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Protective effect of deuterium-depleted water combined with platelet-rich plasma on pancreatic islet cells and TGF-ÿ1 expression in diabetic rats

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[Abstract] Objective: To observe the effect of deuterium-depleted water (DDW) combined with platelet-rich plasma (PRP) on the The protective effect of glutathione on pancreatic islet cells of diabetic rats and the effect on the expression of TGF-ÿ1 were investigated to preliminarily explore its possible mechanism in protecting pancreatic islet cells of diabetic rats and promoting the healing of diabetic ulcers.

Method: The diabetic rat ulcer model was established by feeding with high-sugar and high-fat diet combined with intraperitoneal injection of streptozotocin (STZ) and full-thickness excision of the back skin. The mice were divided into diabetic model group, deuterium-depleted water group (DDW), platelet-rich plasma group (PRP) and deuterium-depleted water combined with platelet-rich plasma group (DDW+PRP). The model was established by intraperitoneal injection of citric acid-sodium citrate buffer and full-thickness excision of the back skin. Random blood glucose was measured on days 3, 7, and 14 after treatment in each group. The pathological changes of pancreatic tissue were observed after HE staining, and the expression of TGF-ÿ1 in wound tissue was detected by enzyme-linked immunosorbent assay. Random blood glucose was lower after 14 days of intervention than before intervention, nd the difference was statistically significant (ÿ0.05). The wound healing rate of the DDW+PRP group was higher than that Pof the DDW group and the PRP group at 14 days after intervention, and the difference was statistically significant (ÿ0.05). The morphology, P ÿ0.05); and compared with the blank control group, there was no statistically significant difference (P ÿ0.05). The histological observation of pancreatic islets increased with the prolongation of intervention time. quantity, arrangement and distribution of stained particles of pancreatic islet cells in the DDW+PRP group were significantly improved compared with those before intervention. T in the DDW group and the PRP group was higher than that in the PRP group, and the difference was P ÿ0.05); however, there was no significant difference between the DDW group and the PRP group (P ÿ0.05ÿÿ statistically significant (Conclusion: Deuterium-depleted water combined with platelet-rich plasma has a significant promoting effect on the healing of ulcer wounds in type 2 diabetic rats, and deuterium-depleted water has an effect on the pancreatic islet cells of type 2 diabetic rats The mechanism of promoting wound healing may be related to lowering

random blood sugar in diabetic rats, improving pancreatic islet cell function, and increasing the content of TGF-ÿ1 in wound tissue.

Quantity related.

[Keywords] Deuterium-depleted water; Platelet-rich plasma; Diabetes; Islet cells; Ulcer wound; Transforming growth factor ÿ1

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Effects of Deuterium-depleted Water Combined with Platelet-rich Plasma on Islet

Cell Protection and TGF-ÿ1 Expression in Diabetic Rats

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Abstract: Objective: To observe the effect of deuterium-depleted water (DDW) combined with platelet-rich plasma (PRP) on the islet cell protection and TGF-ÿ1 expression in diabetic rats, and explore its possible mechanism to protect islet cells of diabetic rats and promote the healing of diabetic ulcer. Methods The ulcer model of diabetic rats were established by feeding high-sugar and high-fat feed combined with intraperitoneal injection of streptozotocin (STZ) full-thickness resection of back skin. After modeling, they were divided into diabetes model group, deuterium-depleted water group (DDW), platelet-rich plasma group (PRP) and deuterium-depleted water combined with platelet-rich plasma group (DDW+PRP) according to random numbers. The blank control group was modeled by intraperitoneal injection of citric acid-sodium citrate buffer+full-thickness resection of the back skin.Rats in each group were tested for random blood glucose and wound healing rate 3d, 7d, 14d after treatment. The pathological changes were observed after HE staining of pancreatic tissue.Enzyme-linked immunosorbent assay was used to detect the expression of TGF-ÿ1 in wound tissue. The pathological changes were observed. Enzyme-linked immunosorbent assay was used to detect the expression of TGF-ÿ1 in wound tissue. Results The random blood glucose in the DDW group and DDW+PRP group after 14 days of intervention was lower than before, the difference was statistically significant (Pÿ0.05). The wound healing rate of the DDW group, PRP group, and DDW+PRP group was higher than the diabetes model group after 7d and

