Clinical Effects of Orally Administered Lipopolysaccharide Derived from *Pantoea agglomerans* on Malignant Tumors

ATSUTOMO MORISHIMA[1] and HIROYUKI INAGAWA[2,3]

[1]*Lavita Medical Clinic, Hiradai-cho, Moriguchi-shi, Osaka, Japan;*
[2]*Faculty of Medicine, Kagawa University, Miki-cho, Kita-gun, Kagawa, Japan;*
[3]*Research Institute for Healthy Living, Niigata University of Pharmacy and Applied Life Sciences, Niitsu-shi, Niigata, Japan*

Abstract. *Background/Aim: It has been reported that oral administration of lipopolysaccharide (LPS) recovers an individual's immune condition and induces the exclusion of foreign matter, inflammation and tissue repair. We orally administered LPS from the wheat symbiotic bacteria Pantoea agglomerans, which has been ingested and proven to be safe, to cancer patients. Our observation of clinical improvements resulting from this treatment are reported. Patients and Methods: Sixteen cancer patients who exhibited declined small intestinal immune competence were treated between June and September, 2015. Diagnosis was based on our evaluation on small intestinal immune competence and macrophage activity. Results: The state of malignant tumors at 3 months after starting this treatment was complete recovery for 3 cases, remission for 7 cases, maintenance for 4 cases, exacerbation for 1 case and death for 1 case (total response rate=62.5%). Small intestinal immune competence and macrophage activity recovered in all cases, suggesting that oral administration of LPS contributes to disease improvement. No clear side-effects that appeared to be related to LPS intake were noted. Conclusion: Intake of an appropriate level of Pantoea agglomerans LPS recovers small intestinal immune competence and macrophage activity, contributing to improvement of malignant tumors' therapy.*

In recent years, it has been reported that oral administration of lipopolysaccharide (LPS) activates immune cells through toll-like receptor (TLR) 4, inducing foreign body exclusion, inflammatory action and tissue repair action (1). We reported that a hot water extract of wheat flour (oral administration) contains macrophage-activating substances derived from concomitant Gram-negative plant-associated bacteria, such as *Pantoea agglomerans*. LPS of this bacterium is a major macrophage-activating substance (2, 3). It has been reported that *Pantoea agglomerans* were found to be symbiotic in many plants, such as wheat and brown rice (4, 5). Oral administration of LPS from *Pantoea* aggomerans amplified phagocytic activity of peritoneal macrophages through TLR 4 (6). It has been reported in animal models and clinical studies of humans that oral intake of LPS from *Pantoea agglomerans* can prevent the onset of type I diabetes, control blood glucose levels in type II diabetes and decrease low-density lipoprotein (LDL) cholesterol (7, 8). Furthermore, when treating a mouse melanoma transplantation model with an anti-carcinogenic agent (doxorubicin), oral administration of *Pantoea agglomerans* LPS improves the survival of the B16 melanoma-inoculated murine model (9). Therefore, oral administration of LPS is thought to lead to superior improvement in treatment outcomes (9).

At our hospital, we have implemented immunity-improving therapies in the form of intestinal flora using *kampo* medicine or lactic acid bacteria supplements. However, as these mainly recover functioning of the large intestine and do not recover small intestinal competence, they do not completely increase immunity. To sufficiently increase immunity, small intestinal immune competence also needs to be recovered. Therefore, we focused on the substance called LPS, which recovers small intestinal immune competence.

Here, we used LPS from wheat symbiotic bacteria, *Pantoea agglomerans*, which has been ingested and confirmed as safe. In the present article, we report on the effects observed in our evaluation of the oral administration of LPS to 16 patients with malignant tumors who requested alternative therapy.

Materials and Methods

Patients. Our cohort comprised 16 malignant tumor patients who presented at our hospital as outpatients between June and September 2015 and who exhibited declined small intestinal immune competence.

