ORIGINAL ARTICLE

Serum parabens and its correlations with immunologic and cellular markers in Southern Taiwan industrialized city systemic lupus erythematosus patients

Kun-Siang Huang[a,b], **Chun-Yu Chen**[b,c,d], **Chiao-Yin Sun**[b,c,d], **Yu-Jih Su**[b,e,f,g,*]

[a]Department of Family Medicine, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan, ROC; [b]College of Medicine, Chang Gung University, Taoyuan, Taiwan, ROC; [c]Department of Nephrology, Chang Gung Memorial Hospital, Keelung Branch, Keelung, Taiwan, ROC; [d]Community Medicine Research Center, Chang Gung Memorial Hospital, Keelung Branch, Keelung, Taiwan, ROC; [e]Department of Internal Medicine, Division of Rheumatology, Allergy and Immunology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan, ROC; [f]Center for Mitochondrial Research and Medicine, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan, ROC; [g]Institute of biopharmaceutical science, National Sun Yat-Sen University, Kaohsiung, Taiwan, ROC

Abstract

Background: Although the immune systems of patients with systemic lupus erythematosus (SLE) are affected by both personal characteristics and environmental factors, the effects of parabens on patients with SLE have not been well studied. We investigated the indirect effects of four parabens—methylparaben (MP), ethylparaben (EP), propylparaben (n-PrP), and butylparaben (n-BuP)—on several immunological markers.

Methods: We assessed the serum levels of MP, EP, n-PrP, and n-BuP in 25 SLE patients and correlated the concentration of each paraben with available clinical and laboratory markers, including intracellular markers of antiviral immunity and apoptosis.

Results: The expression of aryl hydrocarbon receptor (AhR) was significantly negatively correlated with n-PrP levels ($p = 0.03$, $r = -0.434$). In monocytes, APO2.7 was significantly positively correlated with n-BuP levels ($p = 0.019$, $r = 0.467$). Glutathione levels were significantly negatively correlated with n-BuP levels ($p = 0.019$, $r = -0.518$). Anti- β2 glycoprotein I IgM was significantly positively correlated with both MP ($p = 0.011$, $r = 0.585$) and EP levels ($p = 0.032$, $r = 0.506$). Anti–cardiolipin IgA was significantly positively correlated with both MP ($p = 0.038$, $r = 0.493$) and n-PrP levels ($p = 0.031$, $r = 0.508$). On CD8 T cells, the early apoptotic marker annexin V was significantly negatively correlated with both MP ($p < 0.05$, $r = -0.541$) and n-BuP levels ($p = 0.02$, $r = -0.616$), and L-selectin was significantly positively correlated with both MP ($p < 0.05$, $r = 0.47$) and n-PrP levels ($p = 0.02$, $r = 0.556$).

Conclusion: Our findings suggest that higher parabens levels were associated with lower AhR expression in leukocytes, increased monocyte apoptosis, lower serum glutathione levels, reduced annexin V expression on CD8 T cells, and higher L-selectin levels on leukocytes.

Keywords: Immune; Paraben; Systemic lupus erythematosus

1. INTRODUCTION

Parabens are industrial byproducts, sometimes referred to as environmental hormones, with the potential ability to alter hormone metabolisms in humans. Some studies have reported that paraben exposure affects mitochondrial function in sperm. The toxic effects of exposure to high parabens concentrations remain unknown, especially among those with autoimmune disease, despite the presence of parabens in cosmetics, food preservation agents, soaps, and other materials that humans come into contact with during daily living. Purified parabens are present as white crystalline powders or colorless crystals that are soluble in alcohol and ether. The parabens family contains several members, some of which can be found in natural materials, such as the detection of methylparaben (MP) in blueberries; however, most commercial parabens are chemically synthesized, including MP, ethylparaben (EP), propylparaben (n-PrP), isopropylparaben, butylparaben (n-BuP), heptylparaben, benzylparaben, and pentylparaben. For decades, parabens have been widely used as preservatives in cosmetics, foods, and medicines. Parabens are favored by manufacturers due to their good antiseptic properties and low cost. We obtained serum samples from patients with systemic lupus erythematosus (SLE) and measured the serum levels of MP, EP, n-PrP, and n-BuP to determine whether paraben exposure was associated with changes in immune markers.

*Address correspondence. Dr. Yu-Jih Su, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital, Chang Gung University College of Medicine, 123, Ta Pei Road, Kaohsiung 833, Taiwan, ROC. E-mail address: bensu8@gmail.com (Y.-J. Su).

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2022) 85: 993-999.

Received January 29, 2022; accepted July 27, 2022.

doi: 10.1097/JCMA.0000000000000802.

Copyright © 2022, the Chinese Medical Association. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

CA9_V85N10_Text.indb 993 03-Oct-22 13:56:05

Parabens contain a phenolic hydroxyl group structure, which confers more effective antibacterial properties than those associated with other organic acid preservatives, such as benzoic acid and sorbic acid, resulting in high toxicity.[1] Parabens primarily act by disrupting the cell membranes and reducing the activities of intracellular respiratory and electron transfer enzymes in microorganisms. Without protein substrates, microorganisms are incapable of rapid growth, preventing their invasion of foods or other commercial products. Parabens are commonly found in shampoo, lotion, toothpaste, shaving cream, lubricant, topical medicine, spray solvent, cosmetics, sunscreen, and canned foods, such as soy sauce, jam, mayonnaise, and fruit juice.

Parabens can be absorbed through both dermal application and oral intake and are metabolized via esterase hydrolysis and glucuronidation in the liver. Parabens metabolites are excreted into urine both as free forms and as glucuronide and sulfate conjugates.[2,3] Parabens have been detected in breast tissue, serum, urine, semen, adipose tissue, placental tissue, amniotic fluid, and breast milk.[4] Higher paraben levels have been associated with increased risk of breast cancer, DNA damage in sperm, and the upregulation of oxidative stress biomarkers.[3] A correlation has also been reported between a decline in kidney function and the parabens concentration in urine.[5]

Aryl hydrocarbon receptor (AhR) acts as an environmental hormone receptor, and previous studies have indicated that AhR activity can have differential effects on different immune cells.[6] For example, AhR activation can prevent the activation of antigen-presenting cells or bias T cells toward inactivation.[7] Studies have also indicated that AhR activation by environmental hormones might affect immune cells.[8] AhR activity has been shown to influence the activation of T-helper cells (Th22) in the intestine.[9] However, the effects of parabens exposure in patients with SLE have not been well studied.[8] In this preliminary study, we enrolled 25 SLE patients to examine the correlations between four specific parabens—MP, EP, n-PrP, and n-BuP—and clinical and laboratory immunological markers.

SLE is a systemic autoimmune disease, and patients with SLE are susceptible to environmental stimuli. The activation of the immune system can potentially worsen the outcomes of minimal cellular damage, resulting in disastrous outcomes in patients with SLE. In the current study, we investigated correlations between the levels of parabens and clinical serology, autoantibody, and laboratory markers, including AhR expression; mitochondrial apoptosis and activation markers; reactive oxidative stress markers, including lipid peroxidation and glutathione levels; general cellular apoptosis markers; and cell surface adhesion molecules. The results of this preliminary study may provide clues regarding the extent to which the immune system is affected by exposure to environmental hormones and identify the specific pathways activated by exposure to parabens.

2. METHODS

2.1. Study patients

Twenty-five SLE patients were prospectively evaluated and followed for at least 6 months at the Rheumatology Outpatient Clinic. SLE was diagnosed according to the 1997 American College of Rheumatology classification criteria for SLE,[10] and SLE activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2000).[11] The Institutional Review Committee on Human Research approved the study protocol (103-7505B, 104-5433B, 104-9764B, and 201901809B0), and all participants provided informed consent. Patients were excluded if they had autoimmune diseases other than SLE or if they had fever or any infectious disorder that could affect results.

2.2. Clinical assessments and specific biomarkers

All study participants underwent a complete medical examination upon enrollment. Clinical data were obtained from chart review, including serum C3 and C4 levels, erythrocyte sedimentation rate (ESR), complete blood cell counts, hemoglobulin, hematocrit, C-reactive protein, anti–double-stranded DNA (dsDNA), and SLE disease activity. Treatment for patients with SLE was limited to steroids (typically less than 10 mg prednisolone or equivalent) and corticosteroid-sparing drugs, such as azathioprine or hydroxychloroquine. Available information regarding autoantibody levels was also obtained via chart review, including anti–β2 glycoprotein I IgG, anti–β2 glycoprotein I IgM, anti–cardiolipin IgA, anti–cardiolipin IgG, anti–cardiolipin IgM, anti-dsDNA, anti-Mi2, perinuclear anti–neutrophil cytoplasmic antibodies (p-ANCA), cytoplasmic anti–neutrophil cytoplasmic antibodies (c-ANCA), anti–ribosomal P protein (anti–Rib-P), anti–U1 ribonucleoprotein (anti-U1RNP), anti-Ro, anti-Ro52, anti-Ro60, anti-La, anti–Scl-70, and anti-Sm.

Blood sampling and the assessment of leukocyte apoptosis biomarkers were performed as described previously.[12–17] In brief, intracellular AhR levels were determined by western blot (Biomol Research Laboratories, Plymouth Meeting, PA). Apoptotic markers, such as APO2.7, annexin V, and 7-amino-actinomycin D (7AAD), were detected by flow cytometry. Mitochondrial pathway markers, including mitochondrial antiviral signaling (MAVS), phosphorylated interferon regulatory factor 7 (pIRF7), and melanoma differentiation-associated protein 5 (MDA5), were detected by western blot. Several serum markers, such as serum MDA5, intracellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), p-selectin, L-selectin, thiobarbituric acid reactive substances (TBARS), red blood cell (RBC)-superoxide dismutase (SOD), RBC-glutathione peroxidase (GPX), and serum glutathione levels, were detected using commercial kits according to the manufacturer's instructions as described previously.[12–17] Blood samples were collected by venipuncture of the patient’s forearm vein before 10 AM.

2.3. Liquid chromatography–mass spectrometry

Serum levels of MP, EP, n-PrP, and n-BuP were determined by liquid chromatography–mass spectrometry (LC–MS). For the detection and quantification of all analytes, a Waters ACQUITY UPLC system (Waters Corporation, Milford, MA) coupled with a tandem MS (Finnigan TSQ Quantum Ultra triple-quadrupole MS; Thermo Electron, San Jose, CA) was used with Xcalibur software (Thermo Finnigan, Bellefonte, PA). The LC–MS–MS system was equipped with an electrospray ion source and run in positive mode. The injection volume was 10 μL on an ACQUITY UPLC BEH C18 Column (130 Å, 1.7 μm, 2.1 mm × 50 mm; Waters Corporation, Milford, MA) equipped with a filter (Waters ACQUITY UPLC™ BEH C18 column,1.7 μm, 2.1 mm × 5 mm) before the column.

3. RESULTS

3.1. Correlations between clinical markers, MP, EP, n-PrP, or n-BuP

Clinical data were obtained via chart review, including serum C3 and C4 levels, ESR, complete blood cell counts, hemoglobulin, hematocrit, C-reactive protein, anti-dsDNA, and SLE disease activity. None of these clinical factors were significantly correlated with any of the four tested parabens (all $p > 0.05$, Table 1).