14d intervention (Pÿ0.05). The wound healing rate of the DDW+PRP group was higher than DDW group and PRP group (ÿ0.05), Pand compared with the blank control group, the difference was not statistically significant (ÿ0.05). With Pthe extension of the intervention time, compared with the previous groups, the islet cells in the DDW group and DDW+PRP group were significantly improved in their morphology, number, arrangement, and distribution of stained particles. The content of TGF-ÿ1 in the DDW+PRP group was higher than the DDW group and PRP groups at 7d and 14d after intervention (Pÿ0.05), but there were no significant difference between the DDW and PRP groups (ÿ0.05). Conclusion Deuterium-depleted water combined with PRP can significantly promote the healing of ulcer wounds in type 2 diabetic rats, and deuterium-depleted water can protect and repair the islet cells of type 2 diabetic rats. The mechanism of promoting wound healing may be related to reducing the random blood glucose of diabetic rats, improving the function of islet cells, and increasing the content of TGF-ÿ1 in the wound tissue.

Key words: deuterium-depleted water; platelet-rich plasma(PRP); diabetes; islet cells; ulcer wounds; transforming growth factor-ÿ1

According to the World Health Organization, there are currently about 422 million people with diabetes worldwide. The number of diabetic patients is expected to reach 693 million by 2045[1]. May develop into diabetic foot ulcers, small skin wounds that can cause chronic Refractory ulcers may eventually lead to infection, gangrene, and even amputation[2], causing huge The diabetic ulcer has a great social and economic burden [3]. The level of growth factors decreases, and the chemotactic ability of recruiting inflammatory cells to the wound surface decreases. Low, poor wound vascularization and chronic inflammatory state limit diabetic foot ulcer The main goal of current treatment is to close the wound.

In such cases, conventional treatments including dressing changes and debridement cannot achieve satisfactory results. As a result, new strategies to promote healing of diabetic foot ulcers are urgently needed [5]. However, the ratio of deuterium to hydrogen in water (D/H) is about 1:6 600, that is, the volume fraction of deuterium is 0.015%. Water with a deuterium concentration below 0.015% is usually called deuterium-depleted water (DDW) [6]. It has antioxidant, anti-depressant, anti-tumor, Biological effects such as hypoglycemia can be used to treat related diseases [7-8]. Deuterium-depleted water alleviates and repairs damaged pancreatic ÿ cells in diabetic rats [9] Platelet-rich plasma (PRP) is

The whole blood is centrifuged and contains high concentrations of platelets, white blood cells, and fibrinogen. Related scholars have found that PRP can effectively treat chronic ulcers in diabetes mellitus. It has

unique advantages in the healing of ulcer wounds and can significantly promote healing[10]. This study aims to observe the effect of deuterium-depleted water combined with platelet-rich plasma on pancreatic islets in type 2 diabetic rats The protective effect of cells and its impact on ulcer tissue healing.

1 Materials and methods

1.1 Experimental animals: 120 healthy clean male SD rats, weight (180±10) g, provided by Liaoning Changsheng Biotechnology Co., Ltd., with permission No. SCXK (Liao) 2015-0001. The animals were kept at room temperature of 20ÿÿ25ÿ.

The relative humidity was 50% to 70%. Approved by the Ethics Committee, ethics number: 2020376.

1.2 Main reagents and instruments: Streptozotocin STZ), chloral hydrate, glucose, citric acid, trisodium citrate (Beijing So Leybold Company); Rat TGF-ÿ1 Elisa kit (Shanghai ELISA Biotechnology Co., Ltd. Technology Co., Ltd.); rat ordinary feed (Chengdu Dashuo Biotechnology Co., Ltd.Co., Ltd.); high-sugar and high-fat diet for rats (Beijing Keao Xieli Co., Ltd.); Blood glucose meter + test strips (vitality type); high-speed refrigerated centrifuge (Eppendorf Germany); Pipette (Finnpipette, 20-200 ÿl Shanghai Jinruike Scientific Instrument Co., Ltd.); Electric Thermostatic Box (Wuhan-Hengsujing Scientific Instrument Co., Ltd. Co., Ltd., 37°C); microplate reader (Rayto, RT-6100, 450nm); ZMX- 998B ELISA plate washer; deuterium-depleted water was provided by Luzhou Harold Health Technology Co., Ltd.

Image Pro Plus 6.0 professional image analysis software (Media Cybernetics, USA) was provided by Media Cybernetics.