Correspondence to: Dr. Atsutomo Morishima, Medical director, Lavita Medical Clinic, Hiradai-cho, Moriguchi-shi,Osaka, 570-0078, Japan. Tel/Fax: +81 669911000, e-mail: atsu5426@yahoo.co.jp

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Treatment. After receiving written consent from the subjects to participate in this study, administration of LPS from *Pantoea agglomerans* (wheat symbiotic bacteria LPS; Macrophi Inc., Kagawa, Japan) commenced with tablets containing 0.25 mg/tablet. Intake volume was adjusted based on testing in accordance with the status of each patient. For these tests, we used frequency measuring equipment (Rayocomp PS 1000 Polar; Layonex, Lennestadt Germany) and evaluated small intestinal immune competence and macrophage activity at the initial examination and 3 months after starting administration. Evaluation of malignant tumor treatment was also performed at the same time points as above.

Apparatus for diagnosis. The Rayocomp PS 1000 Polar is a type of frequency measuring equipment made by Leyonex. It was fundamentally developed in 1976 by Paul Schmidt, a German physicist, who discovered that each cell and tissue exhibited individual frequency (vibration), which could change depending on its status. A treatment device that resonated these individual frequencies using electromagnetic vibration was developed and related treatment methods were established. Currently, various programs have been developed based on individual vibration data from tens of thousands of measurement results that are, currently, being applied clinically and in research (10). Measurement results are divided into six classifications (resonance present (large): very good, resonance present (small): good, resonance absent: mild, moderate, severe or extreme). These were digitized and used as bioresonance indices (very good, 1; good, 2; mild, 3; moderate, 4; severe; 5; extreme, 6) for evaluation.

Statistical analysis. The Student's *t*-test with StatView (https://en.wikipedia.org/wiki/StatView) was used to statistically analyze test results. The level of significance was set at $p<0.05$.

Results

Malignant tumor therapy. The male-to-female ratio of the 16 subjects was 5:11. When classified according to primary lesion, the malignant tumors included 4 cases of lung cancer, 4 cases of breast cancer, 1 case of thyroid gland cancer, 2 cases of gastric cancer, 1 case of colorectal cancer, 1 case of uterine cancer, 1 case of melanoma and 2 cases of kidney cancer. When classified by stage, 3 cases were stage 1, 4 cases were stage 2, 1 case was stage 3 and 8 cases (50%) were stage 4. Metastatic lesions included 1 case in the liver, 1 case in the brain, 2 cases in the lungs, 2 cases in the lymph nodes, 3 cases in the bones and 1 case in the peritoneum. There were 11 cases (68.8%) that had received conventional tumor treatment from a previous doctor of which 8 cases received surgery, 2 cases radiotherapy and 8 cases chemotherapy. There were 2 cases (12.5%) that were receiving treatment from our department while continuing to receive treatment from their previous doctor. Treatment involved mega vitamin C infusion therapy (25 g~75 g/person/day) for 16 cases, ozone therapy for 12 cases and alpha-lipoic acid infusion for 11 cases. As immunity-improving therapy works in combination with these infusion therapies, all 16 cases were administered *daio kanzo to* or Pro5®, a probiotic bacteria supplement, to improve their intestinal environment. After one month of this treatment, oral administration of LPS was started with the aim of recovering small intestinal competence. To improve the intestinal environment before LPS administration, *daio kanzo to* was administered to 11 cases and Pro5® was administered to 5 cases. The daily oral dose of LPS was 0.75 mg/day/person for 1 case, 1 mg/day/person for 14 cases and 1.5 mg/day/person for 1 case. The average initial dosage of LPS was 1.02 mg/day (Figure 1).

The mean number of days of oral administration of LPS until bioresonance testing indicated recovered small intestinal immune competence was 18.3 days. Mean small intestinal immune competence, which was 4.88 at the initial examination, had improved to 1.69 at 3 months after starting LPS administration ($p<0.05$). Macrophage activity, which was 4.94 at the initial examination, was 2.25 at 3 months after starting LPS administration ($p<0.05$). These two indices showed a statistically significant difference together (Figure 2). The state of malignant tumors at 3 months after starting LPS treatment was complete recovery for 3 cases (1 case each of kidney cancer, uterine cancer and breast cancer) (Figure 3), remission for 7 cases (3 cases of breast cancer, 1 case of gastric cancer, 1 case of colorectal cancer and 2 cases of lung cancer), maintenance for 4 cases (1 case each of thyroid gland cancer, melanoma, lung cancer and gastric cancer), exacerbation for 1 case (lung cancer) and death for 1 case (kidney cancer). Thus, outcomes of remission or better were achieved for 10 cases (62.5%). No clear side-effects that appeared to be related to LPS intake were noted.

