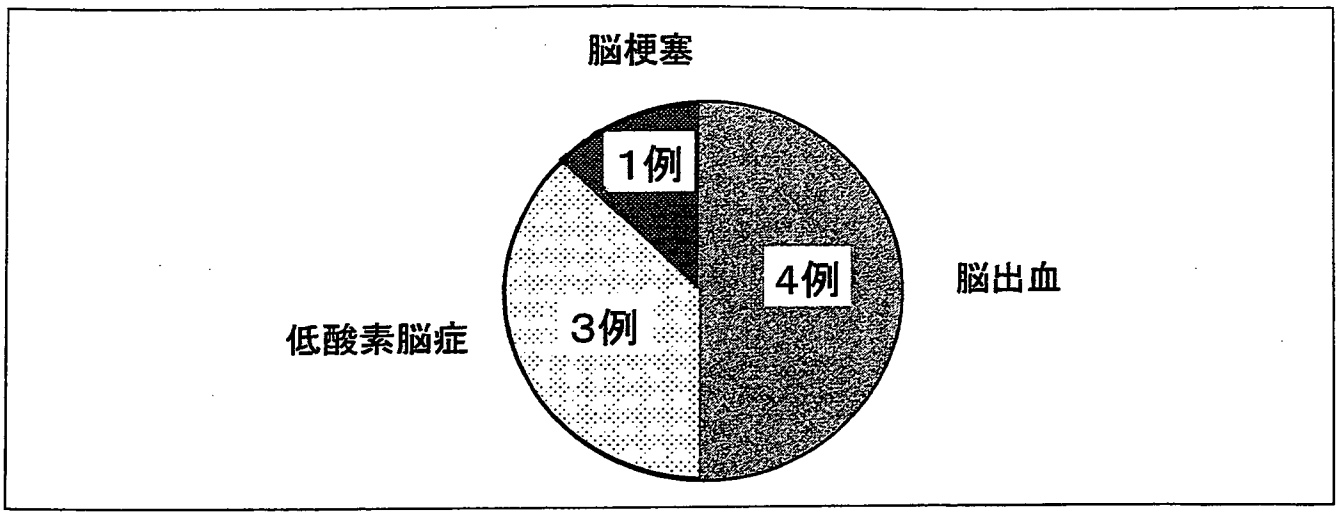

図2　原疾患の内訳

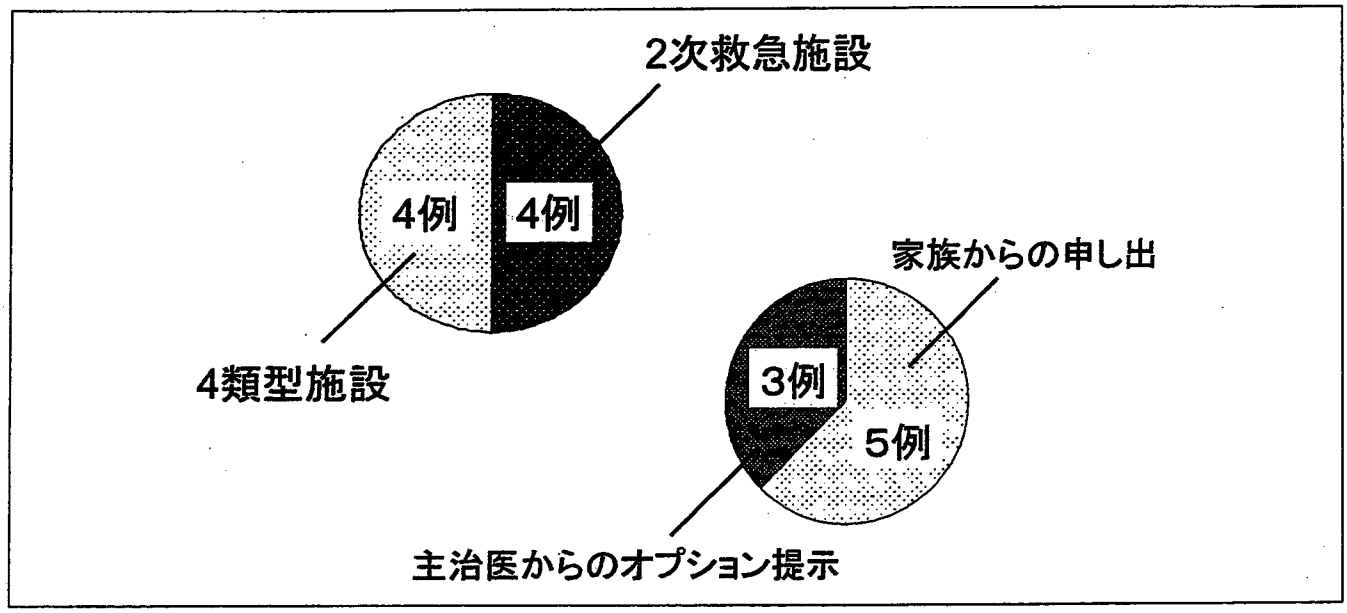

図3　提供施設・提供の契機

受けたが，組織提供の説明を希望しない例があった。②中国地方・四国地方からの情報が少なかった。③脳神経外科を診療科目として標榜する2次救急施設からの提供が多かった。④主治医からのオプション提示によって提供に至った症例があった。以上が明らかとなった。

①に関しては，組織提供のために，臓器提供のICに加えて,改めて組織提供のICを行う現在の方法は，家族の立場からは，さまざまな意味で負担が大きいといえる。今後は，JOTCo，県Coおよび西日本組織移植Coの，いっそうの理解，協力のもとに，一度で，臓器・組織両方の提供のICとなるような方策を考える必要があると思われた。日本組織移植学会では，認定Co制度を発足させており，このような制度を有効に使用し，家族に負担のない効率的なICを考慮することが，移植医療全体の発展のために必須であると思われた。②に関しては，組織バンクとして，個別病院訪問等の啓発活動の拡大を計画中である。神戸大学では，膵島移植の開始にあたり，兵庫県を中心とした近畿地方(兵庫，和歌山，大阪)において，組織移植Co，時には県Co，JOTCoとともに，移植医も病院訪問を行い，膵島移植の説明とドナー情報等の協力を依頼してきた。そして，まだまだ十分とはいえないが，3年間で8例の膵臓提供が実現した。今後は，このような

啓発活動を，中国地方・四国地方にも広げていく予定である。また，その際には③で示したように脳神経外科を標榜する2次救急施設を中心とした病院訪問に重点をおくべきであることが，今回の検討から示唆された。最後の④の主治医によるオプション提示に関しては，臓器移植の分野で一定の効果を上げているいわゆるドナーアクションプログラムを各提供施設において展開することで，提供側の施設，特に主治医の認識の変化が期待できるといった報告がある[5]。今後は，JOTCo，県Coさらには組織移植Coとも連携して，このような運動も視野にいれ，組織バンクとしてさらなる提供数の増加に繋がるよう活動を強化していきたい。

謝　辞：本稿の内容の一部は，2003年度文部科学省の21世紀COEプログラム「糖尿病をモデルとしたシグナル伝達病拠点」の助成金による支援を受けています。

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□ 症例報告

摘出膵をヘリコプターで搬送し，膵島移植を施行した1例

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Successful islet transplantation using a pancreas shipped by a helicopter

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【Summary】

Background: To reduce cold and warm ischemic injury is paramountly important in islet transplantation in Japan. This is a case report of a successful islet transplantation using a pancreas shipped by helicopter.

Case: The donor was a 50-year-old man who was hospitalized at Wakayama prefecture due to hypoxic enchephalopathy. Before admission he suffered at least 10 minutes of cardiac arrest. Cannulas for in situ cooling were inserted via femoral artery and vein before cardiac arrest through the appropriate consent from the kin. Total pancreatectomy was performed preceded by bi-lateral nephrectomy. Excised pancreas was preserved by the two-layer method and shipped to Kobe University by helicopter. The pancreas was digested by the method described by Ricordi, et al at Kobe University.

Results: It required only an 80-minute flight fiom Wakayama to Kobe University. This is one third of the time required with usual vehicle or railway transportation. Final islet yield was 352,000 IEQ with>30% purity, 99.6% viability, 9 ml of total tissue volume, negative Gram staining, and negative endotoxin level.

Recipient: The selected recipient was a 60-year-old man who was on insulin due to his labile type 1 diabetes. His body weight was 63 kg. Islets were infused into the liver via portal vein. The immunosuppressive protocol was almost the same as that of the Edmonton protocol. Three weeks after the transplant his glycemic lability was improved. Daily insulin requirement was two thirds the pre-transplant level.

Conclusion: shipping by helicopter helps in useing marginal pancreas in islet transplantation.

