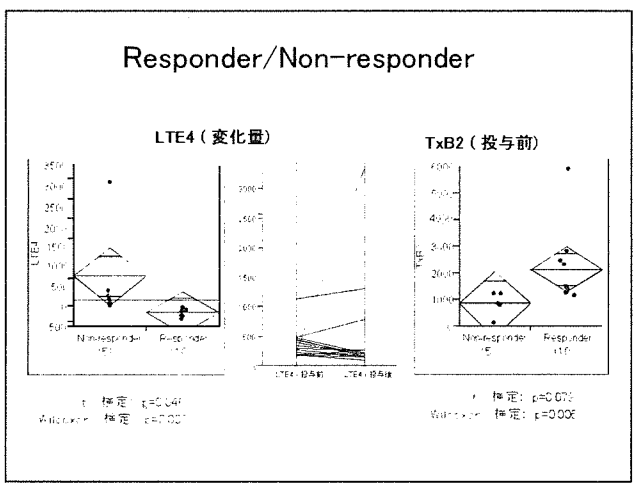
C. 研究結果

① アレルギーの系統的遺伝子診断キットをインベーダーアッセイ法を利用して構築した。

② ロイコトリエン受容体拮抗薬のうち、モンテルカスト投薬前後における尿中アラキドン酸代謝産物の変化について検討した。対象は、6歳以上の気管支喘息患者とした。尿中のLTE4排泄量をモンテルカスト投与前後で検討したところ、効果のみられた症例では、モンテルカスト内服4週後にLTE4排泄量が減少しており LTE4排泄量の変化量は有効例と無効例の間に有意差を認めた（図1）。また、さらに、有効例では、無効例に比較して投与前の尿中TxB2の排泄量が低下していることが明らかになった。

図1



③ 遺伝子多型とモンテルカストの有効性との関連を検討した（表1）。表1に示すように今回遺伝子検出キットを用いて解析したSNPsと有効性との間に有意差を認めたSNPsは見られなかった。

表1

SNPsと効果との関連

Gene	SNP	n	Genotypes	Non-responder	Responder	P value
IFN-GR1	IFN-GR1	29	A/A	7 (100%)	13 (93%)	
			A/G	0 (0%)	1 (7%)	0.466
IL12RB1	IL12RB1 (3'UTR)	29	T/T	4 (57%)	6 (43%)	
			T/C	3 (43%)	6 (43%)	
			C/C	0 (0%)	2 (14%)	0.545
IL1R	IL-1(3737)	29	T/T	3 (43%)	4 (29%)	
			T/C	4 (57%)	8 (57%)	
			C/C	0 (0%)	2 (14%)	0.526
	IL-1(-1160976)	29	G/G	6 (86%)	12 (86%)	
			G/T	1 (14%)	2 (14%)	1.000
LTC4S	LTC4S(-444)	29	A/A	4 (57%)	10 (71%)	
			A/C	3 (43%)	4 (29%)	
			C/C	0 (0%)	0 (0%)	0.275
IL4RA	IL-4RA	29	G/G	2 (33%)	8 (57%)	
			G/A	3 (50%)	5 (36%)	
			A/A	1 (17%)	1 (7%)	0.585
IL18	IL-18	29	G/G	2 (29%)	5 (36%)	
			G/A	3 (43%)	8 (57%)	
			A/A	2 (29%)	1 (7%)	0.415

そこで、ジーンチップ（500K Mapping assay）によるロイコトリエン受容体拮抗薬の効果と遺伝子多型解析を行った。表2に結果を示すが、GATA3などの免疫関連遺伝子領域に存在する22種の多型、IL-8, IL-19, TNFなどのサイトカイン関連遺伝子領域に存在する20種類の多型が、有効性と関連（$p<0.001$）が認められた。

表2

P＜0.001でLTRAの有効性と関連のみられた免疫関連リストと一致したSNPs一覧

dbSNP RS ID	Chromosome	Cyto band	Transcript	Gene Relationship	Gene
rs3821821	9	q26.31	NM_003810	intron	TNFSF10
rs9774915	9	q26.31	NM_003810	intron	TNFSF10
rs1172801	4	q13.3	NM_000584	upstream	IL8
rs865474	3	q12.1	NM_007013	downstream	WNT1
rs2419117	1	q24.2	NM_002995	downstream	XCL1
rs2384818	-	q32.2	NM_006147	upstream	IRF6
rs13139200	4	q15.31	NM_025221	downstream	KCNIP4
rs1335231	10	p14	NM_001002295	downstream	GATA3
rs1111958	-	q32.1	NM_013371	intron	IL19
rs4244185	-	q25.1	NM_003326	upstream	TNFSF4
rs1111958	-	q32.1	NM_013371	intron	IL19
rs1111960	-	q32.1	NM_013371	intron	IL19
rs4284450	-	q25.1	NM_003326	upstream	TNFSF4
rs3934575	-	q25.1	NM_003326	upstream	TNFSF4
rs7530469	-	q25.1	NM_003326	upstream	TNFSF4
rs12042283	-	q32.1	NM_013371	intron	IL19
rs10185293	2	q37.3	NM_006037	downstream	HDAC4
rs17168022	7	q33	NM_002825	intron	PTN
rs4721726	3	p21.1	NM_058176	intron	HDAC9
rs6977685	7	p14.1	NM_002192	upstream	INHBA

D. 考察

増加を続けるアレルギー疾患患者の個々の病態を遺伝子レベルで整理し、分類することは、テーラーメイド医療を考える上で、非常に重要なことであると考えられる。多数報告のみられるアレルギー関連遺伝子多型情報を、臨床レベルで活用するためには、多くのサンプルを迅速に処理する必要があり、これを実現するために、遺伝子診断キットは、非常に有用であると考えられた。さらに、どの目的のためには、どの遺伝子をどのように組み合わせるべきかについて検討を加え、実用化に向けて検討を続けたい。また、治療への応用として、得られた遺伝子情報を活用し、より適切な治

療薬を選択していきたいと考える。

E. 結論

アレルギー疾患の遺伝子診断キットを開発した。一般臨床へ応用するため、さらに、今後、適格な診断、治療法の確立により医療効果が向上し、アレルギー疾患の治癒、軽快率が向上し、QOL の改善がはかるため、診断、治療に着目した感受性、特異性の向上、薬理遺伝学的見地からの十分な検討が必要である。

