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·综述·

基于再生机制的淫羊藿黄酮在骨细胞微环境的药代动力学研究思路与方法

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摘要: 随着全球人口老龄化的加剧以及人民生活水平的提高,越来越多的人期望并致力于让身体组织焕发新生,这致使再生药物成为了当今医学领域研究的前沿与热点。而骨骼作为人体的重要组成部分,起着支持,保护,运动等多项作用。伴随着衰老,骨密度逐渐降低,渐而演变成骨质疏松,人变得弯腰驼背且不灵活,骨骼变得易断且断后难以恢复,严重影响着人们的生活质量及生命安危。利用再生药物实现骨再生有望从根本上攻克骨质疏松这一难题。研究表明,不同糖基数量/种类/位置淫羊藿黄酮(Different glycosylation Number/Type/Location of epimedium flavonoids, DGEFs)具有基于再生机制的抗骨质疏松作用,但其作用强度存在显著差异。通过查阅国内外文献及研究,我们推测淫羊藿黄酮骨再生作用的强度与其药代动力学过程密切相关,糖基数量与溶解性呈正相关,与渗透性呈负相关,糖基种类/位置决定膜转运方式,透膜后的胞内处置改变了糖基结构,并转化为活性更强的黄酮。本文从药代动力学视角,深入解析糖基数量/种类/位置的不同对淫羊藿黄酮在骨细胞微环境中的药物代谢动力学过程及骨再生作用的影响。

关键词: 骨细胞药代动力学;再生药物;糖基数量/种类/位置;淫羊藿黄酮

Research ideas and methods for the pharmacokinetics of epimedium flavonoids with different glycosylation number, type or location in bone cell microenvironment

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Abstract: With the aging of the world population and the improvement of people's living standards, more and more people are looking forward to and making efforts to let the body tissue regenerate, which made regenerative medicine a frontier and hot spot in medical research. As an important part of the human body, the skeleton plays a role in supporting, protecting, movement and so on. With aging, bone density decreases gradually, and may evolve into osteoporosis. People develop kyphosis and become not flexible. Bones break easily and are hard to restore, which seriously affects individuals' quality of life and safety. Using regenerative drugs to achieve bone regeneration is expected to overcome the problem of osteoporosis fundamentally. Studies had shown that epimedium flavonoids with different glycosylation number, type or location could exert anti-osteoporosis action, however their strength of activity were significant different. By studying Chinese and international literature and research, we hypothesize that the intensity of epimedium flavonoids' bone regenerate mechanism is closely related to its pharmacokinetics: the number of glycosylation and membrane permeability are negatively correlated; the number of glycosylation and solubility are positively correlated; glycosylation type and position determine membrane transport mode; the intracellular disposal changes glycosylation structure, and transforms them into more active flavonoids. In this review, from the perspective of pharmacokinetics, the influences of different glycosylation number, type or location on membrane transport and osteogenic differentiation in the bone cells

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microenvironment were discussed in depth.

Key words: Bone cell pharmacokinetics; Regenerative drugs; Glycosylation Number, Type, or Location; Epimedium flavonoids

再生药物是当今医学领域研究的前沿与热点，对其再生机制的深入研究将成为人类医学史上的一大革命。近年来，众多研究表明再生药物能够通过影响细胞微环境和(或)细胞信号通路，影响细胞的自我更新能力和定向分化方向，进而体现相应细胞功能[1]，实现组织器官的再生，如加味丹参饮能促进坏死心肌组织心肌特异性蛋白表达，诱导骨髓间充质干细胞向心肌样细胞分化，促进心肌组织再生[2]；鹿茸多肽能够抑制成骨样细胞分泌肿瘤坏死因子TNF和白细胞介素-α，促进软骨和成骨样细胞再生[3]等。目前，骨再生因为显著的代表性、可操作性和实用性，成为了深入研究再生药物机制的切入点。大量文献报道不同糖基数量/种类/位置淫羊藿黄酮(DGEFs)单体均具有基于再生机制的抗骨质疏松作用，但作用强度存在显著差异，而药物穿透细胞膜并与细胞内靶点结合是影响其药效强度的重要环节，因此阐述不同糖基数量/种类/位置淫羊藿黄酮在骨细胞微环境的药物代谢动力学是深入解析其骨再生机制的重要研究内容。本文在多年的研究基础上，结合前期实验数据，提出了全新的假说和研究思路，以期深入阐明不同糖基数量/种类/位置淫羊藿黄酮抗骨质疏松的科学内涵。

