Bioavailability Evaluation of a New Compound Bone Peptide Formula

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Abstract: To investigate the contents of hydroxyproline (Hyp) and Glycine-Proline- Hydroxyproline (Gly-Pro-Hyp) in the serum of C57/BL mice after the new compound bone peptide formula was gavaged. The bioavailability and utilization efficiency of the new compound bone peptide formula were evaluated by the contents of above substances in the serum. Moreover, the area under the drug time curve (AUC) was analyzed and compared to explore the effective exposure amount of the new bone peptide formula. The results showed that the contents of Hyp and Gly-Pro-Hyp increased in a dose-dependent manner in the serum. The contents of Hyp and Gly-Pro-Hyp were the highest after 3 h and remained in 24 h. The results indicated that the utilization rate of bone peptide samples was significantly higher than that of bone peptide tablet and bone peptide injection. The AUC of Hyp and Gly-Pro-Hyp in the low-dose bone peptide sample group were significantly higher than that of clinical dose bone peptide samples. AUC of Hyp and Gly-Pro-Hyp in the low-dose bone peptide sample group was significantly higher than those in the clinical dose bone peptide injection and bone peptide tablet groups, indicating that the clinically dose low-dose bone peptide tablet had a good effective exposure to efficacy.

1 INTRODUCTION

Collagen is one of the main macromolecules that constitutes the extracellular matrix. Bovine bone peptide is an active peptide obtained by enzymatic digestion of bovine collagen, which completely opens the triple helix structure of bovine collagen, and the peptide chain is degraded into short peptide chains to obtain a peptide mixture with a molecular mass of several thousand daltons.

These small molecule peptides are compatible with living organisms and have better nutritional functions than proteins and amino acids (Ahn 2019, Bello 2006, Cao 2020, Lee 2019). At the same time, it has good solubility and stable physical properties. The small molecular peptides obtained from different sources and different enzymatic hydrolysis processes have different biological activities, such as immune regulation, lowering blood pressure, regulating blood sugar and blood lipid, anti-aging, anti-oxidation, anti-cancer, anti-microbial, anti-toxin, increasing bone density and improving bone toughness (Li, Wu 2018, Pountos 2016, RosanoBraun 1987). This study investigated the bioavailability of bovine osteoptin in mice after administration.

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Bone peptide is rich in a variety of non-essential amino acids, such as glycine (Gly), proline (Pro) and hydroxyproline (Hyp). Hyp is the main component of collagen tissue, and it is a specific amino acid in collagen, accounting for about 13% of the total collagen amino acid (SatoAsai 2020, Wang 2020, Watanabe 2010). Hyp in blood is a degradation product of various collagen peptides and is used as a marker of bone absorption. By measuring the content of hyp in the serum, the absorption and bioavailability of bone peptide in mice after sample administration can be measured (Yazaki 2017). In addition, previous studies have shown that mice can directly absorb collagen Gly-Pro-Hyp in the bone peptide sample polypeptide mixture. Therefore, the measurement of collagen peptides in mice is another key indicator to evaluated bone peptide absorption and bioavailability. In this study, the contents of Hyp and Gly-Pro-Hyp in mouse plasma were measured after administration for different times. The bioavailability of various bone peptide samples was evaluated by simulating the area model under the curve of drug time, which provided basic research basis for the development and application of new bovine bone peptide products.

2 MATERIALS AND METHODS

2.1 Material

Bone peptide composite powder (Hangzhou baibeiyou Biotechnology Co., Ltd.); Bone peptide tablet (Jilin Huakang Pharmaceutical Co., Ltd. National medicine quasi H20058927); Injection of compound bone peptide (Nanjing Xinha Pharmaceutical Co., Ltd. National medicine quasi H20003533). All samples are stored at 4℃.

2.2 Determination of Serum Index

Preparations of serum: the blood was taken from the aorta of mouse eyeball and placed in a centrifugal tube with plug. After standing at room temperature for 30 min, the blood was centrifuged at 4000 rpm for 10 min. The upper serum was slowly taken out by pipette and placed in another centrifuge tube with plug for determination of serum indexes.