1.3 Experimental methods

1.3.1 Experimental grouping and model preparation: 120 healthy clean male SD rats After one week of adaptive feeding, the mice were randomly divided into a blank control group of 20 and a diabetic There were 80 rats in the disease group and 20 rats in the blood collection group (not involved in modeling and grouping). The body weight of rats in each group was measured, and the blood glucose in the tail vein of rats in each group was measured. The diabetic rats were fed with a normal diet for 4 weeks, and the diabetic rats were fed with a high-sugar and high-fat diet (67% vitamin C). Feed 4 days Before modeling, the rats in each group were fasted for 12 h, but not water- estricted. Rats in the blank control group were intraperitoneally injected with citric acid-sodium citrate buffer (with The same volume as the diabetic group), 7 days later, the tail was cut off and blood was collected to measure the blood Sugar. Anesthetize with 7% chloral hydrate intraperitoneal injection (0.5ml/100g). The rats were fixed in a rat holder, their skins were prepared, disinfected, and their backs were

marked before making the surface The wound area is 3cm×3cm, and the wound tissue reaches the fascia layer. The model is successfully created. Then 18 rats were randomly selected as blank control group. Cover the wound with cloth and fix it with adhesive tape.

The diabetic rats were given a single intraperitoneal injection of 30 mg/kg 1% streptozotocin. Strept zotocin (STZ) solution (0.1mmol/L pH 4.5 lemon Citric acid-sodium citrate buffer, low temperature, avoid light, prepare before use), injection The rats were then fed with a high-sugar and high-fat diet for 7 days. Random blood glucose, random blood glucose ÿ16.7mmol/L indicates type 2 diabetes rat model The blood sugar level did not reach the target level. After 3 days, 10 mg/kg of 1% chain Streptozotocin (STZ) solution, blood test again after 7 days The model was successfully established when random blood glucose ÿ16.7mmol/L[11]. 7% chloral hydrate was injected intraperitoneally (0.5 ml/100 g) for anesthesia. Set the rat holder, prepare the skin, disinfect, and mark the back to make the area size The wound surface is 3cm×3cm, and the wound tissue reaches the fascia layer. After taking a photo with a digital camera The wound surface was covered with sterile gauze and fixed with adhesive tape. The subjects were randomly divided into diabetic model group, DDW group, PRP group and DDW+PRP group.

There were 18 mice in each group.

1.3.2 Platelet-rich plasma (PRP)

Preparation: 3 to 4 SD rats were taken from each blood collection group, weighed and diluted with 7% hydrated chlorine. The rats were anesthetized by intraperitoneal injection of aldehyde (0.5 ml/100 g) and each rat was anesthetized by intraperitoneal injection of aldehyde (0.5 ml/100 g). About 10 ml of blood was collected from the aorta and pre-coagulated with ACD anticoagulant (1 ml of citrate glucose Glucose solution) vacuum centrifuge tube and disposable blood collection needle to extract whole blood, The platelet count of 1 ml of whole blood was 628 × 109. Platelet-rich plasma was prepared by the Landesberg method. The first centrifugation was performed at 4°C and 200 g. Centrifuge for 15 minutes. After centrifugation, the blood in the centrifuge tube is divided into three layers. The upper layer is the supernatant, the middle layer is the The middle layer is rich in platelets, and the lower layer is the red blood cell layer. Mark 2mm below the cell layer and use a pipette to extract all the liquid on the marked line.

Transfer to another blank centrifuge tube and shake well. Centrifuge for the second time at 4°C and 500g Centrifuge for 10 minutes. The liquid is divided into two layers after centrifugation. The upper layer is platelet-poor plasma. (Platelet-poor plasma, PPP), the lower layer contains a large number of blood cells PRP is a mixture of platelets, plasma, and a

small amount of red blood cells. Anemia is removed with a pipette The platelet plasma layer and the rest is PRP. Number 4 163×109 ÿ 1.3.3 Intervention method: blank control group, 0.9% sodium chloride solution gavage, drink Use ordinary water, disinfect the ulcer locally, and then bandage it with 0.9% sodium chloride solution gauze; sugar Diabetes model group, 0.9% sodium chloride solution was given by gavage, ordinary water was drunk, and local ulcer After disinfection, the 0.9% sodium chloride solution was used for gauze bandage; in the DDW group, deuterium-depleted water was administered orally. Use deuterium-depleted water, disinfect the ulcer locally, and then bandage it with 0.9% sodium chloride solution gauze; PRP Group A, 0.9% sodium chloride solution was administered orally, and the patients drank normal water. After local disinfection of the ulcer Apply PRP and then bandage with sterile gauze; DDW+PRP group, oral administration of deuterium-depleted water, drinking Use deuterium-depleted water to disinfect the ulcer locally, apply PRP, and then bandage it with sterile gauze. The oral dose for rats was 0.01 ml/g/d. Rats in each group were housed in a single cage. Change the dressing on the ulcer wound once a day, and apply PRP to the ulcer wound every other day (the dosage is 100 ÿl per wound surface), and the clean pad was replaced once a day.