1 不同糖基数量/种类/位置淫羊藿黄酮(DGEFs)具有基于再生机制的抗骨质疏松作用

淫羊藿为小檗科植物淫羊藿、箭叶淫羊藿、柔毛淫羊藿或朝鲜淫羊藿的干燥叶，具有补肾阳、强筋骨、祛风湿作用[4]。从淫羊藿中分离得到的化合物超过270种，其中淫羊藿黄酮含量最高[5,7]。这类黄酮成分主要包括，宝藿苷I(Baohuside I)、淫羊藿苷(Icariin)、朝藿定A、B、C(Epimedin A、B、C)[8]。前期研究发现，朝藿定A、B、C、淫羊藿苷、宝藿苷I经大鼠口服给药后，在其血清、胆汁或者粪便中能检测到淫羊藿素(Icaritin)，如此淫羊藿素也不可忽视地成为来源于淫羊藿的重要活性成分。这些黄酮类化合物以淫羊藿素为母核，选择性地在C-7位连接葡萄糖(glucose, glc)或者在C-3位连接不同数量的鼠李糖(rhamnose, rha)、glc、木糖(xylose, xyl)。此外，淫羊藿素还有一个同分异构体苷元-环淫羊藿素(C-icaritin)[5]。这些黄酮成分化学结构及其相互关系如图1所示。

研究发现，朝藿定B、淫羊藿苷、宝藿苷I能够促进雌激素依赖的成骨细胞(osteoblast, OB)活力而发挥骨保护作用[9,10]；朝藿定B、淫羊藿苷、宝藿苷I能刺激OB形成，抑制骨质疏松小鼠破骨细胞分化[11]。淫羊藿总黄酮及其中所含淫羊藿苷和宝藿苷I能调节破骨细胞形成及其活性，抑制破骨细胞的分化和骨吸收[12]；淫羊藿总黄酮还能促进骨间质干细胞(mesenchyma stem cells, MSCs)的成骨分化，促进骨再生和修复功能[13]。淫羊藿总黄酮及微量的淫羊藿苷和朝藿定B能对抗泼尼松龙诱导的斑马鱼骨质疏松[14,15]。因此，淫羊藿黄酮是通过促进MSCs的成骨性分化和OB的分化成熟，抑制破骨细胞的分化和骨吸收活性，实现骨再生的，在骨质疏松治疗方面具有重要的研究价值和应用前景。

2 在骨细胞微环境的药代动力学过程是淫羊藿黄酮发挥促骨再生作用的关键

骨质疏松症是慢性疾病，临床患者需长期服药才能在骨组织、骨细胞中达到有效的治疗浓度，现有靶向技术也注重将药物提送至骨细胞[16]。基于血药浓度的经典药动学研究不能完全解释药物在特定组织(如肿瘤、脑组织等)中的药理学作用，或难以真实预测药物的药效[17]。药物通常须穿透多重生物屏障，且与细胞内靶点结合而发挥药效。2013年诺贝尔生理医学奖颁发给了研究“细胞内分子运输调节机制”的学者，细胞内物质运输调控是重大科学问题，细胞内药物转运调控也同样重要[18]。

骨质疏松症发病的根本原因是骨吸收和骨形成动态平衡失调[19]。其中，骨形成是由OB介导的骨合成代谢活动，OB移行至被吸收陷窝部位，分泌骨基质，骨基质矿化而形成新骨[20]，而MSCs能够分化成OB和脂肪细胞(Adipocytes, AC)[21]，且是OB的前体细胞[22]，如图2所示。在临床治疗中，促进MSCs成骨分化是有助于促进骨形成而抗骨质疏松的重要手段。药物发挥促成骨分化作用，必先透过MSCs细胞膜，经细胞内处置，与相关靶点结合而发挥药效。因此，促骨再生药物透过MSCs膜转运机制、MSCs内的处置过程、动态分布及外排机制均是影响其促MSCs成骨分化的重要因素。