2.3 Detection of Hyp in Serum

Healthy C57/BL mice were selected and divided into 6 groups: blank control group, positive pharmaceutical control group 1 (bone peptide injection group), positive pharmaceutical control group 2 (bone peptide group), low (1.6 g/kg), medium (3.2 g/kg) and high (4.8 g/kg) dose of bone peptide. After adaptive for a week, 20 mice in each group were gavaged once. 4 mice were sacrificed at 1, 3, 6, 12, 24 h in in each group and the plasma of each mouse was obtained. Then the content of hyp in the serum was determined, and the bioavailability of the samples was analyzed. The AUC of Hyp was calculated to compare the effective exposure amount of drug absorption.

2.4 Detection of Gly-Pro-Hyp

The mice of positive pharmaceutical control groups and the experimental groups were gavaged and sacrificed the same as above. The content of Gly-Pro-Hyp in the serum was determined, and the bioavailability of the samples was analyzed. The AUC of Gly-Pro-Hyp was calculated to compare the effective exposure amount of drug absorption.

3 RESULTS AND ANALYSIS

3.1 Determination of Hyp Content in the Serum of Mice

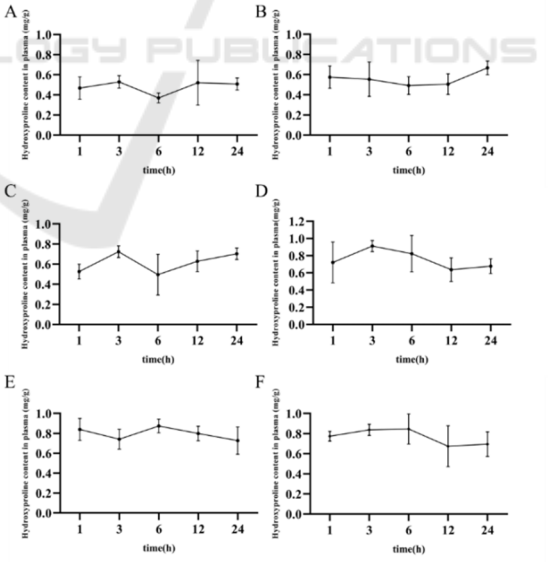
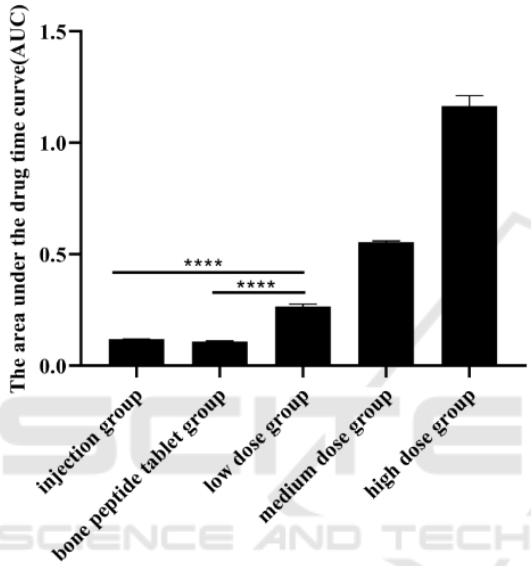


Figure 1: The content of Hyp in serum A: Blank group; B: Low dose group (1.6 g/kg); C: Medium dose group (3.2 g/kg); D: High dose group (4.8 g/kg); E: Bone peptide tablet group (576 mg/kg); F: Injection group (90 mg/kg).

The content of Hyp in the serum of the experimental group can be obtained in Figure 1. The level of Hyp

was increased in a dose-dependent manner in the serum. The content of Hyp in the serum of bone peptide samples was reached the highest level after 3 h, and the Hyp level remained high with the extension of time, and no significant decrease of Hyp occurred after 24 h. It indicated that the utilization rate of compound bone peptide samples was significantly higher than that of bone peptide tablet and bone peptide injection.

3.2 Calculation of AUC of Serum Hyp in Mice



(**** P <0.0001)

Figure 2: The AUC of Hyp in the serum of mice.

The AUC is the area under the curve obtained by taking the blood concentration after administration as the ordinate and time as the abscendant. In this study, the integral method is used to obtain AUC, which indicate that the drug absorbs in a certain amount of time after taking a certain dose drug and is proportional to the amount of drug absorbed by the organism.

As shown in Figure 2, the AUC of Hyp was increased in a dose-dependent manner. And the AUC of low, medium, high-dose bone peptide samples of Hyp is much higher than the clinical dose bone peptide injection group and the bone peptide tablet group. It proved that the advantage of effective absorption of bone peptide samples. The results showed that the effective exposure of clinical dosage - low dose groups was significantly higher than that of the bone peptide injection and bone peptide tablet.

