Needle-Free Insulin Drug Delivery

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For most patients with type 1 diabetes, the worst part of the disease is to tolerate needle after needle, both for glucose measurement and to deliver insulin. In the last two decades, concept of insulin therapy by multiple-dose injection has undergone a miraculous change. Needle-free insulin delivery appeared to be a wonderful approach, and its allure rested in being comfortable and safe. In today's era, insulin delivery by alternative route is a topic of current interest in the design of drug delivery system. Major global pharmaceutical companies are showing encouraging progress in their attempts to develop alternative insulin delivery technologies. Many such drug delivery systems have been developed for oral, buccal and nasal route. This review article discusses, in brief, the novel and emerging technologies that are in pipeline, including insulin inhalers, insulin spray, insulin pill, insulin analogues, insulin complement, islet cell transplant, implantable insulin pumps and guardian continuous glucose monitoring system.

Diabetes is a major public-health problem and is emerging as a pandemic. As estimated, 135 million people worldwide had diagnosed diabetes in 1995, and this number is expected to rise to at least 300 million by 2025[1]. Diabetes mellitus represents a group of diseases of heterogeneous etiology, characterized by chronic hyperglycemia and other metabolic abnormalities. The etiological classification of diabetes includes type 1, type 2, those due to specific mechanisms or diseases, and gestational diabetes. Diabetes mellitus type 1 is characterized by destructive lesions of pancreatic β cells by an auto-immune mechanism. Type 2 diabetes is characterized by a combination of decreased insulin secretion and sensitivity[2]. Attempts to attain strict glucose control when managing diabetes have traditionally utilized daily subcutaneous injections of human insulin. This strategy has offered improvements in glycemic control but is unable to replicate fully the normal, diurnal plasma profile of endogenous insulin[3]. The development of novel non-invasive routes of insulin administration promises to further improve diabetes management. Many barriers to initiate insulin therapy include need for frequent insulin injection, fears that insulin injections will be painful and difficult to administer, concerns about hypoglycemia and weight gain. Thus, each measure that reduces these barriers will help to prevent inappropriate delays in starting insulin therapy as well as to promote better compliance with therapy[4].

New developments are happening all the time, and the new technologies, some of which are under development and some which are developed to hit the market, are insulin inhalers, insulin spray, insulin pill, insulin analogues, insulin complement, islet cell transplant, implantable insulin pumps and guardian continuous glucose monitoring system.

NEW TECHNOLOGIES FOR INSULIN DELIVERY

Insulin inhalers:

Inhaled insulin appears to be a non-invasive, well-tolerated and liked modality of treatment with potential for both type 1 and 2 diabetes[5]. Results of short-term studies indicate that glycemic control achieved with an inhaled insulin regimen is comparable with a subcutaneous insulin regimen in patients with type 1[6] and type 2[7] diabetes. It has been determined in patients with type 1 diabetes that improvement in overall patient satisfaction with inhaled insulin is rapid and sustainable compared with conventional subcutaneous insulin, and the reduced treatment burden has a positive impact on psychological well-being[8]. Inhaled insulin greatly enhances patient satisfaction, quality of life and acceptance of intensive insulin therapy in a diabetic patient[9,10]. Several drug

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delivery systems in various stages of development are given below:

