Proteomics System of MEBO in Treating Diabetic Foot of Silkworm

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Abstract: Objective: Moist Exposed Burn Ointment (MEBO) is a kind of external preparation widely used in skin burn. We found that MEBO can also promote the healing of diabetes mellitus in silkworm feet. In order to further understand the mechanism of MEBO promoting skin healing, lable-free quantitative proteomics analysis is used to detect differential protein between MEBO groups and blank groups.

Results

After MEBO treatment, the proportion of diabetic foot in the MEBO treatment group was 14.63%, that in the high glucose control group was 26.88%, and that in the blank control group was 10.00%. The histological study showed that 254 differentially expressed proteins were obtained in the blank control group as compared with the MEBO-treated group, of which 193 were up-regulated by more than 2.0-fold and 61 were down-regulated by less than 0.5-fold. Gene Ontology and KEGG analysis shows that these differential proteins are related to many signaling pathways. Finally, it was confirmed by parallel reaction monitoring that MEBO intervention in diabetic foot by affecting nitrogen metabolism signaling pathway, promoting wound healing.

Conclusion

MEBO promotes skin healing of diabetic silkworm feet, which is very similar to the mechanism of MEBO in promoting skin healing of diabetic mice. This will provide a new low-cost animal model for screening diabetic foot drugs or therapies in the future, and will be conducive to overcoming diabetic foot. CLC number: R285. 5 Document code: A

Introduction

Diabetic foot is one of the common refractory chronic complications of diabetes, which has an incidence rate up to 25% [1], and more than 15% of the patients are at risk of amputation or even death at any time [2]. Such disease brings heavy economic pressure to the society. Recent years witness increasing incidence of diabetic foot ulcers. Because of the complex causes of diabetes, there are currently no radical medicines or treatments. Therefore, it is very urgent to develop a therapy or a drug for diabetic foot ulcer treatment with good effects. At present, scholars and medical workers have proposed a variety of drugs and therapies [3-4]. Where, MEBO-DFU regenerative therapy has achieved wound healing in the treatment of diabetic foot with significant curative effect achieved, thus avoiding amputation [5-6]. The proteomic label-free analysis technique was used to study the blank control group, high glucose control group and MEBO treatment group [7-8], in order to understand the action mechanism of MEBO in promoting wound healing of diabetic foot skin ulcers in silkworm, thereby establishing a low-cost, fast and reliable animal model on therapy or drugs for screening treatment of diabetic foot.

1. Results

2.1 Establishment of animal model

   The silkworms were divided into three groups. One blank group was fed with common mulberry leaves, and the other groups were fed with glucose solution-sprayed mulberry leaves to establish a diabetes model. After feeding the glucose solution-sprayed mulberry leaves for 12 hours, the tissue glucose increased and the model was established. Wounds were formed in the legs for all the two groups to create diabetic foot infection conditions in the first day of fifth instar penetrating silkworm feet, and MEBO group smeared with MEBO, the thickness of the wounds was 0.1mm. The tissue glucose was measured and the MEBO was applied once every 12 hours. After the experiment, the number of diabetic foot and rotten foot in the two groups of silkworms were numerically compared.
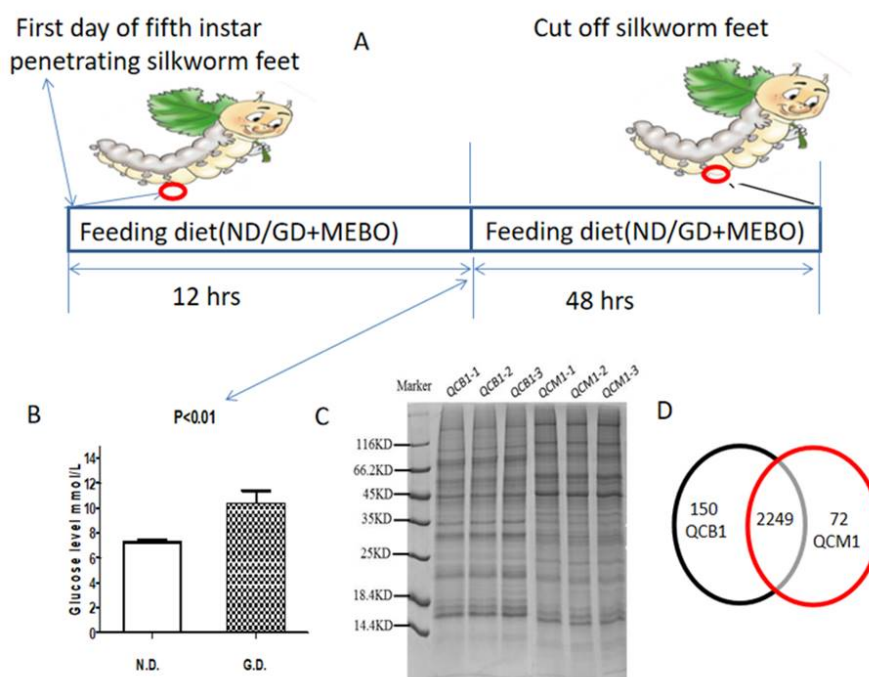