F. 健康危険情報

なし

G. 研究発表

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H. 知的財産権の出願、登録状況

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2、実用新案登録 特になし

3、その他 特になし

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表（平成18年度）

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Ⅳ. 研究成果の刊行物・別冊

Toxicology and Industrial Health 2006; **22:** 131–136
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Effects of dioxins on the quantitative levels of immune components in infants

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Dioxins (polychlorinated dibenzo-*p*-dioxin (PCDD)+polychlorinated dibenzofuran (PCDF)) and polychlorinated biphenyls (PCBs) are potentially hazardous compounds and have structural similarity with thyroid hormones. Animal studies have demonstrated that PCDDs, PCDFs and PCBs can alter immune functions. However, in humans it is not yet elucidated whether dioxins contained in breast milk have any effects on the immune functions in infants. To investigate the effects of dioxins on the immune system, we compared the quantitative levels of immune components between a breast-fed group and bottle-fed group, in which dioxin concentration is almost zero. Ratios of immune cells, such as CD4+ and CD8+ T-lymphocytes, as well as B-lymphocytes (CD19+ and/or CD20+) and NK cells (CD16+, CD56+) in peripheral blood lymphocytes, serum immunoglobulin level, and level of specific IgE antibody to allergens in the venous blood at 12 months of age were assessed in a subgroup of 281 infants. The relationship of post-natal dioxin exposure via breast feeding with the ratio of immunological markers and the level of humoral antibodies up to 12 month of age was not demonstrated. In conclusion, it would appear that the content of dioxins in breast milk in the Japanese general population is not enough to induce any change in theses-examined immunological parameters during the first year of life, although long-term effects remain to be evaluated. *Toxicology and Industrial Health* 2006; **22:** 131–136.

Key words: *breast feeding; bottle feeding; dioxins; IgE; lymphocytes subsets*

Introduction

Polychlorinated-dibenzo-*p*-dioxin (PCDD), polychlorinated-dibenzofuran (PCDF), and coplanar-polychlorinated biphenyl (Co-PCB) compounds, hereafter referred to as dioxins, are tricycle aromatic compounds. They are mainly formed as byproducts of the synthesis of organochlorine chemicals and from the combustion of municipal and hazardous waste. In the late 1970s, the production and use of these compounds were banned because their adverse health effects had become evident.

Immune suppression is a common and extensively characterized sequela associated with acute 2,3,7,8,-tetrachloro-dibenzo-*p*-dioxin (TCDD)

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exposure in laboratory animals. Comprehensive cell-type fractionation-reconstitution studies have previously demonstrated the profound inhibition of B-cell function by TCDDs. According to direct addition studies utilizing primary cultured murine B cells, there is evidence suggesting that the suppression of antibody production by TCDDs may be closely associated with altered B-cell differentiation (Suh *et al.*, 1983). This finding is further supported by the observation that TCDDs only modestly inhibit B-cell proliferation.

There is a paucity of *in vivo* studies on the effects of dioxins on the immune system of humans (Tryphona *et al.*, 1998). Initial studies, showing that PCB and dioxins may be toxic to human immune function, were carried out on individuals accidentally exposed to these compounds (Patterson *et al.*, 1988). Weisglas-Kuperus *et al.* (1995) demonstrated that prenatal PCB/dioxin exposure was associated with changes in T cell subpopulations in the blood in Dutch infants.

It is not yet clearly determined, however, whether pre- and post-natal exposures to high background levels of PCDD, PCDF and PCB can alter the immune system in human infants, and whether the health of infants is adversely affected by these pollutants. In this study, we investigated the effects of dioxins contained in breast milk on the quantitative levels of various immune components in Japanese infants from birth to 12 months of age.

Subjects and methods

We collected breast milk from 415 mothers in 20 prefectures and cities in Japan at 30 days post-partum and quantified 14 isomers for PCDDs, 15 for PCDFs and 12 for coplanar PCBs (Co-PCBs). To express the toxic potency of the mixture of dioxins in breast milk samples, the toxic equivalency (TEQ) calculation, based on the new TEF re-evaluated by WHO in 1997, was used. The ages of the mothers were limited to 25–34 years, and all mothers were primiparous and resided in the same area for more than five years.

At one year of age, blood samples were obtained from 281 breast-fed infants (breast-fed group) for the evaluation of immune functions. The breast-fed group was infants who had mainly received breast feeding until one year of age. Blood samples were also obtained from 20 infants who were bottle-fed at one year of age, as a control group (bottle-fed group).

The fat content in human milk was determined by weighing, as described by Patterson *et al.* (1988). In brief, breast milk (50 mL) was mixed with saturated potassium citrate (10 mL), ethanol (100 mL), diethylether (50 mL) and hexane (120 mL) in a 500-mL separatory funnel and shaken vigorously for 10 min. The hexane phase was then removed and washed first with 2 mol/L NaOH followed by sulfuric acid. The hexane phase was then dried and weighed. The fat content of breast milk at five days post-partum was $3.0 \pm 1.4\%$, and at 30 days post-partum was $3.8 \pm 1.2\%$, and did not change thereafter (Matsuura, 2001a,b).

PCDDs, PCDFs and Co-PCBs in human milk were identified by GC/MS conducted at the Japan Food Research Laboratory (Matsuura, 2001b). Surface markers of peripheral blood monocytes (PBMCs) were quantified by flow cytometry (SRL, Tokyo, Japan) (Ip *et al.*, 1982).

Serum IgE concentrations were determined by chemiluminescent enzyme immunoassay (Matsui *et al.*, 2000). Specific IgE antibodies for house dust, milk and egg white were quantified by fluoroenzyme assay (CAP RAST FEIA, Pharmacia & Upjohn, Sweden). All parents who participated in this study gave their written informed consent.

The ratio of CD3, CD4, CD8, CD4/8, CD19, CD20 and CD86 between the breast-fed and bottle-fed groups was analysed by Student's t-test. The serum immunoglobulin levels of IgG, IgA, IgM and IgE between the breast-fed and bottle-fed groups were analysed by Student's t-test. Distribution of CAP-RAST scores between 0 and 1–6 was analysed by Fisher's exact test. Probability (P) values <0.05 were considered to be statistically significant.

