論 文 内 容 の 要 旨
Synopsis of Thesis

氏　名 Name	バル　ゼイネップ Bal Zeynep
論文題名 Title	A novel nano-hydroxyapatite/synthetic polymer/bone morphogenetic protein-2 composite for efficient bone regeneration [有能な骨再生のために、ナノハイドロクシアパタイト、PLA-PEG及びBMP-2を用いた新規複合体を作成すること]

論文内容の要旨

〔目　的(Purpose)〕

Efficient bone regeneration using recombinant human bone morphogenetic protein-2 (BMP-2) is needed so as to reduce side effects caused by high-dose BMP-2 use. Synthetic copolymer polylactic-acid polyethylene glycol (PLA-PEG) is known as a good carrier for BMP-2. Osteoconductive properties of nano-sized hydroxyapatite (nHAp) is reported to be superior to conventional hydroxyapatite. The composite material of PLA-PEG (for sustained release) and osteogenic nHAP can contribute to efficient bone regeneration by BMP-2. In the literature no study is reported with PLA-PEG/ BMP-2 / nHAp. So the purpose of this study is to produce a novel biocomposite of PLA-PEG/nHAp/BMP-2 and to investigate the effectiveness of a novel composite material of PLA-PEG and nHAp as a carrier for BMP-2.

〔方法ならびに成績(Methods/Results)〕

方法[Methods]

The release kinetics of BMP-2 from the composites was investigated by ELISA. Thirty-six males Sprague-Dawley rats underwent posterolateral spinal fusion on L4-5 transverse processes with 3 different doses of BMP-2 (0μg [control], 3μg [low dose], 10μg [high dose]). Weekly μCT and manual palpation - histology [by Haematoxylin & Eosin (H&E) and Safranin O (SO)] at 8 weeks postoperatively were used to assess spinal fusion.

成績 [Results]

ELISA demonstrated the sustained release of BMP-2 from the composite until day 21. μCT and manual palpation results demonstrated a solid fusion in 91.6% (11/12) of specimens in both the low- and high-dose groups. No mice in the control group attained bony fusion (0%, 0/9). nHAp was resorbed between 2 and 4 weeks postoperatively and regenerated fusion mass at 8 weeks postoperatively consisted of only newly formed bone. Histological evaluation with H&E and SO staining was coherent with μCT results. The μCT evaluation of the newly formed bone mass of low- and high-dose BMP-2 groups demonstrated the newly formed fusion mass was larger in size (BV and TV) in the high-dose BMP-2 group, however the low-dose BMP-2 group formed more solid fusion mass with a higher BV/TV value.

〔総　括(Conclusion)〕

The novel nHAp/PLA-PEG composite enabled efficient bone regeneration with low-dose BMP-2. The sustained release of BMP-2 by PLA-PEG and the osteogenic and biodegradable scaffold of nHAp might contribute to efficient bone regeneration. This novel composite material has potential in clinical applications(spinal fusion, large bone defect and non-union) by enabling efficient bone formation by BMP-2.

[Format-H07]

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

As far as it is known this is the first study in the literature by nano-hydroxyapatite/PLA-PEG and BMP-2 in bone regeneration. Although BMP-2 is one of the widely used growth factors in both bone regeneration studies and clinics, the supraphysiological doses are reported to be correlated as side effects such as tumour formation, ectopic bone formation, soft tissue edema, inflammation, inappropriate adipogenesis and so on. Thus it is a must to decrease the required amount of BMP-2 for bone regeneration.

First of all this novel composite of PLA-PEG/nHAp/BMP-2 is shown to provide the sustained release of BMP-2 for 3 weeks in vitro and the release was shown to be still in continue in the end of 21 days.
As the first aim of the study after achieving the sustained release from the composite with a proper polymer:nanohydroxyapatite ratio, the bone formation capacity of the composites were tested on rat posterolateral spinal fusion model, in vivo. For this 36 male SD rats were undergone the fusion operation. The composites including the same amount of BMP-2 were placed on L4-L5 transverse processes on both sides and animals were sacrificed at 8 weeks. The BMP-2 study groups were 0 μg BMP-2 (negative control), 3 μg BMP-2 (low dose) and 10 μg BMP-2 (his dose/ positive control). Weekly μCT data, μCT evaluation for bone volume (BV) - tissue volume (TV) - BV/TV, manual palpation (for mechanical testing) and histology with hematoxylin & eosin and safranin O staining were done for evaluation.

The μCT data at operation day confirmed the distribution of nHAp through the composites as opacity in μCT data. The bone formation was started to be seen around the transplanted composites at 2 weeks in low- and high-dose BMP-2 study groups. At 4 weeks the spinal fusion (defined as bony bridging between L4 -L5 transverse processes on both sides) was observed in low- and high- dose BMP-2 groups however there were a slight or no bone formation in negative control group. At 8 weeks spinal fusion in 11 of 12 animals for both low- and high- dose BMP-2 groups with a 91.6% fusion rate was achieved while there was no fusion body observed in negative control group. There observed an increased bone volume with lower opacity in high-dose BMP-2 group compared to low-dose BMP-2 group. Manual palpation, which was done in a blinded manner, confirmed the μCT data with a fusion rate of 91.6% both for low- and high- dose BMP-2 groups. μCT evaluation for BV and TV confirmed the increased bone volume of the high dose BMP-2 group however the BV/TV ratio showed the newly formed fusion mass in low dose BMP-2 was denser and stiffer compared to high dose BMP-2. SO and H&E staining confirmed the osseous bridging between L4-L5 transverse processes for low- and hig- dose BMP-2 groups. Histology results showed the increased bone volume in high dose BMP-2 group was because of the fatty bone marrow formation. In none of the low- and high- dose BMP-2 groups there observed any remnants of nHAp while in some of the samples of negative control group there were some slight remnants of nHAp.

These data showed that the novel composite of PLA-PEG/nHAp can provide the sustained release of BMP-2 at least for 21 days. nHAp is shown to increase the surface area because of its small size which is lower than 100nm is reported to enhance cell and protein adhesion and mechanical properties concomitantly.Thus in this study with the the sustained release of BMP-2 the composites and as well as with the presence of nHAp it is achieved to decrease the required amount of BMP-2 for spinal fusion from 10μg to 3μg with a more stiffer and denser fusion body formation in all μCT data, μCT evaluation and histology compared to fatty bone marrow filled 10 μg BMP-2 results. nHAp was shown to be replaced to new bone ultimately even in the low dose BMP-2 group was shown to be proved with no remnant observation in both low- and high- dose BMP-2 groups.

As a clinical significance of this study, we think that the efficient bone formation capability of this novel composite of PLA-PEG/ nHAp and BMP-2 makes it a potential candidate in clinics for spinal fusion, large bone defects and non-union.

本研究は学位に値すると考える。