CA9_V85N10_Text.indb 994 03-Oct-22 13:56:05

Table 1

Correlation between clinical markers and methylparaben, ethylparaben, n-propylparaben, and n-butylparaben

	Methylparaben (MP)		Ethylparaben (EP)		n-Propylparaben (n-PrP)		n-Butylparaben (n-BuP)	
N = 25	*p*	Spearman's rho	*p*	Spearman's rho	*p*	Spearman's rho	*p*	Spearman's rho
c3	0.11	0.43	0.19	0.36	0.66	0.12	0.94	0.02
c4	0.23	0.33	0.24	0.33	0.62	−0.14	0.44	−0.22
Erythrocyte sedimentation rate	0.60	0.40	0.60	0.40	0.20	0.80	0.60	0.40
Hemoglobulin, mg/dL	0.41	0.22	0.94	0.02	0.64	−0.13	0.65	−0.12
Hematocrit, %	0.67	−0.50	0.67	−0.50	0.67	−0.50	0.67	−0.50
C-reactive protein	0.80	0.20	0.60	−0.40	0.80	0.20	0.64	0.22
White blood cells, ×1000/mL	1.00	0.00	0.61	−0.14	0.80	−0.07	0.85	−0.05
% granulocyte	0.42	−0.23	0.93	−0.02	0.97	0.01	0.11	0.44
% lymphocyte	0.43	0.23	0.97	0.01	0.85	−0.06	0.16	−0.39
% monocyte	0.69	−0.12	0.88	−0.05	0.78	−0.08	0.12	−0.43
Platelet counts, ×10 000/mL	1.00	0.00	0.58	−0.15	0.42	0.22	0.74	−0.09
SLEDAI2k	0.43	−0.21	0.58	−0.15	0.81	0.07	0.79	0.07
Anti-dsDNA	0.56	0.16	0.55	0.16	0.44	0.21	0.68	0.11

SLEDAI2k = Systemic lupus erythematosus disease activity index-2000

3.2. Correlations between mitochondrial apoptotic and activation markers, reactive oxidative markers, and MP, EP, n-PrP, and n-BuP

AhR expression levels (Fig. 1) were significantly negatively correlated with n-PrP (p = 0.03, correlation coefficient (r) = –0.434) but not with the other three parabens (Table 2).

Mitochondrial apoptosis pathway markers were examined by intracellular APO2.7 staining across various cell types, including granulocytes, lymphocytes, monocytes, total leukocytes, CD4 T cells, CD8 T cells, and CD19 B cells. Mitochondrial apoptosis markers in lymphocytes were not correlated with any of the four tested parabens (all $p > 0.05$); however, APO2.7 on monocytes was significantly correlated with n-BuP levels (p = –0.019, r = 0.467) but not with the other three parabens. Intracellular apoptosis markers, including caspase 9 and caspase 10, were also determined, but neither was correlated with parabens (both $p > 0.05$).

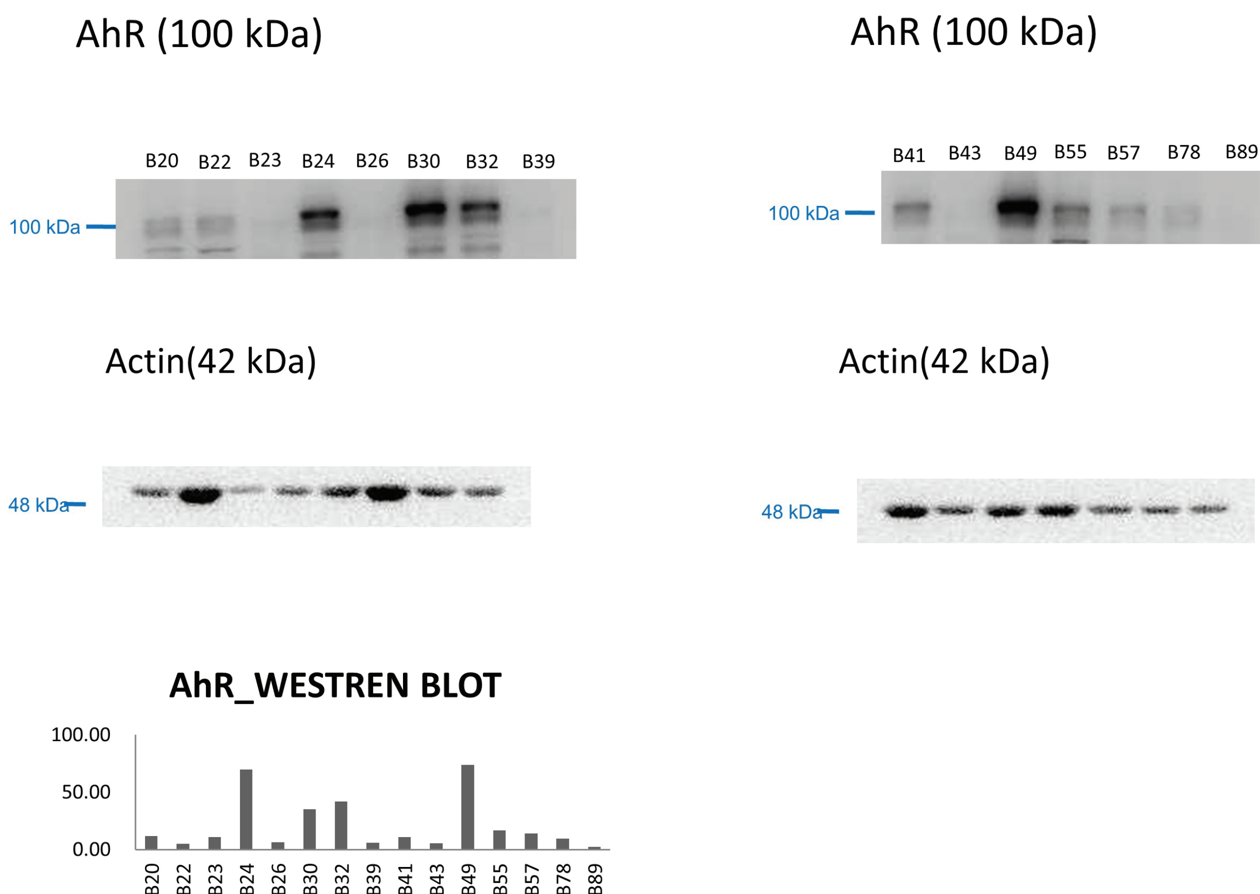

Fig. 1 Western blot showing aryl hydrocarbon (AhR) receptor expression levels among our 15 patients with systemic lupus erythematosus. The results were transformed into a histogram for comparison.