Figure 1 General workflow of Diabetic foot ulcer model

(Note: A: Establishment of animal model B: Changes in tissue fluid glucose of silkworm in each group. C: SDS-PAGE electrophoresis pattern of each sample (loading amount 300μl). D: QCB1 represents the first blank group, QCM1 represents the MEBO group, and the following 1, 2 and 3 represent three parallel samples for detection.)

2.2 Preliminary verification of differences

2.2.1 Changes in tissue fluid glucose of silkworm

   As shown in the figure 1B, the data show that MEBO has a significant ($P<0.01$ (student T test or One-way ANOVA)) effect on tissue glucose of diabetic silkworm, the glucose level of tissue increased exceeded 10 mmol/L after 12 hours of feed GD [9].

2.2.2 SDS-PAGE electrophoresis pattern of each sample (loading amount 300μl).

   The SDS-PAGE shows preliminary analysis of protein abundance distribution map of each group by electrophoresis. As shown in the figure 1C, when protein molecular weight of the diabetic group and the MEBO group is significantly higher than that of the blank group at 116 KD, 45 KD segments, and significantly lower than that of blank group at 35 KD and 14.4 KD segments.

2.2.3 Summary of Protein Identification Results

   A total of 2471 proteins were identified in this project. Results Venn diagram shows the overlap of protein identification between the two groups as shown in Fig 1D [10].

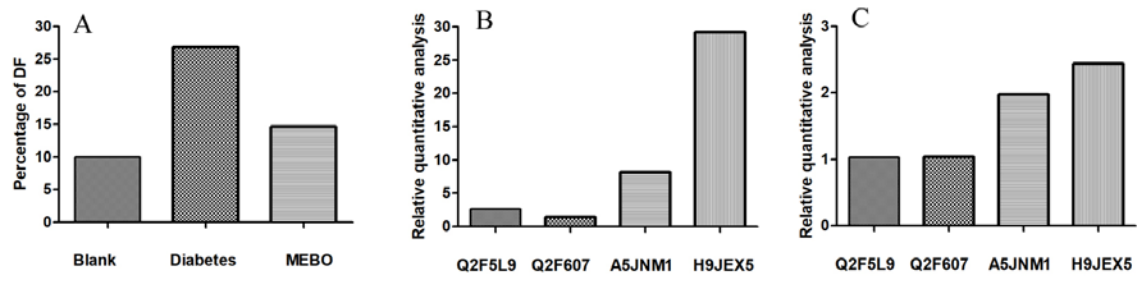## 2.2.4 Changes in the prevalence rate of silkworm foot ulcers in each group after administration

Figure 2 Confirmation and analysis of diabetic foot in silkworm

(Note: A: The percentage of diabetic foot in blank group, Diabetes and MEBO group. B: Differential protein by Parallel Reaction Monitoring between blank and diabetic. C: Differential protein by Parallel Reaction Monitoring between blank and MEBO.

After puncture, good skin healing of silkworm pupae is defined as non-diabetic foot, and diabetic foot is defined as the occurrence of black and necrosis of foot skin tissue. The percentage of diabetic foot (DF) is equal to the number of silkworms with diabetic foot divided by the total number of silkworms alive.