Nektar Therapeutics (formerly Inhale Therapeutics Systems, Inc.) completed their initial phase III clinical trials of insulin inhaler (Exubera) in 2002 in partnership with Pfizer Inc. and Aventis Pharma. Pfizer and Aventis are currently carrying out further long-term trials looking at the safety and efficacy of Exubera (www.nektar.com; 15 Jan. 2005). The rationale behind developing a pulmonary drug delivery system is to ensure that insulin powder is delivered deep into the lungs, where it is easily absorbed into the bloodstream, in a hand-held inhalation device. The device converts the insulin powder particles into an aerosol cloud for the patient to inhale. In March 2004, Pfizer and Aventis announced that the European Medicines Evaluation Agency (EMEA) accepted the filing of the Marketing Authorization Application (MAA) for inhaled insulin (Exubera) for the treatment of type 1 and type 2 diabetes mellitus. Pfizer is also conducting phase III clinical trials with inhaled insulin in paediatric patients aged 6-17 years. Nektar Therapeutics is using its Advanced PEGylation technology to develop a dry powder-inhaled polyethylene glycol (PEG) formulation for delivering peptides efficiently across the lungs and to promote prolonged serum concentration of the peptide[11]. Exubera represents a novel prandial insulin delivery method. Good glycemic control, comparable to modern subcutaneously administered insulin preparations, has already been demonstrated, and no unexpected safety concerns have been reported with inhaled insulin[12]. Novo Nordisk and Aradigm Corporation are beginning phase III clinical trials of their insulin inhaler, the AER_X Diabetes management system. The AER_X system is an electronic inhaler that releases a blister pack of liquid insulin deep into the lungs of the patient (www.novonordisk.com, www.aradigm.com; 15 Jan. 2005). Alkermes and Eli Lilly are collaborating for a system as AIR (www.jdrf.org/index.cfm; 15 Jan. 2005).

Among all the above, the most studied one is Exubera. All the products are at least several years away from government approval and marketing but are suggesting that insulin can be delivered through the lungs.

Hallschmid reported that after intranasal administration, insulin enters the cerebrospinal fluid compartment and alters brain function. Insulin acts in CNS to reduce food intake and body weight and is considered a major adiposity signal in men[13]. Studies conducted by Harrison on 38 individuals suggest that intranasal insulin administration is safe as it not accelerate loss of β-cell function in individuals at risk of type 1 diabetes and induces immune changes consistent with mucosal tolerance to insulin. This finding justifies that intranasal insulin is immunotherapeutic and retards progression to clinical diabetes[14].

Insulin spray:

The buccal route is another promising alternative for insulin delivery. With the buccal area having an abundant blood supply, it offers some advantages such as a means to deliver the acid labile insulin, and elimination of insulin destruction by first pass metabolism[15]. The buccal spray formulation being developed by Generex Biotechnology, based in Toronto, delivers insulin to the buccal cavity as a fine spray using company's 'rapidmist' device. The company's leading product is Oralin. It is currently in phase II B clinical trial (www.newsrx.com; 18 Jan. 2005). The patient does not inhale with the buccal spray device; instead, the drug is sprayed onto the buccal mucosa. The high-speed spray allows the drug to be rapidly absorbed into the bloodstream. The deposition of the drug onto the buccal mucosa also allows the developers to bypass earlier concerns about any risks to lung tissue that have been raised regarding investigative inhaled insulin formulation (www.newsrx.com; 18 Jan. 2005).

Insulin pill:

To adequately control postprandial glycemia, several daily injections of insulin are necessary. However, insulin therapy via subcutaneous or other parenteral route is known to result in peripheral hyperinsulinemia. In addition to the risk of hypoglycaemia, some studies have suggested that peripheral hyperinsulinemia may be associated with coronary artery disease, hypertension, dyslipidemia and weight gain[16]. There is strong evidence suggesting that an oral insulin product would provide insulin in a more physiological manner, with a resultant decrease in peripheral insulin concentration and that it would more adequately insulinize the liver[17,18].