Keywords: islet transplantation, helicopter transportation, two-layer method, non-heart beating donor

I. はじめに

膵島移植においては，摘出膵搬送に要する冷阻血時間は，膵島収量を低下させる主要な要因の1つである[1]。また，わが国の膵島移植で使用できる膵臓は，心停止後摘出膵にほぼ限定される[2]が，摘出前後の温阻血傷害も膵島収量を低下させる因子の1つである[3]。これらの阻血傷害をいかに最小限にするかが，移植成績向上のためには非常に大切である。

われわれはこれらの問題に対し，①膵グラフトの温阻血傷害からの機能回復，冷阻血傷害の軽減を目的として，膵臓の搬送・保存に二層法を使用する[4]。②摘出時の温阻血時間を可及的に短縮するために，心停止前にカニュレーションが行われる腎提供症例に限定する，等の対策を行っている。

今回，摘出膵をヘリコプターで搬送し，冷阻血時間

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を従来よりもさらに短縮でき，膵島移植を行いえた症例を経験したので報告する。

II. ドナー

ドナーは，窒息による低酸素脳症で和歌山県内の病院に入院中の50歳代の男性で，病院搬送前に10分以上の心停止があり，いわゆるマージナルドナーと考えられた。入院中の血液検査では膵の虚血傷害を反映すると思われるアミラーゼ値，リパーゼ値，血糖値等に異常な上昇を認めなかったため，開腹所見で摘出の可否を判断することとした。心停止と同時に，あらかじめ大腿部より挿入したカニューレから冷保存液（初期はEC液続いてUW液）を灌流しながら手術室に搬送，腎摘出チームが腎臓の摘出を開始した。開腹所見で，膵に肉眼的な異常を認めなかったため摘出することとし，腎摘出に引き続いて，神戸大学の膵摘出チームが十二指腸温存膵全摘を行った。

III. 膵の搬送

摘出膵は二層法を使用して提供病院から神戸大学の膵島分離施設まで搬送した。二層法に使用するperfluorochemical（PFC）は，腎摘出中の時間を利用して酸素化した。

通常われわれは，摘出施設から神戸大学の膵島分離施設までの輸送手段として車（神戸大学から業務使用許可を得た車両）を使用している。今回の場合は，帰路の一般道区間が長くまた雨天であったこと等，車での搬送には長時間を要する可能性があった。そこで，和歌山から神戸大学までヘリコプターを利用することになった。

ヘリコプターの運航は，患者搬送等の実績が豊富な民間のボランティア団体（スカイワン，代表：田井秀治氏）が担当した。運航に必要な手続きもすべてスカイワンの担当者が行った。提供病院から県内のヘリポートまでは緊急車両により搬送し（所要時間およそ44分），そこから神戸大学病院屋上のヘリパットまでの飛行時間はおよそ36分であり，搬送に要した時間は80分であった。

IV. 膵島分離

摘出膵の温阻血時間（心停止から冷灌流開始まで）は0分，冷阻血時間（冷灌流開始から膵島分離開始まで）は406分であった。膵島分離はRicordi法に準じて行い[5]，純化はCOBE 2991にEuro-Ficollの連続密度勾配を作成して行った。結果を表1に示す。膵島収量は352,000 IEQであり，レシピエント候補者の体重が70.4 kg未満であれば新鮮膵島移植が可能である膵島収量が得られた。

表1　膵島分離結果

摘出膵	重　量	183 g
	温阻血時間（WIT）	0分
	冷阻血時間（CIT）	406分

	結　果	新鮮膵島移植基準*
膵島収量	352,000 IEQ	>5,000 IEQ/kg
純　度	>30%	>30%
Viability	99.6%	>70%
組織量	9 ml	<10 ml
エンドトキシン	測定感度以下	<5 EU/ml
グラム染色	陰性	陰性

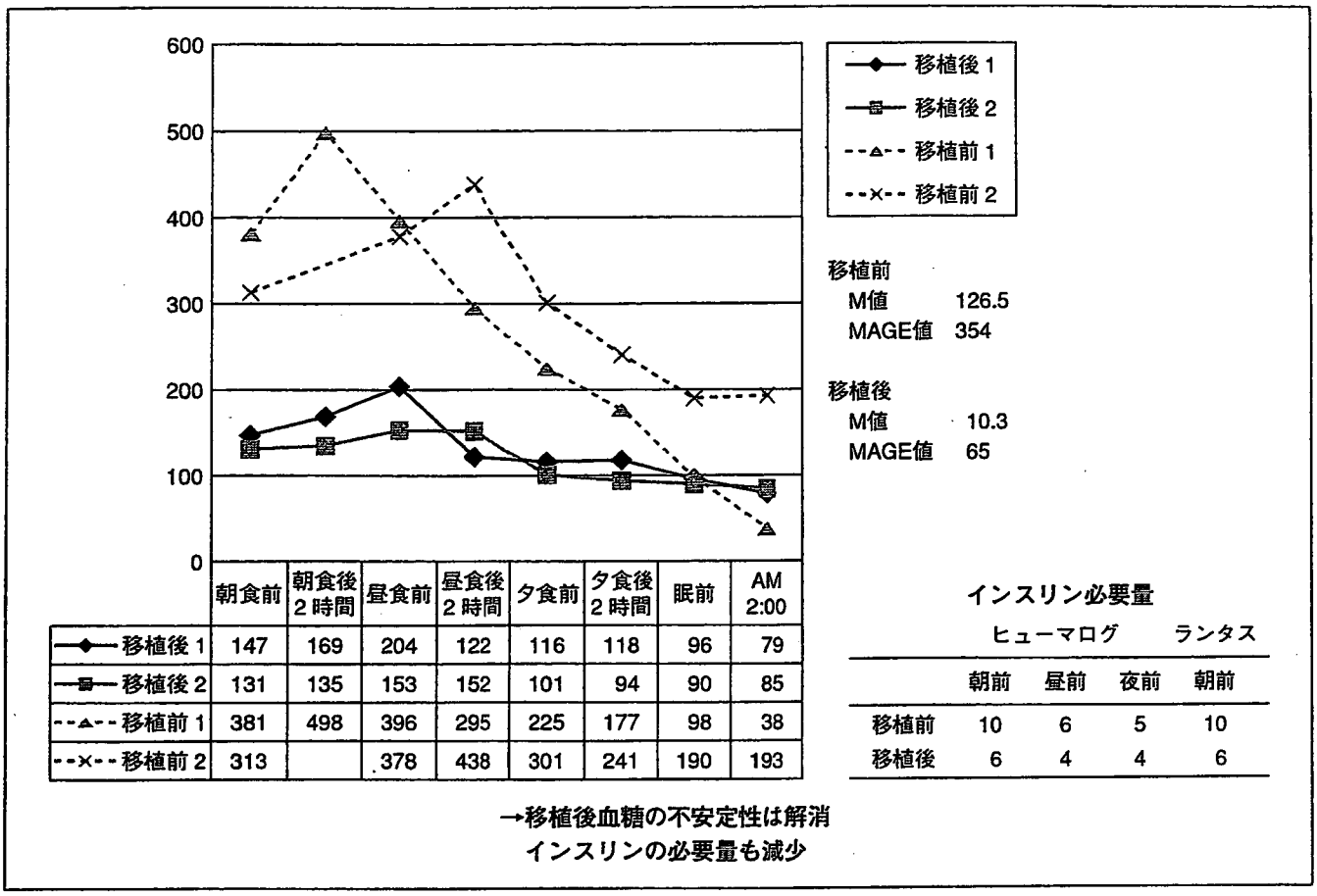
*：『膵島移植マニュアル』（膵・膵島移植研究会編）による

V. レシピエント

膵島移植班事務局で選定されたレシピエント候補者は，1型糖尿病で，27歳時よりインスリン治療中の60歳男性で，2003年8月18日から膵島移植待機中であった。体重は63 kgであり，結果的に膵島収量は5,587 IEQ/kgとなり，移植基準をすべて満たすため，本人の意見を確認の上移植することになった。移植は，神戸大学病院中央放射線部の血管造影室にて行った。放射線科医により超音波ガイド下門脈穿刺を局所麻酔下に行い，門脈本幹に5 Frカニューレを挿入した。門脈造影を行い，カニューレの位置および造影剤の漏出がないことを確認した後，点滴法にて膵島を門脈内に注入した。この間，門脈圧を経時的にモニターした。

術後経過はおおむね良好で，穿刺部からの出血や肝機能異常等の移植に起因すると思われる合併症は認めなかった。なお術後の血糖管理は，糖尿病内科の協力の下に行った。免疫抑制はエドモントンプロトコールに準じ[6]，IL-2レセプター抗体（シムレクト®移植前，移植後4日後にそれぞれ20 mg静注），sirolimus（ラパマイシン®移植前0.2 mg/kg，その後0.1 mg/kg経口，薬物血中濃度12–15 ng/ml），tacrolimus（プログラフ®1 mg経口，トラフ値3–6 ng/ml）の3剤にて行った。