3.3 Determination of Gly-Pro-Hyp Content in the Serum of Mice

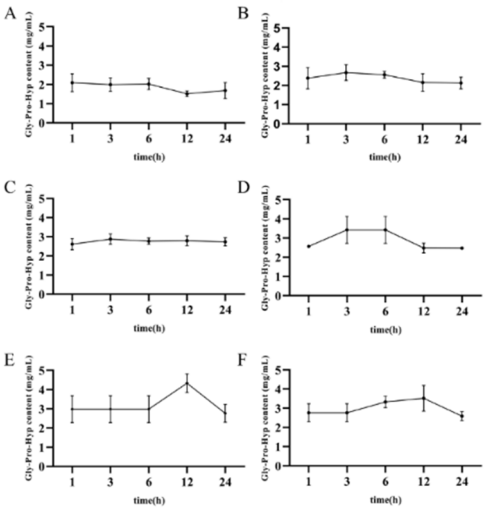
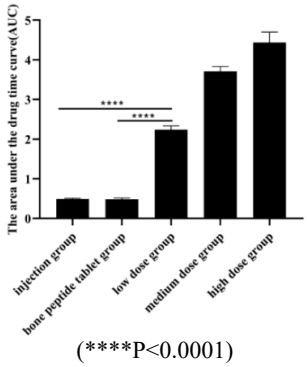


Figure 3: The content of Gly-Pro-Hyp in serum A: Blank group; B: Low dose group (1.6 g/kg); C: Medium dose group (3.2 g/kg); D: High dose group (4.8 g/kg); E: Bone peptide tablet group (576 mg/kg); F: Injection group (90 mg/kg)

There is a significant increase in the content of Gly-Pro-Hyp in each experimental group mouse serum. The level of Gly-Pro-Hyp was increases in a dose-dependent manner in the serum. And the level of Gly-Pro-Hyp content is reached the highest after 3 h. The Gly-Pro-Hyp content was remained high, and no significant decrease of Gly-Pro-Hyp occurred after 24 h. It indicated that the utilization rate of the bone peptide samples is significantly higher than the bone peptide tablet and bone peptide injection.

3.4 Calculation of AUC of Serum Gly-Pro-Hyp in Mice



(****P<0.0001)

Figure 4: The AUC of Gly-Pro-Hyp in the serum of mice.

Using the same principle of Figure 2, the AUC of Gly-Pro-Hyp was obtained using the integration method. As shown in Figure 4, the AUC of Gly-Pro-Hyp of bone peptide samples increased in a dose-dependent manner. And the AUC of low, medium and high-dose bone peptide samples was higher than the clinical dose bone peptide injection group and bone peptide tablet group, which proved the advantage of effective absorption of bone peptide samples. It indicates that the effective exposure of clinical dosage - low dose groups was significantly higher than that of the bone peptide injection and bone peptide tablet.

4 CONCLUSIONS

In summarize, the results showed that compared with the blank negative control group, the levels of Hyp and Gly-Pro-Hyp in the serum of the experimental groups (positive medicinal bone peptide tablet, positive medicinal bone peptide injection, low-dose bone peptide samples and high-dose bone peptide samples) were significantly increased.

The levels of Hyp and collagen Gly-Pro-Hyp in mouse serum can reach the highest after at gavaged for 3 h of the new bone peptide formulation sample, and the levels of Hyp and collagen Gly-Pro-Hyp was still remained at high level in 24 h. It indicates that the utilization rate of mouse bone peptide samples is significantly higher than that of bone peptide tablet and bone peptide injection, and the action time of bone peptide samples is significantly longer than that of bone peptide tablet and bone peptide injection.

The AUC was obtained by simulating the pharmacokinetic mathematical model of Hyp and Gly-Pro-Hyp using the integration method. The results revealed that the AUC of the new bone peptide formulation samples increased in a dose-dependent manner, indicating that the effective exposure of the mice absorbing the bone peptide samples increased. And the AUC of Hyp and Gly-Pro-Hyp of low-dose bone peptide sample group was significantly higher than those in the clinical dose bone peptide injection and bone peptide tablet groups. It indicates that the drug exposure of clinical use dose - low dose bone peptide samples was significantly higher than that of bone peptide injection and bone peptide tablet.

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