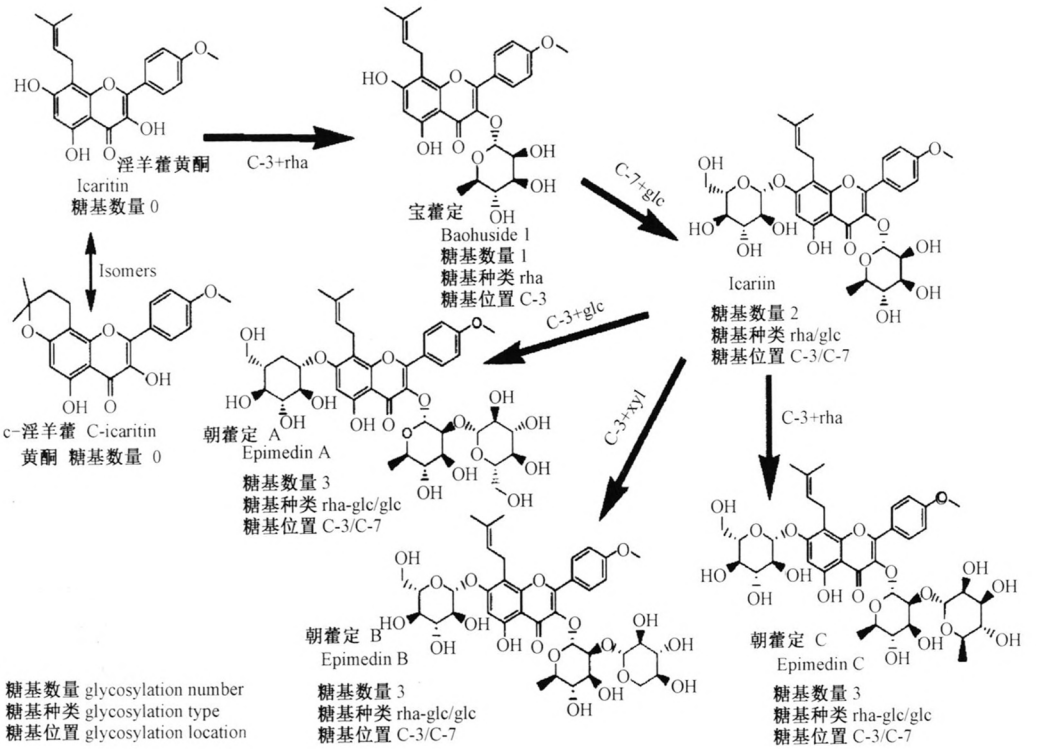

图 1 不同糖基数量/种类/位置淫羊藿黄酮成分化学结构及其相互关系

Fig. 1 Chemical structure of epimedium flavonoids with different glycosylation number, type or location of and their relationships

注：rha：鼠李糖（rhamnose）；xyl：木糖（xylose）；glc：葡萄糖（glucose）

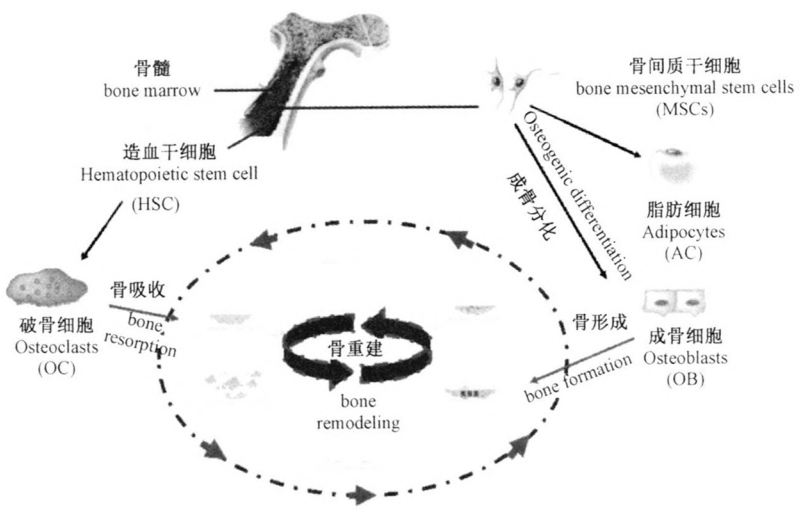

图 2 骨细胞参与的骨吸收及骨形成机制示意图

Fig. 2 Diagram of bone resorption and formation mechanism

3 糖基数量/种类/位置影响淫羊藿黄酮的生物药剂学性质及膜转运机制

研究表明，淫羊藿素结构中不含有糖基，属于难溶性药物，但却有较好的渗透性[23]。槲皮素苷是槲皮素的糖苷，槲皮素苷的溶解度高于槲皮素，但槲皮素苷的肠吸收透过率显著低于槲皮素[24]。黄芩苷是在黄芩素的糖苷，黄芩素为不含糖基的苷元，黄芩苷的溶解度是黄芩素的 2 倍以上，但通过油水分配系数测定发现黄芩苷在水溶液的 lg$P$ 值接近 0，黄芩