移植後3週間目の1日血糖の推移を図1に示す。移植前には1日およそ30単位のインスリン注射を必



	朝食前	朝食後2時間	昼食前	昼食後2時間	夕食前	夕食後2時間	眠前	AM 2:00
移植後1	147	169	204	122	116	118	96	79
移植後2	131	135	153	152	101	94	90	85
移植前1	381	498	396	295	225	177	98	38
移植前2	313		378	438	301	241	190	193

インスリン必要量

	ヒューマログ			ランタス
	朝前	昼前	夜前	朝前
移植前	10	6	5	10
移植後	6	4	4	6

図1　移植前後の一日血糖

要としていたが，術後は2/3の20単位まで減量することができた。また，血糖の不安定性を示すM値は移植前の126.5から移植後は10.3と著明に改善し，血糖の不安定性が解消したことが示された。レシピエントは移植後1カ月目に退院となった。

VI. 考　察

移植手技が簡便で，レシピエントに対する安全性が高い膵島移植は，膵臓移植に代わる治療法として期待されたが，従来その成績は著しく不良で実験的医療の域を出ないものであった。1996年のITR (International Islet Transplant Registry) のreportによれば，移植成功の要因として，①臓器搬送に要する膵保存時間が8時間以内であること，②レシピエント体重あたり6,000 IEQ以上の膵島が移植されること，③門脈経由で肝臓に移植されること，④免疫抑制として，T細胞に対する抗体を用いたinduction therapyが，移植前になされていること。以上4点が挙げられていた[7]。これらの問題点に対し，新しい免疫抑制法を軸としたいわゆるエドモントンプロトコールと呼ばれる独自の膵島移植法を考案し，膵臓移植に匹敵する短期成績を発表したのがアルバータ大学であり[6]，これ以降膵島移植がにわかに脚光を浴びた。しかし，エドモントンプロトコールにおいても，膵の保存許容時間は従来と同じく8時間以内とされ，膵島移植においては冷阻血傷害の軽減は解決すべき課題として残されていた。これに対し，われわれが開発した二層法の臨床応用によって，ある程度の効果を示すことができ[4]，これ以降世界の臨床膵島移植で使用され，膵島分離前の膵保存の標準法とみなされるようになった[8]。

一方，わが国の膵島移植は組織移植に分類され，使用できるのは心停止後摘出膵にほぼ限定される。このため，搬送時の冷阻血傷害のみならず，摘出前後の温阻血に対する対策も必要となり，二層法の使用が強く推奨されている[2]。とはいえ，冷阻血傷害のみならず温阻血傷害にもさらされている心停止後摘出膵を使用するわが国の膵島移植においては，これら阻血時間を可及的に短縮することは非常に重要であるといえる。今回の症例では，搬送にヘリコプターを使用したことにより，他の交通機関を使用した場合と比較し，搬送

時間は1/2～1/3に短縮されたことになり，これが膵島分離の成功に繋がった要因の1つであると考えている。ドナーの入院前には，膵島収量を低下させる因子の1つである心停止の既往[9,10]があったことから，今回のように冷阻血を可能な限り短縮できた意味は大きいといえる。

神戸大学での膵島移植は2004年度に第1例目の膵島分離を行って以来現在（2007年5月末）までに，8例の膵臓提供が実現し，7回の膵島分離を行い3名の患者におのおの1回の移植を行った。しかしいまだに移植を受けていない待機患者は14名で，移植を受けた3名も追加移植が必要である。従って現状以上に移植機会を増やす努力が求められている。そのような意味で今回の症例は，提供された膵臓を有効に移植でき，貴重な経験となった。

謝　辞：ヘリコプターの運航にあたりご尽力いただいたスカイワン(代表：田井秀治氏)に深甚なる感謝を表します。

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Improved Quantity and In Vivo Function of Islets Isolated by Reduced Pressure-Controlled Injection of Collagenase in a Rat Model

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In islet transplantation, insufficient yield is a major obstacle to one-donor/one-recipient transplant. Collagenase, which is injected via a pancreatic duct to separate islets from acini, can so easily distribute into the islet core that it may result in disruption of islets. The purpose of this study was to evaluate the superiority of reduced pressure-controlled collagenase injection (RPCI) at 80 mmHg on islet isolation to injection at 180 mmHg by examining in vivo transplant experiments besides the yield and the glucose stimulation test in a rat model. Lewis rat pancreases were distended with collagenase solution at 80 mmHg pressure as the RPCI group (group 1) and at 180 mmHg (group 2), followed by isolation. The yield in group 1 (1100 ± 160 islets with 2750 ± 530 IEQ) was significantly higher than that in group 2 (900 ± 130 islets with 1570 ± 350 IEQ, $p < 0.01$) due to the significant difference of the number of islets sized >150 μm in diameter, although the purity was not significantly different between the two groups. Stimulation indices in the glucose stimulation tests were 2.88 ± 1.12 in group 1 and 1.93 ± 0.62 in group 2 ($p < 0.05$). The cure rate by transplantation of 100 islets to diabetic nude mice in group 1 (8/10) was significantly higher than that in group 2 (3/10, $p < 0.05$). In a syngenic transplant model of 90% of islets isolated from one donor, the cure rates were 100% and 67% in groups 1 and 2, respectively (NS). The area under the curve on the graph of IPGTT on postoperative day 28 in group 1 was significantly smaller than that in group 2 ($p < 0.05$). In conclusion, our data show that RPCI at 80 mmHg could contribute to consistently high islet yield and in vivo function in a rat model. It was suggested that the current human protocol should be reviewed from this viewpoint.

Key words: Islet isolation; Islet transplantation; Reduced pressure-controlled injection of collagenase

INTRODUCTION

Islet transplantation has demonstrated great success since the clinical report from the Edmonton group (13, 14). However, one of the major obstacles to one-donor/one-recipient transplantation is the insufficient yield of islets from a donor pancreas. In the Edmonton protocol, islets were isolated from two or more donors for each recipient (14,16). In addition, even in the best preparations the process recovered only 20–50% of the potential islet mass (12). Obtaining a consistently high yield is prerequisite to islet transplantation becoming a standard therapy for type 1 diabetes, from the viewpoints of donor shortage and economics.

One methodology, the collagenase perfusion method via a pancreatic duct, has become a standard technique to achieve high yields in islet isolation. The Edmonton group described that controlled collagenase perfusion permitted the gradual opening of the fine pancreatic ductal system slowly, resulting in improved collagenase delivery to all regions of the pancreas (8). They determined that the pancreas was best perfused under controlled conditions with collagenase solution while maintaining a constant pressure of 60–80 mmHg for the first 5 min and 160–180 mmHg for the next 5 min in the clinical setting, based on the principle reported by Horaguchi and Merrell (5).

On the other hand, van Suylichem et al. reported that collagenase could easily distribute into the islet core through its capsule even at <80 mmHg injection pressure and that the extent of its distribution depended on the this pressure (17,18). They suggested that injection of collagenase into the duct may easily result in disruption of islet integrity and, therefore, has to be performed with care (17).

It is hypothesized that excessive collagenase within

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islets due to injection at relatively high pressure such as at 180 mmHg might deteriorate islets during digestion, resulting in insufficient yield. It is well known that the pressure of collagenase injection is one of the keys to high yield and in vitro quality of isolated islets. But no one has previously reported on either the difference of quantity and quality of islets isolated following collagenase injection between 80 and 180 mmHg, such as in a clinical setting, or the difference of in vivo function.

The purpose of this study was to evaluate the superiority of reduced pressure-controlled collagenase injection (RPCI) at 80 mmHg on islet isolation to injection at 180 mmHg by examining in vivo transplant experiments besides the yield and the glucose stimulation test in a rat model, before a preclinical study in a large animal model.

MATERIALS AND METHODS

Animals

Male Lewis rats weighing 250–300 g (Oriental Yeast Co., Ltd, Tokyo, Japan) were used as donors and recipients, and 6- to 7-week-old male Balb-c nude mice (Oriental Yeast Co., Ltd) were used as recipients in this study. Diabetes was induced in recipient rats and mice by IV and IP injection of streptozotocin (Roche, Indianapolis, IN) dissolved in a freshly prepared citrate buffer (pH 4.5). The streptozotocin dose was 65 mg/kg body weight (BW) for a rat and 240 mg/kg BW for a nude mouse. Recipients were considered diabetic if their nonfasting blood glucose levels exceeded 350 mg/dl for 3 consecutive days. All animals were maintained in animal care facilities in accordance with the Guidelines for Animal Experimentation at Kobe University Graduate School of Medicine.

Isolation of Islets

Middle abdominal incision was performed under ether anesthesia. After a donor rat was killed by cutting the abdominal aorta, the pancreas was removed with the duodenum and spleen following cannulation of the common bile duct with a PE50 polyethylene tube (Becton Dickinson and Company, Sparks, MD). Each pancreas was distended by intraductal injection of 6 cc collagenase solution (1 mg/cc of Collagenase P (Cat. No. 1 213 873; Roche, Indianapolis, IN) in Hanks' balanced salt solution (HBSS; Sigma, St. Louis, MO)) on a cooled tray at 4°C. The two groups were divided according to the injection pressure. While maintaining the injection pressure with a manometer, the pancreases were distended at 80 mmHg in group 1 ($n = 14$), and at 180 mmHg in group 2 ($n = 14$). After distension, the spleen and duodenum were removed and the distended pancreases were incubated in a 50-cc conical tube at 37°C for 20 min (chemical digestion) without shaking. Subsequently, the chemical digestion was stopped by addition of 20 cc cold HBSS and the conical tube was shaken strongly 20 times (mechanical digestion). The suspension filtrated through a metallic filter net was washed with HBSS three times by centrifugation ($150 \times g$, 2 min, 4°C) and then purified with a discontinuous density gradient by using Histopaque 1077 (Sigma) and HBSS. The islets were collected and washed in RPMI-1640 (Gibco, Burlington, Ontario). The crude number of islets in each diameter class was determined by counting after diphenylthioncarbazone staining using an optical graticule. This number was then converted to the standard number of islets equivalents (IEQ; diameter standardizing to 150 μm) (9,11).

Stimulation Index (SI)

To determine the in vitro potency of isolated islets, the insulin secretary response to glucose ($n = 13$) was measured using a modification of the method previously described (15). Briefly, after incubation in RPMI-1640 solution containing 3.3 mM glucose at 37°C for 120 min, 20 islets of 150–250 μm in diameter were hand-picked and transferred to a Cell Culture Insert (Falcon, Franklin Lakes, NJ). Thereafter, they were suspended three times for 60 min at 37°C in RPMI-1640 solution with addition of various glucose concentrations (basal I: 3.3 mM, stimulation: 20 mM, basal II: 3.3 mM, respectively). The supernatants were collected and stored at −20°C. The insulin level was measured using a Rat Insulin Enzyme-Linked Immunosorbent Assay Kit (Mercodia AB, Uppsala, Sweden). The stimulation index (SI) was calculated as the insulin released into the stimulation medium divided by insulin released into the basal I medium.

Transplantation of Islets to Diabetic Nude Mice

Transplantation to diabetic nude mice was performed as an in vivo assessment of islet potency ($n = 10$). Diabetic nude mice were anesthetized by avertin, and the left kidney was exposed through a lumbar incision. A breach was made in the kidney capsule, and a polyethylene catheter was introduced through the breach and advanced beneath the kidney capsule to generate a subcapsular space. One hundred islets with a diameter range of 150–250 μm, which were hand-picked immediately after purification, were slowly injected through the PE50 tube into the subcapsular space. After removing the PE50 tube, the opening was cauterized, and the kidney was repositioned, followed by suturing of muscle and skin. Blood was sampled daily from the tail vein for the determination of blood glucose levels for 2 weeks after transplantation. On day 14 posttransplant the graft was removed by nephrectomy. Success of transplantation was defined as maintenance of normoglycemia (<200

mg/dl) for at least 3 consecutive days and return of hyperglycemia (>250 mg/dl) after nephrectomy (15).

Transplantation of Islets to Diabetic Rats

To evaluate the islet isolation results comprehensively without interference from any artificial factors, 90% of islets isolated from one donor, except 10% for counting, were transplanted into the portal vein of diabetic syngenic rats anesthetized by pentobarbital sodium (Dainippon Pharmaceutial Co., Ltd, Osaka, Japan) (n = 6). They were injected with 0.3 ml HBSS solution using a 23-gauge sure shield SV set (Terumo, Co., Ltd, Tokyo, Japan) connected to a 1-ml syringe. After islets were infused, the portal vein was compressed manually with a cotton bud to stanch bleeding, followed by suturing of muscle and skin. Blood was sampled daily from the tail vein for the determination of blood glucose levels for 4 weeks after transplantation. Success of transplantation was defined as maintenance of normoglycemia (<200 mg/dl) for at least 3 consecutive days. On day 28 posttransplant intraperitoneal glucose tolerance tests (IPGTTs) were examined. In brief, normal saline containing 2.5 g/kg BW of glucose was injected IP into overnight-fasting recipient rats 28 days posttransplantation. Their blood glucose levels were measured at 10, 30, 60, 90, and 120 min after injection. Glucose clearance was calculated after IP bolus by calculating the area under the curve (AUC) of the glycemic profile (6). Naive nude mice (n = 5) were also tested as a control for this procedure.

Histological Examination

After counting the number of purified islets, we examined islet purity and morphology under a microscope.

In another series, five pancreases immediately after distention with collagenase solution and five pancreases after subsequent incubation at 37°C for 20 min in each group were carefully harvested to fix in 10% formalin, embedded in paraffin, and stained with hematoxylin-eosin in order to assess the morphological damage against islets during digestion.

Statistical Analysis

Results were expressed as mean ± SD, and the statistical differences between groups were determined using Fisher's exact test and the Mann-Whitney U-test, where appropriate. Values of $p < 0.05$ were considered significant.

RESULTS

Islet Yield and Islet Size Distribution

The islet yield in group 1 (1100 ± 160 islets, 2750 ± 530 IEQ) was significantly higher than that in group 2 (900 ± 130 islets, 1570 ± 350 IEQ: $p = 0.0026$ and $p < 0.0001$, respectively) (Fig. 1). The numbers of isolated islets sized at 150–250 and >250 μm in diameter in group 1 were significantly greater than those in group 2 ($p = 0.0026$ and $p = 0.0078$, respectively) (Fig. 2). However, there were no significant differences in the numbers of islets in the sediment after purification in every diameter class between the two groups (data not shown).

Stimulation Index (SI)

Twenty islets with a diameter range of 150–250 μm were picked up and challenged using a glucose stimulation test as an in vitro function assessment. SI in group 1 (2.88 ± 1.12) was significantly higher than that in group 2 (1.93 ± 0.62, $p = 0.0225$ vs. group 1) (Table 1).

Transplantation of Islets to Diabetic Nude Mice

One hundred picked-up islets with a diameter range of 150–250 μm were transplanted into athymic diabetic nude mice as an in vivo function assessment. The success rate in group 1 (8/10) was significantly higher than that in group 2 (3/10, $p = 0.0349$ vs. group 1) (Table 1).

Transplantation of Islets to Diabetic Rats

In a syngenic transplant model, the crude numbers of transplanted islets were 990 ± 60 and 850 ± 110 in group 1 and 2 (NS), respectively. The purity of transplanted islets was 92.5 ± 2.7% and 90.0 ± 5.5% in group 1 and 2 (NS), respectively. Transplant success rate in group 1 (6/6) was higher, but not significantly higher, than that in group 2 (4/6).

To further investigate the function of these islets, IPGTTs were performed 28 days after transplantation (Fig. 3). The rate of normoglycemia at 120 min in group 1 (5/6) was significantly higher than that in group 2 (1/6, $p = 0.0400$) and was comparable with that in the control group (6/6) (Table 2). Moreover, the calculated AUC in group 1 was significantly smaller than that in group 2 ($p = 0.0163$), although it was also significantly different from the control group ($p = 0.0062$) (Table 2).

Histological Examination

We morphologically assessed the isolated islets after purification (Fig. 4A, B). The shape, border, and integrity of islets were not different between groups 1 and 2, except the size. But some single cells and fragments of islets were seen in group 2, in contrast to almost none in group 1. We also assessed the morphology of islets in each pancreas after chemical digestion before mechanical digestion in another series (Fig. 4C, D). Most of islets sized >150 μm in diameter in group 1 were well rounded and compact, and intracellular integrity was maintained while acinar cells were digested moderately. In contrast, a lot of fragile islets sized >150 μm in diameter were observed in group 2 and their intracellular integrity was severely broken. However, there was no sig-

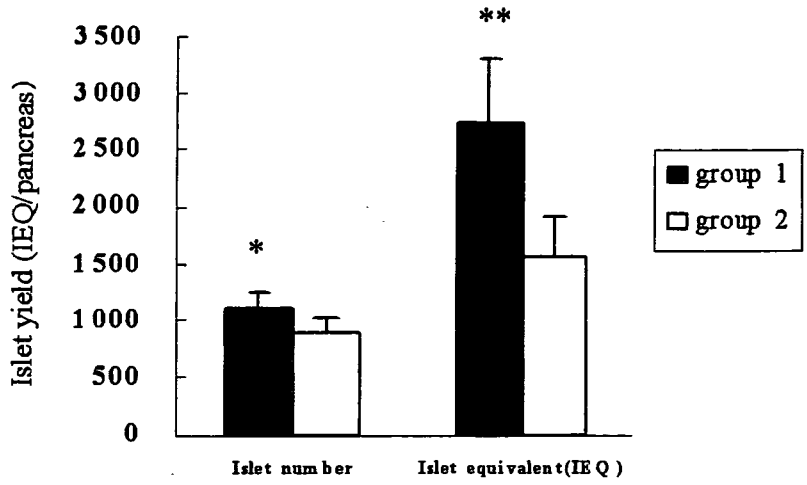

Figure 1. Islet yield. Data are expressed as mean ± SD. The islet yield in group 1 ($n = 14$) was significantly higher than that in group 2 ($n = 14$) [crude islet number: $*p < 0.01$, yield (IEQ): $**p < 0.0001$].

nificant difference in morphological damage against smaller ones between the two groups (data not shown).

