

Identification of transplanted pancreatic islet cells by radioactive Dithizone- ^{131}I -Histamine conjugate. Preliminary report

Piotr Garnuszek¹, Iwona Licińska¹, Agnieszka Mrozek², Agata Wardawa², Piotr S. Fiedor², Aleksander P. Mazurek¹

¹Drug Institute, Warsaw, Poland

²Department of General and Transplantation Surgery, Transplantation Institute, Warsaw, Poland

Abstract

BACKGROUND: The unique mechanism of dithizone action in the interior of the viable pancreatic islet suggests the possible development of a specific radiopharmaceutical that may have a potential clinical application in the diagnosis of the pancreatic organ allografts or islets rejection.

The radiodiagnostic properties of the newly developed radioactive analogue of dithizone, i.e. Dithizone- ^{131}I -Histamine conjugate have been evaluated in the present study.

METHODS: The four islet cells transplantation models were chosen for this purpose.

The most important feature of the Dithizone- ^{131}I -Histamine conjugate is the ability of zinc chelation. As was presented in the recent study, the conjugate stains pink-reddish the isolated pancreatic islets in vitro. Among the studied transplantation models, only the islets grafting under testis capsule enabled determination of the pancreatic islets in rats by radioactive Dithizone- ^{131}I -Histamine conjugate. The level of the radioactivity in the recipient testis (right) was almost two times higher compared to the controls (0.24 vs. 0.13% ID/g, respectively).

CONCLUSIONS: These preliminary data demonstrate the ability of the developed radioactive analogue of dithizone for in vivo

identification of transplanted pancreatic islets, and suggests a potential clinical application of the radiodithizone in the diagnosis of the pancreatic islet rejection.

Key words: dithizone, iodine radioisotopes, pancreatic islet cells, transplantation, monitoring

Introduction

At present, there is no specific method available for the identification of pancreatic islet grafts and monitoring their function. Dithizone, a powerful zinc chelator, applied for in vitro staining of the viable pancreatic islet cells, has been recently proved to stain crimson the islets in vivo (1–3). These observations suggest a possible application of the radioactive analogue of dithizone for in vivo monitoring of the transplanted islet cells survival.

Our previous study (4) has shown that the radioactive analogue of dithizone (Dithizone- ^{131}I -Histamine conjugate, Figure 1)

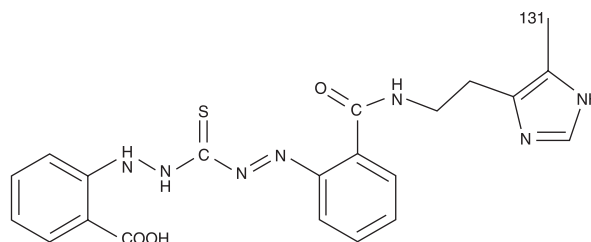


Figure 1. Scheme of Dithizone- ^{131}I -Histamine Conjugate.

could be efficiently prepared by conjugation of the carboxylic derivative of dithizone (i.e. 2,2'-dicarboxy dithizone) to the carrier of iodine radioisotopes i.e. Histamine. The mixed anhydride conjugation technique has been applied for the amide bond formation between the carbonyl atom of 2,2'-dicarboxy dithizone and the side chain amine group of iodohistamine. Radiochemical properties of the conjugate, as well as preliminary biodistribution in animals have been investigated previously (4).

Correspondence to: Piotr Garnuszek
Radioisotope Drugs Department, Drug Institute,
ul. Chełmska 30/34, 00-725 Warsaw, Poland
Tel: (+ 48 22) 7180741, fax: (+ 48 22) 7180740
e-mail: pg@il.waw.pl

The aim of this study was to evaluate the utility of the Dithizone- ^{131}I -Histamine conjugate for survival staining of transplanted pancreatic islets following intravenous administration.

Materials and Methods

Two radioactive analogues of dithizone, i.e. Dithizone- ^{125}I -Histamine and Dithizone- ^{131}I -Histamine conjugates were prepared according to the procedure described previously (4) by linkage of 2,2'-dicarboxy dithizone with one form of the radioiodinated histamine i.e. ^{125}I -Histamine or ^{131}I -Histamine. In our studies, we used Dithizone- ^{125}I -Histamine conjugate of specific activity $A_{\text{SA}} \sim 1.92 \text{ GBq}/\mu\text{mol}$, and Dithizone- ^{131}I -Histamine conjugate of specific activity in the range from 1.7 to 2.0 $\text{GBq}/\mu\text{mol}$.

The pancreatic islets (both for in vitro and for transplantation studies) were isolated using modified Lacy et al. method (5). Fresh islets were isolated from WAG rats by digestion of pancreas using collagenase P (Boehringer Mannheim, Indianapolis, IN, USA), followed by islets purification on Ficoll's (Sigma) gradient, and hand-picking under stereomicroscope (6).

