Hyperhomocysteinemia disturbs fracture healing in mice

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ABSTRACT INTRODUCTION:

Homocysteine (Hcy) is the degradation product of the essential amino acid methionine. Hcy is either metabolized to cysteine and glutathione by a vitamin B6 dependent pathway or remethylated to methionine by a vitamin B12 and folate dependent pathway. In the Western world hyperhomocysteinemia (HHcy) represents a widespread metabolic disorder leading to atherosclerosis and osteoporosis. The main reasons for HHcy are deficiencies of folate, vitamin B6, and vitamin B12, renal dysfunction, as well as a disturbed methionine metabolism. A Hcy plasma concentration of 12–30 µmol/l, which is commonly found in individuals with a vitamin deficiency, is considered as moderate HHcy. In contrast, a Hcy concentration of 30–100 µmol/l, which is primarily found in patients with homozgyous enzyme defects or chronic kidney disease, has been termed intermediate HHcy. A plasma Hcy concentration > 100 µmol/l is defined as severe HHcy and occurs almost exclusively in individuals with congenital disorders or homocystinuria (1).

During the last years, several studies have reported that HHcy is capable to stimulate the proliferation of osteoclasts resulting in a negative bone turnover. In addition, HHcy has been demonstrated to impair extracellular bone matrix by disturbing enzymatic crosslinking during the synthesis of type I collagen. Further, an Hcy-induced apoptosis of pre-osteoblastic cells has been suggested to affect bone metabolism (2). While HHcy has been indicated as a new risk factor for osteoporosis and osteoporotic fractures, there is no information on whether these metabolic alterations affect also fracture healing. The formation of woven bone by osteoblasts represents a critical component of secondary fracture healing. During endochondral ossification, osteoclasts contribute to the resorption of calcified cartilage, which allows the invasion of osteoblasts. Type I collagen synthesis plays a crucial role in this stage of fracture healing (3). All these events during fracture healing might be affected by HHcy. Therefore, we analyzed the effect of HHcy, which was induced by a Hcy-supplemented, a methionine-supplemented, or a vitamin-deficient diet, on bone repair in mice.

METHODS:

All experiments were performed according to the National Institute of Health guidelines for the use of experimental animals and were approved by the national legislation on the protection of animals. For the present study CD-1 mice (30–40 g bw) were fed a Hcy-supplemented diet (C1000 = 1.5 % homocysteine, Altromin, Lage, Germany), a methionine-supplemented diet (C1000 + 2.5 % L-methionine, Altromin) or a vitamin-deficient diet (TD 06250, Harlan, Indianapolis, IN). Controls were fed the according standard diet (1324, Altromin or TD 06422, Harlan). Four weeks after stable fixation of a closed femoral fracture, animals were sacrificed to prepare bones for histomorphometric and biomechanical analyses. In addition, blood samples were obtained to evaluate plasma concentrations of Hcy, vitamin B12, and folate.

Plasma concentration of Hcy was calculated by means of gas chromatography mass spectrometry. Plasma concentrations of vitamin B12 and folate were measured with commercial, competitive chemiluminescence immunoassays on an ADVIA Centaur automated analyzer ( Bayer Diagnostics, Fernwald, Germany). The flexural rigidity of the healed femurs was evaluated using a biomechanical three-point bending test (1454, Zwick, Ulm, Germany). For histomorphometric analyses the healed femurs were embedded in PMMA. Structural indices were calculated using undecalciﬁed Paragon-stained sections of 70 µm thickness: callus diameter [mm], bone callus fraction [%], cartilage callus fraction [%], and ﬁbrous callus fraction [%].

All data are given as means ± standard deviation (SD). After proving normal distribution and equal variance, comparison between the experimental groups was performed by one-way ANOVA followed by Student-Newman-Keuls test.

RESULTS SECTION:

Animals, which were fed the Hcy-supplemented diet, the methionine-supplemented diet, and the vitamin-deficient diet showed a significantly increased plasma concentration of Hcy when compared to the according controls (Hcy-supplementation: 102.3 ± 61 8 µmol/l, n = 13 vs. 2.6 ± 1.3 µmol/l, n = 12; methionine-supplementation: 38.4 ± 29.5 µmol/l, n = 13 vs. 2.6 ± 1.3 µmol/l, n = 12; vitamin deficiency: 14.6 ± 4.8 µmol/l, n = 14 vs. 2.5 ± 0.7 µmol/l, n = 13; p < 0.05 each). Thereby, animals, which were fed the Hcy-supplemented diet showed a severe HHcy (>100 µmol/l), while animals, which were fed the methionine-supplemented diet demonstrated an intermediate HHcy (30–100 µmol/l). In animals, which received the vitamin-deficient diet, however, we found only a moderate HHcy (12–30 µmol/l). This was associated with significantly decreased plasma concentrations of vitamin B12 and folate in animals, which received the vitamin-deficient diet when compared to controls (vitamin B12: 0.5 ± 0.2 ng/ml vs. 12.4 ± 3.8 ng/ml; folate: 45 ± 12 ng/ml vs. 141 ± 49 ng/ml, p < 0.05 each).

Four weeks after fracture, flexural rigidity of the callus was significantly decreased in animals, which were fed the Hcy-supplemented diet when compared to animals, which received the control diet (45.5 ± 17.4 N/mm, n = 13 vs. 65.2 ± 15.7 N/mm, n = 12, p < 0.05). In contrast, we found no significant differences in flexural rigidity of the callus between animals, which received the methionine-supplemented or the vitamin-deficient diet and animals, which received the according control diet. The histological analysis demonstrated in all animals a typical pattern of secondary fracture healing with osseous bridging of the fracture gap after four weeks of bone repair. Callus was dominated by woven bone undergoing extensive remodeling. In contrast, animals of all study groups showed a comparable callus diameter without significant differences in the tissue composition of the callus (data not shown).

DISCUSSION:

Plasma analyses in this study conﬁrmed the ﬁndings of previous animal studies on rats demonstrating that a Hcy-supplemented diet generates a severe HHcy, while a methionine-supplemented and a vitamin-deficient diet cause an only moderate to intermediate HHcy (2). Thereby, we could show that a severe HHcy leads to an alteration of bone repair as indicated by the biomechanical analyses, however, without affecting the diameter and tissue composition of the callus. These results suggest that a severe HHcy might impair the formation of extracellular bone matrix, which is known to determine the biomechanical properties of bone (2). In contrast, a moderate and intermediate HHcy, which was induced by a methionine-supplemented and a vitamin-deficient diet, had no impact on fracture healing. Studies on rats have indicated that a moderate HHcy leads to an only minor alteration of bone metabolism, while on the other hand a markedly increased Hcy plasma concentration critically disturbs bone metabolism (2). In conclusion, a moderate to intermediate HHcy is not capable to impair bone repair, while a severe HHcy affects the biomechanical stiffness of the healing callus.

REFERENCES:

