Magnetic Microspheres (MMS) Coupled to Selective Lectins: A New Tool for Large-scale Extraction and Purification of Human Pancreatic Islets

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DUE TO the strong allograft immunogenicity of the exocrine pancreas, it is important to remove these cells from islet preparations before transplantation. In rodent models, hand-picking provides sufficiently purified preparations. But, for the number of islets needed in human transplantation, this method is far too time-consuming and laborious. A new approach for solving this problem has been established in rat models.[1] It was shown that magnetic microspheres (MMS) coupled to a lectin with binding specificity for the exocrine tissue portion, namely UEA-I, can trap the latter in an electromagnetic field, thus providing effluent islets of a high degree of purity. It was the aim of this study to adapt this principle to human islet preparations. In this context, our primary interest was focussed on lectins of adequate specificity for human pancreatic tissue, and on the functional integrity of β-cells after magnetic separation.

MATERIALS AND METHODS

Human Islet Preparation

Crude human islet preparations were isolated from cadaver pancreata according to the technique of Gray et al[2] and stored in liquid nitrogen until further use.

Preparation of MMS and Lectin Coupling

MMS were prepared by modification of the basic procedure described recently.[3] They are activated with 2.5% glutaraldehyde and, after several washings, incubated with a lectin (20 μg lectin/mg MMS) in phosphate-buffered saline (PBS) for 2 hours. Finally, they are washed with PBS and stored in PBS + 0.02% sodium azide + 1% BSA + 0.05% Tween at 4°C. Before use, lectin-coupled MMS are washed several times with sterile PBS and resuspended in RPMI/10% FCS.

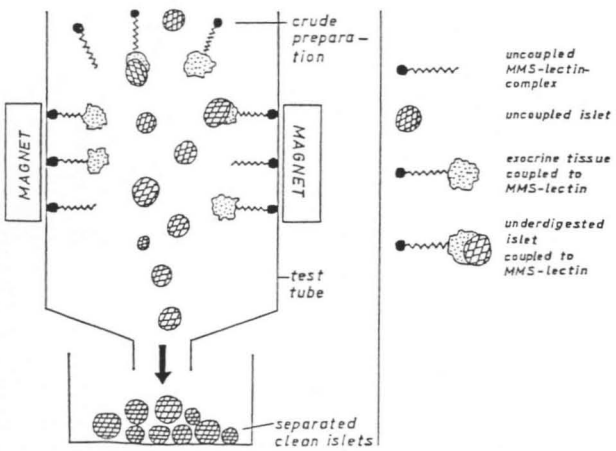

Fig 1. Principle of islet separation and composition of the various magnetic microsphere complexes.

Fluorescence Studies of Isolated Islets

Crude islet preparations were tested with 19 different lectins for their binding specificity to exocrine tissue: 100μl of crude islet suspension was incubated with 100 μl of fluorescein (FITC)-labeled lectin (diluted 1:10 with PBS, 40 min, 4°C in the dark) and after three washings prepared for microscopic evaluation.

MMS Binding Studies of Isolated Islets

We incubated 200 μl of crude suspension with 20 μl (0.2 mg) lectin-coupled MMS for 40 minutes on ice. MMS-binding was evaluated microscopically.

Magnetic Separation Procedure

We incubated 200-300 crude islets with 2-3 mg lectin-coupled MMS in 2ml RPMI/10% FCS for 40 minutes at 4°C in a 10 ml plastic tube (Fig 1). MMS-binding was evaluated microscopically. The suspension was separated by flow through an electromagnetic field and purified islets were collected as eluate according to the procedure of Winoto-Morbach, et al.[4] To distinguish islets from exocrine cells, separated islets were stained with the dye dithizone (diphenylthiocarbazone), which forms a red-coloured complex with zinc ions of the endocrine cells. Thus, separated islets could be analyzed microscopically for purification status.

Functional Integrity of Separated Islets

In in vitro rat islet studies (rat model study for human islets) a new highly sensitive ELISA[5] to determine total immunoreactive insulin (IRI) was established. This ELISA is based on the principle of competitive saturation of an anti-insulin antibody with either unlabeled or peroxidase-labeled insulin. Our magnetic separation method was compared to hand-picking with regard to the function of β-cells after separation. Insulin secretion was determined in separated rat islets by cultivation in glucose of various concentrations (CMRL medium, 10% FCS).

RESULTS

Lectin Binding Patterns of crude human islet preparations with FITC- or MMS-coupled lectins show that only 1 out of

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Table 1. Binding Patterns of Different Lectins to Different Human Pancreas Portions

		Groups of Lectins					
		1	2	3	4	5	6
		UEA-I	AAA HPA PNA GSA-I GSA-II BPA LPA DBA	Succ.ConA Succ.WGA SBA RCA-I	ECA LCA ConA WGA	MPA	WFA
A: Lectins coupled to FITC	exo.	+++	−	+	+	+	+++
	endo.	+	−	−	−	−	−
B: Lectins coupled to MMS	exo.	++	n.d.	n.d.	−	−	++
	endo.	+			−	−/+	−

+++ strong; ++ medium; + weak; − no detectable binding; n.d. not done; exo. exocrine; endo. endocrine.

