

Supplemental Information

Hydrogen Attenuates Inflammation by Inducing Early M2 Macrophage Polarization in Skin Wound Healing

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Results

H₂ inhibited the systemic inflammatory response

Skin defects may cause systemic inflammatory responses, so we performed routine blood tests to determine the number of leukocytes and the percentages of neutrophils, lymphocytes, and monocytes in the blood of each group on the first three days (Fig. 4). The number of leukocytes in the blood corresponds to the state of systemic inflammation of the organism. From day 1 to day 3, no significant difference was observed in Leukocyte counts in all groups (Fig. 4A). Neutrophils remove cellular debris and release cytokines to create conditions for subsequent repair. The proportion of neutrophils in the H₂ group gradually decreased from 24 h to 72 h; at 48 h, the proportion of neutrophils was higher in the H₂ and NAC groups than in the control group ($P < 0.01$ between the NAC group and the control group), while at 72 h, the proportion of neutrophils in the H₂ group was lower than in the control group (Fig. 4B). The proportion of lymphocytes in the NAC group was significantly lower than in the control group ($P < 0.01$) and no significant difference was observed compared with the H₂ group at 48 h; at 72 h, the proportion of lymphocytes was higher in the H₂ group than in the other two groups (Fig. 3C), and no significant differences observed. At 72 h, monocyte counts in the H₂ group were lower than those in the control groups ($P < 0.052$). Therefore, taking insight into the blood cell count results, systemic inflammatory responses in the H₂ group have no obvious changes at the first 72 h, while the monocytes number in H₂ group was slightly decreased on the 72 h.

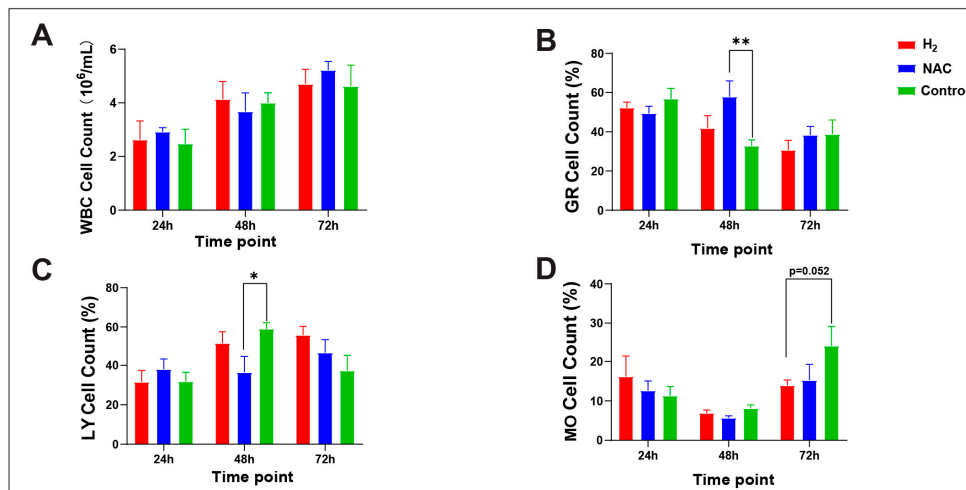


Figure S1. Routine blood tests during the inflammatory stages of wound healing. Routine blood tests of the three groups. **(A)** White blood cell (WBC) count. **(B)** Granulocytes (GR). **(C)** Lymphocytes (LY). **(D)** Monocytes (MO). Percentages in whole blood at 24, 48, and 72 h after wounding. Data were analyzed by two-way ANOVA and plotted as mean±SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.