Ancillary Experiments on Four Single Herbs

1. Materials and Methods

1.1Protective effects of four single herbs for containing sera against $Na_2S_2O_4$-Induced Hypoxia damage in PC12 Cells

The PC12 cells were inoculated onto 96-well plates for experiments after growth to logarithmic growth phase. All cells were randomly divided into fifty groups. Each group was inoculated into four wells, and RPMI-1640 culture solution containing 5% corresponding serum was added. The groupings were as follows: (1) blank control group; (2) model control group; (3)~(14): Da Huang-containing serum groups; (15)~(26): Ren Shen-containing serum groups; (27)~(38): Ge Gen-containing serum groups; and (39)~(50): Chuan Xiong-containing serum groups. Groups (1) and (2) were incubated with RPMI-1640 medium containing 5% blank serum, while groups (3)~(50) were incubated with RPMI-1640 medium containing 5% of the corresponding drug-containing serum at different time points and preprotected for 2 h. In addition to the blank control group, groups (2)~(50) were treated with $Na_2S_2O_4$ solution to a final concentration of 15 mmol/L for 4 h to induce PC12 cell damage. Subsequently, the original cell culture medium containing $Na_2S_2O_4$ solution was removed, and RPMI-1640 medium without calf serum was added to every well. The cells were maintained at 37ºC in a humidified atmosphere containing 5% CO_2 for 24 h. Cell viability was determined by MTT reduction assay, and the efficacy-time curves were plotted. The experiment was repeated three times.

1.2 Protective effects of four single herbs for containing sera against Glutamate-induced neurotoxicity damage in PC12 Cells

The groupings in this experiment are the same as those described above. After all drug-containing serum groups were preprotected for 2 h, in addition to the blank control group, groups (2)~(50) were treated with Glu solution to a final concentration of 25 mmol/L for 1 h to induce neurotoxicity damage. Subsequently, the original cell culture medium containing Glu solution was removed, and RPMI-1640 medium without calf serum was added to every well. The cells were maintained at 37ºC in a humidified atmosphere containing 5% CO_2 for 24 h. Cell viability was determined by MTT reduction assay, and the efficacy-time curve was plotted. The experiment was repeated three times.

1.3 Protective effects of four single herbs for containing sera against Potassium chloride -induced calcium overload injury in PC12 Cells

The groupings in this experiment are the same as those described above. After all drug-containing serum groups were preprotected for 12 h, in addition to the blank control group, groups (2)~(50) were treated with KCl solution to the final concentration of 75 mmol/L for 4 h to induce calcium overload damage. Subsequently, the original cell culture medium containing KCl solution was removed, and RPMI-1640 medium without calf serum was added to every well. The cells were maintained at 37ºC in a humidified atmosphere containing 5% CO_2 for 24 h. Cell viability was determined by MTT reduction assay, and the efficacy-time curve was plotted. The experiment was repeated three times.

1.4 Protective effects of four single herbs for containing sera against Hydrogen peroxide-induced free radical damage in PC12 Cells

The groupings in this experiment are the same as those described above. After all drug-containing serum groups were preprotected for 12 h, in addition to the blank control group, groups (2)~(50) were supplemented with H_2O_2 solution to a the concentration of 150 μmol/L for 2 h to induce free radical damage in PC12 cells. Subsequently, the original cell culture medium containing H_2O_2 solution was removed, and RPMI-1640 medium without calf serum was added to every well. The cells were maintained at 37ºC in a humidified atmosphere containing 5% CO_2 for 24 h. Cell viability was determined by MTT reduction assay, and the efficacy-time curve was plotted. The experiment was repeated three times.

2. Results

2.1Protective effects of four drug-containing sera on PC12 Cell damage induced by Sodium Dithionite

Cell viability of PC12 cells, measured by MTT reduction assay. As shown in Fig. 1, by observing the efficacy-time curves, it was found that rhubarb-containing serum had a weak protective effect on hypoxic injury within 5 min~1 h, and the effect began to decrease after 1.5 h. Ginseng-containing serum had a protective effect within 0.5 h ~ 6 h; there was only one peak, at 1.5 h, which demonstrated a significant improvement of 16.17% in PC12 cell viability compared with that of the control group. The experimental results show that Pueraria-containing serum had different degrees of protection at all time points on cells suffering hypoxic injury. There are three effect peaks, and the cell viability at 15 h was increased by 10.51% compared with that of the control group. Chuanxiong-containing serum had a weaker protective effect on cells suffering hypoxic injury, and after 2 h the effect gradually reduced to zero. The results are shown in Table 1 and Fig. 1.

2.2 Protective effects of four drug-containing sera on PC12 Cell damage induced by Glu

The cell viability of PC12 cells was, measured by MTT reduction assay. As shown in Fig. 2, by observing the efficacy-time curve of Rhubarb, it was found that there is only one effect peak at 1 h, which illustrates that the protective effect has reached the maximum efficacy. The results showed that the cell survival rate at the effect peak improved significantly, by 15.21%, compared with that of the control group. The protective effect of Ginseng-containing serum on anti-glutamate neurotoxicity was not obvious. The drug works quickly, but the effect was gradually reduced to none after 0.5 h, and the cell survival rate at the effect peak at 15 min was slightly higher than that of the control group. The Pueraria-containing serum had no obvious protective effect on Glu-damaged cells at all time points. The efficacy-time curve of Chuanxiong xhibited three effect peaks, especially the peak at 1 h. The results showed that the cell survival rate of the effect peak at 1 h improved significantly, by 20.71%, compared with that of the control group. The results are shown in Table 2 and Fig. 2.

2.3 Protective effects of five drug-containing sera on PC12 Cell damage induced by Potassium chloride

Cell viability of PC12 cells, was measured by MTT reduction assay. As shown in Fig. 3, rhubarb-containing serum has a weak protective effect on anti-calcium overload injury within 0.5~1.5 h and no protective effect two hours later. The protective effect of

Ginseng-containing serum was not obvious, and almost no protection was observed two hours later. Pueraria-containing serum also had no obvious protective effect against KCl-induced cell injury. For Chuanxiong, it was found that there was only one effect peak at 1.5 h. The main protective effect of Chuanxiong was observed within 0.25~4 h and gradually reduced after 6 h. The results showed that the cell survival rate at the effect peak at 1.5 h improved significantly, by 18.49%, compared with that of the control group. The results are shown in Table 3 and Fig. 3.

2.4 Protective effects of four drug-containing sera on PC12 Cell damage induced by Hydrogen peroxide

Cell viability of PC12 cells, was measured by MTT reduction assay. As shown in Fig. 4, by observing the efficacy-time curve of Rhubarb, it was found that there are two effect peaks. The cell protection rate of the effect peak at 45 minutes was significantly higher, by 18.49%, than that of the control group. There is a weak protective effect of Ginseng-containing serum against H_2O_2-induced cell injury within 5 min~1 h. The protection rate was maintained at a high level up to 1h and disappeared after 1.5 h. The results show that Pueraria-containing serum had an obvious protective effect against H_2O_2-induced cell injury within 15 min~2 h. The cell protection rate of the effect peak at 1.5 h was significantly higher, by 19.05%, than that of the control group. The Pueraria-containing serum worked quickly, and the protection rate was maintained at a high level within 2 h. The experimental results show that Chuanxiong-containing serum had no obvious protective effect against H_2O_2-induced cell damage. The results are shown in Table 4 and Fig. 4