Azopolymer coated pellets to deliver insulin to the colon region were studied earlier. The azopolymer protects the entrapped therapeutic agent till the pellets reach the colon. As only the bacteria inhabiting the colon secrete enzymes that can breakdown the azopolymer, insulin release will be initiated once the pellets reach the large intestine[19]. Microencapsulation of insulin in polymeric

microspheres coated with pH responsive polymers such as alginate is also known. Alginate coating protects the spheres in the acidic pH of the stomach but dissolves in the intestine where the pH increases to above 7 and liberates the entrapped insulin[20]. Recently several biotech companies have been conducting pilot trials in the effort to develop an insulin pill as a potential alternative to injected or pumped insulin. The attempt requires the development of novel delivery technology. For example, Nobex Corporation has developed hexyl-insulin monoconjugate 2 (HIM- 2) in which single amphiphilic oligimer is covalently linked to the free amino group on the Lys-β 29 residues of recombinant human insulin via an amide bond. This alters the physical- chemical characteristics, leading to enhanced stability and resistance to intestinal degradation of ingested insulin[21]. Oral HIM-2 is safe[22] and reproduces the physiological pathway of insulin secreted by pancreas[23]. Also Depomed, Inc. is developing oral medications using its Gastric Retention (GR) system, an advanced polymer-based, oral drug delivery formulation. Initially small enough to be easily swallowed by the patients, the pill swells following its ingestion. Simultaneously, the system begins a period of extended drug release. This sustained delivery could some day lead to an insulin pill that provides steady release into the bloodstream, minimizing the number of doses required per day (www.newsrx.com; 18 Jan. 2005).

Insulin analogues:

Traditional insulin preparations such as NPH (Neutral Protamine Hagedom) insulin have duration of action 14 h and plasma insulin peak level 4-6 h after administration[24]. As a consequence, NPH insulin may need to be administered up to three times daily in type 1 diabetic patients to provide sufficient insulin supply throughout the day[25]. Multiple dosing regimens are less optimal in terms of adherence, flexibility and choice for the patients to adapt treatment to their individual lifestyle[24]. To satisfy the need for optimized basal insulin, recombinant human insulin analogues have been developed, like Glargine[26] and Aspart[27]. Glargine-treated patients experienced significantly less weight gain than those treated with NPH insulin[26], which had a lower risk of nocturnal hypoglycemia[28,29] and was well tolerated, whether it is injected once daily before breakfast, dinner or at bedtime in Type 1 diabetic patients[30]. Similarly, Aspart is also now well established as an effective and convenient means of providing glycaemic control[27]. Table 1 (www.fda.gov/fdac/features/2002/chrt_insulin.html; 20 Feb. 2005) lists some of the more common insulin available today.

In the study done by Radermecke and Scheen, it was found that rapidly absorbed insulin analogues, such as insulin Lispro or Insulin aspart, may offer an advantage over regular human insulin for insulin pumps. Continuous subcutaneous insulin infusion with insulin lispro provided a better control of postprandial hyperglycaemia and a slightly but significantly lower glycated haemoglobin level, with lower daily insulin requirement and similar or even less hypoglycemia or ketoacidosis in diabetic patients[31].

Very recently, one or more analogue insulin Glusidine[32] (Apidra, brand name) from Aventis got approval in April 2004 (www.drugdel.com; Feb 2005). Another one, Insulin Detemir by Novo Nordisk, is under phase IIIb trial. Studies showed that a single injection of insulin Detemir had duration of action of 20 h. Also insulin Detemir decrease body weight, which is of potential clinical benefit and in contrast with other insulin, which can increase body weight (www.rf.org/index.cfm; 3 Feb. 2005).

Insulin complement:

Apart from the new insulin, one new drug, Symylin, is ready to be launched by Amylin Pharma, San Diego. Symylin is a synthetic version of the human hormone amylin, which moderates the glucose lowering effect of insulin. Symylin has been designed to complement insulin action and has been shown to reduce blood glucose

TABLE 1: COMMON INSULIN AVAILABLE TODAY

Type of insulin	Examples (Brand name)	Name of Company	Onset of action (min)	Peak of action (min)	Duration of action (h)
Rapid acting	Humalog	Eli Lilly	15	30-90	3-5
	Novolog	Novo Nordisk	15	40-50	3-5
Short acting (Regular)	Humulin R	Eli Lilly	30-60	50-120	5-8
	Novolin R	Novo Nordisk	30-60	50-120	5-8
Intermediate acting (NPH)	Humulin N	Eli Lilly	60-180	480	20
	Novolin N	Novo Nordisk	60-180	480	20
	Humulin L	Eli Lilly	60-150	420-900	18-24
	Novolin L	Novo Nordisk	60-150	420-900	18-24
Long acting	Ultalente	Eli Lilly	240-480	480-720	36
	Lantus	Aventis	60	_	24

without causing an increase in hypoglycemic episodes. It could provide a potential adjunct to insulin therapy in both type 1 and type 2 diabetics (www.newsrx.com; 18 Jan. 2005).