1.4 Observation indicators

1.4.1 Random blood glucose: After the model was successfully established, the rats in each group were tested without intervention. Random blood glucose levels were measured by tail-cutting blood sampling at 3, 7, and 14 days after intervention.

1.4.2 Wound healing rate: after ulcer wound creation (without intervention),

The wound surface was imaged 3d, 7d, and 14d after intervention, and the wound area was measured and wound healing was calculated using Image Pro Plus 6.0 professional image analysis software.

Wound healing rate = (wound area before intervention - wound area after intervention) / Wound area before intervention × 100%.

1.4.3 Observation of pancreatic tissue: 3d, 7d, and 14d after ulcer wound creation.

Six rats were randomly selected from each group by lottery, and the whole pancreas was quickly removed after anesthesia. Tissue, 4% formaldehyde fixation, dehydration, paraffin embedding, sectioning, hematoxylin-eosin staining The cells were stained and sealed, and the pathological changes of pancreatic islet tissue were observed under an ordinary optical microscope.

1.4.4 Detection of TGF-ÿ1 in ulcer wound tissue:

On days 3, 7 and 14, 6 rats were randomly selected from each group

and anesthetized after random blood sugar measurement. Take 2-3 pieces of tissue from the ulcer surface and the 5mm surrounding tissue. Immediately cool them in liquid nitrogen. The ulcer wound tissue was tested by enzyme-linked immunosorbent assay. The expression of TGF-ÿ1 in the culture medium was measured, and the process was carried out strictly according to the instructions of the kit.

1.5 Statistical analysis: SPSS 20.0 statistical software was used for statistical analysis.

The data were expressed as mean ± standard deviation, and the comparison among the groups was performed by single factor method. Difference analysis, pairwise comparison using t test, Pÿ0.05 means the difference is statistically significant Academic significance.

2 Results

2.1 General condition and weight changes of rats: Rats in the blank control group responded well. sensitive, energetic, shiny hair, thick sebum, and fast weight gain.

Compared with the control group, the hair of diabetic mice was rough, less shiny, and yellowish. Easy to fall off, symptoms of polydipsia, polyphagia, polyuria, slow reaction, and low mobility Poor, obvious defecation reflex when picked up, slow weight gain.

2.2 Changes in blood glucose levels in rats of each group: blank control group, diabetic model

Comparison of random blood glucose of rats in group and PRP group at 3d, 7d, and 14d after intervention with that before intervention There was no statistically significant difference (P ÿ0.05); DDW group, DDW+PRP group After the intervention with low-deuterium water, random blood glucose began to decrease slowly. Compared with the random blood glucose reduction, the difference was statistically significant (P ÿ0.05).

Table 1.

Table 1 Random blood glucose levels of rats in each group at different intervention time points ($\bar{x}$±s, mmol/L)

Number of groups Before intervention 3 days after intervention 7 days after intervention 14 days after intervention

Blank control group6 7.06±0.49 7.35±0.64 6.68±0.76 7.22±0.66

Diabetes model group 6 24.20±3.75 23.80±5.49 23.22±2.38 23.97±4.96

DDW Group 6 24.42±5.24 22.73±4.67 21.80±4.70 19.50±4.66a

PRP group 6 22.67±4.29 21.23±3.70 21.50±3.98 22.68±2.76 DDW+PRP group 6 26.00±4.17 24.25±4.19 22.50±2.93 22.10±3.34b

Note: a means comparison with the DDW group before intervention. t =2.59Ã¿ P =0.049; b indicates the difference with DDW+PRP group

