



Title	A combined autologous cellular cardiomyoplasty with skeletal myoblasts and bone marrow cells in the canine hearts for ischemic cardiomyopathy
Author(s)	メモン, イムラン アハマド
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The University of Osaka

氏名 メモン イムラン アハマド
 Memon Imran Ahmed

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学位論文名 A combined autologous cellular cardiomyoplasty with skeletal myoblasts and bone marrow cells in the canine hearts for ischemic cardiomyopathy.
 (虚血性心筋症犬に対する自己筋芽細胞と骨髄単核球細胞を用いた細胞左室形成術に関する研究)

論文審査委員 (主査)
 教授 松田 輝

(副査)
 教授 堀 正二 教授 白倉 良太

論文内容の要旨

〔目的〕: Objectives:

Cellular cardiomyoplasty with isolated skeletal myoblasts (SM) and bone marrow mononuclear cells (BM-MNCs) is an encouraging therapeutic strategy for heart failures. We investigated the combined achievements accomplished by SM and BMCs transplantation therapy in enhancing the regeneration of the impaired heart.

〔方法ならびに成績〕: Methods:

The impaired heart was created in 16 canines by ligating left anterior descending coronary artery for 2 weeks. SM (1×10^8) and BMCs (3×10^6) were injected directly into the damaged myocardium. Groups: Combine cell therapy (CCT, n=4), Myoblasts (M, n=4); Bone marrow mononuclear cells (B, n=4) and Medium only (C, n=4). Angiogenic factors expression by SM and BMCs was analyzed in additional rat experiments.

First week cultures contained 70-80% myoblasts and at the time of cell transplantation, the cultures contained $>60\%$ myoblasts as checked by desmin staining. 1×10^8 cultured myoblasts were transplanted to canine hearts after 14 days of LAD ligation.

About twenty milliliter of collected heparinized canine autologous bone marrow was centrifuged at $800 \times g$ for 20 min. The cells were collected, washed several times, and then suspended at concentration of 3×10^6 in 1 ml DMEM for transplantation.

Results:

Combined cell therapy hearts showed reduced left ventricular dilation with thickened anterior wall and significantly improved ejection fraction (CCT: $55.37 \pm 8.6\%*$; M: $47.4 \pm 7.4\%$; B: $44.4 \pm 6.7\%$; C: $34.4 \pm 5.4\%$; $*P < 0.05$). Percentage fractional area shortening (%FAS) was significantly improved in CCT group as compared

to all other groups. M-group also showed improvement in cardiac performance but was not significant as compared to CCT. There was no significant difference in improvement of cardiac function between the B-group and C-group.

With combined cell therapy, sections showed patches of grafted multinucleated myofibers with significant high number of neo-vessels as compared to the other groups (Neo-vessels/mm² ; CCT : 45.5±11.9* ; M : 26.5±8.1 ; B : 30.7±14.8 ; C : 7.1±1.4 ; *P<0.05). Compared to CCT, M and B groups showed almost half the number of vessels. After 4 weeks, fast-myosin heavy chain expressing myoblasts also expressed slow-myosin heavy chain showing a phenotype conversion to slow twitch myofibers. Grafted myoblasts showed negative staining for cardiac specific marker, Troponin T. These myofibers were all grafted parallel, following hosts cellular organization. Although, a few surviving host cardiomyocytes in the scar area stained positive for MHC slow, both MHC fast and slow stained negative for skeletal myoblasts in B and C groups.

In the CCT group, significant higher expression of vascular endothelial growth factor and hepatocyte growth factors were measured by ELISA analysis as compared to the other groups. In vivo bFGF expression level was lower but detectable in all 3 cell transplantation groups and was not significant between cell therapy groups. Infarcted hearts without any cell transplantation showed minimal detectable VEGF and bFGF expression

[総 括] : Conclusion :

This combined autologous cell therapy demonstrates to be a compatible potential therapy of clinical relevance. It provides advantageous supportive angiogenesis and myogenesis for the striving grafts and improves global left ventricular function.

論文審査の結果の要旨

高齢化、虚血性心疾患の増加に伴い本邦でも心不全患者数が増加しつつあるが、重症例では補助人工心臓や心臓移植などの置換型治療でしか救命できず、未だ普遍的な治療とはいひ難いのが現状であり、新たな治療法の開発が切望されている。

近年、自己筋芽細胞移植や骨髄単核球細胞移植など心不全に対する様々な再生型治療が研究されその有用性が報告されている。

本研究では、心筋梗塞のモデル犬に対し、骨格筋筋芽細胞と骨髄単核球細胞の併用治療を行い、それら単独治療と比較して心機能・心筋組織の改善効果が得られるかを検討した。

成犬の冠動脈の左前下行枝を結紮し、2週間後に筋芽細胞移植群、骨髄単核球細胞移植群、それらの併用群および培養液投与（コントロール）群の4群を作成し、移植後の心機能・心筋組織を比較検討した。その結果、併用療法では、それぞれ単独療法に比べ心機能が有意に回復し、心筋組織は左室壁厚の増大や梗塞領域での新生血管増加といった心筋組織のリモデリングの抑制効果が認められた。また、これらの効果には内因性 VEGF や HGF といった血管増殖因子の発現増加が関与していることが示唆された。

本研究の結果は、筋芽細胞と骨髄単核球細胞の併用治療が重症虚血性心筋症に対する新たな治療戦略となる可能性について言及した点で非常に意義深く、学位の授与に値すると考えられる。