Islet cell transplant:

In contrast to conventional insulin treatment, islet transplantation is far superior for achieving a constant normoglycaemic state and avoiding hypoglycaemic episodes. Using a novel protocol established by the Edmonton Centre, Canada, the insulin dependence rates have improved, reaching 50-80% level[33]. Thus, islet transplantation typically offers stabilization of blood glucose control and elimination of problematic hypoglycaemia[34-36] and is being increasingly used worldwide[37-39]. The development of the Edmonton protocol dramatically transformed clinical outcomes in islet transplantation in recent years through the introduction of a more potent, less diabetogenic corticosteroid for immunosuppressive regimen consisting of sirolimus, low dose tacrolimus and induction anti-interleukin-2 receptor antibody. While insulin independence rates under this protocol have been highly successful, patients must be maintained on lifelong immunosuppression[40]. Clinical studies confirmed the efficacy of the Edmonton immunosuppressive regimen and indicate that insulin independence can often be achieved by a single transplant of sufficient islet mass[41]. Procedure for islet transplantation involves enzymatic digestion of the pancreatic tissue, purification of the islets from exocrine tissue infusion of the islets into the portal vein and implantation in the liver[42]. The percutaneous transhepatic approach for the implantation of islet cells into the portal vein is a safe procedure and together with the use of current cell separation techniques and an immunosuppressive regimen, offers a marked advance in the type 1 diabetes mellitus treatment[43]. For islet transplants to become a widespread clinical reality, diabetes reversal must be achieved with a single donor to reduce risks and cost and increase the availability of transplantation. Researchers in their study find out that tested transplant protocol restored insulin independence and protected against hypoglycemia after single donor, marginal-dose islet transplantation. These findings may have implications for ongoing transition of islet transplantation from clinical investigation to routine clinical care[44]. There is a spectrum of outcomes after islet transplantation. For measuring clinical success after an islet transplant, which includes insulin independence or the need for insulin, glucose control, and graft survival, scientists have developed a β-score. β-score provides integrated measure of β-cell function after islet transplantation using this parameters[45].

An alternate strategy to immunosuppression and immune tolerance is immuno-isolation. Here the technological goal being pursued is encapsulation of islet cells in microcapsules with semipermeable membrane that permits access to nutrient and oxygen but are impermeable to cells and products of the immune system. A review article discusses the latest advancement in use of biocompatible, nonporous silicone membrane as a tool to deliver living cells. This article describes the development of a novel technology using living cells, preferably pancreatic islet cells, to treat diabetes using micro-fabricated immuno-isolating biocapsule (Silicone). This approach may permit human islet transplantation without immunosuppression[46].

An approach to prevent autoimmune diabetic recurrence after islet transplantation is the use of novel anti-inflammatory agent lisofylline. Study done by Yang in protected non-obese diabetic (NOD) mice demonstrates that autoimmune diabetes recurrence after islet transplantation could be prevented by treatment with lisofylline. Lisofylline and its analogues may have the potential to prevent islet autoimmune destruction in clinical transplantation[47]. Thus islet cell transplantation offers the advantage of being performed as a minimally invasive procedure, in which islets can be perfused percutaneously into the liver via the portal vein. Finally, the concept of islet cell or stem cell transplantation is most attractive since it offers many perspectives [33].