Discussion

The 16 patients with malignant tumors included 8 stage 4 cases in a terminal state and 7 cases that were stage 1 or 2. Of the 3 cases for which complete recovery was achieved, 2 cases were stage 1 and 1 case was stage 4. Many patients come to our hospital to receive preventive treatment after being treated for cancer or to receive alternative therapies for terminal cancer after being told that their condition is untreatable. This range of case types is reflected in the results.

At our hospital, mega vitamin C infusion (25 g~75 g/person/day), ozone therapy and alpha-lipoic acid infusion are used as alternative therapies for cancer patients who are difficult to treat with conventional treatment (surgery, chemotherapy and radiation) or for patients who do not wish to receive these conventional treatments. There have been proposals made for immunity-improving therapy to be conducted together with the above therapies that, when used jointly with infusions, improve the patients' immunity.

To improve the intestinal environment for immunity-improving therapy, we first administer *daio kanzo to* (*kampo*)

No.	Age	Sex	Stage	Primary tumor	Metastasis	History of conventional therapy	Intestinal therapy	Day of improvement on intestine activity	Quantity of LPS／day(mg)	State of oncology 3 months after intake of LPS	Index of bioresonance			
											Small intestine before intake of LPS	Small intestine after intake of LPS	Macrophage before intake of LPS	Macrophage after intake of LPS
1	56	Male	4	Kidney	Lung	Chemotherapy	Probiotic	20	0.75	Death	5	2	5	2
2	62	Female	1	Uterine corpus	None	None	Probiotic	16	1	Complete cure	5	2	5	3
3	31	Female	4	Breast	Bone, liver	Operation chemotherapy Radiation	Kampo	18	1	Remission	5	2	5	4
4	41	Female	2	Thyroid	None	None	Kampo	30	1	Conservation	5	1	5	2
5	78	Male	2	Stomach	None	Operation	Kampo	19	1	Remission	5	2	5	3
6	36	Male	4	Lung	Brain	Operation chemotherapy	Kampo	7	1	Exacerbation	5	2	5	2
7	49	Female	2	Colon	None	Operation chemotherapy	Probiotic	14	1	Remission	4	1	5	1
8	56	Female	2	Melanoma	None	Radiation	Kampo	21	1	Conservation	6	2	6	3
9	61	Female	4	Lung	Bone	Chemotherapy Radiation	Probiotic	19	1.5	Conservation	5	2	5	3
10	66	Male	4	Kidney	Lung	Operation Chemotherapy	Kampo	21	1	Complete cure	5	1	5	1
11	73	Female	4	Lung	Lympatic node	Chemotherapy	Kampo	15	1	Remission	5	2	5	2
12	68	Female	3	Breast	None	Operation	Kampo	19	1	Remission	5	2	5	2
13	53	Female	4	Lung	Bone	Chemotherapy	Kampo	12	1	Remission	4	2	4	2
14	59	Female	1	Breast	Lympatic node	Operation	Kampo	22	1	Complete cure	4	1	4	1
15	36	Female	1	Breast	None	None	Kampo	21	1	Remission	5	2	5	3
16	50	Male	4	Stomach	Peritoneum	Operation	Probiotic	18	1	Conservation	5	1	5	2

Figure 1. *Background of 16 malignant tumors patients.*

or Pro5® to recover large intestinal competence. Patients (11/16 cases) were prescribed *kampo* by determination based on Oriental medical diagnosis. Patients determined unsuitable (5/16 cases) for *kampo* were prescribed a probiotic bacteria supplement. At our hospital, we implement treatment to recover small intestinal competence after recovering large intestinal competence.