Compared with before intervention, t=2.86ÿ P=0.035

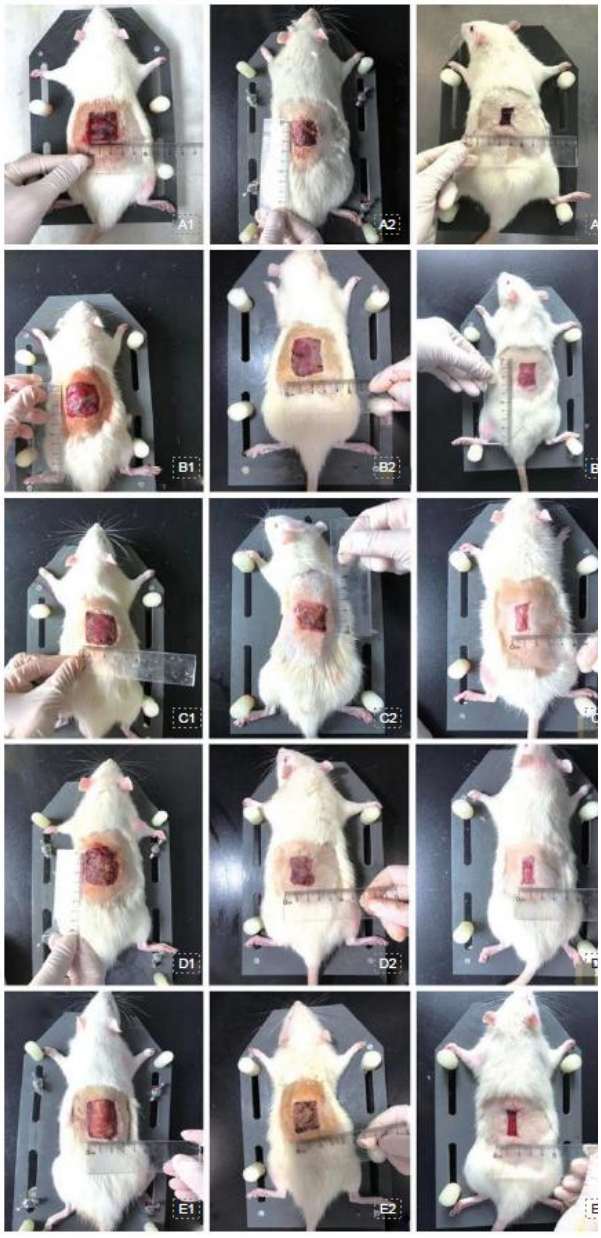
2.3 Comparison of wound surface gross condition and wound healing rate in each group: After 3 days of intervention, The wound surface of rats showed no obvious shrinkage in gross observation, and scattered skin

Island, wound edge showed different degrees of exudation, edema and a small amount of bleeding, diabetic model A small amount of purulent secretion was observed under the wound margins of rats in the group; after intervention for 7 days, The wound surface of rats in the group was reduced visibly, and some wounds were covered with a thin layer of scab. A small amount of granulation tissue can be seen, and the exudation and edema have improved to varying degrees compared with before. The number of skin islands increased compared with the previous observation, with the blank control group having the highest degree, and the diabetic model The wounds of rats in the dry type group were the lowest, and the wounds of rats in each group gradually healed over time. On day 14 of the pre-treatment period, the wounds in the blank control group, DDW group, PRP group, and DDW+PRP group showed The wound surface shrunk, the exudation and edema subsided significantly, and some wounds dried and scabbed. The wound surface of rats in the diabetic model group was significantly reduced, and there was still a small amount of The wound surface edema was reduced compared with the previous group, and the degree of healing was lower than that of the other groups. Figure 1.

After 3 days of intervention, the diabetic model group, DDW group, PRP group, and DDW+PRP group The wound healing rates of the two groups were significantly lower than those of the blank control group, and the difference was statistically significant. yP ÿ0.05); the wound healing rate in the DDW+PRP group was higher than that in the diabetic model group, The difference was statistically significant (P ÿ0.05); diabetic model group, DDW group, There was no statistically significant difference between the two PRP groups. P ÿ0.05). Each group The healing order was blank control group > DDW+PRP group > PRP group > DDW group > diabetes

Disease model group.

At 7 days after intervention, the wound healing rate of the blank control group was higher than that of the diabetic model group and the DDW group. There were significant differences among the PRP group, DDW+PRP group (ÿ0.05);



Note: A. Blank control group; B. Diabetes model group; C. DDW group; D. PRP group; E. DDW+PRP group Group. 1.3d; 2.7d; 3.14d

Fig.1 Wound healing of rats in each group at different time points

The wound healing rate of the diabetes model group was lower than that of the DDW group, PRP group, and DDW+PRP group. There is statistical significance (Pÿ0.05); Pairwise comparison among DDW group, PRP group, and DDW+PRP group The difference was not statistically significant (Pÿ0.05). Control group > DDW+PRP group > PRP group > DDW group > diabetes model group.