Results

Effect of dioxins in breast milk on T cell ratio in PBMCs

The ratios of CD3+, CD4+, CD8+ or CD4+/CD8+ cells in PBMCs were compared between the breast-fed and bottle-fed groups (Table 1). No significant differences were demonstrated. The correlation between the concentration of dioxins in human milk and T cell ratio was also investigated

Table 1. The ratio of lymphocyte subsets in the breast-fed and bottle-fed groups.

	Breast-fed group (N=281)	Bottle-fed group (N=20)	P
CD3 (%)	73.1±7.2	69.9±6.1	0.053
CD4 (%)	50.1±8.4	48.6±6.7	0.436
CD8 (%)	24.1±5.8	24.1±7.4	0.861
CD4/CD8	2.2±0.9	2.3±1.2	0.718
CD19 (%)	14.7±5.6	15.9±6.7	0.362
CD20 (%)	14.3±5.6	15.9±5.8	0.242
CD86 (%)	0.8±0.5	1.2±0.9	0.078
IgG (mg/dL)	645.1±182.1	694.0±186.2	0.248
IgA (mg/dL)	34.2±22.6	36.6±19.1	0.644
IgM (mg/dL)	105.9±33.6	106.9±41.4	0.899
IgE (U/mL)	54.4±89.9	58.2±105.9	0.857

and no significant correlation was found between them (Figure 1A).

Effect of dioxins in breast milk on B cell ratio in PBMCs

The ratios of CD19+, CD20+ or CD86+ cells, which are the surface markers of activated B cells, were compared between the breast-fed and bottle-fed groups (Table 1). There was no significant correlation. The correlation between the concentration of dioxin in human milk and B cell ratio was also investigated and no significant correlation was found between them (Figure 1A).

Effect of dioxins in breast milk on NK cell ratio in PBMCs

The correlation of the ratio of NK cells (CD16+/CD56+) with the concentration of dioxins in human milk was examined and no significant correlation was found (Figure 1A).

Effect of dioxins in breast milk on the serum immunoglobulin levels

The serum immunoglobulin levels of IgG, IgA, IgM and IgE were compared between the breast-fed and bottle-fed groups (Table 1). No significant differences were demonstrated between them. The correlation between the concentration of dioxins in human milk and the serum immunoglobulin levels was also investigated and no significant correlation was found between them (Figure 1B).

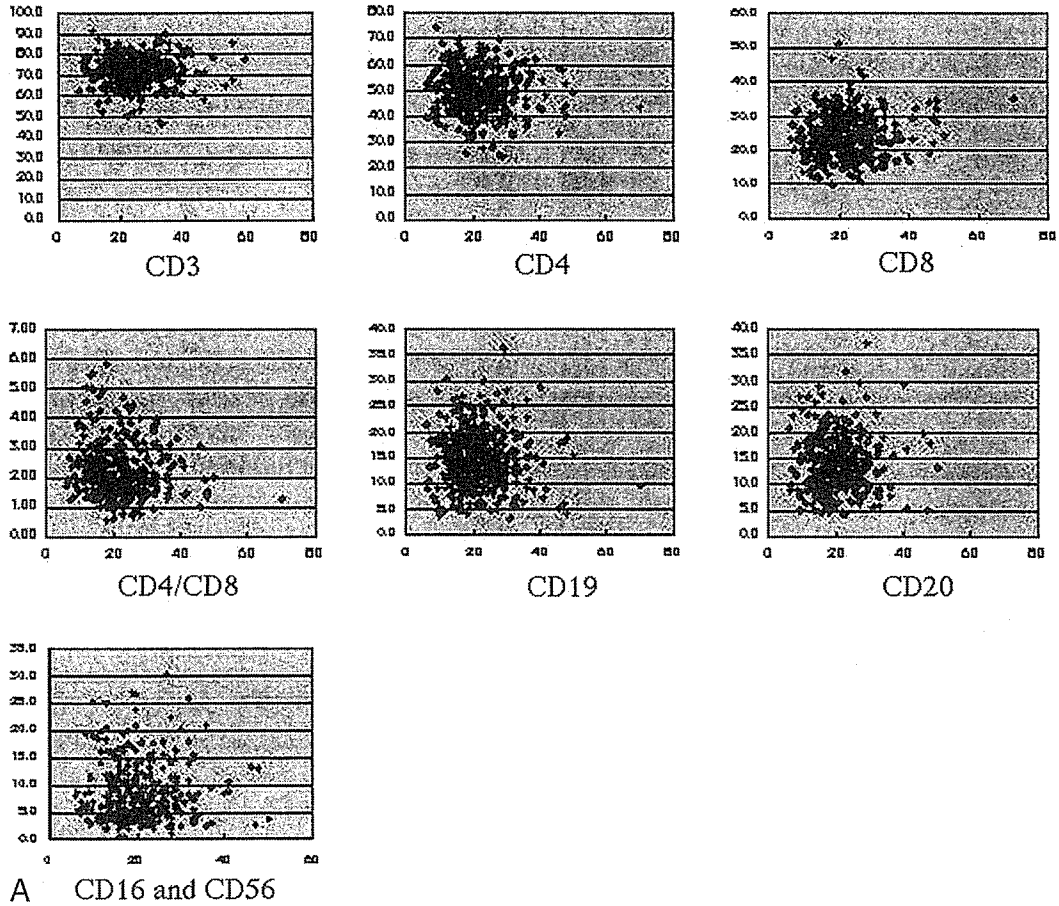
The specific IgE antibody to house dust, milk and egg white was quantified (Table 2). It was not demonstrated that there was no significant correlation between the breast-fed and bottle-fed groups.

Discussion

In this study, we investigated the relationship of the concentration of dioxins contained in breast milk with the ratios of immune cells and immunoglobulin levels. It has been reported that *in vitro* dioxins suppress B cell differentiation. Furthermore, there are some *in vivo* studies suggesting an effect of dioxins on immune functions (Forawi *et al.*, 2004). Smoger *et al.* (1993) reported that for children born to mothers living in a TCDDs-contaminated environment in Time Beach (MO) during and after pregnancy, a decrease in CD4+ T cells and an increase in CD8+ T cells was detected in children from nine to 14 years of age. In one preliminary study conducted in Northern Quebec, the CD4+/CD8+ T cell ratio of Inuit infants, whose mothers have increased levels of PCB and dioxins in their breast milk, decreased at six and 12 months of age (Dewailly *et al.*, 1993).