Lectins (Agglutinins): Anguilla anguilla (AAA), Bauhinia purpurea (BPA), Concanavalia ensiformis (ConA), Dolichos biflorus (DBA), Erythrina cristagalli (ECA), Griffonia simplicifolia I (GSA-I), Griffonia simplicifolia II (GSA-II), Helix pomatia (HPA), Lens culinaris (LCA), Limulus polyhemus (LPA), Maclura pomifera (MPA), Peanut-Arachis hypogaea (PNA), Ricinus communis I (RCA-I), Soy Bean-Glycine max (SBA), Succinyl Concanavalia ensiformis (Succ. ConA), Succinyl Wheat Germ-Triticum vulgaris (Succ.WGA), Ulex europaeus I (UEA-I), Wheat Germ-Triticum vulgaris (WGA), Wistaria floribunda (WFA). Lectins were purchased from Medac, Hamburg, FRG.

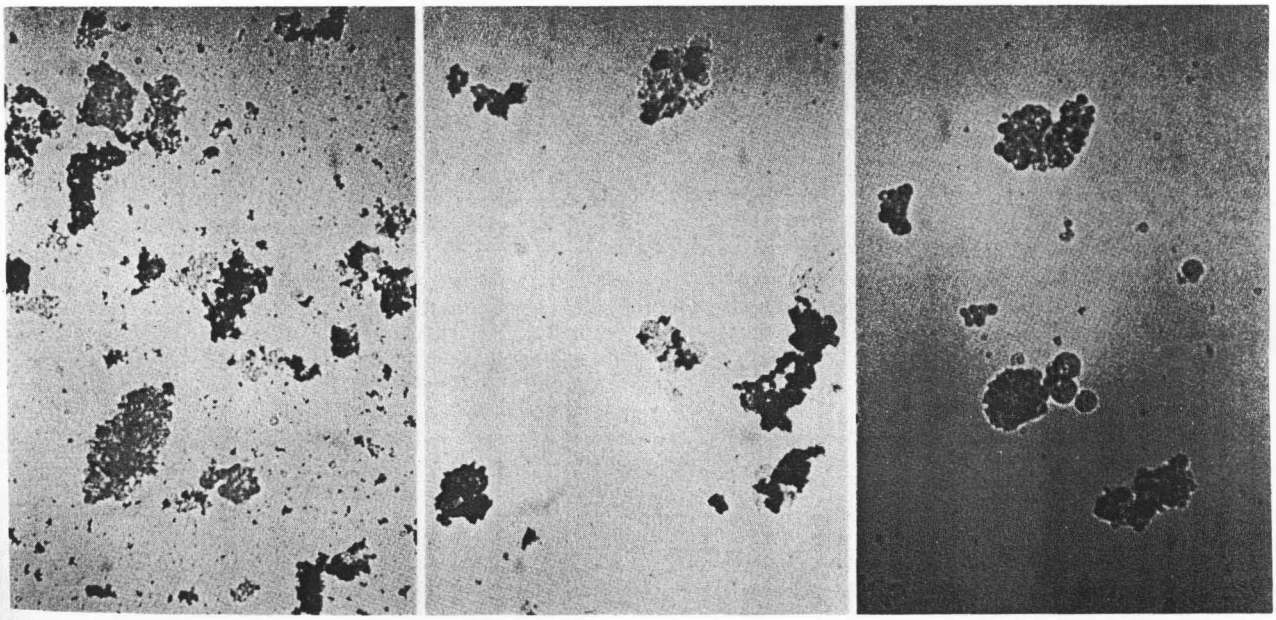

Fig 2. Human islet samples: (Left) Crude human islet preparation incubated with WFA-coupled MMS before magnetic separation. (Center) After magnetic separation exocrine cells are trapped in the magnetic fraction. (Right) Purified islets are collected in the eluate.

Table 2. Functional Integrity of Separated Rat Islets: Comparison Between Hand-picking and Magnetic Microspheres

Purification Procedure	Glucose (mM)	Insulin Secretion Per Islet (ng/ml)			
		24 h	48 h	72 h	96 h
Handpicking	5	206 ± 20	207 ± 15	201 ± 17	204 ± 3
	20	470 ± 20	459 ± 32	455 ± 22	452 ± 27
Magnetic separation	5	155 ± 32	153 ± 38	131 ± 17	135 ± 16
	20	500 ± 15	513 ± 25	495 ± 25	501 ± 12

19 lectins, namely WFA, reacted strongly and selectively in fluorescence and MMS-binding studies with human exocrine tissue (Table 1). Therefore only WFA was usable for MMS-separation studies.

Purity of WFA-MMS-separated human islets in the eluate was in the order of 90-95% when they were analyzed with the dithizone staining technique (Fig 2).

Viability before and after separation was studied by staining with propidium iodide and fluorescein diacetate and found to be in the order of more than 90%.

Functional integrity of magnetically separated rat islets is demonstrated in Table 2. The results indicate that the function of insulin secretion of β-cells is unaltered by the technical procedure of magnetic separation.

CONCLUSIONS

Our experience with lectins in studies on rat and human pancreas indicates that each islet donor species requires a new search for a lectin which selectively binds to the pancreas exocrine portion.

Magnetic separation does not alter the biological integrity of endocrine cells. Thus it is suggested that islet purification by WFA-coupled MMS is applicable to human islet separation without loss of function.

The electromagnetic flow separation principle is effective, simple, fast and applicable to large volumes in a standardized manner. Thus, it may be helpful in solving the problems of quantity and purity in human islet transplantation.

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