DISCUSSION

Ductal injection or perfusion of the pancreas with a solution containing collagenase prior to islet isolation has been reported to result in high islet yields since Lacy introduced an islet isolation technique in rats that involved the ductal distension of the pancreas and collagenase digestion (7). In theory, either method seems an obvious way of delivering collagenase to the exocrine tissue without reaching the islets, because this ductal administration of collagenase could mechanically separate islets from acini and could chemically digest only acinar cells without any damage to islets. However, as many investigators have reported (1,3,17), collagenase administered via the ductal system can easily be distributed around and even within the islets themselves. Studies of the dispersion of India ink within the pancreas after intraductal injection have suggested that the initially injected collagenase solution is relatively selectively distributed to the exocrine tissue, sparing the islets, but that subsequent continued injection tends to produce increasing penetration of the solution into the islets (17,18). Moreover, the collagenase solution could easily penetrate into the islets at low injection pressures such as 40–90 mmHg (18).

Although Gray et al. suggested that it is unknown

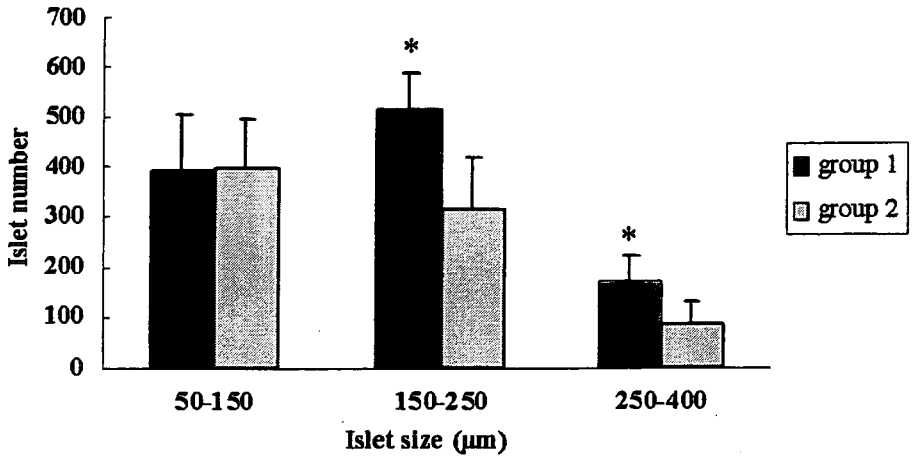

Figure 2. Islet number in each diameter class. Data are expressed as mean ± SD. The numbers of isolated islets sized at 150–250 and >250 μm in diameter in group 1 were significantly greater than those in group 2 (both $*p < 0.01$).

Table 1. Quality Control of Islets

Group	In Vitro Function: SI in the Glucose Stimulation Test	In Vivo Function: Transplant Success Rats to Nude Mice
1	2.88 ± 1.12* (n = 13)	80% (8/10)*
2	1.93 ± 0.62 (n = 13)	30% (3/10)

*$p < 0.05$ versus group 2.

Table 2. Intraperitoneal Glucose Tolerance Tests in a Syngenic Transplant Model

Group (No. of Tx Islets)	Normal Blood Sugar Level at 120 min	AUC
Group 1 (990 ± 60)	5/6*	31000 ± 6700*†
Group 2 (850 ± 110: NS vs. group 1)	1/6	43600 ± 5700†
Control (naive)	5/5*	15700 ± 700

*$p < 0.05$ versus group 2.
†$p < 0.01$ versus control.

whether penetration of the collagenase into the islets is a good or bad thing (4), there is no doubt that the amount of collagen within and at the periphery of the islets is related to the stability of the islets during chemical digestion by collagenase at 37°C, as well as the shape of the islets and the capillary density of the islets, and results in disruption of islet integrity, as van Suylichem et al. described (17). Because the extent of its distribution depends on the pressure of injection (18), the pressure of injection must be a key factor in successful islet isolation. On this point, it is easily hypothesized that higher pressure injection results in more collagenase within islets and consequently more damage to the construction of islets during the activation of collagenase.

The present study demonstrated that RPCI results in higher yield and higher quality in both in vitro and in vivo experiments compared to the high-pressure controlled collagenase injection at 180 mmHg (HPCI) in a rat model. Changes in the pancreatic morphology immediately after collagenase injection were not different macro- or microscopically between the RPCI group and the HPCI group (data not shown). Macroscopically the pancreases were just as well distended in the RPCI group as in the HPCI group, although it took a longer time to accomplish loading by RPCI (298 ± 28 s) compared to by HPCI (82 ± 16 s). A microscopic widening of the interlobar, interlobular, and interacinar spaces was seen in both groups, but no difference in the widening depending on the injection pressure was observed. However, after activation of collagenase by incubation at 37°C for 20 min following the collagenase injection, most islets in the distended pancreas in the RPCI group were morphologically intact, with compact and well-rounded shapes, compared with a lot of fragile islets sized >150 μm in diameter in the HPCI group. After purification, the number of isolated islets of >150 μm in the HPCI group decreased significantly and some single cells and fragments were seen in this group.

We also found that the number of the islets in the

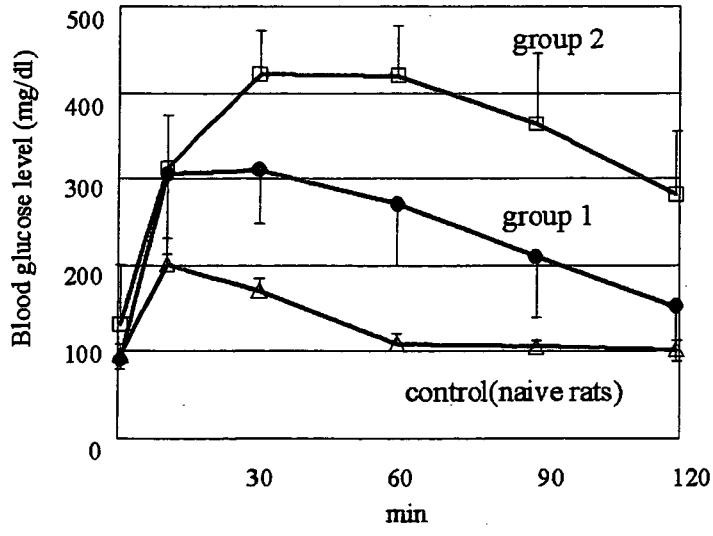

Figure 3. Intraperitoneal glucose tolerance test (IPGTT) 28 days after islet transplantation from a syngenic donor. Data are expressed as mean ± SD. Filled circle: group 1 (n = 6), open square: group 2 (n = 6), open triangle: naive mice (n = 5) used as a control.