Svensson *et al.* (1994) reported that the consumption of fatty fish species, such as salmon and herring, from the Baltic Sea is an important source of human exposure to persistent organochlorine compounds, eg, PCDDs, PCDFs and co-PCBs. The high fatty-fish consumers had lower ratios and numbers of NK cells, identified by the CD56 marker, in peripheral blood than the non-consumers. The weekly intake of fatty fish correlated negatively with the ratio of NK cells. This indicates that accumulation of persistent organochlorine compounds in high fatty-fish consumers may adversely affect NK cell ratios. Weisglas-Kuperus *et al.* (1995) demonstrated that a high post-natal PCB/dioxin exposure is associated with an increase in the number of TcR$\gamma\delta$+ T cells at birth and with an increase in the number of CD8+, TcR$\alpha\beta$+ or TcR$\gamma\delta$+ T cells at 18 months of age. Nagayama *et al.* (1998) reported that the ratios of CD4+ to CD8+ T cells had a significant increasing tendency with the estimated total TEQ intakes.

In our study, it was not demonstrated that the ratios of T cell subpopulation and CD16+ CD56+ cells (NK cells) in PBMCs correlated with the concentration of dioxins in human milk, although the number of CD4+ and CD8+ T cells were not



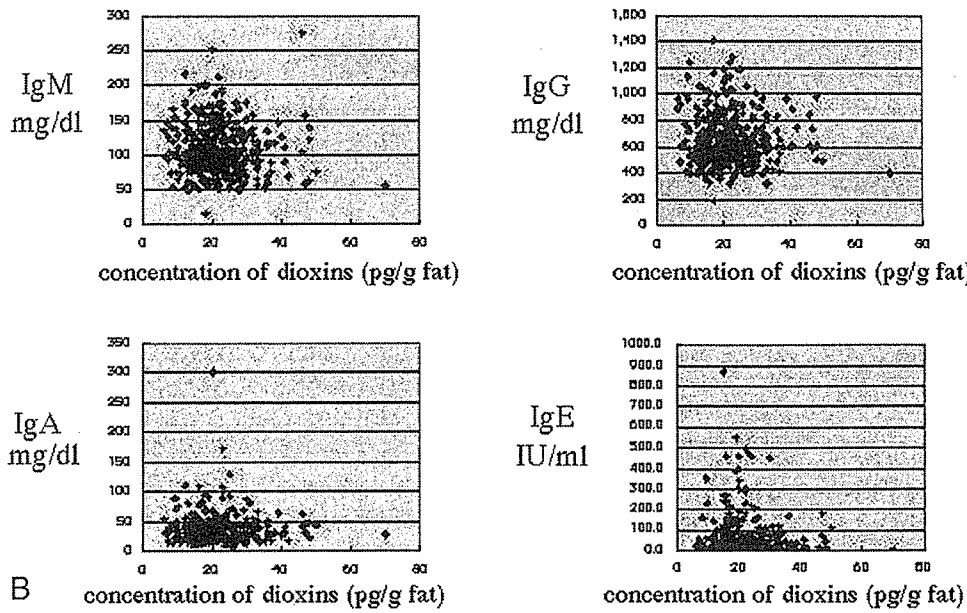


Figure 1. (A) Relationships between ratios of CD3+, CD4+, CD8+, CD4+/CD8+, CD19+, CD20+ and CD16+ CD56+ cells in PBMCs and the concentration of dioxins in human milk at 30 post-partum days. X-axis means the concentration of dioxins (pg/g fat). Y-axis means the percent of surface marker positive cells except CD4/CD8. In CD4/CD8, Y-axis means the ratio of CD4 per CD8. (B) Relationships between ratios of serum IgM, IgG, IgA and IgE and the concentration of dioxins in human milk at 30 post-partum days. X-axis means the estimated intake of dioxins (pg/g fat). Y-axis means the serum concentration of IgM (mg/dL), IgG (mg/dL), IgA (mg/dL) and IgE (IU/mL).

Table 2. Specific IgE antibody of breast-fed and bottle-fed groups.

	Breast-fed group							Bottle-fed group							*P*
	0[a]	1	2	3	4	5	6	0	1	2	3	4	5	6	
House dust	251[b]	8	10	5	1	1	0	19	0	1	0	0	0	0	0.568
Milk	238	12	20	5	1	0	0	17	0	2	1	0	0	0	0.558
Egg white	180	24	44	22	2	3	0	15	3	2	0	0	0	0	0.264

[a]Number (0–6) indicates CAP-RAST scores to each allergens.
[b]Number indicates the number of persons who have the CAP-RAST score.
Distribution of CAP-RAST scores between 0 and 1–6 was analysed by Fisher's exact test.

analysed. We could not find that the levels of serum immunoglobulins and specific IgE to allergen were significantly different between the breast-fed and bottle-fed groups. The difference between our data and those of Weisglas-Kuperus may be due to the following: the time at which immunological analysis was performed, and the amount of dioxins to which the subjects were exposed, that is, a higher concentration of dioxins in the early days after birth; the content of dioxins in breast milk was almost 2-fold higher in the Netherlands (30.75 pg TFQ/g fat) than in our study (14.8 ± 6.1 pg TFQ/g fat) (Matsuura *et al.*, 2001b).

The sample size in this study was 281 in the breast-fed group and 20 in the bottle-fed group, which is a maximum size considering the budget for this study and the co-operation of the mothers. When we consider the value between the breast-fed and bottle-fed groups (shown in Table 1 as true difference), the power of CD3 and CD86 was higher than 50%, however, CD8, CD4/8, IgA, IgM and IgE was lower than 10%.

On the basis of the results of this study, we conclude that, although the infants were exposed to some amounts of dioxins in the breast milk in Japan, we could not find that the quantitative levels of immune components at one year of age was seriously impaired. However, long-term effects remain to be evaluated.