At 14 days after intervention, the wound healing rate of the blank control group was higher than that of the diabetic model group and There was a statistically significant difference between the DDW group and the PRP group (P ÿ0.05); diabetes. The wound healing rate in the model group was lower than that in the DDW group, PRP group, and DDW+PRP group. There is statistically significant difference (P ÿ0.05); wound healing rate in DDW+PRP group. Higher than the DDW group and PRP group, the difference was statistically significant (P ÿ0.05ÿÿ Although the wound healing rate of the DDW+PRP group was lower than that of the blank control group, the two groups There was no statistically significant difference (P ÿ0.05), at which time the wound healing rate reaches a high The healing order of each group was blank control group > DDW+PRP group > PRP group > DDW Group>diabetes model group. See Table 2 and Figure 2.

Table 2 Comparison of wound healing rates in rats of each group at different intervention time points ($\bar{x}$±s, %)

Number of groups		3 days after intervention	7 days after intervention	14 days after intervention
Blank control group	6	10.87±1.40	50.42±6.23	84.49±1.38
Diabetes model group	6	3.60±2.12a	23.45±4.65a	61.25±2.71a
DDW Group	6	5.40±2.79a	40.84±2.04ab	71.09±4.76ab
PRP group	6	5.64±0.78a	41.56±3.05ab	71.80±4.43ab
DDW+PRP Group	6	6.34±0.32ab	43.78±3.49ab	80.53±4.56bcd
F_{value}		14.76	34.85	34.05
P_{value}		ÿ0.05	ÿ0.05	ÿ0.05

Note: a means compared with the blank control group. P ÿ0.05; b means compared with the diabetes model group, P ÿ0.05; c means compared with the DDW group, P ÿ0.05; d means compared with the PRP group, P ÿ0.05

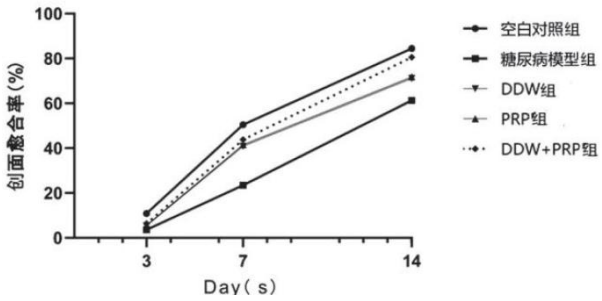


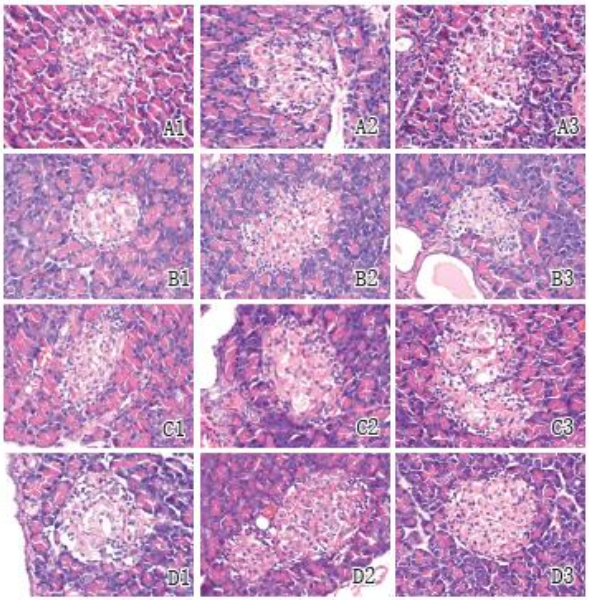
Figure 2 Schematic diagram of wound healing rate of rats in each group at different intervention time points

2.4 Histological changes of pancreatic islets in each group of rats: HE staining of pancreatic tissue of rats Under the microscope, the number of pancreatic islets in the blank control group was large, and no islet rupture was observed. The number of pancreatic islet cells is large, and they are evenly and closely arranged, with clear boundaries between cells.