素的 lg*P* 值大于 2，表明不含糖基的黄芩素较连接糖基的黄芩苷具有更好的渗透性[25]。淫羊藿苷和朝藿定 A/B/C 分别含有 2 和 3 糖基，具有较好的溶解性，但是渗透性较差。宝藿苷 I 是单糖基黄酮，具有较好的溶解性的同时其渗透性 > 淫羊藿苷 > 朝藿定 A/B/C[26]。因此，糖基数量/种类/位置影响淫羊藿黄酮的溶解性和渗透性，且糖基数量与溶解性呈正相关、与渗透性呈负相关。

朝藿定 A、B、C 具有相同的糖基数量(3 糖基)，但其糖基种类/位置存在差异，Caco-2 细胞模型研究表明朝藿定 A、B、C 的渗透性均较差，且有严重的外排现象，朝藿定 A 和 C 的外排机制主要是有 P-糖蛋白和乳腺癌耐药蛋白参与，而朝藿定 B 的外排机制只有乳腺癌耐药蛋白参与[27]。可见，糖基种类/位置影响其膜转运机制。

4 透膜后的处置过程促进淫羊藿黄酮糖基数量/种类/位置结构转化

肠道菌转化研究表明，不同糖基数量/种类/位置淫羊藿黄酮在肠道菌及其 LPH 酶的作用下可部分被转化成淫羊藿素，且转化速率为淫羊藿苷 > 朝藿定 B > 朝藿定 A > 朝藿定 C > 宝藿苷 I[28]。采用 UPLC-Q/TOF-MS 技术解析朝藿定 A、朝藿定 B、朝藿定 C、宝藿苷、淫羊藿苷的处置过程，发现其结构中的糖基数量/种类/位置均发生了改变，且朝藿定 B、淫羊藿苷、宝藿苷的吸收转运过程中均能发现淫羊藿素[5,31]。已有报道，采用 UPLC-MS/MS 技术能检测到淫羊藿素在大鼠脊柱骨中的动态分布，结果发现淫羊藿素在脊柱骨中的半衰期($t_{1/2}$)为 10.68 h，曲线下面积(area under curve, AUC)为 642 hng/g，分布容积(distribution volume, V)为 238 683 g/kg，系统清除率(system clearance, CLS)为 15 486 g/hkg，平均滞留时间(mean recidence time, MRT)为 45.02 h[32]。可见，透膜后的处置过程促进了淫羊藿黄酮糖基数量/种类/位置结构转化。

5 骨细胞内处置过程可促进不同糖基数量/种类/位置淫羊藿黄酮转化成活性更强的黄酮成分

淫羊藿素能促进骨质疏松大鼠骨形成、抑制骨吸收而发挥很好的抗骨质疏松作用[33,34]。环淫羊藿素能显著改善骨质疏松大鼠的骨密度、生物力学性质、促进骨形成、抑制骨吸收而发挥抗骨质疏松作用[35]。研究表明，朝藿定 A、B、C、淫羊藿苷、淫羊藿素的抗骨质疏松活性强弱顺序为：淫羊藿素 > 淫羊藿苷 > 朝藿定 A、B、C[36]。

有效成分透膜转运后与对应靶点相互作用而展现出相应的药理活性[37]。骨形态发生蛋白(bone morphogenetic protein, BMPs)能调节 MSCs 向 OB 分化，如 BMP-2、BMP-4[38]。Runx2 是 BMP 信号通路的下游调控因子，Runx2 也被称为 OB 的主要转录因子，并参与 OB 成熟过程[39]，BMPs 能诱导 Runx2 表达，然后进一步促进 MSCs 向 OB 分化和成熟[40]。如图 3 所示。