CA9_V85N10_Text.indb 995 03-Oct-22 13:56:06

Table 2

Correlation between intracellular aryl hydrocarbon receptor levels, intracellular mitochondria-related apoptotic or activation pathway markers, reactive oxidative markers, and the four parabens, methylparaben, ethylparaben, n-propylparaben, and n-butylparaben

	Methylparaben (MP)		Ethylparaben (EP)		n-Propylparaben (n-PrP)		n-Butylparaben (n-BuP)	
N = 25	*p*	Spearman's rho	*p*	Spearman's rho	*p*	Spearman's rho	*p*	Spearman's rho
Aryl hydrocarbon receptor	0.14	−0.30	0.30	−0.22	0.03*	−0.434*	0.95	−0.01
CD19-B APO2.7, %	0.39	−0.25	0.29	−0.30	0.98	0.01	0.87	−0.05
CD4-T APO2.7, %	0.15	−0.41	0.55	0.18	0.90	−0.04	0.70	0.11
CD8-T APO2.7, %	0.05	−0.53	0.54	−0.18	0.97	−0.01	0.63	0.14
Granulocyte-APO2.7, %	0.27	0.23	0.40	0.18	0.10	0.34	0.55	0.13
Lymphocyte-APO2.7, %	0.90	−0.03	0.44	0.16	0.81	−0.05	0.14	0.30
Monocyte-APO2.7, %	0.91	−0.02	0.35	0.20	0.58	0.12	0.019*	0.467*
Total leukocyte-APO2.7, %	0.25	0.24	0.12	0.32	0.11	0.33	0.22	0.26
Mitochondria-related pathway proteins								
Intracellular caspase 10	0.76	0.09	0.73	0.11	0.39	0.26	0.49	0.21
Intracellular caspase 9	0.77	−0.07	0.87	0.04	0.94	−0.02	0.69	−0.10
Intracellular MAVS-57	0.94	−0.02	0.62	−0.12	0.71	0.10	0.96	−0.01
Intracellular MAVS-75	0.69	−0.10	0.37	−0.23	0.86	0.04	0.87	−0.04
Intracellular MDA5 protein	0.84	0.05	0.94	0.02	0.86	0.05	0.38	0.23
Intracellular pIRF7	0.65	0.12	0.51	0.17	0.23	0.30	0.43	0.20
Serology markers								
Serum MDA5, pg/mL	0.11	−0.54	0.60	−0.19	0.25	−0.40	0.83	−0.08
Serum mtDNA, ng/mL	0.70	0.08	0.41	0.18	0.90	0.03	0.90	−0.03
Serum nuclear DNA, ng/mL	0.15	0.31	0.87	0.04	0.13	0.33	0.53	0.14
RBC-GPX, unit/mL	0.71	0.09	0.94	−0.02	0.99	0.00	0.54	−0.15
RBC-SOD, units/mL	0.82	−0.06	0.94	−0.02	0.08	−0.41	0.63	−0.12
TBARS	0.06	−0.43	0.30	−0.25	0.05	−0.44	0.44	−0.18
Glutathione	0.85	0.05	0.54	−0.15	0.29	−0.25	0.019*	−0.518*

The APO 2.7-PE antibody reacted with a 38-kDa mitochondrial membrane protein (7A6 antigen), which was detectable on nonpermeabilized cells in the late apoptotic state.
GPX = glutathione peroxidase; MAVS = mitochondrial antiviral signaling protein; MDA5 = melanoma differentiation-associated protein 5; MFI = mean fluorescence intensity; pIRF7 = phosphorylated interferon regulator factor 7; mt DNA = mitochondrial DNA; RBC = red blood cells; SOD = superoxide dismutase; TBARS = 2-thiobarbituric acid reacting substances test.
*$p < 0.05$.

Intracellular markers of the mitochondrial activation pathway, including pIRF7, MAVS, and MDA5, were also examined, but none of these correlated with parabens (all $p > 0.05$).

Cell apoptosis markers, including free serum mitochondrial DNA and nuclear DNA, were examined, but neither correlated well with parabens (both $p > 0.05$).

Oxidative stress in patients with SLE was assessed using several markers, including the antioxidant capacity of RBCs, as indicated by RBC-GPX and RBC-SOD levels; overall levels of lipid peroxidative stress, as indicated by TBARS; and serum glutathione levels, as a measure of the reductive capacity of the blood. Serum glutathione levels were significantly negatively correlated with n-BuP levels (p = 0.019, r = ±0.518) but not with the other three parabens.

3.3. Correlation between autoantibodies and MP, EP, n-PrP, and n-BuP

Autoantibodies are hallmarks of SLE, and different autoantibodies represent different disease subsets or clinical manifestations. Data regarding several clinically available autoantibodies were obtained via chart review, including anti–β2 glycoprotein I IgG, anti–β2 glycoprotein I IgM, anti–cardiolipin IgA, anti–cardiolipin IgG, anti–cardiolipin IgM, anti-dsDNA, anti-Mi2, p-ANCA, c-ANCA, anti–rib-P, anti-U1RNP, anti-Ro, anti-Ro52, anti-Ro60, anti-La, anti–Scl-70, and anti-Sm.

The results showed that anti- β2 glycoprotein I IgM was significantly positively correlated with both MP (p = 0.011, r = 0.585) and EP (p = 0.032, r = 0.506) levels. Anti–cardiolipin IgA was significantly positively correlated with both MP (p = 0.038, r = 0.493) and n-PrP levels (p = 0.031, r = 0.508, Table 3).

Other antibodies against extractable nuclear antigens, including anti-Ro, anti-La, anti–Scl-70, anti-Sm, anti-Mi2, anti-U1RNP, anti-Rib-P, p-ANCA, c-ANCA, and anti-dsDNA, were also assessed, but none were significantly correlated with any of the four parabens (all $p > 0.05$, Table 3).

3.4. Correlation between general apoptosis markers, adhesion molecules, and MP, EP, n-PrP, and n-BuP

Both early and late cellular apoptosis markers were determined in different cell types. Cells were characterized as early apoptotic cells if they were positive for annexin V-FITC but negative for 7-AAD. Late apoptotic cells were defined as positive for annexin V-FITC and 7-AAD. Only the early apoptosis marker Annexin V in CD8 T cells was significantly negatively significantly associated with MP ($p < 0.05$, r = −0.541) and n-BuP levels (p = 0.02, r = −0.616, Table 4).

Leukocyte surface markers, including ICAM-1, L-selectin, P-selectin, and VCAM-1, were tested. Only L-selectin was found to be significantly positively associated with both MP ($p < 0.05$, r = 0.47) and n-PrP levels (p = 0.02, r = 0.556, Table 4).

4. DISCUSSION

This preliminary study identified several positive findings, including a significant negative correlation between AhR expression and n-PrP levels (p = 0.03, r = −0.434, Table 2), a significant positive correlation between APO2.7 in monocytes and n-BuP levels (p = 0.019, r = 0.467, Table 2), a significant negative correlation between serum glutathione levels and n-BuP levels (p = 0.019, r = −0.518, Table 2), significant positive correlations between anti-β2 glycoprotein I IgM and both MP (p = 0.011,

CA9_V85N10_Text.indb 996	03-Oct-22 13:56:06

Table 3

Correlation between serological autoantibodies and methylparaben, ethylparaben, n-propylparaben, and n-butylparaben

	Methylparaben (MP)		Ethylparaben (EP)		n-Propylparaben (n-PrP)		n-Butylparaben (n-BuP)	
N = 25	p	Spearman's rho	p	Spearman's rho	p	Spearman's rho	p	Spearman's rho
Anti–β2 glycoprotein I IgG	0.84	0.05	0.53	−0.16	0.49	0.17	0.94	−0.02
Anti–β2 glycoprotein I IgM	0.011*	0.585*	0.032*	0.506*	0.11	0.40	0.48	0.18
Anti–cardiolipin IgA	0.038*	0.493*	0.51	0.17	0.031*	0.508*	0.27	0.28
Anti–cardiolipin IgG	0.06	0.46	0.96	−0.01	0.09	0.41	0.30	0.26
Anti–cardiolipin IgM	0.79	0.07	0.44	0.20	0.48	0.18	0.37	0.23
Anti-dsDNA	0.56	0.16	0.55	0.16	0.44	0.21	0.68	0.11
Anti-MI2	0.55	0.19	0.49	−0.22	0.15	0.45	0.96	−0.02
p-ANCA	0.92	0.03	0.80	0.08	0.45	0.24	0.23	0.37
c-ANCA	1.00	0.00	0.60	0.17	0.31	−0.32	0.53	−0.20
Anti–rib-p	0.42	−0.26	0.70	−0.12	0.35	−0.30	0.82	−0.07
Anti-U1RNP	0.68	0.13	1.00	0.00	0.46	0.24	0.27	−0.35
Anti-Ro	1.00	0.00	0.51	0.21	0.93	−0.03	0.70	−0.12
Anti-Ro52	0.97	−0.01	0.78	0.09	0.73	−0.11	0.67	−0.14
Anti-Ro60	0.55	−0.19	0.58	0.18	0.50	−0.22	0.66	−0.14
Anti-La	0.40	−0.27	0.75	−0.10	0.42	−0.26	0.61	−0.16
Anti–Scl-70	0.47	−0.23	0.20	−0.40	0.89	−0.05	0.08	−0.53
Anti-Sm	0.61	−0.16	0.83	−0.07	0.97	0.01	0.61	−0.16

c-ANCA = cytoplasmic anti–neutrophil cytoplasmic antibody; p-ANCA = perinuclear anti–neutrophil cytoplasmic antibody; rib-p = ribosomal p IgG; U1RNP = U1 ribonucleoprotein; dsDNA = double-strand DNA.
*$p < 0.05$.

r = 0.585) and EP levels (p = 0.032, r = 0.506, Table 3), significant positive correlations between anti–cardiolipin IgA and both MP (p = 0.038, r = 0.493) and n-PrP levels (p = 0.031, r = 0.508, Table 3), significant negative associations between the early apoptosis marker annexin V on CD8 T cells and both MP (p < 0.05, r = −0.541) and n-BuP levels (p = 0.02, r = −0.616, Table 4), and significant positive associations between L-selectin and both MP (p < 0.05, r = 0.47) and n-PrP (p = 0.02, r = −0.556, Table 4). Our findings suggest that higher parabens levels are associated with reduced AhR expression in leukocytes, increased monocyte apoptosis, reduced serum glutathione levels, reduced annexin V expression on CD8 T cells, and L-selectin upregulation on leukocytes.