The cytoplasm is evenly lightly stained, the nucleus is clear and oval, and the nucleolus is visible. Prognosis 3d, diabetes model group, DDW group, PRP group, DDW+PRP group and control group Compared with the control group, the number of islets decreased, the size became smaller, the boundaries were unclear, and some Hyalinized material can be seen under the capillary basement membrane, and the cells in the islands are sparsely arranged. Sparse, cytoplasmic vacuoles increased, cytoplasm light color, nuclear condensation, some cells The nucleus is missing, and the chromatin is unevenly distributed and marginalized. Seven days after intervention, the islets in the DDW group and DDW+PRP group were higher than those before intervention. The number of cells increased slightly, the size decreased, and the cell arrangement structure was different from that before the intervention. The cell boundaries are clearer than before, the cytoplasmic vacuolar changes are reduced, and the staining particles are The distribution of granulocytes was slightly improved, and the lymphocyte infiltration around the islets was reduced. 14 days after intervention, the islets in the DDW group and DDW+PRP group were higher than those before intervention.

The number of cells increases, the cells are evenly arranged, the borders are clearly visible, and the cytoplasmic vacuoles The cytoplasm is evenly lightly stained, the nucleus is clear, and the chromatin is evenly distributed. The lymphocyte infiltration around the islets decreased. The cells showed obvious differences in morphology, number, arrangement, and distribution of staining particles. Significant improvement. After 3d, 7d, and 14d of intervention, the pancreas of rats in the diabetes model group and PRP group The islet tissue is severely damaged, the islet cells are enlarged, and the arrangement of cells is obviously disordered. The cytoplasm is vacuolated or loosely reticular, with granular staining. The granules are unevenly distributed, some nuclei are condensed and lost, and the lymphocytes around the pancreatic islets There is more infiltration, and

there is no obvious improvement compared with the previous one. See Figures 3-4.



Note: A. Diabetes model group; B. DDW group; C. PRP group; D. DDW+PRP group. 1.3d; 2.7dÿ3.14d

Fig. 4 Pathological changes of pancreatic islets in rats of each group at different intervention time points (HE, 400×)

2.5 Effects of TGF-ÿ1 on wound tissue of rats in each group: diabetic model group, The levels of TGF-ÿ1 in wound tissues of rats in DDW group, PRP group and DDW+PRP group were significantly different on 3d, The levels at 7d and 14d were lower than those in the blank control group, and the difference was statistically significant (ÿ0.05); The rats in the DDW group, PRP group, and DDW+PRP group had The amount was higher than that of the diabetic model group, and the difference was statistically significant (Pÿ0.05ÿÿ)

The content of PRP group was higher than that of DDW group 3 days after intervention, and the difference was statistically significant. righteous(Pÿ0.05), there was no statistically significant difference between the two groups at 7d and 14d after intervention. (Pÿ0.05). There was no statistical difference between the DDW+PRP group and the PRP group 3 days after intervention. Calculation significance (P ÿ0.05); DDW+PRP group had a difference in wound surface area at 7d and 14d after intervention The content of TGF-ÿ1 in the tissue was significantly higher than that in the DDW group and PRP group. Significance P(ÿ0.05). See Table 3 and Figure 5.

3 Discussion

Wound healing is a complex and orderly biological process, including the initial Injury and rapid hemostasis, inflammation, proliferation, and maturation stages, Under normal coordination, the healing process is highly orderly and complete[12]. Correlation between delayed wound healing and neurovascular lesions and low expression of multiple growth factors Insulin resistance and hyperglycemia further delay healing.

Table 3 Changes of TGF-ÿ1 content in wound tissue of rats in each group at different intervention time poi

Number of groups	3 days after intervention	7 days after intervention	14 days
Blank control group	6 2 315.44±270.34	2 349.06±187.07	2 311.18±153.03
Diabetes model group	6 860.13±208.29a	930.20±227.82a	854.65±138.81a
DDW Group	6 1 420.62±55.81ab	1 506.27±71.21ab	1 516.32±80.84ab
PRP group	6 1 598.01±69.53ab	1 576.69±126.53ab	1 493.46±64.03ab
DDW+PRP group	6 1 767.48±108.26a	b c 1 969.73±173.74abcd	1 771.57±108.3
F value	61.66	61.54	127.7
P value	ÿ0.05	ÿ0.05	ÿ0.05

Note: a means compared with the blank control group. P ÿ0.05; b means compared with the diabetes model

c indicates comparison with DDW group, P group, ÿ0.05; d means compared with the PRP group, P ÿ0.05

