Primer (GCCP)	A liquid: ethanol, silane B liquid: ethanaol, MMA, UDMA, organic acid	0601262	GC Corp., Tokyo, Japan
Porcelain Liner M (PLM)	A liquid: MMA, 10% 4-META, other B liquid: MMA, silane (γ - MPTS) 10%, other	MR1	Sun Medical Co., Ltd, Moriyama, Japan
Resin luting agent:			
Variolink II (VLII)	A3, Resin matrix: Bis-GMA, UDMA, TEGDMA. Filler: content 72.3%, mean particle size 0.7 μm, Ba-Al-F-Si-glass	Base: H23580 Catalyst: H23432	Ivoclar-Vivadent, Schaan, Liechtenstein
Linkmax HV (LMHV)	Universal, Resin matrix: UDMA, TEGDMA. Filler: content 70%, mean particle size 0.8 μm, F-Al-Si-glass	Base:0601131 Catalyst: 0601131	GC Corp., Tokyo, Japan

1. 1.5 mm thick of ceramic plates (13 X 11 mm squares) Ceramic plate preparation

2.The measurement of contact angle between resin bond agent and silane ceramic surface

3. The determination of pH value in five silane coupling agents

4. The FT-IR analysis of siloxane quantity in five silane coupling agents

5. Micro-shear test of bond strength between dual-cured resin luting agent and silaned ceramic surface before and after 10,000 and 30,000 thermal cycling time.

結果：

Three-way ANOVA analysis showed that bond strength was significantly affected by silane coupling agent and thermal cycling, not by composite luting agent. The interactions were significant between silane coupling agent/thermal cycling, resin

luting agent/thermal cycling, silane coupling agent/reisn luting agent, silane coupling agent/resin luting agent/thermal cycling.

Table 2 pH value, siloxane signal absorbance strength and mean contact angle for five silane coupling agents.

Silane coupler	Contact angle θ	pH value	Siloxane signal absorbance strength
MBS	34.0 (1.5)	4.0	0.022
RCP	27.7 (1.2)	4.0	0.035
CCP	21.7 (1.2)	1.0	0.116
GCCP	20.7 (1.1)	1.4	0.053
PLM	21.5 (0.9)	2.2	0.075

Table 3 Mean bond strength (SD) of ten combinations between five silane coupling agents and two resin luting agents after 0 10,000 and 30,000 time thermal cycling.

* Same letters at bond strength for each resin luting agent were not significantly difference by post HocTukey test (p<0.05).

Resin luting agent	Silane coupler	Thermal cycling time		
		0	10,000	30,000
VL II	MBS	24.1 (2.2)^{f}	18.6 (4.3)^{de}	3.6 (3.5)^{a}
	RCP	18.6 (2.7)^{de}	7.3 (2.9)^{ab}	5.2 (3.7)^{a}
	CCP	21.7 (2.0)^{ef}	23.0 (3.3)^{ef}	10.8 (5.3)^{bc}
	GCCP	24.0 (4.7)^{f}	23.3 (4.6)^{ef}	13.9 (2.6)^{cd}
	PLM	25.1 (5.4)^{f}	15.3 (2.9)^{cd}	12.1 (4.2)^{bc}
LMHV	MBS	22.5 (2.7)^{f}	19.6 (4.5)^{ef}	5.1 (5.2)^{a}
	RCP	19.8 (2.2)^{ef}	12.3 (3.3)^{bc}	10.1 (3.3)^{b}
	CCP	20.2 (2.2)^{ef}	17.7 (3.0)^{def}	10.9 (3.3)^{b}
	GCCP	18.7 (2.6)^{def}	17.9 (2.9)^{def}	14.2 (3.1)^{bc}
	PLM	22.1 (4.1)^{f}	22.3 (2.6)^{f}	15.8 (3.4)^{cde}

Table 5 Pearson's correlation coefficient and respective p value between two properties. Correlation is significant at p<0.05 (2-tailed).