A recent study reported that maternal paraben exposure might promote adipocyte differentiation from hematopoietic stem cells in humans, increasing the risk of overweight in childhood. However, this effect was only observed for n-BuP levels but not for MP, EP, or n-PrP levels.[18] In our study, selective proapoptotic effects in monocytes (represented by APO2.7) and antiapoptotic effects in CD8 T cells (represented by annexin V) were associated with n-BuP levels. The maternal effects of immune dysregulation caused by n-BuP, such as the antiapoptotic effects on CD

Table 4

Correlation between common apoptotic markers (annexin V, 7-AAD), adhesion molecules and methylparaben, ethylparaben, n-propylparaben, and n-butylparaben

	Methylparaben (MP)		Ethylparaben (EP)		n-Propylparaben (n-PrP)		n-Butylparaben (n-BuP)	
N = 25	p	Spearman's rho	p	Spearman's rho	p	Spearman's rho	p	Spearman's rho
CD19-early apoptosis	0.78	−0.08	0.65	0.13	0.94	0.02	0.81	−0.07
CD19-late apoptosis	0.24	−0.34	0.48	−0.21	0.28	−0.31	0.80	0.08
CD4-early apoptosis	0.38	−0.25	0.52	−0.19	0.49	−0.20	0.32	−0.29
CD4-late apoptosis	0.27	−0.32	0.13	−0.42	0.23	−0.34	0.31	−0.29
CD8-early apoptosis	0.05*	−0.541*	0.63	−0.14	0.30	−0.30	0.02*	−0.616*
CD8-late apoptosis	0.53	−0.19	0.24	−0.34	0.22	−0.35	0.31	−0.30
Granulocyte-early apoptosis	1.00	0.00	0.22	0.26	0.38	0.19	0.28	0.23
Granulocyte-late apoptosis	0.43	−0.17	0.14	−0.31	0.44	−0.16	0.45	−0.16
Lymphocyte-early apoptosis	0.12	−0.32	0.70	−0.08	0.99	0.00	0.64	0.10
Lymphocyte-late apoptosis	0.19	−0.27	0.65	−0.10	0.88	−0.03	0.54	0.13
Monocyte-early apoptosis	0.200	−0.27	0.565	0.12	0.763	0.06	0.318	0.21
Monocyte-late apoptosis	0.05	−0.40	0.23	−0.25	0.43	−0.17	0.81	−0.05
WBC-early apoptosis	0.09	−0.35	0.84	−0.04	0.99	0.00	0.63	0.10
WBC-late apoptosis	0.12	−0.32	0.08	−0.36	0.40	−0.18	0.56	−0.12
ICAM-1	0.89	−0.04	0.73	−0.09	0.58	0.14	0.54	0.16
L-selectin	0.05*	0.470*	0.93	0.02	0.02*	0.556*	0.45	−0.19
P-selectin	0.13	−0.37	0.10	−0.40	0.30	−0.26	0.81	0.06
VCAM-1	0.73	0.09	0.87	0.04	0.23	0.30	0.20	0.32

Early apoptotic cells were defined as positive for annexin V but negative for 7-amino-actinomycin D (7-AAD); late apoptotic cells were defined as positive for both annexin V and 7-AAD.
ICAM-1 = intercellular adhesion molecule 1; VCAM-1 = vascular cell adhesion molecule 1.
*$p < 0.05$.

CA9_V85N10_Text.indb 997 03-Oct-22 13:56:06

8 T cells, may contribute to the development of autoimmune diabetes in children.[19] In addition, increased monocyte activation, represented by mitochondrial activation and APO2.7 positivity, might result in pregnancy complications[20] and increased risks of neonatal diabetes.[21]

In a recent sophisticated study[22] from Korea, Lee et al demonstrated that only n-PrP urinary levels correlate well with the symptoms of atopic dermatitis in children, but no such correlations were identified for the levels of MP, EP, or n-BuP. They also found that an AhR signaling pathway–related product, picolinic acid, was significantly increased with higher urinary n-PrP levels than with lower n-PrP levels. (1.69-fold change between the lowest and highest quintile urinary n-PrP groups, $p < 0.001$).[22] Picolinic acid might be a neuroprotective agent[23,24] produced by kynurenine, which is also involved in tryptophan pathway activation. Our data show that the n-PrP is significantly negatively associated with intracellular AhR levels in leukocytes (Table 2), implying that the kynurenine pathway behaves in an opposite manner from the AhR pathway during tryptophan metabolism, which echoes the findings of the prior study.

Finally, L-selectin, also known as CD62L, is a leukocyte surface adhesion molecule, and its ligands range from MadCAM-1 on the endothelial cells of gut-associated lymphoid tissue[25] to GlyCAM-1 in the endothelial venules of lymph nodes,[26] which can affect lymphocyte flow. L-selectin overexpression on leukocytes correlates with lupus nephritis and proteinuria.[27] We found that the L-selectin levels on leukocytes were positively correlated with MP ($p < 0.05$, $r = 0.47$) and n-PrP ($p = 0.02$, $r = -0.556$) levels, which may indicate a direct effect for parabens on leukocytes[28] or indicate indirect insults due to the effects of parabens on adaptive immunity[29]; however, discerning which mechanism is responsible for these changes requires further investigation.