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PEDIATRIC ALLERGY AND IMMUNOLOGY

Suppression of IFN-gamma production in atopic group at the acute phase of RSV infection

Kaneko H, Matsui E, Asano T, Kato Z, Teramoto T, Aoki M, Kawamoto N, Lian LA, Kasahara K and Kondo N. Suppression of IFN-gamma production in atopic group at the acute phase of RSV infection. Pediatr Allergy Immunol 2006: 17: 370–375.
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Several studies have suggested that respiratory syncytial virus (RSV) bronchiolitis induced the change of cytokine production profile in childhood. We sought to determine whether the RSV-induced cytokine production was affected by the patient's atopic background. We quantified interferon-gamma (IFN-gamma) and interleukin (IL)-4 in the supernatant of peripheral blood mononuclear cells (PBMCs) cultured for 24 h and in the presence of phytohemaglutinin (PHA), IL-12, or IL-18, from 14 infants who were divided into two groups, those who are non-atopic and an atopic group. In RSV-infected infants with atopic diseases, IFN-gamma production from IL-12- or especially IL-18-stimulated PBMCs was subtotally suppressed in the acute phase, whereas in RSV-infected infants without atopic diseases IFN-gamma production was not suppressed on acute phase. The IFN-gamma suppression observed in the atopic group is not caused by the immaturity of an infant's immune system since reduced IFN-gamma production to RSV is not observed in the infants of non-atopic group. IFN-gamma suppression in regard to RSV infection might be caused by some genetic factor involved in the development of atopic disease such as IL-18 signal cascade.

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Key words: respiratory syncytial virus; interferon-gamma; atopy; interleukin-4; interleukin-12; interleukin-18

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Respiratory syncytial virus (RSV) remains the most prevalent infectious cause of lower respiratory tract illness in both infants and children (1–3). Most infections with RSV are symptomatic; however, the range of severity in infants and young children varies greatly. Several studies have suggested that infants in whom RSV bronchiolitis develops will have recurrent wheezing and asthma later in childhood (4). The reasons why this occurs are still unknown; whether RSV is the actual causal factor for asthma or simply targets children who are predisposed to bronchial obstructive disease is still speculation (5). Further complicating the picture is the question of whether there is a link between RSV infection in infancy and the development of wheeze or asthma later in childhood. Results from clinical studies have been contradictory, with some results supporting a link, and others disproving a link (6, 7).

It has been shown in animal studies that viral infections can increase the risk of allergic sensitization. A viral influence causing a switch from Th2 to Th1 lymphocytes, and thus a lower production of IgE antibodies, has been suggested, but a more complex mechanism may operate in RSV infection, judging from animal studies and the results of the present and other clinical studies (8). It has been hypothesized that an early RSV infection may perpetuate Th2-dominant immune responses (9).

Aberle et al. tried to compare both the levels of IFN-gamma mRNA expression in peripheral blood mononuclear cells (PBMCs) and the distribution of lymphocyte subpopulations in

infants with severe RSV bronchiolitis and those with a milder clinical course of illness (10). Their data indicated that reduced IFN-gamma expression might be an important factor in the pathogenesis of severe RSV disease in infancy.

In this study we tried to determine whether lymphocytes in children with atopy or some genetic background show a different immune response to RSV compared with non-atopic children. Our results showed that the reduced IFN-gamma expression was observed specifically in the atopic group.

Subjects and methods

Subjects

Fourteen children between 1 month and 4 yr of age, were admitted for respiratory tract infection to our hospital during the epidemic period of 2002–2003. All of our cases were admitted in our hospital according to the dyspnea and/or the exhaustion because of the continuance of the high fever. They had infections with a fever and coughing with and without bronchial obstruction. Their nasopharyngeal secretions were positive for the RSV antigen by enzyme-linked immunosorbent assay (ELISA) kit (Directigen RS, Becton, Japan). They were divided into two groups: (i) with no allergic disease or history thereof, and (ii) with allergic disease or a family history and/or a past history of allergic disease. The atopic group in this study consisted of subjects with bronchial asthma and atopic dermatitis or a family history within one generation of allergic diseases. There were no significant differences in age, sex and clinical severity between the non-atopic and atopic group. The diagnosis of bronchial asthma was made according to the criteria of the American Thoracic Society, and that of atopic dermatitis was made according to the criteria of Hanifin. Informed consent was obtained from their parents. All of the subjects were randomly selected among patients in our hospital. This study was approved by Gifu University ethical committee.

Cell preparation and culture

Peripheral blood mononuclear cells were isolated from the heparinized blood of patients by gradient centrifugation in Ficoll-Paque (Pharmacia AB, Uppsala, Sweden). PBMCs were suspended to give a density of 10^6/ml in the culture medium which consisted of RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, L-glutamine (2 mmol/l), penicillin (100 U/ml) and streptomycin (100 μg/ml). PBMCs (10^6/ml) were cultured in the presence of 10 μg/ml phytohemagglutinin (PHA) (Gibco BRL, Grand Island, NY, USA), interleukin (IL)-12 (5 IU/ml), or IL-18 (400 ng/ml) in a volume of 1 ml in a round-bottom tube (Falcon 2059, Becton Dickinson Labware, Lincoln Park, NJ, USA) at 37°C in a humidified atmosphere containing 5% CO_2. The recombinant IL-18 had been prepared in our laboratory through use of an *Escherichia coli* expression system [according to a method developed by Kato et al. (11)].

Assays for IFN-gamma and IL-4

The test-tube culture supernatants were spun to remove the cells after culturing and were stored, frozen at −80°C until assayed. IFN-gamma concentrations were measured with a human IFN-gamma ELISA kit (Ohtsuka, Tokyo, Japan), and the detection limit was 15.6–1000 pg/ml. IL-4 concentrations of the culture supernatants were measured with a human IL-4 US ELISA kit (Bio Source, Camarillo, CA, USA). When cytokine values were not detectable, for statistical analysis the minimum detectable level was used.

IgE assay

Plasma samples obtained from heparinized blood were kept at −30°C. Plasma IgE concentrations were determined by chemiluminescent enzyme immunoassay. The values were regarded as the serum IgE concentrations. Specific IgE antibodies for house dust and *Dermatofagoides farinae* were determined by fluoloenzyme assay (CAP RAST FEIA, Pharmacia & Upjohn, Sweden).

Statistical analysis

The significance of the difference between the control and atopic group was analyzed by the Wilcoxon Mann Whitney test. A statistical significance was assumed for p-values < 0.05.

