Ultraviolet blood irradiation and oxygenation affects free radicals and antioxidase after rabbit spinal cord injury

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Keywords： *ultraviolet therapy* · *autologous blood transfusion* · *spinal cord injury* · *free radicals* · *antioxidase*

Objective To investigate the effects of ultraviolet blood irradiation and oxygenation (UBIO) on free radicals and antioxidase after spinal cord injury in rabbits.

Methods Totally, 186 rabbits were used and divided randomly into four experimental groups: control (n = 6), blood transfusion (n = 24), injured (n = 96) and treatment (n = 60) groups. The relative intensity of free radical (FR) signals, malondialdehyde (MDA) content, as well as the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) were compared among the four groups at 6, 24, 48, and 72 hours and 6 days after injury.

Results The relative intensity of FR signals in spinal cord tissue in the injured group increased at 48 hours and showed a striking difference compared with the control group; in the treatment group, it decreased and showed a striking difference compared with the injured group. MDA content in blood in the injured group increased and showed a striking difference at 6, 24 and 48 hours and showed a significant difference at 72 hours and 6 days after injury compared with the control group. In the treatment group, MDA content in blood decreased and showed a significant difference at 48 hours compared with the injured group. MDA content in spinal cord tissue increased in the injured group and showed a striking difference compared with the control group; in the treatment group, it decreased and showed a striking difference compared with the injured group at the corresponding times. The activity of SOD in blood and spinal cord tissue decreased in the injured group and showed a striking difference compared with the control group; in the treatment group, it increased and showed a striking difference compared with the injured group at the corresponding times. The changes in activity of GSH-PX in blood and spinal cord tissue were similar to that in SOD. No significant difference was observed between the blood transfusion and control groups.

Conclusion UBIO can ease free radical damages and elevate the activity of antioxidases after spinal cord injury in rabbits.

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Ultraviolet blood irradiation and oxygenation (UBIO) is a form of physical therapy, during which a certain volume of venous blood is drawn from a patient and processed with a specific dose of ultraviolet irradiation and oxygenation in a blood therapy instrument, it is then infused back into the same patient's veins to treat many nonspecific diseases in clinics.[1-6] Since 1990, we started the first research treatment with UBIO in spinal cord injury. Our progress is reported here.

METHODS

Groups and models

186 rabbits weighing from 2.5 to 3.0 kg were randomly divided into 31 subgroups for different measurements. Each subgroup consisted of six rabbits. Under general anesthesia, the rabbit's vertical skin was incised 2 cm long along the spinous process T_{13}, and both sides of the sacrospinal muscle were pushed aside. Then the spinous process T_{13} and vertebral lamina were removed, and 0.6 cm × 0.6 cm of the spinal cord was exposed. All subgroups were further arranged into four experimental groups: control, blood transfusion, injured and treatment groups. For the control group (n = 6),

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the spinal cord was exposed and not stricken. The incision was then sutured (sham control). For the blood transfusion group (n = 24), the method of spinal cord exposure was the same as that of the control group and the incision was sutured. Their venous blood was drawn (3.5 ml per kilogram weight), mixed thoroughly with a quarter of the estimated blood volume of ACD preservative fluid, and then quickly infused back into the animals' veins. For the injured group (n = 96), the method of spinal cord exposure was the same as that of the control group. The spinal cord was stricken using a modified Allen's method (the force causing injury was 12 g × 10 cm) and incision was sutured. In accordance with the death time after injury, the rabbits were divided into the 6, 24, 48, and 72 hours and 6 days subgroups. For the treatment group (n = 60), the methods of spinal cord exposure and strike were the same as those of the injured group. In accordance with the death time after injury, the rabbits were further divided into the 48 and 72 hours and 6 days subgroups (Table 1).

Table 1. Experimental groups (n)

Group (n)	FR	MDA	SOD	GSH-PX
Control (6)		Totally 6		
Blood transfusion (24)	6	6	6	6
Injured (96)				
6 h		6	6	6
24 h		6	6	6
48 h	6	6	6	6
72 h		6	6	6
6 d		6	6	6
Treatment (60)				
48 h	6	6	6	6
72 h		6	6	6
6 d		6	6	6

FR：free radical；MDA：malondialdehyde；SOD：superoxide dismutase；GSH-PX：glutathion peroxidase.