Implantable insulin pumps:

Continuous improvements in microelectronics, as well as in the development of biomaterials and stable insulin solutions, have led to the availability of implantable pumps able to infuse insulin by the peritoneal route, in a continuous and programmable way, for several years[48].

The Medtronic/Minimed 2007 system may offer treatment advantages for diabetic patients who have difficulty in maintaining consistent glycaemic control. This system delivers insulin into the peritoneal cavity in short, frequent burst or "pulses" similar to how pancreatic β cells secrete insulin. This system is placed external to the rectus muscle. Current model has eight years battery life expectancy. The system's reservoir is refilled with fresh insulin every two or three months. Different insulin delivery algorithms used in implantable pumps automatically infuse more basal insulin during 'dawn phenomenon,' compensating the increasing need for

insulin during this period. This system is limited in the USA for investigational use only (www. Minimed.com; 15 Feb. 2005).

The human insulin used in implantable pumps, regardless of how long it had remained in the pump reservoir, did not induce macrophage activation in diabetic patients treated through intraperitoneal insulin delivery[49]. Improved implantable pumps and insulin solutions show both long-term safety and effectiveness of this treatment in type 1 diabetic patients, following improvement in infused insulin solutions and catheter[50].

Transdermal patch:

Ozin and Landskron announced recently that they had created an unusual material using manmade molecules called dendrimers. It can store drugs and, when spread on the skin as a film, allow them to dissipate into a patient's bloodstream like a new type of patch. The problem with current drug delivery systems is that it is either injected in such a manner that acquires too high concentration to ensure that it stays in the system but can be toxic, or it is injected too little into a person such that it is not effective. The new material, Periodic Mesophorus Dendrisillicus (PMD) would let drugs seep through a person's skin in just the right amount and stay at that level (www.Defeatdiabetes.org; 3 Mar. 2005).

Three-dimensional model of insulin receptor:

Scientists have created a three-dimensional model of insulin receptor to help in designing molecules to treat diabetic patients by oral delivery. A research team, comprising seven scientists, has reported that newly developed molecules mimicking insulin can bind to the patient's insulin receptors. The scientists have successfully constructed a complex of insulin- insulin receptor using Electron Cryomicroscopy (EM)[51].

Glucose monitoring device:

Medtronic Inc. got USFDA approval on February 11, 2004 for Guardian continuous glucose monitoring system. It is an external system that warns patients of unusual blood sugar level by sounding an alarm (www.Medtronic.com; 6 Mar. 2005).

CONCLUSIONS

The development of technologies in the last decade have brought to limelight the strategies that hold some promise in turning non-injectable insulin delivery from theory to reality. However, further elaborate investigations in humans are required. The approaches that seem to hold potential must be consolidated and converted to a working protocol. Among the various alternative delivery systems, each have their own set of favourable and unfavourable properties. Some unfavourable aspects have to be circumvented to make this alternative insulin delivery system a reality and make them to reach the market.