Composite luting agent	VL II			LMHV		
Properties of silane couplers	Contact angle	pH value	Siloxane absorbance	Contact angle	pH value	Siloxane absorbance
Bond strength (TC0)	-0.154 (p=0.805)	-0.318 (p=0.603)	0.052 p=0.933	0.525 (p=0.364)	0.428 (p=0.473)	-0.164 (p=0.792)
Bond strength (TC10,000)	-0.353 (p=0.560)	-0.738 (p=0.154)	0.458 p=0.437	-0.175 (p=0.778)	-0.331 (p=0.586)	0.240 (p=0.697)
Bond strength (TC30,000)	-0.947 (p=0.014)*	-0.898 (p=0.039)*	0.622 p=0.262	-0.910 (p=0.032)*	-0.639 (p=0.245)	0.474 (p=0.420)

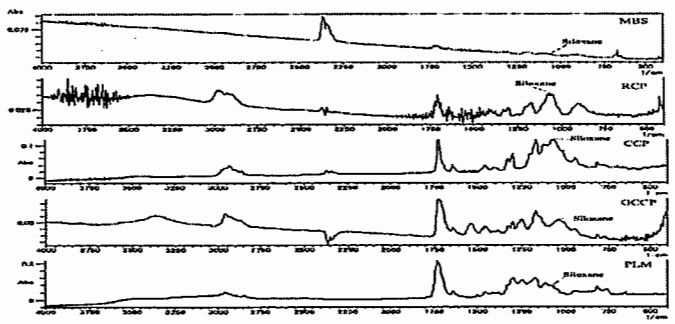

Fig 1-The FT-IR spectra of the five dental silane coupling agents. X-axis: wave number in cm^{-1}; Y-axis: absorbance in arbitrary unit.

考察：

In this study, no silane coupling agents could maintain their role in resin bond of ceramic, and significant interaction between silane coupling agents and thermal cycling suggested that they had different hydrolytic degradation process of silane. The diffefrence of composition in silane coupling agents could affect the hydrolytic rate of silane.

In this study, we simulated the silane film of ceramic on the KBr plate, and found that different siloxane absorbance strengths occurred in five dental silane coupling agents. This difference was not correspond their silane concentrations demonstrated by manufacturers. RCP with <1% silane had higher has siloxane absorbance strength than MBS with 1% silane, which suggested that the the silane degree activation and condensation could not only depended by silane concentration, but also depended by other factor such as pH value, solvent type, hydrolysis time. Within the range of siloxane absorbance strength in this study, the difference of siloxane quantity might not cause a substantial influence on the hydrolytic degradation of silane coupling agent, because the siloxane absorbance strength of five dental silane coupling agents had any correlation with their bond strength at TC0 and 10,000 in two resin luting agents.

Five silane coupling agents have different activation modes: MBS and RCP activated by water, while CCP, GCCP and PLM activated by organic acid. So CCP, GCCP and PLM have lower pH value than MBS and RCP. Previous research showed pH value of ceramic surface and silane coupling agents could affect primary resin bond strength.[16] In this study pH value of silane coupling agents had no correlation with their bond strength (TC0). After TC 30,000, the significant correlation between pH value and bond strength occurred in VL II. It might support at some extent the thought of Olmos etal[12], in which higher pH of silane coupling agent could catalyze the hydrolytic degradation of siloxane. However the correlation effect of pH value was lower than that of contact angle in VL II; even did not occur in LMHV.

Contact angle is a consequence of the inter-molecular interaction between the probing medium and solid. When two media of similar chemical composition are used to probe the surface, the one with a lower surface tension should yield a lower contact angle. VL II and LMHV are dual-cured composite resin luting agents with rather low fluidity. So we used a resin bond agent (Heliobond) as probing medium to reference the wettability of five dental silane coupling agents to resin luting agent. Comparing with pH value, contact angle showed more significant correlation with bond strength (TC 30,000) in two resin luting agents. This result further suggests that the water-resistance ability of resin bond interface to ceramic could be depended on the combination between silane coupling agent and resin luting agent. High wettability of silane coupling agent to resin luting agent helps to form their interpenetrating polymer network (IPN) and copolymerization. MBS and RCP activated by water showed lower wettability than those activated by the use of organic acid such as phosphate acid monomer. Even if under the same activation mode, solvent type and solution concentration also affected their wettability, in which RCP had higher wettability than MBS. To improve the lower wettability of MBS, the manufacturer recommends the additional use of flowable resin bond agent (Heliobond) follows the treatment of MBS. However our and other researches showed that this resolution was not more effective than the only use of MBS.[17,18] Besides the use of organic acid, two-liquid silane coupling agents (GCCP and PLM) blend functional monomer such as MMA, UDMA into silane before the use of silane, and obtained better bond durability. This beforehand use of functional monomer might be an effective way to obtain better interpenetrating polymer network (IPN) and copolymerization between silane coupling agents and resin luting agents.