All animal experiments were approved by the Ethics Committees of the Institutes and were carried out in accordance with principles of good laboratory practice.

In vitro staining of the isolated rats' pancreatic islets

In vitro staining of the isolated pancreatic islets was investigated using two of the Dithizone- ^{131}I -Histamine conjugates of a similar specific activity ($A_{\text{SA}} \sim 1.92$ and $1.85 \text{ GBq}/\mu\text{mol}$), although containing different iodine isotope, i.e. I-125 and I-131, respectively. Approximately the 1500 islets dispersed in 23 ml of Hank's balancing salt were concentrated to ca 200 islets in 1ml by centrifugation and decantation. 50 ml of the radioactive conjugate (C_A 5 MBq/ml) was then added to the suspension of 200 islets in 1ml Hank's balancing salt, followed by incubation at 37°C for 10 and 30 min. The islets were isolated by centrifugation and decantation, and then they were washed with PBS solution. The washing procedure was repeated, then the islets were transferred to clean tubes, and the radioactivity was measured in the gamma counter. The staining experiment was carried out in triplicate for each of the radioactive conjugates used.

Localisation of the transplanted islets in vivo

Localisation of the transplanted islets in vivo was tested using four models of islet cells transplantation. Rats (WAG, 250–280g) were used as donors and recipients. Diabetes was induced by intravenous streptozocin injection (65 mg/kg body weight). Approximately 1200–1500 islets were transplanted: to liver (into the portal vein), under kidney capsule, to mesentery and under testis capsule. Blood glucose level was monitored daily after transplantation. The biodistribution study was done within two weeks of remission of diabetes (blood glucose level below 105–110 mg%). The ^{131}I -radiolabelled conjugate (0.2 ml, C_A 10 MBq/ml, $A_{\text{SA}} \sim 2 \text{ GBq}/\mu\text{mol}$) was injected intravenously to tail vein. 30-min p.i.v., the rats were anaesthetised, and the organs were selected for radioactivity determination in gamma-counter. Biodistribution of the conjugate in the transplantation models (from 5 to 7 animals for each model) was compared to the

controls (six Wistar rats for each daily series). An unpaired Student t-test (at 95% confidence interval) was applied for data evaluation.

Results and Discussion

The most important feature of the Dithizone- ^{131}I -Histamine conjugate is the ability of zinc chelation (4). In the present study we have observed that most of the fresh isolated rats' islets stained pink-reddish just 10 minutes following exposure to a medium containing the Dithizone- ^{131}I -Histamine solution. The colour of the stained islets was similar to that observed after dithizone or di-iododithizone exposure (1–3), lasting during the washing with PBS, and while storing at room temperature for ca 3–4 hrs. Additionally, the staining was accompanied by the radioactivity found (for example over 300 Bq of ^{125}I in 200 islets). Regarding the specific activity of the conjugate, the content of the chelated zinc(II) in the interior of the islet cells could be estimated as ca 0.2 pmol in 200 islets, although this calculation might have only a qualitative value.

In spite of the fact that all of the tested grafts reversed diabetes in the proper time after transplantation, it seems evident that the quantity of the transplanted islet cells was too small for its distinct radiographic visualisation, especially in the regions of a high activity background (liver, kidney). Among the studied transplantation models, only the islets grafting under testis capsule enabled determination of the pancreatic islets in rats by radioactive Dithizone- ^{131}I -Histamine conjugate (Figure 2). In the cases of other recipient organs, the activity background was too high, and prevented the differentiation of the transplanted cells.

As presented in Figure 2, the level of the radioactivity in the recipient testis (right) was almost two times higher compared to the controls ($0.24\% \pm 0.05$ vs. $0.13\% \pm 0.02 \text{ ID/g}$, respectively, $t_{\text{Stat}} = 6.66$, $df = 15$). Equally, the radioactivity accumulation in the pancreas of the animals that had been exposed to streptozotocin, was approximately one-half of that found in the pancreas of the controls ($0.30\% \pm 0.09$ vs. $0.66\% \pm 0.14 \text{ ID/g}$, respectively $t_{\text{Stat}} = 4.94$, $df = 9$).

The changes of islet zinc content occurred mainly on account

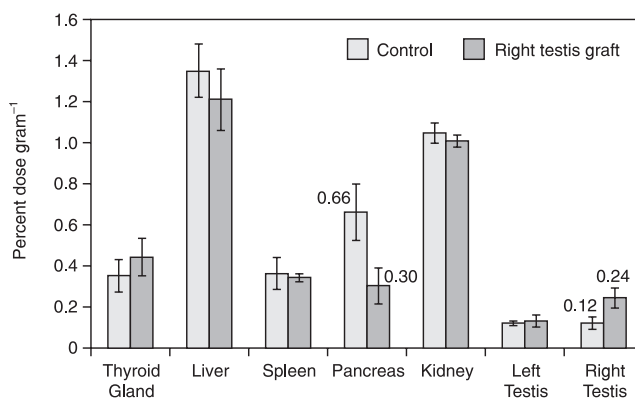


Figure 2. Comparison of Dithizone- ^{131}I -Histamine accumulation in the selected tissues of normal rats (control, $N = 6$) and the recipients of transplanted pancreatic islet cells (right testis graft, $N = 5$). 30 min p.i.v administration, % ID gram^{-1} .