REFERENCES

1. Narayan, K.M., Gregg, E.W., Faqot-Campagna, A., Engelgau, M.M. and Vinicor, F., **Diabetes,** 2000, 50, S77.
2. Kuzuya, T., Nakagawa, S., Satoh, J., Kanazawa,Y., Iwamoto,Y., Kobayashi, M., Nanjo, K., Sasaki, A., Seino, Y., Ito, C., Shima, K., Nonaka, K. and Kadowaki, T., **Diabetes Res. Clin. Pract.,** 2002, 55, 65.
3. Verge, D., **Med. Sci.,** (Paris), 2004, 20, 986.
4. Heinemann, L., **Eur. J. Endocrinol.,** 2004, 151, T-23.
5. Quattrin, T., **Expert Opin. Pharmacother.,** 2004, 5, 2597.
6. Skyler, J.S., Cefalu, W.T., Kourides, I.A., Landschulz, W.H., Balagtas, C.C., Cheng, S.L. and Gelfand, R.A., **Lancet,** 2001, 357, 331.
7. Cappelleri, J.C., Cefalu, W.T., Rosenstock, J., Kourides, I.A. and Gerber, R.A., **Clin. Ther.,** 2002, 24, 552.
8. Su, M., Testa, M.A., Turner, R.R. and Simonson, D.C., **Diabetes,** 2002, 51, A-448.
9. Testa, M.A.,Turner, R.R., Hayes, J.F. and Simonson, D.C., **Diabetes,** 2001, 50, A-45.
10. Testa, M.A., Turner, R.R. and Simonson, D.C., **Diabetes,** 2002, 51, A-135.
11. Wigley, F.M., Londona, J.H., Wood, S.H., Shipp, J.C., Waldman, R.H., **Drug Res. Develop.,** 2004, 5, 166.
12. Barnett, A.H., **Int. J. Clin. Pract.,** 2004, 58, 394.
13. Hallschmid, M., **Diabetes,** 2004, 53, 3024.
14. Harrison, L.C., **Diabetes Care,** 2004, 27, 10.
15. Narayani, R., **Trends Biomater. Artif. Organs,** 2001, 15, 12.
16. Haenni, A., Reneland, R., Lind, L. and Lithell, H., **J. Hypertens.,** 2001, 19, 107.
17. Hoffman, A. and Ziv, E.**, Clin. Pharmacokinet.,** 1997, 33, 285.
18. Gwinup, G., Elias, A.N. and Domurat, E.S., **Gen. Pharmacol.,** 1991, 22, 243.
19. Guevara, A.J. Guevera, M., Saavedra, J., Mihic, M. and Modi, P., **Diabetes Technol. Ther.,** 2004, 6, 1.
20. Saffron, M., Kumar, G.S., Savarian, C., Burnham, J.C. and Williams, F., **Science,** 1986, 233, 1081.
21. Still, J.G., **Diabetes Metab. Res. Rev.,** 2002,18, 29.
22. Kipnes, N., Dandona, P., Tripathy, D., Still, J.G. and Kosutic, G.**, Diabetes Care,** 2003, 26, 421.
23. Clement, S., Still, J.G., Kosutic, G. and Mcallister, R.G., **Diabetes Technol. Ther.,** 2002, 4, 459.
24. Lepore, M., Pampanelli, S., Fanelli, C., Porcellati, F., Bartocci, L., Divincenzo, A., Cordoni, C., Costa, E., Brunetti, P. and Bolli, G.B., **Diabetes,** 2000, 49, 2142.
25. Bolli, G.B., Di Marchi, R.D., Park, G.D., Pramming, S. and Koivisto, V.A., **Diabetologia,** 1999, 42, 1151.
26. Raskin, P., Klaff, L., Bergenstal, R., Halle, J.P., Donley, D. and Mecca, T., **Diabetes Care,** 2000, 23, 1666.
27. Chapman, T.M., Noble, S. and Goa, A., **Drugs**, 2003, 63, 512.
28. Pieber, T., Eugene-Jolchine, I. and Derobert, E., **Diabetes Care,** 2000, 23, 157.
29. Ratner, R.E., Hirsch, I.B., Neifing, G., Garg, S.K., Mecca, T.E. and