The lack of correlations between SLE markers, such as SLEDAI-2K, C3 or C4 levels, or cytopenia, and serum parabens levels is demonstrated in Table 1. The clinical symptoms and disease activity of SLE are the result of a mixture of humoral and cellular immunity factors, with differences in the contributing proportions across patients. Serum paraben levels may not influence every immune pathway equally, resulting in a poor correlation between paraben levels and SLE biomarkers. The relationships between parabens and antiphospholipid antibodies are shown in Table 3, which reveals interesting findings. Only two antiphospholipid antibodies, anti–β2 glycoprotein I IgM and anti–cardiolipin IgA, were positively correlated with parabens (MP and EP; and MP and n-PrP, respectively). These two antiphospholipid antibody isotypes might link these parabens to different types of systemic autoimmunity[30–35] and suggest potential vasculopathy among these patients.[36–38] The negative correlation between serum glutathione and n-BuP levels ($r = -0.518$, $p = 0.019$) indicates that n-BuP contributes to oxidative stress in patients with SLE, and glutathione fluctuations represent dynamic antioxidant consumption.[39,40]

This is a preliminary study of a small cohort of patients with SLE that examined the correlations between four parabens, MP, EP, n-PrP, and n-BuP, and extensive markers of immune activity. Although the case number was small, and our study did not include any control population, the results of our study contribute to our understanding of paraben exposure outcomes that are consistent with the findings of other studies.[5,41] We observed several interesting findings that are consistent with both clinical evidence and previous studies. A large cohort study remains necessary to verify these results in the future.

In conclusion, higher parabens levels were associated with lower AhR expression levels in leukocytes, increased monocyte apoptosis, lower serum glutathione levels, reduced CD8 T cell annexin V levels, and upregulated L-selectin on leukocytes. These findings may provide clinical clues regarding the immunological response to parabens among patients with SLE.

ACKNOWLEDGMENTS

Y.-J.S. thanks Prof. Jau-Ling Suen, from the Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, for determining parabens levels. This research is supported by Chang Gung Memorial Hospital funding CRRPG8K0063.

REFERENCES

1. Fransway AF, Fransway PJ, Belsito DV, Yiannias JA. Paraben toxicology. *Dermatitis* 2019;**30**:32–45.
2. Abbas S, Greige-Gerges H, Karam N, Piet MH, Netter P, Magdalou J. Metabolism of parabens (4-hydroxybenzoic acid esters) by hepatic esterases and UDP-glucuronosyltransferases in man. *Drug Metab Pharmacokinet* 2010;**25**:568–77.
3. Shin MY, Shin C, Choi JW, Lee J, Lee S, Kim S. Pharmacokinetic profile of propyl paraben in humans after oral administration. *Environ Int* 2019;**130**:104917.
4. Petric Z, Ružić J, Žuntar I. The controversies of parabens - an overview nowadays. *Acta Pharm* 2021;**71**:17–32.
5. Chen CY, Sun CY, Hsu HJ, Wu IW, Chen YC, Lee CC. Xenoestrogen exposure and kidney function in the general population: results of a community-based study by laboratory tests and questionnaire-based interviewing. *Environ Int* 2021;**155**:106585.
6. Yu H, Jiang L, Liu R, Yang A, Yang X, Wang L, et al. Association between the ratio of aryl hydrocarbon receptor (AhR) in Th17 cells to AhR in Treg cells and SLE skin lesions. *Int Immunopharmacol* 2019;**69**:257–62.
7. O'Driscoll CA, Owens LA, Hoffmann EJ, Gallo ME, Afrazi A, Han M, et al. Ambient urban dust particulate matter reduces pathologic T cells in the CNS and severity of EAE. *Environ Res* 2019;**168**:178–92.
8. Nowak K, Jabłońska E, Ratajczak-Wrona W. Immunomodulatory effects of synthetic endocrine disrupting chemicals on the development and functions of human immune cells. *Environ Int* 2019;**125**:350–64.
9. Azizi G, Yazdani R, Mirshafiey A. Th22 cells in autoimmunity: a review of current knowledge. *Eur Ann Allergy Clin Immunol* 2015;**47**:108–17.
10. Smith EL, Shmerling RH. The American College of rheumatology criteria for the classification of systemic lupus erythematosus: strengths, weaknesses, and opportunities for improvement. *Lupus* 1999;**8**:586–95.
11. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992;**35**:630–40.
12. Su YJ, Cheng TT, Chen CJ, Chiu WC, Hsu CY, Chang WN, et al. The association among leukocyte apoptosis, autoantibodies and disease severity in systemic lupus erythematosus. *J Transl Med* 2013;**11**:261.
13. Su YJ, Cheng TT, Chen CJ, Chang WN, Tsai NW, Kung CT, et al. Investigation of the caspase-dependent mitochondrial apoptotic pathway in mononuclear cells of patients with systemic lupus erythematosus. *J Transl Med* 2014;**12**:303.
14. Su YJ, Cheng TT, Chen CJ, Chiu WC, Chang WN, Tsai NW, et al. The association among antioxidant enzymes, autoantibodies, and disease severity score in systemic lupus erythematosus: comparison of neuropsychiatric and nonneuropsychiatric groups. *Biomed Res Int* 2014;**2014**:137231.
15. Su YJ, Huang CR, Chang WN, Tsai NW, Kung CT, Lin WC, et al. The association between autoantibodies and peripheral neuropathy in lupus nephritis. *Biomed Res Int* 2014;**2014**:524940.
16. Su YJ, Lin IC, Wang L, Lu CH, Huang YL, Kuo HC. Next generation sequencing identifies miRNA-based biomarker panel for lupus nephritis. *Oncotarget* 2018;**9**:27911–9.
17. Hsu CY, Lin YS, Cheng TT, Syu YJ, Lin MS, Lin HF, et al. Adherence to hydroxychloroquine improves long-term survival of patients with systemic lupus erythematosus. *Rheumatology (Oxford)* 2018;**57**:1743–51.
18. Leppert B, Strunz S, Seiwert B, Schlittenbauer L, Schlichting R, Pfeiffer C, et al. Maternal paraben exposure triggers childhood overweight development. *Nat Commun* 2020;**11**:561.
19. Roy E, Leduc M, Guegan S, Rachdi L, Kluger N, Scharfmann R, et al. Specific maternal microchimeric T cells targeting fetal antigens in β cells predispose to auto-immune diabetes in the child. *J Autoimmun* 2011;**36**:253–62.

