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Electronic Supplementary Information

The Effect of Low Color Temperature Based Yellow Light Source on the Prevention of Phlebitis Induced by Chemotherapy

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Materials and Method

Materials

1900 K yellow light and 3000 K blue light came from National silicon-based semiconductor lighting engineering technology research center in Jiangxi province. Vinorelbine (VNR) tartrate injection was purchased from Qilu Pharmaceutical (Hainan) Co., Ltd. Rabbit serum IL-1β ELISA kit was purchased from Wuhan Yunclone Technology Co., Ltd. Fetal bovine serum (FBS) and Endothelial Cell Medium (ECM) were purchased from ScienCell (Beijing, China). Cell Counting Kit-8 (CCK-8) was purchased from Bestbio Biotech Co., Ltd. (Shanghai, China). Toll4 receptor antibody was from Wuhan Sevier Biotechnology Company (Wuhan, China).

Establishment of phlebitis model in rabbits

New Zealand white rabbits were female, weighs about 2.0 kg ± 0.1 kg. Rabbits were provided by the Department of Animal Science of Nanchang University. Firstly, VNR was dissolved in normal saline at a concentration of 0.6 mg/ml. According to the weight of rabbits, the dosage is 30 mg/kg. Then infused the medicinal solution from

the ear margin vein on one side of the rabbit within 10 minutes, the other ear margin vein was reserved for serum collection. During the puncture process, if a needle punctured a blood vessel leading the VNR solution to penetrate into the surrounding tissues, the rabbit would be discarded and replaced with a new rabbit.

Phlebitis evaluation

After administration, the performance, diet, activity and death of rabbits were observed every day. According to American Intravenous Nurses Society Phlebitis Rating Scale, the severity of phlebitis in these three groups was evaluated. Serum samples were collected on the 1st, 2nd, 3rd, and 5th days, and the serum inflammatory factor IL-1β was measured by using ELISA kits. Finally, rabbits were sacrificed. Starting from the puncture point, 1 cm tissue was taken from the front edge of the ear marginal vein, fixed with formalin solution immediately, and then treated with hematoxylin eosin staining (HE staining) and tumor necrosis factor-α (TNF-α) immunohistochemical staining

The IL-1β in the serum was measured to evaluate the degree of systemic inflammation. Histopathology: under light microscopy, it is judged from 5 aspects: endothelial cell loss; perivascular edema; inflammatory cell infiltration; vascular smooth muscle cell rupture; thrombosis. TNF-α immunohistochemical staining was used to evaluate the content of TNF-α in tissue by absorbance OA value. The larger the OA value, the higher the content of TNF-α in tissue.

Treatment of phlebitis

Rabbits were divided into 6 groups, namely control group, group 1, group 2, group 3, group 4, group 5, random grouping, $n \geq 4$.

Control group

The control group (without light for 7 days), serum samples were collected on the 1st, 2nd, 3rd, and 5th days. And then the rabbits were sacrificed on the 7th day, and collected marginal vein tissue Specimens.

Group 1

In group 1 (continuous exposure to 1900 K yellow light for 7 days), a portable 1900 K

yellow light small lamp bead was fixed at the puncture site and illuminated for 7 days. Serum samples were collected on the 1st, 2nd, 3rd, and 5th days, and the rabbits were sacrificed on the 7th day. Then, tissue specimens were taken out to judge the results.

Group 2

In the group 2 (continuous exposure to blue light for 7 days), serum samples were collected on days 1, 2, 3, and 5, rabbits were sacrificed on day 7, tissue samples were taken out to judge the results.

Group 3

In group 3 (exposure to 1900 K yellow light for 1 h every 12 h during 7 days), serum samples were collected on days 1, 2, 3, and 5, rabbits were sacrificed on day 7, tissue samples were taken out to judge the results.

Group 4

In group 4 (exposure to 1900 K yellow light for 24 hours on the first day, 12 hours on the second day, and 1 hour a day from the third day to the seventh day), serum samples were collected on days 1, 2, 3, and 5, rabbits were sacrificed on day 7, tissue samples were taken out to judge the results.

Group 5

In group 5 (rabbits with phlebitis were irradiated with 1900 K yellow light for 7 days), serum samples were collected on days 1, 2, 3, and 5, rabbits were sacrificed, tissue samples were taken out to judge the results.