Treatment methods

Rabbits in the treatment group were separately treated with UBIO at 47, 60 and 72 hours after injury. The blood sampling and the volume of preservative fluid were the same as that of the blood transfusion group. Blood was put into a quartz bottle for a therapeutic instrument (Type MD-120B, Mei Da Co., China) for ultraviolet irradiation and oxygenation. The ultraviolet radiation wavelength was 253.7 nm；the radiation time 4.5 s per milliliter of blood；the average dose rate 5.68×10^{-3} J/s·m^2；the oxygen flow rate 2 L/min. The treated blood was quickly infused back into the rabbit's vein.

Examination methods

Detection of free radical signals in spinal cord tissue

Living tissue from the injured region of the spinal cord was sampled and frozen immediately in liquid nitrogen. The samples were ground, removed from the liquid nitrogen, and put in quartz test tubes for weighing. Free radical (FR) signals were tested directly with an electronic paramagnetic resonance instrument (Type ER-2000, Brucker Co., Germany)：microwave frequency 9.47 GHz；microwave power 12 mW；modulation 100 kHz；time constant 500 ms；and scan time 100 s. The relative intensity of FR signals from a sample was expressed by the FR amplitude value divided by its wet weight in mm/100 mg wet weight.

Detection of malondialdehyde content in blood and spinal cord tissue

Living tissue from the injured region of the spinal cord was sampled and frozen immediately in liquid nitrogen. At the same time, 4 ml of venous blood was drawn and serum was isolated with a low-temperature centrifuge at −10℃ and kept in a refrigerator at −20℃. The samples of spinal cord tissue were removed from liquid nitrogen for weighing, and a 1:10 (w:v) tissue homogenate was made with cool double-boiled water. After centrifugation, the supernatant was drawn. Serum samples were removed from the refrigerator, and malondialdehyde (MDA) content was assayed according to the technical manual of the kit (Jianchen Biologic Institute, China). MDA content was separately measured with a visible light spectrophotometer (Type 721, Shanghai Medical Instrument Factory, China) in mmol/ml for blood, and mmol/mg for spinal cord tissue.

Detection of superoxide dismutase

Samples were treated in the same way as for the detection of MDA content. According to the technical manual of the detection kit (Jianchen Biological Institute, China), superoxide dismutase (SOD) activity (Nu/ml) was separately measured with a visible light spectrophotometer (Type 721, Shanghai Medical Instrument Factory, China).

Detection of glutathione peroxidase

Samples were treated in the same way as for the detection of MDA content. According to the technical manual of the detection kit (Jianchen Biological Institute, China), glutathione peroxidase (GSH-PX) activity of the samples was separately measured with a visible light spectrophotometer (Type 721, Shanghai Medical Instrument Factory, China) in vital unit/0.1 ml.

Statistical methods

Data were shown as $\bar{x} \pm s$ and analyzed with Student-Newman-Keulst.

RESULTS

Relative intensity of FR signals

The relative intensity of FR signals was 1.938 ± 0.34

mm/100 mg in the control group, and 1.901 ± 0.98 mm/100 mg in the blood transfusion group, and no significant difference was observed between them. The injured group had an FR signal of 7.014 ± 1.173 mm/100 mg at 48 hours after injury, significantly different from the control group ($P < 0.01$). The treatment group had an FR signal of 4.215 ± 0.914 mm/100 mg, and the difference, compared with the injured group, was significant ($P < 0.01$).

MDA content in blood and spinal cord tissue

MDA content in blood and spinal cord tissue revealed no significant differences between the blood transfusion and control groups. In the injured group, MDA content in blood increased and showed a striking difference at 6, 24 and 48 hours and showed a significant difference at 72 hours and 6 days after injury compared with the control group; MDA content in spinal cord tissue increased and showed a striking difference compared with the control group. In the treatment group, MDA content in blood decreased and showed a significant difference at 48 hours compared with the injured group; MDA content in spinal cord tissue decreased and showed a striking difference compared with the injured group at the corresponding time points. MDA content in each group measured at various periods after injury is shown in Table 2.