CA9_V85N10_Text.indb 998 03-Oct-22 13:56:06

20. Groen B, Links TP, van den Berg PP, de Vos P, Faas MM. The role of autoimmunity in women with type 1 diabetes and adverse pregnancy outcome: a missing link. *Immunobiology* 2019;**224**:334–8.
21. Cardwell CR, Stene LC, Joner G, Bulsara MK, Cinek O, Rosenbauer J, et al. Maternal age at birth and childhood type 1 diabetes: a pooled analysis of 30 observational studies. *Diabetes* 2010;**59**:486–94.
22. Lee Y, Lee E, Yon DK, Jee HM, Baek HS, Lee SW, et al. The potential pathways underlying the association of propyl-paraben exposure with aeroallergen sensitization and EASI score using metabolomics analysis. *Sci Rep* 2021;**11**:3772.
23. Adams S, Braidy N, Bessede A, Brew BJ, Grant R, Teo C et al. The kynurenine pathway in brain tumor pathogenesis. *Cancer Res* 2012;**72**:5649–57.
24. Venkatesan D, Iyer M, Narayanasamy A, Siva K, Vellingiri B. Kynurenine pathway in Parkinson's disease-an update. *Eneurologicalsci* 2020;**21**:100270.
25. Low S, Hirakawa J, Hoshino H, Uchimura K, Kawashima H, Kobayashi M. Role of MAdCAM-1-expressing high endothelial venule-like vessels in colitis induced in mice lacking sulfotransferases catalyzing l-selectin ligand biosynthesis. *J Histochem Cytochem* 2018;**66**:415–25.
26. Dwir O, Shimron F, Chen C, Singer MS, Rosen SD, Alon R. GlyCAM-1 supports leukocyte rolling in flow: evidence for a greater dynamic stability of L-selectin rolling of lymphocytes than of neutrophils. *Cell Adhes Commun* 1998;**6**:349–70.
27. Celie JW, Reijmers RM, Slot EM, Beelen RH, Spaargaren M, Ter Wee PM, et al. Tubulointerstitial heparan sulfate proteoglycan changes in human renal diseases correlate with leukocyte influx and proteinuria. *Am J Physiol Renal Physiol* 2008;**294**:F253–63.
28. Miyazawa M, Ito Y, Yoshida Y, Sakaguchi H, Suzuki H. Phenotypic alterations and cytokine production in THP-1 cells in response to allergens. *Toxicol in Vitro* 2007;**21**:428–37.
29. Bairati C, Goi G, Lombardo A, Tettamanti G. The esters of p-hydroxybenzoate (parabens) inhibit the release of lysosomal enzymes by mitogen-stimulated peripheral human lymphocytes in culture. *Clin Chim Acta* 1994;**224**:147–57.
30. Domingues V, Magder LS, Petri M. Assessment of the independent associations of IgG, IgM and IgA isotypes of anticardiolipin with thrombosis in SLE. *Lupus Sci Med* 2016;**3**:e000107.
31. Kawakami T, Watabe H, Mizoguchi M, Soma Y. Elevated serum IgA anticardiolipin antibody levels in adult Henoch-Schönlein purpura. *Br J Dermatol* 2006;**155**:983–7.
32. Austin A, Campbell E, Lane P, Elias E. Nodular regenerative hyperplasia of the liver and coeliac disease: potential role of IgA anticardiolipin antibody. *Gut* 2004;**53**:1032–4.
33. Gupta M, Johann-Liang R, Bussel JB, Gersony WM, Lehman TJ. Elevated IgA and IgM anticardiolipin antibodies in acute Kawasaki disease. *Cardiology* 2002;**97**:180–2.
34. Baleva M, Boyanovsky B, Nikolov K, Kolarov Z, Nikolova M. High levels of IgA anticardiolipin antibodies in patients with systemic lupus erythematosus, Henoch-Schoenlein purpura, Sneddon's syndrome and recurrent pregnancy loss. *Thromb Haemost* 1999;**82**:1774–5.
35. Burden AD, Tillman DM, Foley P, Holme E. IgA class anticardiolipin antibodies in cutaneous leukocytoclastic vasculitis. *J Am Acad Dermatol* 1996;**35**(3 Pt 1):411–5.
36. Chayoua W, Kelchtermans H, Gris JC, Moore GW, Musiał J, Wahl D, et al. The (non-)sense of detecting anti-cardiolipin and anti-β2glycoprotein I IgM antibodies in the antiphospholipid syndrome. *J Thromb Haemost* 2020;**18**:169–79.
37. Senda Y, Ohta K, Yokoyama T, Shimizu M, Furuichi K, Wada T, et al. Microangiopathic antiphospholipid antibody syndrome due to anti-phosphatidylserine/prothrombin complex IgM antibody. *Pediatr Int* 2017;**59**:378–80.
38. Kawakami T, Takeuchi S, Soma Y. The presence of IgM antiphospholipid antibodies in patients with Henoch-Schonlein purpura and recurrent palpable purpura. *Arch Dermatol* 2011;**147**:986–8.
39. Duarte-Delgado NP, Cala MP, Barreto A, Rodríguez C LS. Metabolites and metabolic pathways associated with rheumatoid arthritis and systemic lupus erythematosus. *J Transl Autoimmun* 2022;**5**:100150.
40. Sam NB, Li BZ, Leng RX, Pan HF, Ye DQ. Circulating antioxidant levels in systemic lupus erythematosus patients: a systematic review and meta-analysis. *Biomark Med* 2019;**13**:1137–52.
41. Frederiksen H, Jørgensen N, Andersson AM. Parabens in urine, serum and seminal plasma from healthy Danish men determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). *J Expo Sci Environ Epidemiol* 2011;**21**:262–71.

CA9_V85N10_Text.indb 999 03-Oct-22 13:56:06