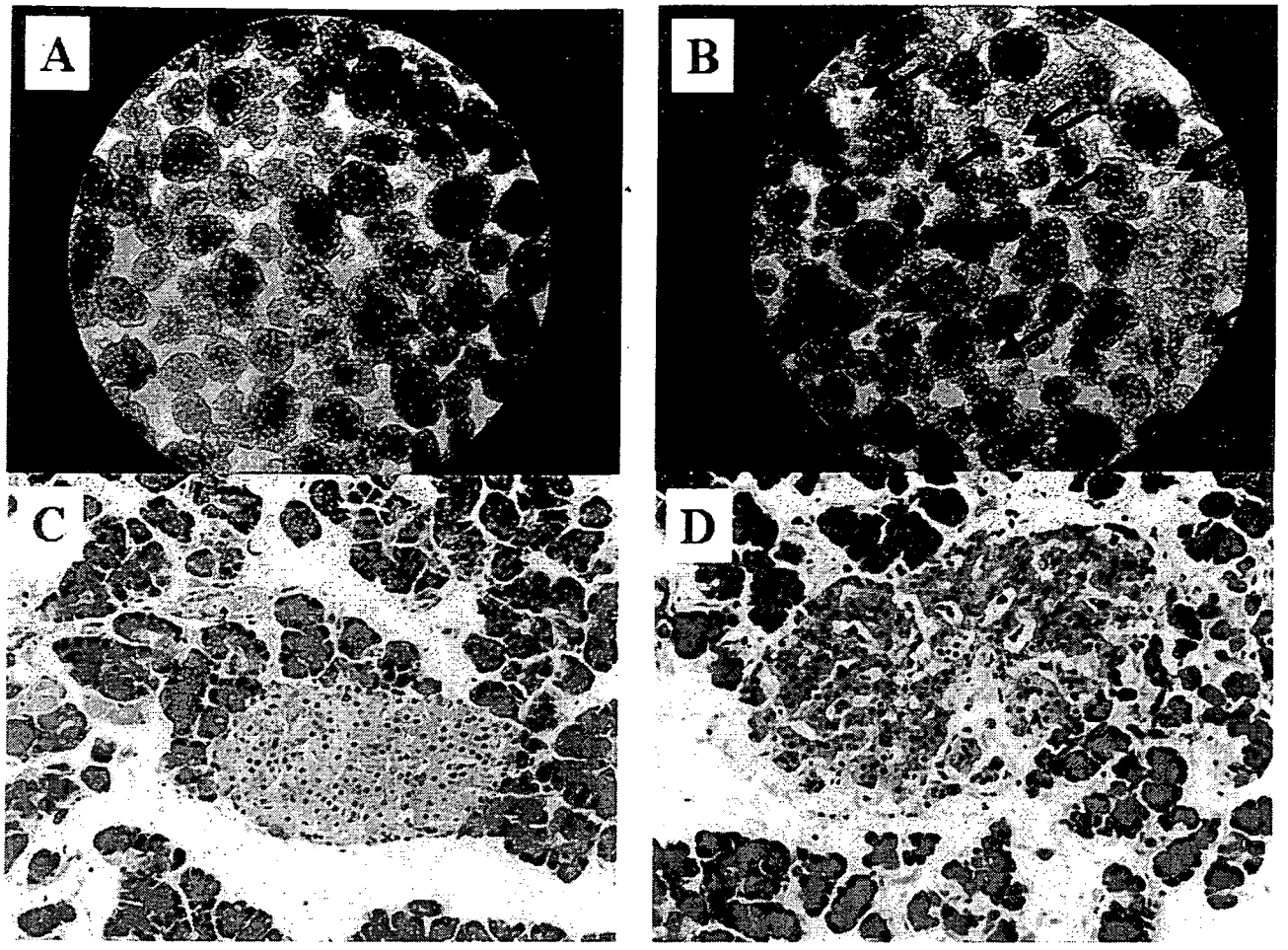

Figure 4. Appearance of isolated islets after purification (A and B: original magnification, 6×) and typical histological appearance after H&E staining of a pancreas after incubation (C and D: original magnification, 400×). The shape, border, and integrity of purified islets were not different between group 1 (A) and group 2 (B). But some single cells (↓) and fragments (↓↓) of islets were seen in group 2 (B), in contrast to almost none in group 1 (A). Most islets of >150 μm in diameter in group 1 (C) were well rounded and compact after incubation at 37°C for 20 min. In contrast, a lot of fragile islets in the same diameter class were observed in group 2 (D).

sediment, which contains acinar cells, after purification was small and not significantly different in each diameter class between these groups (data not shown). We speculate the following from these findings. Distension at 180 mmHg caused more widespread collagenase penetration into islets, compared with RPCI at 80 mmHg. These islets suffered considerable injury from the increased collagenase within islets themselves due to HPCI when it was activated in chemical digestion at 37°C. Consequently, some of these might be destroyed after purification, resulting in a poor yield. Even surviving islets should be significantly damaged, resulting in poor in vivo function. But we could not clarify whether the islets of >150 μm in diameter damaged during chemical digestion in the HPCI group were specifically destroyed and why this phenomenon could occur mainly in them.

The harmful effects of exogeneous isolation enzymes on islets during isolation were also reported by Balamurugan et al. (2). Exposure to Liberase, which is one of the exogeneous isolation enzymes like collagenase, could easily cause its penetrating the cytoplasm of beta cells during islet isolation, resulting in activation of apoptotic pathways and a reduced ability to release insulin in response to glucose without any effects on morphological abnormalities. If even exposure to enzymes could result in detrimental effects on isolated islets, it is more easily understood that the larger amount of collagenase within an islet due to the higher injection pressure could result in the poorer in vivo function of transplanted islets in our series.

In the clinical setting adopted in such a high-volume islet isolation center as Edmonton, Miami, or Minnesota, 80 mmHg for the first several minutes and subsequent

180 mmHg for the next several minutes were selected for their original recirculating perfusion device. This is based on the report by Horaguchi et al. (5) and many investigators have developed isolation methods from the viewpoint of collagenase administration (8,10,19,20). However, no one has previously reported the remarkable differences in islet yield and quality, especially in vivo function, due to the difference in injection pressure between at 80 mmHg and at 180 mmHg, which were both categorized as "low" pressure in previous series. It was reported that collagenase could be easily distributed even into the human islets similarly to rat islets and that relative collagen amounts within and around islets were fairly similar between these two species (17,18). But further investigations concerning the appropriate pressure of collagenase injection in human islet isolation should be performed, because the exact collagen contents in a pancreas are apparently different among species, and human pancreases have a variety of fibrotic components that depend on the donor's age and life style, and various extent of ischemic damage in a clinical setting.

CONCULSIONS

Our data clearly showed the importance of the reduced pressure of collagenase loading not only on islet yield and in vitro function, but also on in vivo function, and that the collagenase loading at 180 mmHg was detrimental to islets in a rat model. The current clinical protocol with loss of islets during isolation should be reviewed from the viewpoint of the pressure of collagenase loading.

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Comparison of M-Kyoto Solution and Histidine–Tryptophan–Ketoglutarate Solution With a Trypsin Inhibitor for Pancreas Preservation in Islet Transplantation

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The use of University of Wisconsin (UW) preservation solution in islet transplantation has some disadvantages, including inhibition of collagenase activity for pancreatic digestion. Histidine–tryptophan–ketoglutarate (HTK) solution has demonstrated an efficacy similar to UW solution for organ preservation in clinical pancreas transplantation. Recently, we reported that islet yield from porcine pancreata was significantly gtreater when they were preserved using M-Kyoto solution compared with UW solution. Here, we compared HTK solution with ulinastatin (M-HTK) and M-Kyoto solution for islet yield. In porcine islet isolation, islet yield after purification was significantly greater in the M-Kyoto/perfluorochemical (PFC) group compared with the M-HTK/PFC group. The M-Kyoto/PFC group had a significantly lower ADP/ATP ratio compared with the M-HTK/PFC group, suggesting that different islet yields might be due to the differences as energy sources of the solutions used. In conclusion, M-Kyoto/PFC solution is better for pancreas preservation before islet isolation than M-HTK/PFC solution.

Keywords: Islet transplantation, Islet isolation, M-Kyoto solution, Histidine–tryptophan–ketoglutarate solution, Trypsin inhibitor.

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Pancreatic islet transplantation represents a viable option for the treatment of patients with unstable type 1 diabetes mellitus who have frequent severe hypoglycemia and hypoglycemia unawareness (*1–6*). Since the Edmonton protocol was announced, more than 500 type 1 diabetes patients in more than 50 institutions have undergone islet transplantation to cure their disease. However, treatment of diabetic patients by pancreatic islet transplantation often requires the use of islets from two to four donors to produce insulin independence in a single recipient (*1, 2, 5, 6*). After isolation and transplantation, islets are susceptible to apoptosis, which limits their function and probably long-term islet graft survival.

Donor pancreata usually are preserved with University of Wisconsin (UW) solution. Recent reports have shown that the two-layer method (TLM), which uses oxygenated perfluorochemical (PFC) and UW solution, is superior to simple cold storage in UW not only for preserving the whole pancreas but also for improved viable islet yield in subsequent islet transplantation (*7, 8*). However, use of UW solution in islet transplantation has some disadvantages. The high potassium concentration in UW solution causes insulin release from pancreatic β cells (*9*), and the high viscosity of UW solution may prevent sufficient flushing. Moreover, UW solution inhibits the activity of Liberase, an enzyme blend used for pancreatic digestion (*10, 11*). Our previous study showed that ET-Kyoto (Kyoto solution®, Otsuka Pharmaceutical, Tokyo, Japan) with ulinastatin (Miraclid®, Mochida Pharmaceutical, Tokyo, Japan) (M-Kyoto) in combination with PFC significantly improved viable islet yields compared with UW/PFC preservation (*12*). The effectiveness of ET-Kyoto solution has also been demonstrated in clinical lung transplantation (*13, 14*) and skin flap storage (*15*). ET-Kyoto solution contains trehalose and gluconate. Trehalose has a cytoprotective effect against stress, and gluconate acts as an extracellular anti-oncotic agent, which prevents cells from swelling (*16*). Histidine–tryptophan–ketoglutarate (HTK) solution (Custodiol®, Alsbach, Hähnlein, Germany), originally developed for cardioplegia, is being used with increasing frequency in cardiac, renal, and hepatic transplantation (*17, 18*). The protective effect of HTK solution is based on the strong buffering capacity of histidine. This solution has a low viscosity, easy handling properties and a relatively low cost. Some studies have demonstrated comparable results between UW and HTK solution for pancreas preservation not only in experimental animal models (*19–21*) but also clinical pancreas transplantation (*22–24*).