Cell culture

Human umbilical vein endothelial cells (HUVECs) are derived from human umbilical cord vein tissue. HUVECs were seeded in 96-well plates with 2000 cells per well and cultured in high glucose medium overnight. Dilute the VNR solution in the culture medium and configure it as a solution with a concentration of 2500ng/ml. The cytotoxicity test was measured the cell growth on the first day, and the seventh day under light conditions, and the effect of 1900 K yellow light on endothelial cells in VNR solution was observed. Human vascular endothelial cells were planted in 6-well plates, 20000 per well, cultured in a high-sugar medium, and exposed to 1900 K yellow light for 24 hours. The effects of yellow light on the structure and morphology

of endothelial cells were observed with live-dead cells stained. Human vascular endothelial cells were planted in 6-well plates with 20,000 cells per well and cultured in a high-sugar medium overnight for the cells being allowed to cling to the wall of the well plates. The cells grew in culture medium containing 0.05 mg/L VNR, after 1 h, the cells were washed with PBS, and then replaced with fresh drug-free medium, cultured for 6 h. Cell was fixed by cell fixation solution, and the expression of Toll 4 receptor was observed by fluorescence staining.

Determination of survival rate and NO release ability of raw 264.7 cells

Raw 264.7 mouse monocyte macrophage leukemia cells, this cell line is derived from the tumor induced by male Abelson murine leukemia virus. Add 200 μL evenly mix the cell suspension to the 96 well plate to make the raw 264.7 cells concentration reach 2×10^4 / well, after overnight culture, remove the medium and add 200 μL LPS. Then, it was illuminated with blue light and 1900 K yellow light for 24 h. The culture medium was collected for NO determination, and then the cell survival rate was determined by CCK-8. The content of NO in the culture medium was tested with NO kit.

All experiments were performed in compliance with the relevant laws and approved by the Institutional Animal Care and Use Committee at Institute of Translational Medicine, Nanchang University.

Table S1. Grading scale for peripheral vein infusion thrombophlebitis. [1]

Grade	Symptoms of phlebitis
0	No symptoms
1	Redness and/or pain in the catheterization site
2	Redness, pain, and/or edema in the catheterization site
3	Redness, pain, and/or edema, red line, and a palpable vein in the form of a cord in the catheterization site
4	Redness, pain, and/or edema, a red line, a palpable vein in the form of a cord and more than 2.5 cm in length and purulent discharge

Table S2. The grade of phlebitis in control group and group 1, group 2.

Group	Quantity	Grade of phlebitis			Phlebitis rate (%)	χ^2	*p*
		0	1-2	3-4			
Control	6	2	1	3	66.7		
Group 1	6	5	1	0	16.7	53.156	0.000**
Group 2	6	3	2	1	50		

*$p < 0.05$, **$p < 0.01$

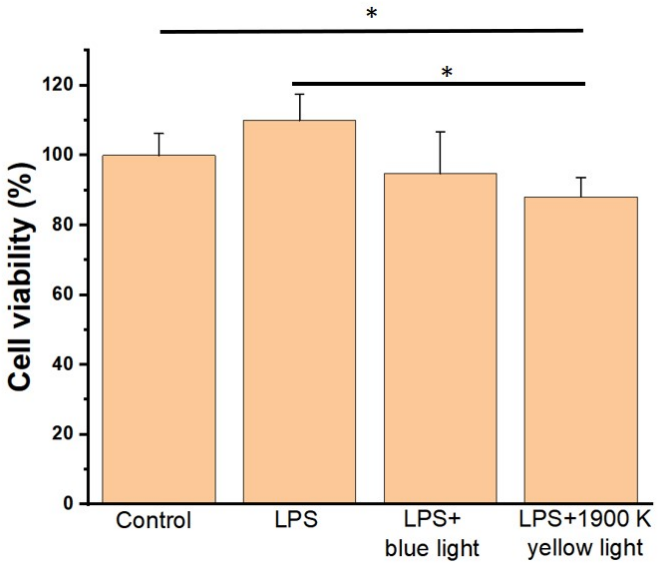

Fig. S1. Effect of 1900 K yellow light on the proliferation of RAW 264.7 cells. *$p < 0.05$.