Table 2. MDA contents in blood and spinal cord tissue after injury

Group	Blood (nmol/ml)	Spinal cord (nmol/mg)
Control	2.31 ± 0.33	5.07 ± 0.56
Transfused	2.22 ± 0.50	4.99 ± 0.74
Injured		
6 h	4.78 ± 0.54**	34.41 ± 0.77**
24 h	4.28 ± 0.45**	28.87 ± 0.73**
48 h	4.07 ± 0.67**	26.90 ± 0.82**
72 h	3.38 ± 0.44*	23.87 ± 0.63**
6 d	3.14 ± 0.59*	21.54 ± 0.71**
Treatment		
48 h	3.09 ± 0.41#	19.95 ± 0.62##
72 h	2.86 ± 0.58	11.55 ± 0.56##
6 d	2.61 ± 0.46	7.13 ± 0.51##

$P > 0.05$, comparison between the transfused and control; * $P < 0.05$, ** $P < 0.01$, vs control; ## $P < 0.01$, # $P < 0.05$, vs injured.

SOD activity in blood and spinal cord tissue

In blood and spinal cord tissue, SOD activity revealed no significant differences between the blood transfusion and control groups. In the injured group, SOD activity decreased and showed a striking difference compared with the control group. In the treatment group, SOD activity increased and showed a striking difference compared with the injured group at the corresponding time points. SOD activity in each group measured at various periods after injury is shown in Table 3.

GSH-PX activity in blood and spinal cord tissue

In blood and spinal cord tissue, GSH-PH activity revealed no significant differences between the blood transfusion and control groups. In the injured group, GSH-PX activity decreased and showed a striking difference compared with the control group. In the treatment group, GSH-PX activity increased and showed a striking difference compared with the injured group at the corresponding time points. GSH-PX activity in each group measured at various periods after injury is shown in Table 4.

Table 3. SOD activities in blood and spinal cord tissue after injury (Nu/ml)

Group	Blood	Spinal cord
Control	110.50 ± 7.94	124.68 ± 8.03
Transfused	109.75 ± 9.06	120.11 ± 6.95
Injured		
6 h	73.67 ± 10.65*	86.35 ± 5.65*
24 h	57.50 ± 7.61*	71.30 ± 5.00*
48 h	56.13 ± 4.84*	75.35 ± 5.63*
72 h	61.22 ± 5.59*	68.95 ± 6.19*
6 d	57.80 ± 5.46*	75.22 ± 5.85*
Treatment		
48 h	115.58 ± 6.16#	160.17 ± 8.13#**
72 h	113.12 ± 7.20#	164.92 ± 8.35#**
6 d	113.18 ± 9.16#	129.60 ± 7.61#

$P > 0.05$, comparison between the transfused and control; * $P < 0.01$, vs control; # $P < 0.01$, vs injured; ** $P < 0.05$, vs control.

Table 4. GSH-PX activities in blood and spinal cord tissues after injury (vital unit/0.1 ml)

Group	Blood	Spinal cord
Control	201.38 ± 13.94	101.72 ± 10.89
Transfused	195.43 ± 9.57	114.26 ± 8.44
Injured		
6 h	109.12 ± 6.64*	85.85 ± 3.26*
24 h	98.50 ± 5.75*	72.87 ± 3.73*
48 h	106.07 ± 7.08*	77.90 ± 2.82*
72 h	102.35 ± 6.99*	75.87 ± 3.53*
6 d	99.42 ± 6.21*	73.68 ± 4.10*
Treatment		
48 h	207.12 ± 7.78#	113.95 ± 6.92#**
72 h	200.28 ± 2.25#	128.55 ± 6.26#**
6 d	203.87 ± 9.82#	101.20 ± 2.87#

$P > 0.05$, comparison between the transfused and control; * $P < 0.01$, vs control; # $P < 0.01$, vs injured; ** $P < 0.05$, vs control.

DISCUSSION

Under normal conditions, the concentration of FR in vivo is extremely low, generally 10^{-7} – 10^{-5} mol/L. Therefore, the lipid peroxidation induced by FR does not cause injury in normal tissues. In vivo antioxidases, such as SOD and GSH-PX and anti-oxidants, such as vitamin A and vitamin C are very effective in removing FR, and further suppress lipid peroxidation. By this mechanism, the structure and function of the organism is protected. Under pathological conditions, either an increase in FR products or

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a decrease in the ability to eliminate FR results in an accumulation of large amount of FR. Both FR and MDA may cause serious damage to the organism.

