Key words: islet of Langerhans, isolation, University of Wisconsin solution, preservation, pig

Porcine Islet Preservation During Isolation in University of Wisconsin Solution

M.P.M. van der Burg, I. Basir, R.P. Zwaan, and E. Bouwman

SUBSTITUTION OF the University of Wisconsin solution (UWS) for the conventional Hanks balanced salt solution (HBSS) during collagenase digestion of the porcine pancreas has been reported to increase the yield of isolated porcine islets. Little is known, however, on the effects of the collagenase solution and different solutions during subsequent steps of the isolation and purification procedure on islet viability. Since the UWS probably also best preserves the islet tissue during the cold steps of the procedure, we compared the use of UWS for all steps of the isolation procedure and also during purification in our novel UWS-based Optiprep gradient[1] vs the use of HBSS for digestion and dispersion and similar Optiprep purification, by in parallel processing of two, paired, segments of the pancreatic body of market-age slaughterhouse pigs.

MATERIALS AND METHODS

Islet Isolation

The design and methods are illustrated in Fig 1. After 19 minutes warm- and 120 minutes cold ischemia the pancreatic body of ~ 6 months old Yorkshire-Pietrain slaughterhouse pigs (n = 7) was transected, and both portions were stationary digested—using UWS for the one and HBSS (containing 10 mmol/L HEPES) for the other segment—for a mean 29 minutes at 37°C with intraductal 2 mg/mL collagenase-P (Boehringer, Mannheim, Germany). The tissue was dispersed in cold isolation solution using forceps and ~ 8 cycles of filtration (400-μm mesh) and shaking of the tissue retained on the sieve. Next, islets isolated in HBSS (with 5% fetal calf serum during washing) were incubated 30 minutes in UWS prior to purification.

From the Department of Surgery (M.P.M.v.d.B., R.P.Z., E.B.), Leiden University, Leiden, The Netherlands; and the Department of Surgery (I.B.), Indonesia University, Jakarta, Indonesia.

Address correspondence to Dr Michel P.M. van der Burg, Department of Surgery Building K6-R, University Hospital, PO Box 9600, NL 2300RC, Leiden, The Netherlands.

A novel density solution of 30% w/v Optiprep™ (Nycomed Pharma, Oslo, Norway) in UWS was introduced for purification[1]. The digest in UWS was mixed with approximately half the volume of the Optiprep-UWS (final density 1.10 g/mL for UWS-isolated islets, and 1.095 for HBSS-isolated islets). The bottom solution of UWS-isolated islets was topped with an 1.095 g/mL Optiprep in RPMI solution, and RPMI. The bottom solution of HBSS-isolated islets was topped with an 1.085 Optiprep-UWS, and UWS. Gradients were centrifuged at 500*g* and 4°C for 5 minutes. Purified islets were aspirated from the top layer following isolation in UWS, and from the two uppermost layers following isolation in HBSS.

The mean diameters of all islets ≥50 μm, were recorded in 25-μm increments for calculation of the number of islet equivalents (IEQs), and the volume-average diameter. Viability was assessed by fluorometry of acridine orange and propidium iodide, and by trypan-blue staining.

Statistical Analysis

Results are expressed as means ± SE. Differences were analyzed by analysis of variance for repeated measures, and considered not significant (NS) at $P > .05$.