Results

The patients with RSV infection were divided into non-atopic and atopic group (Table 1). Clinical severity was determined by the period of fever, wheezing and the hospitalization period. No patients had a prior episode to RSV infection. There were no significant differences in age, sex and clinical severity although the hospitalization period of atopic group seemed to be longer than non-atopic group ($p - 0.079$). In all

Table 1. Clinical features of the patients divided into non-atopic and atopic group

	Sex	Age	FH or PH	Fever (day)	Wheeze	IgE (IU/ml)	RAST	Hospitalization period* (day)
Non-atopic 1	M	1 yr	–	1	–	111.8	HD 0, DF 0	9
Non-atopic 2	M	1 yr	–	4	–	15.7	HD 0, DF 0	13
Non-atopic 3	F	3 month	–	6	+	118.7	HD 0, DF 0	11
Non-atopic 4	F	1 month	–	1	+	0.5	HD 0, DF 0	10
Non-atopic 5	M	3 month	–	2	+	2.1	HD 0, DF 0	6
Non-atopic 6	M	1 yr	–	0	+	19.8	HD 0, DF 0	7
Atopic 1	F	10 month	BA	0	+	57.5	HD 0, DF 0	17
Atopic 2	F	4 yr	BA	1	+	42	HD 0, DF 0	13
Atopic 3	M	1 yr	BA	1	+	200	HD 5, DF 4	6
Atopic 4	M	4 yr	BA	5	+	819.3	HD 3, DF 3	19
Atopic 5	F	4 yr	BA/AD	1	+	6488.4	HD 6, DF 6	9
Atopic 6	M	2 yr	BA	2	+	4.2	HD 0, DF 0	17
Atopic 7	F	1 month	FH	1	+	2.2	HD 0, DF 0	15
Atopic 8	F	1 month	FH	1	+	0.5	HD 0, DF 0	13

Sex: m, male; f, female; FH, family history within one generation of allergic diseases; PH, past history; BA, bronchial asthma; AD, atopic dermatitis; fever, period of fever over 38°C; RAST, CAP RAST scores; HD, house dust; DF, *Dermatophagoides farinae*.
*p-value = 0.079.

patients (n = 14), the IFN-gamma concentration from PHA-stimulated PBMCs between the acute and convalescent phase is shown in Fig. 1a. The concentrations of IFN-gamma in all RSV-infected patients (n = 14) did not show remarkable change between the acute and convalescent phase (Table 2). RSV infected patients were divided into two groups, a non-atopic group (n = 6) and an atopic group (n = 8). In the non-atopic and atopic group, the IFN-gamma concentration from PHA-stimulated PBMCs is shown in Fig. 1b. There was no difference in IFN-gamma production by PHA-stimulated PBMCs in either group. In both groups, the IFN-gamma concentration from IL-12-stimulated PBMCs is shown in Fig. 1c. In the atopic group, the IFN-gamma concentration from IL-12-stimulated PBMCs was significantly lower in the acute phase than that in the convalescent phase ($p = 0.024$). Similarly, in the atopic group the IFN-gamma concentration from IL-18-stimulated PBMCs was significantly lower in the acute phase than that in the convalescent phase ($p = 0.0018$) (Fig. 1d). The change of IFN-gamma production by IL-18-stimulated PBMCs was more significant ($p = 0.0018$) than that by IL-12-stimulated PBMCs ($p = 0.024$). In addition, in the acute phase the IFN-gamma concentrations by IL-12- or IL-18-stimulated PBMCs in the atopic group were significantly lower than those in the non-atopic group ($p = 0.013$ or $p = 0.009$, respectively), while in the convalescent phase the IFN-gamma concentrations by IL-18-stimulated PBMCs in the atopic group were significantly higher than those in the non-atopic group ($p = 0.028$).

The concentrations of IL-4 in all RSV-infected patients (n = 14) between the acute and convalescent phase did not show any remarkable change (Fig. 2). Furthermore, no difference of IL-4 production between the non-atopic and atopic group was detected.

Discussion

In the present study, we have shown that in the atopic group in the acute phase the IFN-gamma concentrations by IL-12- or IL-18-stimulated PBMCs were significantly lower than those in the non-atopic group. Furthermore, the IFN-gamma concentrations by IL-18-stimulated PBMCs in the atopic group were significantly higher than those in the non-atopic group in the convalescent phase. However, no difference of IL-4 production between the non-atopic and atopic group was detected.

We tried to determine whether lymphocytes in children with atopy or a family history of allergic diseases show a different immune response to RSV compared with non-atopic children. Our results suggest that the atopic disease or some genetic background of atopy might be one of the factors which prolongs RSV infection because of a reduced Th1 reaction to RSV, although we could not find any significant differences in the clinical course between the non-atopic group and atopic group.

Our results showed that reduced IFN-gamma expression was observed specifically in the atopic group in the acute phase. As low IFN-gamma expression in RSV is observed specifically in the