The method of recovering small intestinal immune competence employed was administration of LPS from the wheat symbiotic bacteria *Pantoea agglomerans*. Compared to the initial diagnosis using the bioresonance apparatus, it was found that small intestinal competence had improved in all cases after administration of LPS (average 1.02 mg/day/person over 18.3 days). It has been suggested that the mechanism for LPS-recovering small intestinal immune competence involves activation of small intestinal immune tissue through TLR 4 in the small intestinal mucous membrane and, thereby, increasing antimicrobial peptides so that small intestinal mucous membrane returns to a normal state and immune competence is recovered (11). In particular, macrophages are activated to improve innate immune competence (12). We have previously demonstrated that, in mice, oral administration of 10-1,000 μg/kg of LPS from *Pantoea* on consecutive days for a week led to dose-dependent increases in the phagocytic activity of abdominal cavity macrophages, which was found to originate in TLR 4 (6). While it is clear that LPS stimulates TLR 4, it is unknown whether this involves information transmission from intestinal mucosa or through resorption. Other than recovering immune competence, macrophages are also involved in metabolism, wound healing and metabolic regulation. When treating cancer, it is extremely important to improve macrophage function. Tumor-associated macrophages (TAMs) are known to improve two phenotypes (M1 and M2) (13-14). M2 produces interleukin-10, exhibits anti-inflammatory action and improves tissue repair. M1 is activated by bacteria-derived substances and interferon gamma *via* releasing inflammatory cytokines to kill tumors. We have previously demonstrated that intradermal administration of LPS from *Pantoea* can

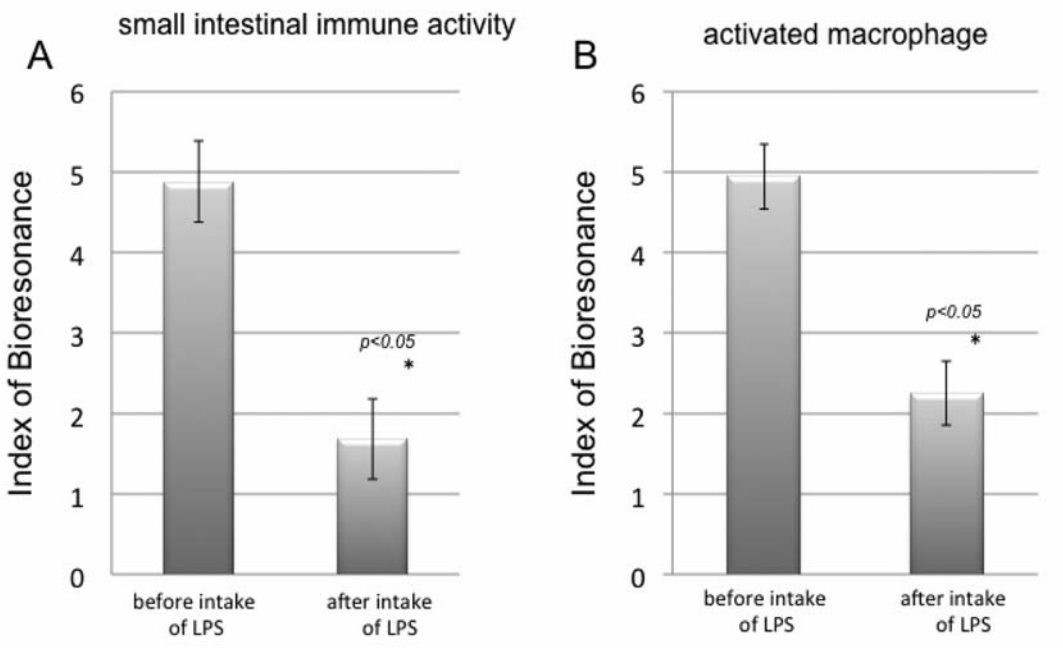

Figure 2. *Improvement of small intestinal immune competence and the degree of activated macrophage by intake of oral LPS. X axis shows the change after a mean of 3 months of oral LPS. Y axis is based on bioresonance indices (very good, 1; good, 2; mild, 3; moderate, 4; severe; 5; extreme, 6) for evaluation. A: Average of 16 patients of small intestinal immune activity index. B: Average of 16 patients of macrophage activating index. Columns and bars indicate mean and standard deviation (n=16), respectively. Average duration and amount of administration of oral LPS is 1.02 mg/person/day over 18.3 days.*