Wilson, C.A., **Diabetes Care,** 2000, 23, 639.
30. Hamann, A., Matthaei, S., Rosak, C. and Silvestre, L., **Diabetes Care,** 2003, 26, 1738.
31. Radermecker, R.P. and Scheen, A.J., **Diabetes Metab. Res. Rev.**, 2004, 20, 178.
32. Naushad, M. and Khan, G., **Drug del.com Newsletter,** 2004, 3, 3.
33. Bretzel, R.G., Eckhard, M. and Brendel, M.D., **Panminerva Med.,** 2004, 46, 25.
34. Ryan, E.A., Lakey, J.R., Paty, B.W., Imes, S., Korbutt, G.S., Kneteman, N.M., Bigam, D., Rajotte, R.V. and Shapiro, A.M., **Diabetes,** 2002, 51, 2148.
35. Hering, B.G., Kandaswamy, R., Harmon, J.V., Ansite, J.D., Clemmings, S.M., Sakai, T., Paraskevas, S., Eckman, P.M., Sageshiva, J., Nakano, M., Sawada, T., Matsumoto, I., Zhang, H.J., Sutherland, D.E. and Bluestone, J.A., **Amer. J. Transplant,** 2004, 4, 390.
36. Ryan, E.A., Shandro, T., Green, K., Paty, B.W., Senior, P.A., Bigam, D., Shapiro, A.M. and Vantyghem, M.C., **Diabetes,** 2004, 53, 955.
37. Goss, J.A., Goodpaster, S.E., Brunicardi, F.C., Barth, M.H., Soltes, G.D., Garber, A.J., Hamilton, D.J., Alejandro, R. and Ricordi, C., **Transplantation,** 2004, 77, 462.
38. Kessler, L., Bucher, P., Milliat-Guittard, L., Benhamou, P.T., Berney, T., Penfornis, A., Badet, L., Thivolet, C., Bayle, F., Oberholzer, J., Renoult, E., Brun, M.J., Rifle, G., Atlan, C., Colin, C. and Morel, P., **Transplantation**, 2004, 77, 1301.
39. Sutherland, D.E., Gruessner, A. and Hering, B.J., **Endocrinol. Metab. Clin. North Amer.,** 2004, 33, 135.
40. Nanji, S.A. and Shapiro, A.M., **Biodrugs**, 2004, 18, 315.
41. Markmann, J.F., Deng, S., Huang, X., Validedeoglu, E.H., Desai, N.M., Frank, A., Lui, C., Markmann, E., Brayman, K., Wolf, B., Bell, E., Vitamaniuk, M., Doliba, N., Palanjian, M., Matschinky, F., Barker, C.F. and Naji, A., **Ann. Surg.,** 2003, 237, 741.
42. Bucher, P., Mathe, Z., Bosco, D., Andres, A., Buhler, L.H., Morel, P. and Berney, T., **Swiss Surg.,** 2003, 9, 242.
43. Owen, R.J., Ryan, E.A., O'Kelly, K., Lakey, J.R ., Mc Carthy, M.C., Paty, B.W., Bigam, D.I., Kneteman, N.M., Korbuttt, G.S., Rajotte, R.V. and Shapiro, A.M., **Radiology,** 2003, 229, 165.
44. Herin, B.J., Kandaswamy, R., Ansite, J.D., Eckman, P.M., Nakano, M., Sawada, T., Matsumoto, I., Ihm, S.H., Zhang, H.J., Parkey, J., Hunter, D.W. and Sutherland, D.E.**, JAMA,** 2005, 293, 830.
45. Edmond, A.R., Breay, W.P., Peter, A., Jonathan, R.T., Bigam, D. and Shapiro, A.M., **Diabetes Care,** 2005, 28, 343.
46. Leoni, L. and Desai, T.A., **Adv. Drug Delivery Rev.,** 2004, 56, 211-229.
47. Yang, Z., Chen, M., Ellett, J.D., Fialkow, L.B., Carter, J.D. and Nadler, J.L., **Transplantation,** 2004, 77, 55.
48. Renard, E., Costalet, G. and Bringer J., **Diabetes Metab.,** 2002, 28, 19.
49. Gin, H., Renard, E., Melki, V. and Boivin S.B., **Diabetes Metab.,** 2003, 29, 601.
50. Kessler, L., Tritscaler, S. and Bohbot, A., **Diabetes Care**, 2001, 24, 302.
51. Tan, C., Wei, L., Ottensmeyer, F.P., Goldfine, I., Maddox, B.A., Yip, C.C., Batey, R.A. and Fotra, L.P., **Bioorg. Med. Chem. Lett.,** 2004, 12, 1407.

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