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論文題目	Preservation effect of imeglimin on pancreatic ß-cell mass: Noninvasive evaluation using ^{111}In-exendin-4 SPECT/CT imaging and the perspective of mitochondrial involvements (イメグリミンによる膵β細胞保護効果の非侵襲的評価とミトコンドリアの関与)		

（論文内容の要旨）

Progressive loss of ß-cell mass (BCM) has a pernicious influence on type 2 diabetes mellitus (T2DM). A study using cadaveric human samples showed the loss of BCM in type 2 diabetes patients. However, evaluation of BCM has conventionally required an invasive method that provides only cross-sectional data. A noninvasive approach to longitudinal assessment of BCM in living subjects using an indium 111–labeled exendin-4 derivative ([Lys12(111In-BnDTPA-Ahx)]exendin-4) (^{111}In-exendin-4) has been developed recently. The injected ^{111}In-exendin-4 probe is accumulated in pancreatic ß-cells due to expression of glucagon-like peptide-1 (GLP-1) receptor. The radioisotope emission then is detected by single-photon emission computed tomography/computed tomography (SPECT/CT). The mitochondrion plays essential roles in energy substrate metabolism and apoptosis in various tissues including pancreatic ß cells. Mitochondrial dysfunction can be responsible for the impairment of glucose-stimulated insulin secretion (GSIS) and insulin sensitivity in T2DM. Imeglimin is a novel antidiabetic agent that is reported to improve glycemic control and GSIS via augmentation of mitochondrial function.　However, the influence of imeglimin on BCM is not fully understood. This study utilized a T2DM animal model, Leprdb (db/db) mice. Db/db mice have been characterized with hyperglycemia accompanied with progressive loss of BCM and excessive mitophagy in pancreatic ß cell.

This study aimed to investigate the effects of imeglimin on BCM in vivo in prediabetic db/db mice using a noninvasive ^{111}In-exendin-4 SPECT/CT and to elucidate mitochondrial involvement in BCM preservation. Db/db mice were treated with imeglimin or vehicle orally twice a day from 4 to 10 weeks of age. The oral glucose tolerance tests (OGTTs) and 111In-exendin-4 SPECT/CT scans were performed at 4, 7, and 10 weeks of age. The pancreas was harvested for immunohistochemical and electron microscopy analysis.

The OGTTs showed that the blood glucose of control had increased significantly in 7 and 10 weeks of age. Plasma insulin level of control showed significant increase on 7 weeks but tended to be reduced at 10 weeks. This is in line with natural course of diabetes development in db/db mice. On the other hand, imeglimin group showed increasing trend of insulin secretion without significant difference of blood glucose between 7 week and 10 weeks. It indicated the attenuation of diabetes progression in db/db mice by imeglimin.

The repeated ^{111}In-exendin-4 SPECT/CT revealed no significant decrease of BCM in imeglimin-treated group. However, BCM of control had decreased significantly at 10 weeks of age. At the end of intervention, imeglimin-treated group had higher BCM compared to that of control, which was further confirmed by immunohistochemical BCM analysis. Furthermore, immunohistochemical analysis revealed tendency of higher ß-cell proliferation and significantly reduced ß-cell apoptosis in the imeglimin-treated db/db mice. The low apoptosis rate was in line with attenuated released cytosolic cytochrome c protein in ß-cells of imeglimin-treated group. These results implied that imeglimin inhibited mitochondrial-mediated ß-cell apoptosis, consequently preserved BCM. Mitochondria membrane potential (MMP) measurement and electron microscopy further confirmed imeglimin action on mitochondria. It was revealed that *in vivo* imeglimin treatment improved MMP in pancreatic islets as well as preserved mitochondrial structure in ß-cells. Moreover, to identify glucose dependency of imeglimin action, isolated islets obtained from 6-week-old db/db mice were incubated in 11.1 mM or 33.0 mM glucose with DMSO or imeglimin. The MMP measurement showed a significant decrease in islets incubated in 33.0 mM glucose and was partly restored by addition of imeglimin. It implied that imeglimin's effect on islet mitochondria is, at least partially, independent of the glucose level.

In summary, the longitudinal observation using ^{111}In-exendin-4 SPECT/CT provides comprehensive evidence on imeglimin action to preserve BCM in db/db mice. In addition, imeglimin prevents ß-cell apoptosis by improving the mitochondrial structure and membrane potentials of ß-cells. Thus, imeglimin may well have beneficial effects on ß-cell mitochondria via improvement of glycemic control as well as glycemic-independent fashions.

（論文審査の結果の要旨）

膵β細胞量の保護は2型糖尿病において重要な治療目標である。イメグリミンはグルコース刺激によるインスリン分泌を促進し血糖降下作用を有する新規糖尿病治療薬であるが、その膵β細胞量への効果は不明である。本論文では、糖尿病モデルマウス(db/db マウス)を用い、イメグリミンの膵β細胞量への効果とその機序を検討した。

生体膵β細胞量評価法である ^{111}In-exendin-4 SPECT/CT を用い、5 週齢の db/db マウスにおいて、5 週間のイメグリミン投与が膵β細胞量の減少を抑制したことを示した。また、イメグリミンを投与した db/db マウスでは、膵病理上で膵β細胞のアポトーシスの抑制、膵島ミトコンドリア構造の保持を認め、単離膵島では細胞質へのチトクローム c 放出の減少とミトコンドリア膜電位の改善を認めた。イメグリミン投与 db/db マウスの単離膵島における細胞質へのチトクローム c 放出の減少とミトコンドリア膜電位の改善は、インスリングラルギン投与にて同程度に血糖コントロールを行った db/db マウスの単離膵島と比較しても有意であった。

以上の研究は、イメグリミンの膵β細胞量保護効果、並びに、その機序としてのイメグリミンの膵β細胞への作用の解明に貢献し、糖尿病学の発展に寄与するところが多い。

したがって、本論文は博士（　医学　）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、令和 5 年 2 月 14 日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日：　　年　　月　　日　以降