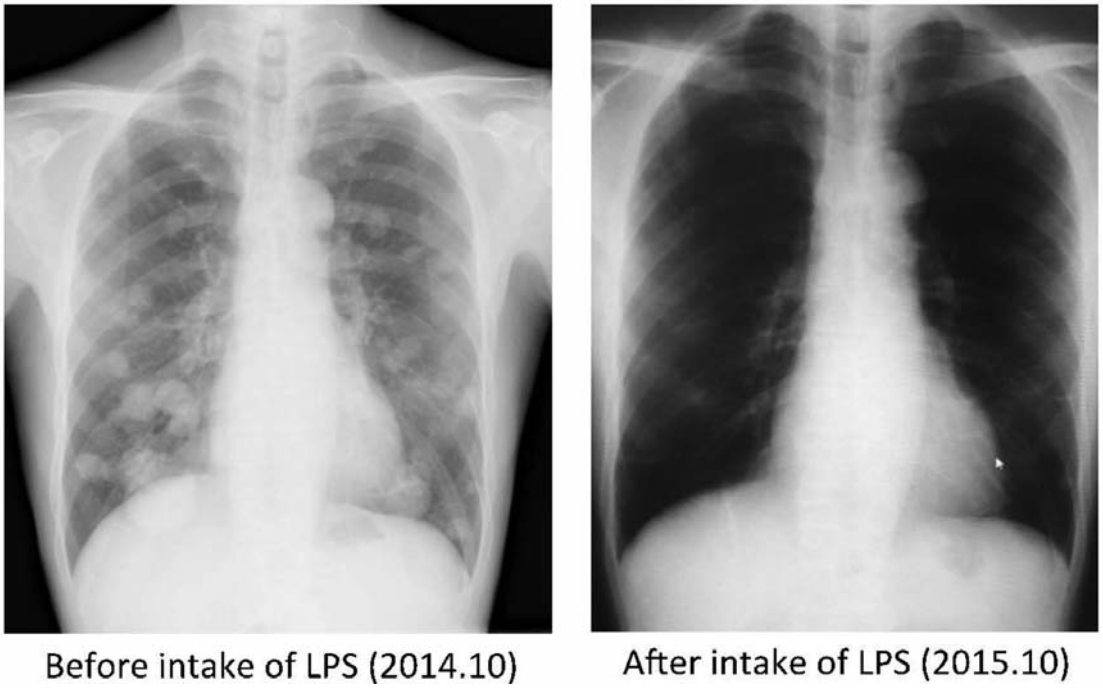

Figure 3. *Comparison of lung X-ray picture before and after oral administration of LPS. The right picture shows the complete regression of multiple metastatic lung tumors (Male, 66-year-old, administered 1.25mg/ day of LPS for 10 months).*

persistently induce tumor necrosis factors at the tumor site (15-16). It is known that anti-tumor effects can be obtained by switching TAMs from M2 to M1 (17) and, since LPS might be able to activate M1 macrophages, it may contribute in killing tumors as well. Accordingly, the use of LPS as an immunity-improving therapy may amplify anti-tumor effects due to immune cells directly impairing the malignant tumor.

Intestinal tracts with normal lactic acid bacterial flora, once the intestinal environment has been improved with lactic acid bacteria, exhibition of increased nitric oxide production and interferon gamma is noticed. This activates macrophages, which are responsible for innate immunity, leading to increased immune competence against pathogens (16). Thus, synergistic immune function effects can be anticipated for lactic acid bacteria and LPS. In the cases of this study, we believe that improving the intestinal environment with *kampo* medicine or probiotic bacteria supplements before adding LPS, greatly recovered immune competence and led to the comprehensive effects observed.

Conclusion

The appropriate oral administration of LPS from the wheat symbiotic bacteria *Pantoea agglomerans* recovers small intestinal immune competence and macrophage activity. Together with an improved intestinal environment, this led to greatly recovered immune competence that may have induced anti-tumor effects against malignant tumors. The present results suggested that oral administration of LPS could also induce synergistic effects with alternative infusion therapies and contribute to treatment of malignant tumors.