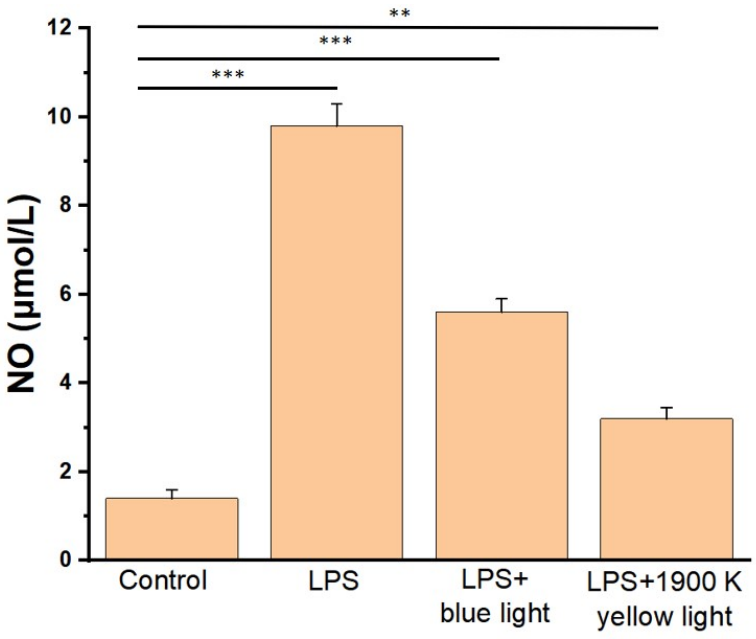

Fig. S2. Effect of 1900 K yellow light on LPS induced NO production in RAW 264.7 cells. $^{**}p < 0.01$, $^{***}p < 0.001$

Table S3. The pathological grades of phlebitis after drugs intervention.

group / grade	Quantity	Loss of venous endothelial cells				Peripheral edema				Inflammatory cell infiltration				Smooth muscle cell necrosis				Extravascular hemorrhage			
		0	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Control	6	2	1	1	2	0	1	3	2	1	1	2	2	2	1	1	2	2	1	2	2
Group 1	6	4	2	0	0	1	4	1	0	3	2	1	0	4	1	1	0	5	1	0	0
Group 2	6	3	2	1	0	1	2	3	0	2	3	1	0	2	2	1	1	3	2	1	0

Table S4. The grade of phlebitis in group 3 and group 4.

Group	Quantity	Grade of phlebitis			Phlebitis rate (%)	χ^2	*p*
		0	**1-2**	**3-4**			
Group 3	4	2	2	0	50	13.333	0.000**
Group 4	4	3	1	0	25		

$^{*}p < 0.05$ $^{**}p < 0.01$

Table S5. The occurrence of phlebitis after treated with yellow light.

Group	Quantity	Grade of phlebitis		
		0	**1-2**	**3-4**
Control	4	0	2	2
Group 5	4	0	2	2

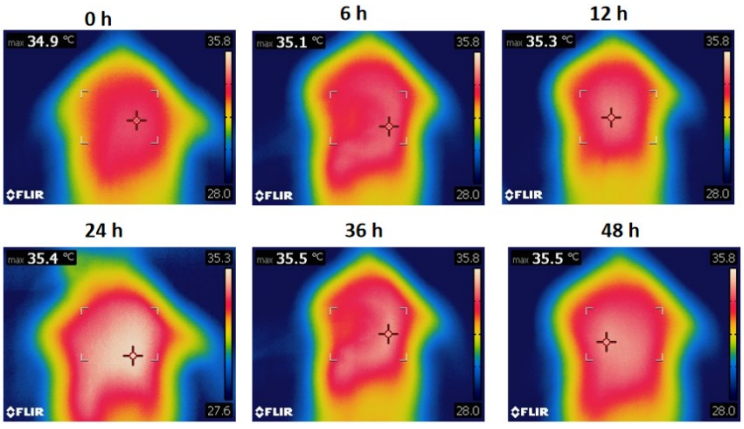

Fig. S3. Effect of long time exposure to 1900 K yellow light on local temperature of

the skin.

Reference

1. V. Tagalakis, S. R. Kahn, M. Libman and M. Blostein, *American Journal of Medicine*, 2002, **113**, 146-151.