In this study, we compared M-Kyoto solution with HTK solution containing ulinastatin (M-HTK) for islet isolation. Animal studies were approved by the Institu-

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TABLE 1. Pig islet isolation characteristics

	M-Kyoto (n=6)	M-HTK (n=4)
Pancreas size (g)	105.2±17.8	108.9±29.0
Operation time (min)	7.8±2.1	7.0±2.2
Warm ischemic time (min)	26.7±3.6	26.0±2.9
Cold ischemic time (min)	123.0±3.5	123.5±3.1
Phase I period (min)	10.2±3.6	9.5±2.1
Phase II period (min)	35.3±6.1	32.8±8.6

Data are expressed as mean±SD.

TABLE 2. Pig islet characteristics

	M-Kyoto (n=6)	M-HTK (n=4)
Islet yield before purification (IE/g)	10,121±1,674	7,904±4,970
Islet yield after purification (IE/g)[a]	6,599±1,854	3,147±1,979
Viability (%)	96.5±2.7	96.4±4.4
Score	9.3±0.6	9.5±1.0
Purity (%)	70.0±16.7	82.2±21.9
Recovery rate (%)	65.6±17.9	43.4±13.3
Stimulation iIndex	2.29±0.67	1.58±0.23

Data are expressed as mean±SD.
[a] Islet yield after purification was significantly greater in the M-Kyoto/PFC group than in the M-HTK/PFC group ($P<0.05$).

tional Animal Research Committees of Kyoto University, Nagoya University, and Fujita Health University. Porcine pancreata were obtained at a local slaughterhouse. About 10 minutes after the cessation of heart beating, the surgery was started. After removing the pancreas, we immediately inserted a cannula into the main pancreatic duct, infused either M-Kyoto or M-HTK preservation solution for ductal protection, and placed the pancreas into the respective two-layer preservation container (M-Kyoto/PFC or M-HTK/PFC). Islet isolation was conducted in accordance with the Kyoto Islet Isolation Method modified in the Edmonton protocol (*1, 4–6, 8, 12*). The characteristics of the porcine islet isolation protocols are shown in Table 1. There were no significant differences in pancreas size, operation time, warm ischemic time, cold ischemic time, Phase I period, or Phase II period between the two groups. Islet yield before purification was higher, but not significantly so, in the M-Kyoto/PFC group (n=6) compared with the M-HTK/PFC group (n=4). Islet yield after purification was significantly higher in the M-Kyoto/PFC group compared with the M-HTK/PFC group (Table 2). Other porcine islet characteristics are shown in Table 2. There were no other significantly different characteristics between the two groups.

Islet function was assessed by the adenosine diphosphate (ADP)/adenosine triphosphate (ATP) ratio, which shows the energy status of islets and correlates with transplantation outcome, according to a procedure described by Goto and colleagues (*25*). The ADP/ATP ratio was measured using the ApoGlowTM kit (Cambrex Bio Science Nottingham Ltd., Nottingham, UK). The ADP/ATP ratio in the M-Kyoto/PFC group was significantly lower than in the M-HTK/PFC group (Fig. 1A). These data suggest that different islet isolation effects between the two preservation solutions might be due to their differences as energy sources. To assess the islet graft function of each group in vivo, mice with severe combined immunodeficiency disease (SCID; CLEA Japan, Inc., Meguro, Tokyo) were used for the experiments. The recipients were rendered diabetic by a single injection of streptozotocin (STZ) at a dose of 220 mg/kg. The 1,500 or 2,000 IE pig islets obtained from each group were transplanted into the renal subcapsular space of the left kidney of diabetic SCID mice as previously described (*26–28*). When 1500 IEs from each group were transplanted below the kidney capsule of STZ-induced diabetic SCID mice, the normoglycemic rate was

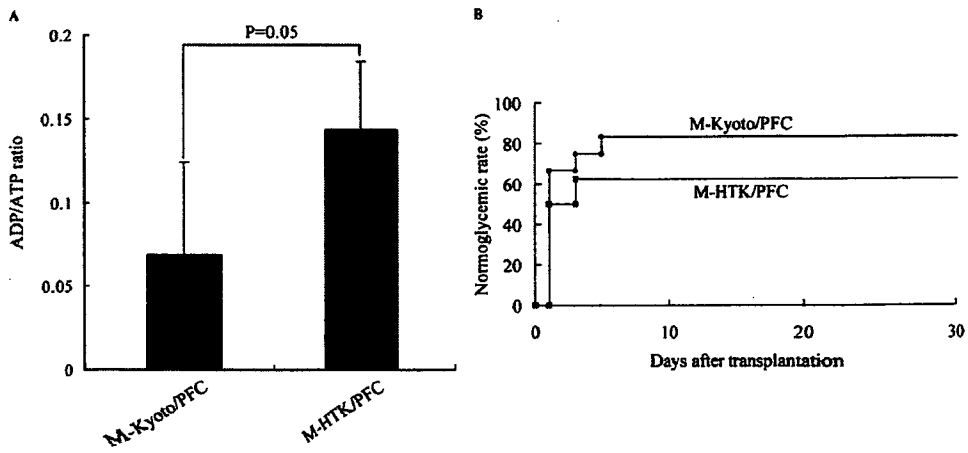

FIGURE 1. ADP/ATP ratio and transplant experiment. (A) The ADP/ATP ratio was measured to evaluate the energy status of cultured islets using the ApoGlowTM kit. The ADP/ATP ratio in the M-Kyoto/PFC group was barely lower than in the M-HTK/PFC group. Data are expressed as the mean±standard deviation. (B) Normoglycemic rate of STZ-induced diabetic SCID mice after islet transplantation. Immediately after isolation, 1,500 IEs were transplanted below the kidney capsule of diabetic SCID mice. Normoglycemia was defined as two consecutive posttransplant blood glucose levels showing less than 200 mg/dl (M-Kyoto group n=12; M-HTK group n=8).

greater, but not significantly so, in the M-Kyoto/PFC group (n=12) compared with the M-HTK/PFC group (n=8) (Fig. 1B). When 2000 IEs from each group were transplanted below the kidney capsule of STZ-induced diabetic SCID mice, the normoglycemic rate was more than 80% in both groups.

Inhibitory effects of collagenase on preservation solutions, such as UW solution, result in poor islet yield and islets of poor viability (*10, 11*). It has been reported that the components in UW solution found to be most inhibitory were magnesium, low Na+/high K+, hydroxyethyl starch (HES), and adenosine. Allopurinol, in combination with either lactobionate or glutathione, was markedly inhibitory, and the most inhibitory solution tested was a combination of three components, raffinose, glutathione, and lactobionate (*11*). M-Kyoto solution has high Na+/low K+ and, of the UW components, it contains only HES at a lower concentration. Moreover, trehalose and ulinastatin in M-Kyoto solution inhibit collagenase digestion less than UW solution (*12*). The M-HTK solution includes magnesium, but does not include HES, adenosine, allopurinol, lactobionate, glutathione, or raffinose. It has also been shown that the adenosine, allopurinol, and glutathione are not essential for the cold storage of pancreatic digests prior to islet purification (*29*). To assess the inhibitory effects on collagenase by M-Kyoto and M-HTK solutions, the rate of inhibition on collagenase digestion was measured in accordance with the modified method as previously described (*11*). The median digestion time was 79.0±2.9 min for M-Kyoto solution and 77.0±1.3 min for M-HTK solution. There are no significant differences between the two solutions on collagenase activity. Therefore, the different islet yields after purification are not due to differences in collagenase inhibition between these two solutions.

The TLM is important for preserving pancreata before islet isolation because it helps to preserve the organ, whereas UW preservation results in the deterioration of both islet isolation efficacy and posttransplant islet function (*7, 8*). The pancreas is directly oxygenated by PFC during pancreas preservation and maintains a high level of ATP in tissues. ATP drives a sodium pump, maintains cell integrity and repairs warm ischemic injury (*30, 31*). After significant warm ischemic injury, adenosine in UW solution is used as a substrate of ATP synthesis, however, without significant warm ischemia, ATP is generated from ADP or AMP located in the cells. We showed previously that ATP levels after preservation in M-Kyoto/PFC were similar to those in UW/PFC preservation and that adenosine is not importance for ATP generation when the warm ischemic time was less than thirty minutes (*12*). In this study, neither the M-Kyoto solution nor the M-HTK solution included adenosine. The ADP/ATP ratio in the M-Kyoto/PFC group was around half of that measured in the M-HTK/PFC group (Fig. 1), suggesting that different islet isolation effects between the two preservation solutions might be because of their differences as energy sources. Because energy status is an excellent predictor of successful pancreas transplantation (*32*), a low ADP/ATP ratio also might reflect effective two-layer preservation. Gluconate as an energy source or trehalose as a cytoprotectant might contribute to the effective M-Kyoto/PFC preservation of warm ischemically damaged pancreata.