RESULTS AND DISCUSSION

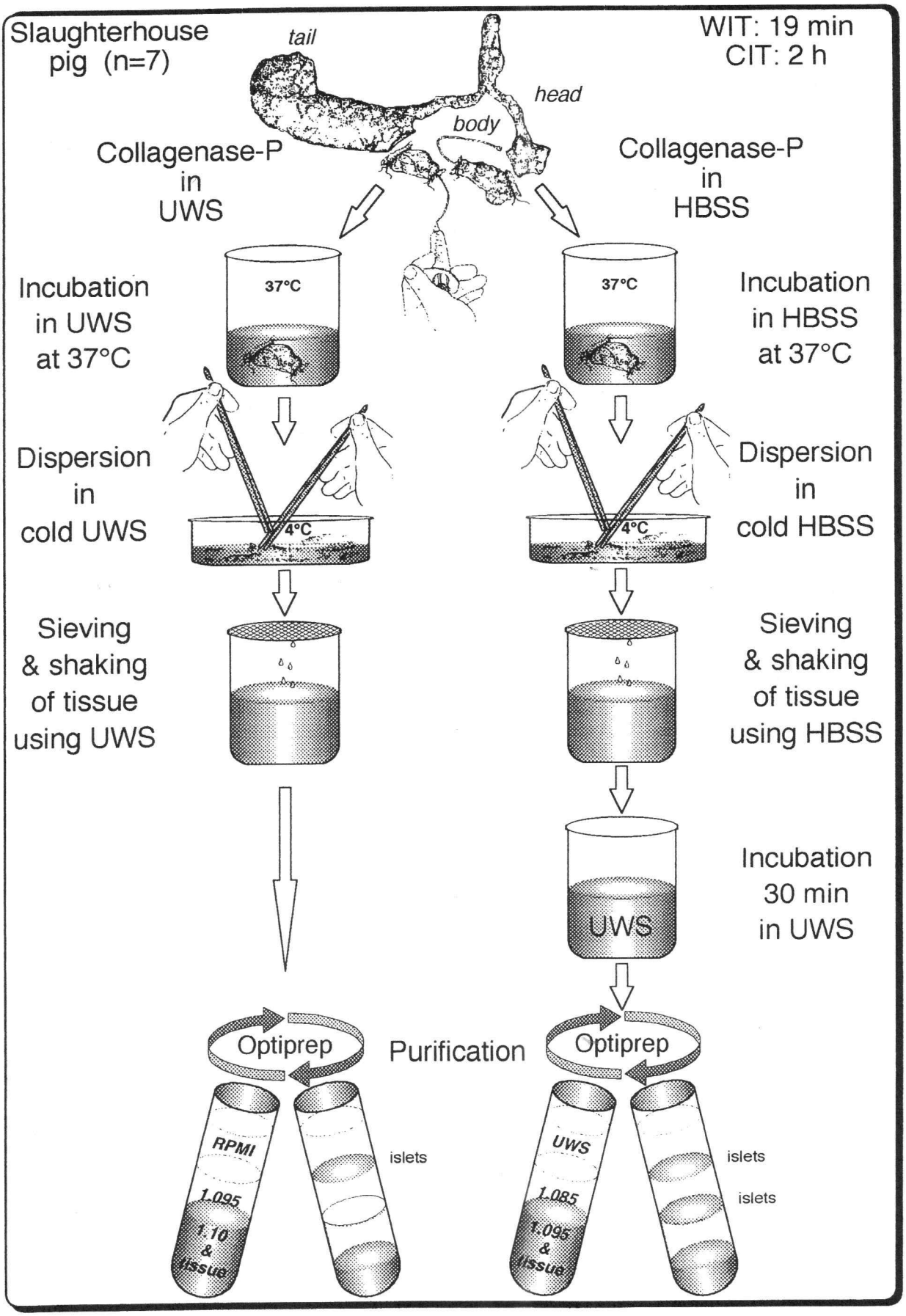
The islet yield in the digest (overall mean 1124 ± 174 IEQs/g pancreas) and size (volume-average diameter 91 ± 6 μm) did not differ after isolation in UWS and HBSS. After purification both islet recovery of the UWS-isolated islets (91 ± 10%) and the HBSS-isolated islets (90 ± 22%), and islet size (106 ± 6% and 100 ± 7%, resp. vs before purification) were also similar, and not significantly different vs before purification. Both the purity of the UWS-isolated islets was superior (81 ± 4 vs 42 ± 14%; $P < .05$), as well as the viability (78 ± 4 vs 45 ± 8%; $P < .05$). In conclusion: in contrast to others, we found no increase of the islet yield following substitution of UWS for HBSS during digestion. Islet viability, however, dramatically increased by using UWS during all isolation and purification steps. Preservation of cell viability in UWS appears to be the key to the successful outcome.

REFERENCES

1. van der Burg MPM, Basir I, Bouwman E: Transplant Proc (this issue)

Legend to figure 1

Fig 1. Illustration of the design of the study, and methods of islet isolation and purification.


Slaughterhouse pig (n=7)
tail
head
body
WIT: 19 min
CIT: 2 h
Collagenase-P in UWS
Collagenase-P in HBSS
Incubation in UWS at 37°C
37°C
37°C
Incubation in HBSS at 37°C
Dispersion in cold UWS
4°C
4°C
Dispersion in cold HBSS
Sieving & shaking of tissue using UWS
Sieving & shaking of tissue using HBSS
UWS
Incubation 30 min in UWS
Optiprep
Purification
Optiprep
RPMI
1.095
1.10 & tissue
islets
UWS
1.085
1.095 & tissue
islets
islets