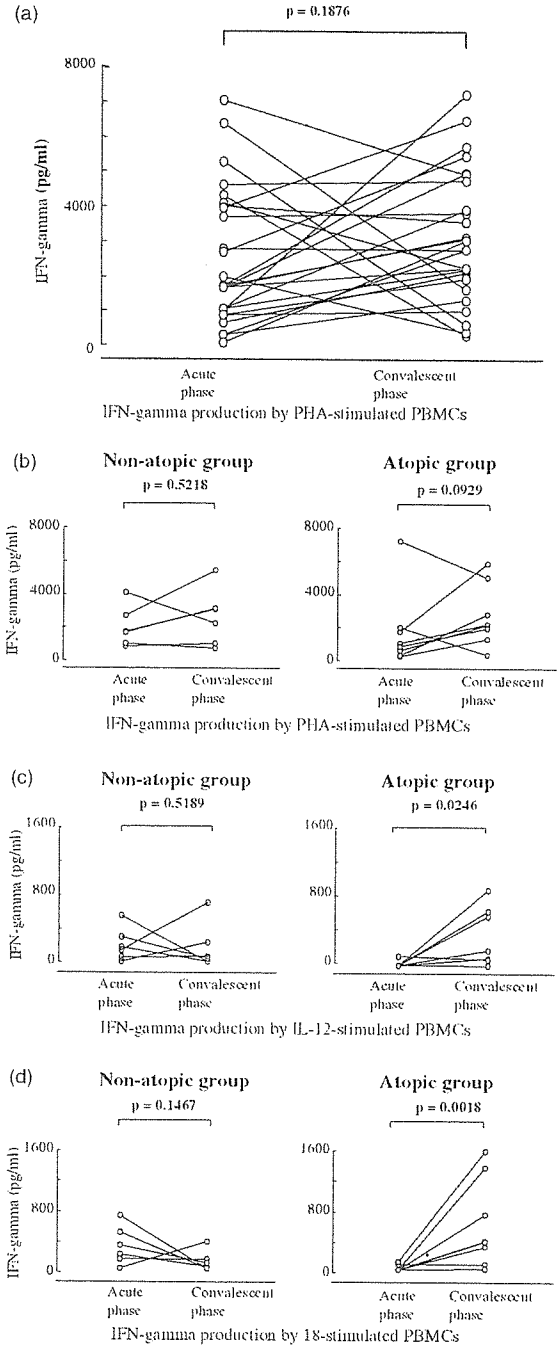

Fig. 1. (a) In all patients (n = 14), interferon (IFN)-gamma concentration from phytohemaglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMCs) between the acute and convalescent phase. (b) In the non-atopic group (n = 6) and atopic group (n = 8), IFN-gamma concentration from PHA-stimulated PBMCs between the acute and convalescent phase. (c) In the non-atopic and atopic group, IFN-gamma concentration from IL-12-stimulated PBMCs between the acute and convalescent phase. (d) In the non-atopic and atopic group, IFN-gamma concentration from IL-18-stimulated PBMCs between the acute and convalescent phase.

atopic group, it may not be due to an inhibitory effect of RSV, which is capable of suppressing both non-specific and RSV-specific lymphocyte proliferation (12). In addition, there was no significant difference about ages between non-atopic and atopic group (Table 1). Reduced IFN-gamma production was observed only in atopic group. Therefore we assumed that a weak IFN-gamma response to RSV may not result from the immaturity of an infant's immune system.

One factor controlling the level of cytokine expression is genotype. Gentile et al. reported that the IFN-gamma genotype is related to the severity of a lower respiratory illness, the duration of intensive care unit stay and the frequency of otitis media (13). In Japanese children the association of IFN-gamma polymorphism with atopic asthma has been reported. It would be intriguing to investigate IFN-gamma polymorphism in our atopic group.

Several lines of evidence have shown the features of cytokine production in RSV infection. Pala et al. reported that enhanced IL-4 responses in children with a history of RSV bronchiolitis in infancy (14). Association of cytokine responses with disease severity in infants with RSV infection was reported (15, 16). van Benten et al., showed that RSV-induced bronchiolitis but not upper respiratory tract infection was accompanied by an increased nasal IL-18 response (17). Joshi et al. showed that RSV is associated with lower IFN-gamma production in young babies compared with upper respiratory tract infections (18). These results suggest that RSV infection changes the cytokine production and the RSV-induced cytokine production might be partly affected by the time and tissues of taking the samples.

The change of IFN-gamma production by IL-18-stimulated PBMCs was more significant ($p = 0.0018$) than that by IL-12-stimulated PBMCs ($p = 0.024$). This result suggests that in RSV infection the IL-18 receptor signal cascade might be specifically inhibited. Our previous reports showed that some atopic diseases are caused by impairment of IL-18Rα chain 950 del CAG, which downregulates IgE production (19). We have not yet determined what mechanism induces the predominant expression of the IL-18Rα chain cDNA in some atopic patients. Viral infection, such as RSV, and environmental factors might be candidate modulators of IL-18Rα chain transcript expression (20).

In conclusion, the suppression of IFN-gamma in the acute phase of RSV infection was observed only in the atopic group. These results suggest

Table 2. Interferon (IFN)-gamma and interleukin (IL)-4 production induced by phytohemaglutinin (PHA), IL-12 or IL-18 stimulation

	PHA acute	PHA convalescent	IL-12 acute	Il-12 convalescent	IL-18 acute	IL-18 convalescent	IL-4 acute	Il-4 convalescent
Non-atopic 1	2669.9	5445.4	177.1	15.6	198.1	37.1	70.73	48.07
Non-atopic 2	860.7	1027.1	15.6	244.6	15.6	362.3	39.1	46.3
Non-atopic 3	1001.2	3063.4	144.8	707.2	132	135.8	9.93	41.61
Non-atopic 4	1726.7	3115	547.9	15.6	308	80.4	36.68	10.63
Non-atopic 5	1685.3	2260	301.6	51.3	701.9	15.6	18.68	10.12
Non-atopic 6	4097	722.3	62.8	74.6	476.4	15.6	34.48	41.91
Mean ± SD	2006 ± 1209	2605 ± 1714	208 ± 193	184 ± 269	305 ± 249	107 ± 132	34 ± 20	33 ± 17
Atopic 1	1724.5	5697.3	15.6	523.9	15.6	34.7	34.57	24.38
Atopic 2	653.6	2122.5	15.6	92.7	15.6	372.6	18.83	87.38
Atopic 3	294.1	1315.7	15.6	15.6	15.6	307.2	17.1	60.81
Atopic 4	1990.4	410.2	116.2	81.3	91	92.7	43.27	105.67
Atopic 5	1063.6	2193.8	15.6	15.6	15.6	698.3	78.34	105.7
Atopic 6	317.9	2778.9	15.6	172.1	15.6	364.9	67.97	38.45
Atopic 7	7053.8	4916.7	23	794.7	119	1484.3	32.29	41.54
Atopic 8	869.9	1945.7	15.6	579.2	15.6	1286.2	33.23	10.59
Mean ± SD	1745 ± 2229	2672 ± 1781	29 ± 35	284 ± 302	37 ± 42	580 ± 538	40 ± 21	59 ± 36

Acute, acute phase; convalescent, convalescent phase.
PHA, IL-12, IL-18 = PHA, IL-12 or IL-18-induced IFN-gamma production (pg/ml); IL-4 = PHA-induced IL-4 production (pg/ml).