Our experiments revealed that the relative intensity of FR signals in the injured group at 48 hours after injury was elevated more than three times compared with the control group. MDA content in blood and spinal cord tissue in the injured group was obviously elevated at various periods after injury. MDA content reached a peak at 6 hours after injury and fell gradually, but it was still higher at 6 days after injury than that of the control group. In addition, the rise of MDA content in spinal cord tissue was much greater than that in blood. In contrast, after treatment with UBIO, the relative intensity of FR signals in spinal cord tissue in the treatment group dropped more than 1.5-fold at 48 hours after injury compared with the injured group. MDA content in blood and spinal cord tissue in the treatment group was substantially lower at various periods after injury. There were two characteristics observed. First, MDA content in blood in the treatment group, compared with the injured group at the corresponding time points, was significantly lower at 48 hours after injury, but not at 72 hours and 6 days after injury, relative to the control group. Thus, UBIO could shorten the rising duration of MDA content in blood. Second, compared with the control group, MDA content in spinal cord tissue in the injured group was elevated 4 – 6 times at 48, 72 hours and 6 days after injury, but only elevated 1 – 4 times in the treatment group at the corresponding time points, This indicates that UBIO may increase the falling range of MDA content in spinal cord tissue, which is an important basis for the positive effect of UBIO.

A great number of studies have demonstrated that UBIO activates in vivo endogenous oxidases[7] and antioxidants[1,2] early, and effectively restrains the lipid peroxide effect,[1,2,4,7] reducing the concentration of FR products. It is also reported that UBIO causes changes in the rheological property of blood,[3,8,9] regulate the scale equilibrium between thromboxane A_2 and prostacyclin I_2,[5] depress the aggregation of blood platelets,[4,9] and improve blood circulation. Accordingly, UBIO reduces the accumulation of FR. Consequently, UBIO could lighten the lipid peroxidation effect induced by FR by regulating production and elimination. This is very important for preventing progressive and secondary damage in spinal cord tissue.

Our study revealed that SOD activity in blood and spinal cord tissue in the the injured group decreased substantially at various periods after injury, and showed a striking difference from the control group. After treatment with UBIO, SOD activity in blood and spinal cord tissue in the treatment group was elevated, and showed a striking difference from the injured group at the corresponding time points. The underlying mechanism might be as follows：(1) To decrease the formation of FR[1,2,4,7] and thus bring on the reduction of SOD consumption. (2) To elevate the mechanical properties of the cellular membranes, to prevent the red blood cells from breaking,[3,9] and also to increase oxygen quantity and efficiency in tissues.[6] Housset et al[10] demonstrated that in intact red blood cell membranes, the synthesis of SOD increased with an increase in the oxygen quantity in tissues. And (3) To directly activate the system of endogenous oxidases, and thereby increase SOD activity.[1,2] Moreover, our experiments revealed that compared with the control group, SOD activity in blood in the treatment group measured at 48 hours after injury had recovered to normal levels. In contrast, SOD activity in spinal cord tissue in the treatment group measured at 48 and 72 hours had gone significantly beyond normal levels, whereas the level measured at 6 days after injury approached normal levels. These results suggest that (1) UBIO could make the SOD activity in blood recover to normal levels. This is very important in improving the level of antioxidases all over the body after spinal cord injury. (2) The mechanism by which UBIO induced the abnormally high SOD activity in spinal cord tissue at 48 and 72 hours after injury remains unknown. It will be necessary to study further. (3) After treatment with UBIO for three times separately at 47, 60 and 72 hours, the activity of SOD in spinal cord tissue measured at 72 hours still remained at the level measured at 48 hours. This suggests that SOD activity in spinal cord tissue could not be raised for a treatment every 12 – 13 hours. However, in the treatment group, the SOD activity in spinal cord tissue measured at 6 days after injury was decreased, compared with the levels measured at 48 and 72 hours. The reason may be that the former was sampled three days after stopping treatment, the latter was immediately sampled after the last treatment. This suggests that the duration of SOD activity in spinal cord tissue beyond normal levels after treatment with UBIO was more than 12, but less than 72 hours. Therefore, in practice the interval between treatments might be prolonged. And (4) The steady production of FR in the treatment group at 6 days after injury consumed the local SOD, and the SOD activity in spinal cord tissue decreased substantially. Even when the treatment was stopped for three days, SOD activity still remained at normal levels, suggesting a lasting biological effect of UBIO.

The changes in GSH-PX activity in blood and spinal cord tissue in the injured and treatment groups were the same as those of SOD, and it seems that their mechanism is basically similar. In addition, our experiments revealed no significant differences between the blood transfusion and

control groups. Therefore, the effect of the transfused blood and ACD preservative fluid can be eliminated.

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