参考文献：

1.Peumans M, Van Meerbeek B, Lambrechts P, Vanherle G. Porcelain veneers: a review of the literature. J Dent 2000; 28: 163-177.

2.Blatz MB, Sadan A, Kern M. Resin-ceramic bonding: a review of the literature. J Prosthet Dent 2003; 89: 268-274.

3.Matinlinna JP, Lassila LV, Özcan M, Yli-Urpo A, Vallittu PK. An introduction to silanes and their clinical applications in

dentistry. Int J Prosthodont 2004; 17:155-164.

4.Kato H, Matsumura H, Tanaka T, Atsuta M. Bond strength and durability of porcelain bonding systems. J Prosthet Dent 1996; 75: 163-168.

5.Foxton RM, Pereira PN, Nakajima M, Tagami J, Miura H. Durability of the dual-cure resin cement/ceramic bond with different curing strategies. J Adhes Dent 2002; 4: 49-59.

6.Foxton RM, Pereira PN, Nakajima M, Tagami J, Miura H. Long-term durability of the dual-cured resin cement/silicon oxide ceramic bond. J Adhes Dent 2002; 4: 125-135.

7.Nakamura S, Yoshida K, Kamada K, Atsuta M. Bonding between resin luting cement and glass infiltrated alumina-reinforced ceramics with silane coupling agent. J Oral Rehabil 2004; 31: 785-789.

8.Ide T, Tanoue N, Yanagida H, Atsuta M, Matsumura H. Effective of bonding systems on bonding durability of a prefabricated porcelain material. Dent Mater J 2005; 22: 257-260.

9.Yoshida K, Yamashita M, Atsuta M. Bond strength between machinable glass-ceramic and dual-cured resin luting cements using silane coupling agents. Am J Dent 2005; 18: 327-330.

10.Ishida H, Koenig JL. A fourier-transform infrared spectroscopic study of the hydrolytic stability of silane coupling agents on E-glass fibres. J Polym Sci Part B: Polym Phys 1980; 18: 1931-1943

11.Nishiyama N, Ishizaki T, Horie K, Tomari M, Someya M. Novel polyfunctional silanes for improved hydrolytic stability at the polymer-silica interface. J Biomed Mater Res 1991; 25: 213-221.

12.Olmos D, Gonzalez-Benito J, Aznar AJ, Baselga J. Hydrolytic damage study of the silane coupling region in coated silica microfibers: pH and coating type effects. J Mater Process Technol 2003; 143-144: 82-86.

13.Miyata N, Matsuura W, Kokubo T, Nakamura T. Mechanical behavior of bioactive composite cements consisting of resin and glass-ceramic powder in a simulated body fluid: Effect of silane coupling agent. J Mater Sci Mater Med 2004; 15: 1013-1020.

14.Matinlinna JP, Lassila LV, Vallittu PK. Evaluation of five dental silanes on bonding a luting cement onto silica-coated titanium. J Dent 2006; 34: 721-726.

15.Kamada K, Taira Y, Yoshida K, Atsuta M. Effect of four silane coupling agents on bonding of two resin-modified glass ionomer cements to a machinable ceramic. Dent Mater J 2007; 26: 240-244.

16.Foxton FM, Nakajima M, Hiraishi N, Kitasako Y, Tagami J, Nomura S, Miura H. Relationship between ceramic primer and ceramic surface pH on the bonding of dual-cured resin cement to ceramic. Dent Mater 2003; 19: 779-789.

17.Reich SM, Wichmann M, Frankenberger R, Zajc D. Effect of surface treatment on the shear bond strength of three resin cements to a machinable feldspathic ceramic. J Biomed Mater Res B Appl Biomater 2005; 74: 740-746.

18.Meng X, Yoshida K, Atsuta M. Micro-shear bond strength of resin bonding systems to machinable ceramic with different surface treatments. J Adhes Dent 2008, in press.

注：本研究は、今欧米雑誌に投稿しています.

作成日：2008 年 2 月 20 日