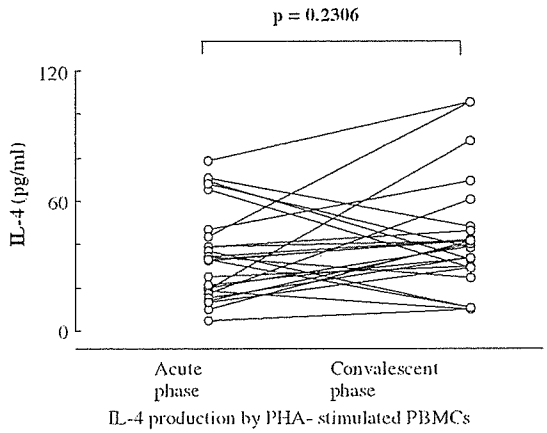

Fig. 2. In all patients (n = 14), IL-4 concentration from phytohemaglutinin-stimulated peripheral blood mononuclear cells between the acute and convalescent phase.

that the significant difference between the non-atopic and atopic group in regard to the immune response to RSV might be caused in part by genetic factors.

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Inosiplex Affects the Spectra of Proton Magnetic Resonance Spectroscopy in Subacute Sclerosing Panencephalitis

ABSTRACT

In vivo magnetic resonance techniques such as magnetic resonance imaging (MRI) and magnetic resonance spectroscopy have been some of the most useful tools for evaluation of neurologic diseases. In subacute sclerosing panencephalitis, magnetic resonance spectroscopy can be an additional tool for evaluation of disease progression or the efficacy of the treatment, such as interferon or inosiplex, compared with MRI. Inosiplex is one of the effective drugs for subacute sclerosing panencephalitis, but our in vivo and in vitro magnetic resonance spectroscopic study indicated that inosiplex affects the spectra, suggesting a possible failure of neurologic evaluation in a patient with subacute sclerosing panencephalitis treated with inosiplex. (*J Child Neurol* 2006;21:177–178; DOI 10.2310/7010.2006.00048).

We previously reported a cerebral magnetic resonance spectroscopic study of a 13-year-old girl with subacute sclerosing panencephalitis.[1] The spectrum clearly demonstrated the early subtle changes in subacute sclerosing panencephalitis, indicating the usefulness of magnetic resonance spectroscopy in evaluation of disease progression or therapeutic efficacy; however, there was an unidentified peak at 2.9 ppm. A further study using a drug solution and the patient's urine indicated that the resonance should be from the methyl of 2-hydroxypropyldimethylammonium. Moreover, the results also cautioned us about the probable contamination of inosiplex resonances on those of the brain itself.

Magnetic resonance spectroscopic examinations were performed using a conventional whole-body system (Signa Horizon 1.5 Tesla, General Electric). Localization of a 20 × 20 × 20 mm region of interest in her right frontal lobe was achieved using short echo time (30 milliseconds) point-resolved spectroscopic sequences. All of the spectra taken from the brain

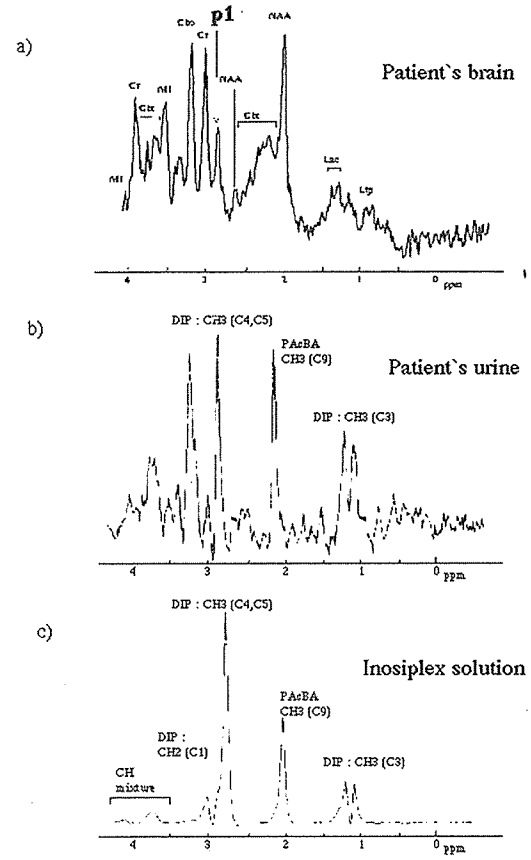

Figure 1. Magnetic resonance spectroscopy. *A,* The spectrum obtained from the region of interest of the left frontal lobe. *B,* The spectrum obtained from the region of interest of the patient's urine. *C,* The spectrum obtained from the region of interest of the inosiplex solution. Cr = creatine and phosphocreatine; Cho = choline; DIP = 2-hydroxypropyldimethylammonium; Glx = glutamate and glutamine; Lac = lactate; Lip = lipids; NAA = *N*-acetylaspartate; MI = *myo*-inositol; p1 = additional peak; PAcBA = *p*-acetamidobenzoate.

showed a decrease in *N*-acetylaspartate resonance, an increase in *myo*-inositol and choline resonances, and the presence of a lactate signal (Figure 1A).[1] An additional peak was observed at the resonances corresponding to γ-aminobutyric acid (GABA; 2.9–3.0 ppm, resonance p1 in Figure 1A).

Resonance of CH2 (C4) in GABA has a triplet splitting pattern at 2.9 ppm, and resonance detection of GABA on in vivo magnetic resonance spectroscopy usually requires specifically edited pulse sequences.[2] The resonance shape and the intensity observed in our patient suggested a contribution of the resonances from the drugs, especially inosiplex (4.8 g/day, oral administration). Inosiplex is a 1:3 complex of inosine and *p*-acetamidobenzoate. Reported resonance assignments of the chemicals are shown in Table 1. The chemical shift value of the observed unknown resonance from the brain is very similar to that of CH3 (C4, C5) in 2-hydroxypropyldimethylammonium, which has a high singlet peak resonance at 2.9 ppm originating from 18 protons per molecule.

Next, spectra were obtained from the patient's urine in a 50 mL tube and inosiplex solution in a 50 mL tube using the same machine and conditions. The spectra from urine clearly showed the presence of a resonance complex similar to that of the inosiplex solution, confirming the intensive excretion of the unchanged inosiplex compound and its metabolites (Figure 1B and C).[3,4] 2-Hydroxypropyldimethylammonium has no detectable binding to the serum proteins and distributes into the brain at a high concentration.[3,4] According to the experimental data in animals, [4] we can assume that 2-hydroxypropyldimethylammonium should be present at around several hundred micromolar concentrations in the brain of a patient