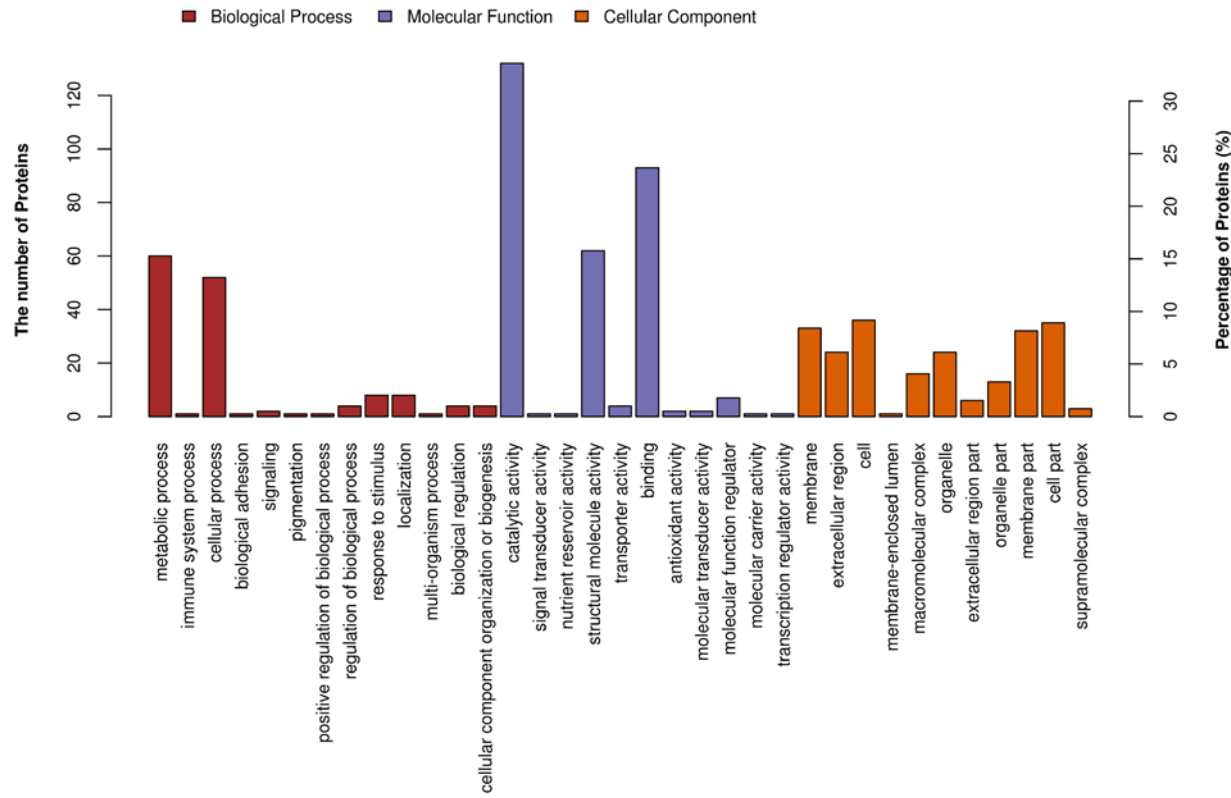## 2.3 Gene Ontology (GO) functional enrichment analysis on differentially expressed protein

Figure 3 Gene Ontology (GO) function enrichment analysis

As shown in Figure 3, the analysis results show that in the comparison groups QCM1 and QCB1, the differential proteins are mainly involved in the important biological processes like metabolic process, immune system processes, cellular processes, etc.

2.4 Verification of protein by Parallel Reaction Monitoring（PRM）

Through literature research, PRM determination of wound healing-related signaling pathways is planned. The principle of selecting target proteins is the conclusion of binding proteomics research, and considering the abundance of differentially expressed proteins, comprehensive consideration is made to determine Q2F5L9, A5JNM1, Q2F607, H9JEX5. The content of protein H9JEX5 in MEBO group was significantly higher than that in other two groups, in the target peptide segment GLLSNLNLPLVDNFR. Same thing as above, the content of protein H9JEX5 in MEBO group was significantly higher than that in other two groups, in the target peptide segment QQSPIAISAR. Similarly, the content of protein Q2F607 in MEBO group was significantly higher than that in other two groups for protein sequences GPGDTSNFDDYEEEALR and VVQLLPFVQHK, respectively.

3 Discussion

In this study, MEBO was used to treat diabetic foot ulcers in silkworms. It was found that it could significantly reduce the occurrence of diabetic foot in silkworm. The possible influence signal pathways are as follows:

Nitrogen metabolism pathway is an important marker affecting diabetic foot [11]. NO is an important potential regulator after burn, and NO content is significantly reduced after burn. This is basically consistent with the results of this study. The content of the proteins Q2F607 and H9JEX5 intervened by MEBO was significantly higher than that of the high glucose group in Figure 2B and 2C. This paper confirmed the existence of nitrogen metabolism-related proteins in the skin of silkworm foot, and the expression changes of such protein was consistent with nitrogen metabolism of wound tissue before and after burn.

Transforming growth factor (TGF-beta) signaling pathway plays an important regulatory role in wound healing. This study found that the TGF-β signaling pathway-associated protein Q2F5L9 extracted from the silkworm foot skin had increased expression on the TGF-β signaling pathway after MEBO treatment [12].

5 Conclusion

MEBO can significantly reduce the probability of silkworm suffering from diabetic foot, and its chance of suffering from diabetic foot is close to that of blank control group. Through proteomic analysis, the mechanism by which MEBO reduces the risk of diabetic foot in silkworm may be through the nitrogen metabolism and TGF-β, promoting wound healing. In the future, our group will conduct further in-depth researches.

Acknowledgments

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