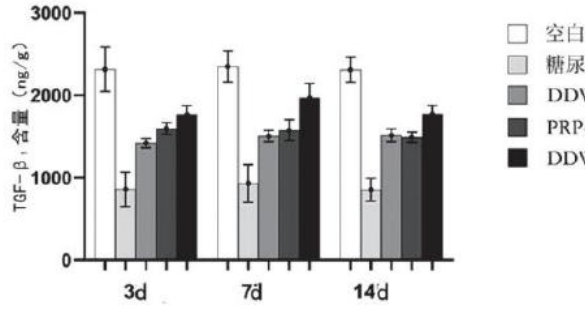


Fig.5 Histogram of TGF-ÿ1 content in wound tissue of rats in each group at different intervention tim

The centrifuged extract is rich in high concentrations of platelets, white blood cells, fibrin, etc. Platelet concentrate. Activated platelets degranulate and release TGF-ÿ, PDGF, IGF, EGF, VEGF and other growth factors work synergistically with each other Promote cell proliferation and differentiation, accelerate angiogenesis and tissue repair[13]. Studies have shown that TGF-ÿ1 expression is decreased in tissues with impaired wound healing[1]. These observations are consistent with the observations in this study. The expression of TGF-ÿ1 in diabetic rats in PRP and DDW groups was low. The expression level was increased, and compared with the diabetic model group in the same period, the granulation tissue of the wound The production increases, exudation and edema subside faster, and the wound healing rate is significantly improved. Compared with the single intervention group, the TGF-ÿ1 expression of rats in the DDW+PRP combined intervention group was significantly higher than that in the single intervention group. The amount of TGF-ÿ1 was found to be involved in Various processes of wound healing, such as the synthesis of extracellular matrix (ECM), ÿ-Expression of smooth muscle actin (ÿ-SMA) and differentiation of fibroblasts. Active TGF-ÿ1 can stimulate fibroblasts to transform into myofibroblasts after skin injury cells, which are characterized by higher expression of ÿ-SMA compared to before injury [14]. TGF-ÿ1 synergistically promotes fibroblast contraction of collagen, thereby accelerating wound healing Tsai et al. [16] reported that activated PRP contains a large amount of fibrin. Interweaving to form a mesh structure to form a biological scaffold, pulling the wound edge to the center of the wound The experiment found that after intervention with deuterium-depleted water The levels of TGF-ÿ1 protein and wound healing rate in rats after 4-6 weeks of follow-up were significantly increased, which was consistent with the results of blood enrichment. There was no statistically significant difference between the two groups in the platelet plasma intervention group.

Deuterium-depleted water has antioxidant, anti-depression, anti-tumor, and blood sugar lowering effects. A series of biological effects

can be used to treat related diseases[7-8]. Zlatska et al.[17] It was found that DDW has a stimulating effect on the proliferation of human dermal fibroblasts in vitro. Studies have shown that [7] DDW can improve the growth of 12-week-old Insulin level in Wistar-Kyoto rats and decreased blood triglycerides and cholesterol.

In another study, plasma glucose and protein levels were found in rats drinking DDW. The total amount of protein and its glycosylation degree were significantly reduced[18]. Zhou Zhenyu et al.[9] found that low Deuterium liquor can reduce the fasting blood glucose level and increase the fasting serum pancreatic Insulin levels; Histological observations showed that low-deuterium liquor can alleviate and repair damage This study found that the diabetic rats treated with deuterium-depleted water The level of TGF-ÿ1 protein in mice increased, and the wound healing rate was compared with that in the diabetic model group. The healing speed was accelerated, and the random blood sugar decreased significantly at 14 days. It can be related to reducing insulin resistance and increasing insulin secretion. The staining showed that with the advancement of intervention time, the morphology, number, The arrangement and distribution of dyed particles have been improved to varying degrees.Deuterium water can protect and repair damaged pancreatic islet cells and reduce the random Blood sugar level, accelerate the healing of ulcer wounds in diabetic rats. There was no significant difference in TGF-ÿ1 protein level and wound healing rate between the PRP group and the PRP group. The DDW+PRP group showed an increase in TGF-ÿ1 protein expression. The most obvious was that at 14 days after intervention, the degree of wound healing was the best compared with the DDW group and PRP group. High, the difference is statistically significant.

In summary, deuterium-depleted water combined with platelet-rich plasma can reduce the Random blood sugar levels have a certain protective and repairing effect on pancreatic islet cells. Significantly promote the healing of ulcer wounds, which may be related to increasing the expression of TGF-ÿ1 in wound tissue The specific mechanism needs further study and exploration.

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