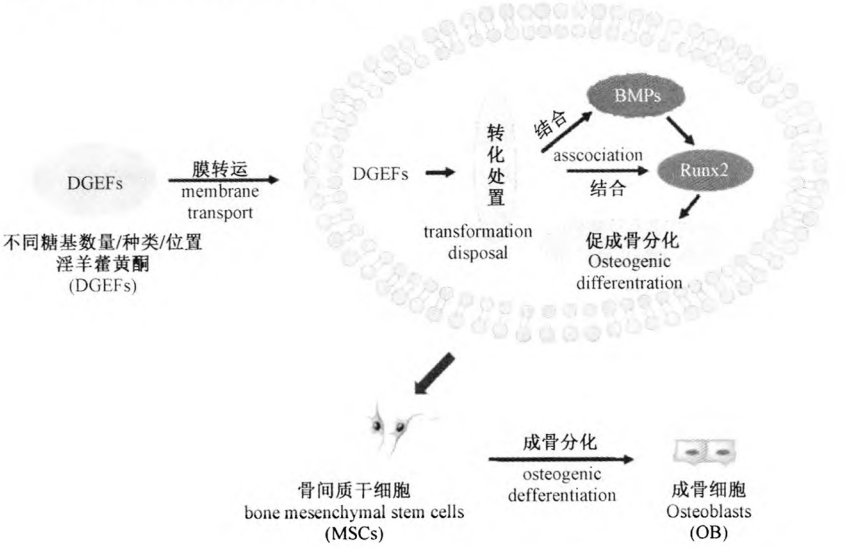

图 3 不同糖基数量/种类/位置淫羊藿黄酮在 MSCs 上的转运转化机制示意图

Fig. 3 Diagram of transport and transform of epimedium flavonoids with different glycosylation number, type or location in MSCs

前期研究表明，淫羊藿苷和宝藿苷 I 均能与 BMP-2、BMP-4、Runx2 牢固结合，从而抑制了 MSCs 向 OB 细胞的成骨分化。而淫羊藿素及环淫羊藿素与 BMP-2、BMP-4、Runx2 的结合显著弱于淫羊藿苷和宝藿苷 I，对 MSCs 向 OB 细胞的成骨分化无抑制作用，可表现更强的促成骨分化作用而抗骨质疏松，如图 4。可见，骨细胞内处置过程可促进不同糖基数量/种类/位置淫羊藿黄酮转化成活性更强的黄酮成分。

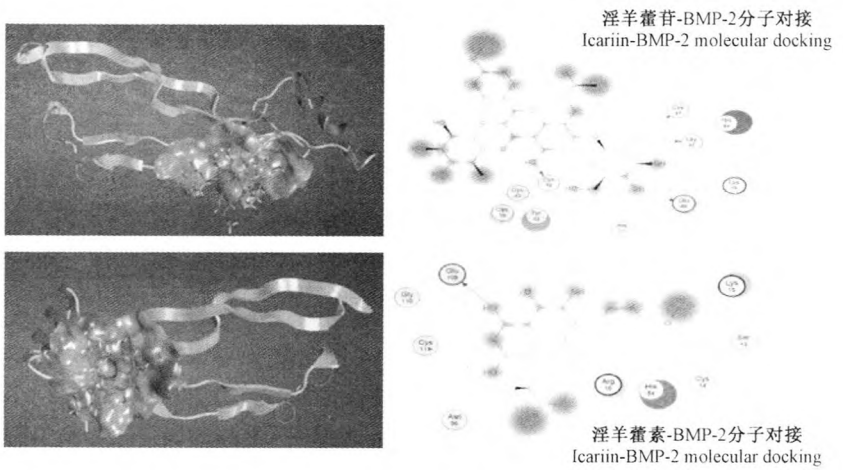

图 4 不同糖基数量/种类/位置淫羊藿黄酮与 MSCs 细胞内靶点相互作用图

Fig. 4 Interaction of epimedium flavonoids of different glycosylation number, type or location with intracellular target in MSCs

6 结语

本课题以细胞药代动力学视角阐述不同糖基数量/种类/位置淫羊藿黄酮在骨细胞微环境的骨再生作用机制，并推测淫羊藿黄酮结构中不同数量/种类/位置的糖基影响其溶解性/渗透性，且糖基数量与溶解性呈正相关，与渗透性呈负相关，糖基种类/位置决定膜转运方式，透膜后的胞内处置改变了淫羊藿黄酮结构中的糖基数量/种类/位置，并促进其转化为活性更强的黄酮。通过深入探究不同数量/种类/位置糖基对淫羊藿黄酮在骨细胞微环境中的药物代谢动力学过程及骨再生作用的影响，可进一步阐明淫羊藿黄酮的骨再生作用机制，开发靶向制剂，提高药效并减轻副作用，深入解决骨质疏松的治疗难题并推动再生药物的发展，实现医学界的大变革。本课题组后续将就不同数量/种类/位置淫羊藿黄酮在 MSCs 的膜吸收转运、胞内动态分布及胞内处置，与胞内靶点亲和力方面进行研究，深入探讨糖基的数量/种类/位置对淫羊藿黄酮促 MSCs 成骨分化的重要影响。

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