In conclusion, M-Kyoto solution is superior to M-HTK solution for islet isolation. The improved pancreas preservation and islet isolation by M-Kyoto solution was not due to differences in collagenase digestion between the two solutions. However, a significantly lower ADP/ATP ratio was observed in the M-Kyoto/PFC group, compared to the M-HTK/PFC group, suggesting that the energy sources might be a factor in the islet yield differences observed between the two solutions. On the basis of these data, we now use M-Kyoto solution for clinical islet transplantation from non-heart-beating donor (NHBD) pancreata. M-Kyoto/PFC preservation makes it feasible to use NHBDs for efficient islet transplantation into type 1 diabetes.

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○先端の治療戦略

膵臓・膵島移植による糖尿病治療

—現況と展望—

岩永康裕*

要　旨

膵臓移植と膵島移植は，どちらもインスリン依存状態糖尿病に対する外科的治療法である．細胞移植である膵島移植は安全性が高く，臓器移植である膵臓移植は高いインスリン離脱率を維持できることが特徴である．現時点では，両者のメリット，デメリットならびに患者の病期を考慮して選択されるべきである．将来的には，低侵襲の治療法である膵島移植の技術がトランスレーショナルリサーチのプロトタイプとして発展していくことが予想される．

はじめに

1型糖尿病などのインスリン依存状態糖尿病に対する外科的治療法には，膵臓移植と膵島移植の2つの方法がある．どちらも膵 β 細胞を補充することで適正なインスリン分泌によって糖代謝を正常化し，糖尿病合併症の発症・進展を予防し，QOL を向上させることを目的としている．前者は膵臓ごと移植する臓器移植であるのに対して，後者は膵臓の中の膵島（ランゲルハンス島）のみを移植する細胞（組織）移植で，通常1人の患者に対して複数回の移植を行う．

膵臓移植は 1966 年米国ミネソタ大学において世界で初めて行われ，2004 年6月の時点で累積症例数は2万 3,000 例を超している．本邦では 1997 年の臓器移植法の施行後，2006 年 12 月までに 32 例が行われた．

膵島移植は，2000 年にカナダのアルバータ大学からいわゆるエドモントンプロトコールが発表されて移植成績が飛躍的に向上し，世界中に広まった．現在では 600 人以上の患者がすでに移植を受けている．本邦では 2004 年4月に第1例目が実施され，2006 年 12 月までに 15 人の患者に 28 回の移植が行われた．

膵臓移植

1．膵臓移植の分類（表1）

1）ドナーによる分類

膵臓移植はドナーによって，① 脳死ドナー膵臓移植，② 心停止ドナー膵臓移植，③ 生体ドナー膵臓移植に分類できる．脳死ドナ

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キーワード：膵臓移植，膵島移植，
インスリン依存状態糖尿病，
移植後合併症，
トランスレーショナルリサーチ

表1　膵臓移植の分類

1．ドナーによる分類
　① 脳死ドナー膵臓移植
　② 心停止ドナー膵臓移植
　③ 生体ドナー膵臓移植

2．術式による分類
　1）複合移植と単独移植
　　① 膵腎同時移植（simultaneous pancreas kidney transplant：SPK）
　　② 腎移植後膵移植（pancreas transplant after kidney：PAK）
　　③ 膵単独移植（pancreas transplant alone：PTA）
　2）膵液ドレナージ法による分類
　　① 膀胱ドレナージ法（bladder drainage：BD）
　　② 腸管ドレナージ法（enteric drainage：ED）
　3）静脈血ドレナージ法による分類
　　① 大循環系ドレナージ法（systemic drainage）
　　② 門脈系ドレナージ法（portal drainage）

一膵臓移植が大多数であるが，心停止ドナー膵臓移植も少数の報告例がある．生体ドナー膵臓移植は健常なドナーの膵体尾部を用いるもので，米国ミネソタ大学を中心に行われている．

2）術式による分類

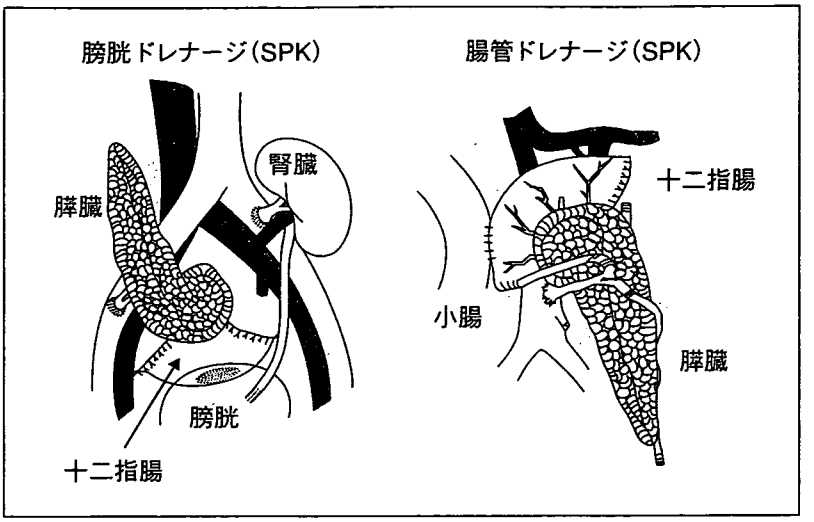
膵腎複合移植と膵単独移植（PTA）があり，さらに前者は膵腎同時移植（SPK）と腎移植後膵移植（PAK）に分類される．

SPK は糖尿病性腎不全患者に対して膵臓と腎臓を同時に移植するものである．PAK は糖尿病性腎不全であらかじめ腎移植を受けた患者に対して膵臓移植を追加するものである．そして，PTA は非腎不全糖尿病患者に対して膵臓のみを単独で移植するものである．各術式数（2000 年 1 月～2004 年 6 月）を見ると，SPK が 71%，PAK が 21%，PTA が 8% を占めている．このように多くの場合 SPK が行われているが，最近 PAK も増加しつつある[1]．

さらに，膵液の誘導法によって膀胱ドレナージ法（BD）と腸管ドレナージ法（ED）に分類できる（図1）．BD は膵頭部に付いている十二指腸をレシピエントの膀胱に吻合して膵液を膀胱内に誘導する方法で，尿中のアミラーゼ値をモニタリングすることで拒絶反応の補助診断が可能という長所がある．しかし，尿路系合併症が多くなる．一方 ED は，十二指腸をレシピエントの小腸に吻合して膵液を腸管内に誘導する生理的な方法である．以前は BD が主流であったが，近年，免疫抑制法が進歩し移植術の安全性が向上したこと，また BD 施行後に尿路系合併症による ED への変更手術（enteric conversion）が必要となる症例がしばしばあることから，ED がよく用いられるようになった．SPK では 81%，PAK では 67%，PTA では 56% に ED が用いられている[1]．

また静脈血の環流法には，グラフトの静脈をレシピエントの腸骨静脈に吻合する大循環系ドレナージ法（systemic drainage）と上腸間膜静脈に吻合して門脈系に環流させる門脈系ドレナージ法（portal drainage）がある．通常は systemic drainage であるが，生理的

図1 膵液ドレナージ（近畿膵移植検討会ホームページより引用）



である portal drainage も増えてきている．

2．膵臓移植後合併症

合併症の中には，移植膵の摘出を余儀なくされたり，レシピエントが死に至るといった重篤なものがある．

1）移植膵臓の血栓症

移植後数日以内に移植した膵臓の静脈（門脈）または動脈内に血栓ができる場合があり，移植膵臓の摘出が必要となる．その原因は，ドナー膵臓が血流を再開するまでに受けた障害度，手技的要因によることが多い．最近では，臓器保存液の改良により欧米での血栓症の頻度は5％以下に低下している．

2）膵液瘻

BD が導入されて以来その発生頻度は減少したが，いったん発生した場合は膿瘍を形成し，血管吻合部への波及と出血の危険性がある．

3）十二指腸・膀胱吻合部縫合不全

BD では，十二指腸への血流不全により吻合部縫合不全が起こることがあり，尿と膵液の腹腔内への漏出で感染が起こる．この場合，再吻合か enteric conversion が必要となる．感染が進むと移植膵臓の摘出が必要になる場合もある．

4）血　尿

膀胱・尿管吻合部位からの出血や，拒絶反応による十二指腸からの出血で血尿となることがある．

5）移植膵膵炎

虚血性膵炎あるいは移植膵臓への尿の逆流で膵炎を起こすことがある．

6）代謝性アシドーシス

BD において，膵液中の重炭酸の尿中への喪失により代謝性アシドーシスを来す．高度な場合は enteric conversion が必要なことがある．

7）尿路感染と排尿障害

糖尿病による神経性排尿障害あるいは膵液の膀胱内での感染のため，膀胱炎を起こしやすくなっている．

8）十二指腸・小腸吻合不全

ED では，十二指腸への血流不全により吻合部縫合不全が起こることがあり，膵液と腸液の腹腔内への漏出で感染が起こる．感染が進むと移植膵臓の摘出が必要になる場合もある．

9）免疫抑制薬の副作用

使用する免疫抑制薬の種類によってさまざまな副作用がある．顆粒球減少症，骨髄抑制，腎毒性による腎機能低下，耐糖能異常，高血