Disclosures

The Authors have no financial conflicts of interest.

References

1 Brandl K, Plitas G, Mihu CN, Ubeda C, Jia T, Fleisher M, Schnabl B, DeMatteo RP and Pamer EC: Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. Nature *455*: 804-807, 2008.

2 Kohchi C, Inagawa H, Nishizawa T, Yamaguchi T, Nagai S and Soma G: Applications of lipopolysaccharide derived from *Pantoea agglomerans* (IP-PA1) for health care based on macrophage network theory. J Biosci Bioeng *102*: 485-496, 2006.

3 Inagawa H, Nishizawa T, Yoshioka N, Taniguchi Y, Kohchi C and Soma G: Preventative and therapeutic potential of lipopolysaccharide derived from edible Gram-negative bacteria to various diseases. Curr Drug Ther *3*: 26-32, 2008.

4 Kadowaki T, Yasui Y, Takahashi Y, Kohchi C, Soma G and Inagawa H: Comparative immunological analysis of innate immunity activation after oral administration of wheat fermented extract to teleost fish. Anticancer Res *29*: 4871-4877, 2009.

5 Inagawa H, Kobayashi Y, Kohchi C, Zhang R, Shibasaki Y and Soma G: Primed activation of macrophages by oral administration of lipopolysaccharide derived from *Pantoea agglomerans*. In Vivo (2016 in press)

6 Inagawa H, Kohchi C and Soma G: Usefulness of oral administration of lipopolysaccharide for disease prevention through the induction of priming in macrophages. Anticancer Res *34*: 4497-4501, 2014.

7 Kohchi C, Inagawa H, Hino M, Oda M, Nakata K, Yoshida A, Hori H, Terada H, Makino K, Takiguchi K and Soma G: Utilization of macrophages in anticancer therapy: the macrophage network theory. Anticancer Res *24*: 3311-3320, 2004.

9 Hebishima T, Matsumoto Y, Watanabe G, Soma G, Kohchi C, Taya K, Hayashi Y and Hirota Y: Oral administration of immunopotentiator from *Pantoea agglomerans* 1 (IP-PA1) improves the survival of B16 melanoma-inoculated model mice. Exp Anim *60*: 101-109, 2011.

10 Schmidt P: Bioresonance According to Paul Schmidt (Heimes, ed). Translated into English. 2007.

11 Vaishnava S: The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine. Science *334*: 255-258, 2011.

12 Meng F and Lowell CA: Lipopolysaccharide (LPS)-induced macrophage activation and signal transduction in the absence of Src-family kinases Hck, Fgr, and Lyn. J Exp Med *185*: 1661-1670, 1997.

13 Saccani A, Schioppa T, Porta C, Biswas SK, Nebuloni M, Vago L, Bottazzi B, Colombo MP, Mantovani A and Sica A: p50 Nuclear Factor-KB Overexpression in Tumor-Associated Macrophages Inhibits M1 Inflammatory Responses and Antitumor Resistance Cancer Res *66*: 11432-11440, 2006.

14 Wang YC, He F, Feng F, Liu XW, Dong GY, Qin HY, Hu XB, Zheng MH, Liang L, Feng L, Liang YM and Han H: Notch signaling determines the M1 *versus* M2 polarization of macrophages in antitumor immune responses. Cancer Res *70(12)*: 4840-4849.

15 Inagawa H, Nishizawa T, Honda T, Nakamoto T, Takagi K and Soma G-I: Mechanisms by which chemotherapeutic agents augment the antitumor effects of tumor necrosis factor: Involvement of the pattern shift of cytokines from Th2 to Th1 in tumor lesion. Anticancer Res *18*: 3957-3964, 1998.

16 Inagawa H, Nishizawa T, Takagi K, Goto S, Soma G-I and Mizuno D: Antitumor mechanism of intradermal administration of lipopolysaccharide. Anticancer Res *17*: 1961-1964, 1997.

17 Hagemann T, Lawrence T, McNeish I, Charles KA, Kulbe H, Thompson RG, Robinson SC and Balkwill FR: "Re-educating" tumor-associated macrophages by targeting NF-B. J Exp Med *205*: 1261-1268, 2008.

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