of insulin-producing cells and in coincidence with glycemia changes. It has been shown that selective damage of insulin producing cells, phase glycaemic fluctuations and permanent diabetes development are connected with chelator zinc binding in the lysosome-segregation apparatus of these cells (7). The insulin producing cells were devoid of zinc in diabetes of long duration with high hyperglycemia, and it is supposed that diabetogenic-chelating agents are capable of producing irreversible diabetogenic affection in those beta-cells that contain a critical concentration of reactant zinc (8).

Dithizone is a recognised diabetogenic agent *in vivo*, however, under controlled conditions (concentration and solvent used), it can play a useful role as a supravital stain for identification of islets to be used for transplantation, as well as *ex vivo* monitoring agent of viability of transplanted islets (1–3, 9, 10). The unique mechanism of dithizone action in the interior of the viable pancreatic islet suggests the possible development of a specific radiopharmaceutical that may have potential clinical application in the diagnosis of the pancreatic organ allografts or the islets rejection.

It seems promising that the strategy described above may be acquired by application of the radioactive analogue of dithizone i.e. Dithizone- ^{131}I -Histamine conjugate. Both the colour and the radioactivity of the islets exposed to the conjugate confirm the transporting of the conjugate through the lipid membrane to the interior of islets, and suggest the specific mechanism of the conjugate action in the interior of the islets. Likewise, the indication of a positive staining and radioactive labelling of the islet cells both *in vitro* and in the testis graft, suggest that the radioactive analogue of dithizone, i.e. the Dithizone- ^{131}I -Histamine conjugate may be a promising diagnostic agent for detecting the viability of transplanted pancreas or the islets by radioisotope scanning techniques. However, regarding a low radioactivity level detected in the graft, further expanded studies using bigger animal transplantation models and a greater amount of transplanted islet cells should be undertaken.

Acknowledgements

Grant No. 4 P05F 01416 from the State Committee for Scientific Research (KBN) is gratefully acknowledged.

References

1. Fiedor P, Sitarek E, Tatarkiewicz K, Sabat M, Orłowski T, Rowiński W. *In vivo* visualization of rat pancreatic islets by intravenous injection of diphenylthiocarbazon (dithizone). *Transplantation Proceedings* 1994; 26 (2): 670–671.
2. Fiedor P, Rowiński W, Licińska I, Mazurek AP, Hardy MA. The survival identification of pancreatic islets of Langerhans. *In vitro* and *in vivo* effects of two dithizone preparations on staining of rat and human islets of Langerhans-Preliminary Study *Acta Poloniae Pharmaceutica-Drug Research* 1995; 52 (5): 431–436.
3. Fiedor P, Oluwole SF, Licińska I, Hardy MA. Localization of endocrine pancreatic islets. *World J Surg* 1996; 20: 1016–1023.
4. Garnuszek P, Licińska I, Fiedor PAP, Mazurek. The synthesis, radioiodination and preliminary biological study of the new carboxylic derivatives of dithizone. *Appl Radiat Isot* 1998; 49 (12): 1563–1571.
5. Shibata A, Ludvigsen CWJr, Naber SP, McDaniel ML, Lacy PE. Standardization for a digestion-filtration method for isolation of pancreatic islets. *Diabetes* 1976; 25 (8): 667–72.
6. Sitarek E, Tatarkiewicz K, Sabat M, Fiedor P, Orłowski T. Methods of rat Langerhans islets isolation. *Bull Pol Ac Biol* 1993; 41: 117.
7. Goldberg ED, Eshchenko VA, Bovt VD. The diabetogenic and acidotropic effects of chelators. *Experimental Pathology*, 1991; 42 (1): 59–64.
8. Bavel'skii ZE, Zaviakina TV, Moiseev IuS, Medvedev VI. Zinc content in pancreatic islets in experimental diabetes induced by chelating agents. *Patol Fiziol Eksp Ter* 1992; 3: 29–32.
9. Latif ZA, Noel J, Alejandro R. A simple method of staining fresh and cultured islets. *Transplantation* 1988; 45: 827–830.
10. Fiedor PS, Jin MX, Zikria BA, Garnuszek P, Licińska I, Mazurek AP, Maroszyńska I, Piaseczna-Piotrowska A, Szymańska K, Rowiński WA, Hardy MA, Oluwole SF. Monitoring of rat islet allografts with dithizone after induction of donor specific transplant tolerance by intrathymic administration of soluble alloantigens. *Ann Transplant* 1998; 3 (4): 21–30.