MODULATORS OF INSULIN ACTION

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ABSTRACT

Although insulin has been in our therapeutic armamentarium for a long time, we are still unable to deliver it physiologically and achieve a precise metabolic control. Recently a large number of drugs have been introduced in the market or are likely to be available soon. These are Insulin analogues (Lispro, Aspart, HOE 901, Arginine insulin), insulin sensitizers (Thiazolidinediones) and insulin mimetics (Vanadium salts, dichloroacetate). These newer drugs are discussed briefly and their role in future drug therapy of diabetics is evaluated.

KEY WORDS: Insulin analogues; Insulin therapy; Insulin sensitizers; Insulin mimetics

MODULATORS OF INSULIN ACTION

Insulin secretion in a normal individual occurs at a constant basal rate, superimposed with meal related peaks. The aim of insulin therapy in a diabetic is to mimic this normal physiology. The initial insulin preparations available (i.e. plain or crystalline insulin) had a short duration of action and initial effort were directed towards prolongation of duration of its action. This involved modification in the structure of the insulin the addition of zinc for complexing it with neutral protamine. In the recent times alteration in the amino acid sequence has also been made, so as to alter the kinetics of insulin. The other potential sites at which the action of insulin can be modulated are at the receptor and post receptor sites, brought out by several drugs, which will be discussed. In addition species, storage, route and site of administration also modifies insulin action. The list of currently available insulin modulators in shown table 1

Table 1: Insulin Modulators

A. INSULIN ANALOGUES

- **Short Acting**
  Lispro (B_{28} lysine – B_{29} proline), X_{14} Analogue (B_{28} Aspartate)
- **Long Acting**
  HOE 901, A_{21} Glycine, $B_{31.22}$ Diarginine, B_{27} Arginine, B_{30} Thr-Nh_2

B. INSULIN SENSITIZERS

Biguanides, Thaizolidinedioness derivatives

C. INSULIN MIMETICS

- **Increase Glucose Metabolism**
  Vitamins K_5, Deoxy Frencilin, Spermine
- **Increase Glucose Oxidation**
  Oxidants, Diamide, Dichoroacetate
- **Trace Elements**
  Vanadium compounds, Zinc, Mg, Mn, Cr, Se, Li.

PROLONGATION OF ACTION

Ever since the discovery of insulin, attempts have been made to prepare an insulin which would be physiologically similar to pancreatic insulin secretion. But unfortunately, we are still far away from the goal. However, to obtain prolongation of insulin action several preparations e.g. isophane insulin, lente insulin (insulin zinc suspection mixed), ultralente (insulin zinc suspension crystalline) and extended acting insulin preparation were introduced (1). Pharmacokinetics of the available insulins are shown in Table 2.

Table 2: Pharmacokinetics of Human Insulin and Analogues following subcutaneous Injection

Insulin	**Onset of Action**	Peak Action (hours)	Duration of Action (hours)
Regular	36-60 Hrs	2-4	6-8
Lispro	5-15 Hrs	1-2	4-5
NPH	1-2 Hrs	5-7	13-18
Lente	1-3 Hrs	4-8	13-20
Ultralente	2-4 Hrs	8-10	18-30

Isophane Insulin

Also known as NPH (Neutral Protamine Hagedron) insulin, is prepared by addition of protamine to insulin. Protamine and insulin are mixed in stoichiometric proportion. The protamine precipitates insulin at neutral pH. A small amount of zinc is added for better stabilization of insulin (2)

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Protamine Zinc Insulin

In this preparation, protamine and zinc both are added to insulin. Zinc inhibits tissue proteins and prevents digestion of protamine and prolongs the duration of action. It is difficult to manufacture protamine zinc insulin. It is highly antigenic, absorption is erractic and is more commonly associated with injection site lipoatrophy.

Lente Insulin

It is a mixture of semilente and ultralente insulin (30:70). Zinc is used as retardant, while acetate buffer is used for stability.

Ultra Lente Insulin

It is a crystalline insulin zinc suspension. In this preparation, crystallization of insulin occur at pH 5.5. one hexamer of insulin contains four zinc atoms. Prolongation of duration of action is due to its slow absorption from the subscutaneous tissues.

Extended Acting Insulins

These are prepared by the addition of basic proteins and local vasoconstrictors

INSULIN ANALOGUES

Insulin analogues are broadly grouped into short acting and long acting insulin analogues.

Short Acting Insulin Analogue-Lispro

It is a short acting insulin analogue which is available for clinical use. Indeed, it has been known for some time that removal of the last five amino acids of the beta chain of insulin stops association, while retaining its potency, but the removal leads to poor chemical stability. To overcome this problem of stability, exchanger of amino acid position in the beta chain of insulin was tried. Here the alternation is B_{28} proline B_{29} proline and B_{28} lysine. This minor alteration in amino acid sequence leads to formation of monomer insulin molecule, instead of usual hexamer insulin molecule. The hexamer of insulin is first converted into its momomeric form in subcutaneous tissue, before absorption. While in momomeric insulin, this step is obviated.

Lispro is a momomer and its onset of action is within 5-10 minutes. It duration of action is also less an compared to regular insulin and it is 4-5 hours (3). Its efficacy and antigenicity is comparable to that of human insulin (4). It is costlier than other insulins. It is specially indicated in postprandial hyperglycemia and in children where food intake may be unpredictable. Insulin lispro can also be used safely in gestational diabetes mellitus.

Long Acting Insulin Analogues

The principle employed in the development of long acting analogue is to reduce solubility at the pH of subcutaneous tissue fluid. This is made by substitution of hydrophobic amino acids within the insulin monomer or addition of arginine residue to the end of beta chain.

The long acting insulin analogue HOE 901 is under trial. This alteration shifts the iso-electric point of the molecule to a slightly acidic pH and renders it less soluble at physiological pH(5). As a result HOE 901 forms a precipitate and subsequently results in a relatively constant and peakless delivery of insulin over approximately 24 hours. Instead of zinc, cobalt can also be used for prolongation of insulin action.

INSULIN SENSITIZERS

Insulin sensitivity can be increased by several pharmacological (metformin, thiazolidinedione derivatives, hHg fragment) and non-pharmacological measures, (exercise, and weight reduction)

Metformin

Metformin is a drug with the unusual distinction of having been rediscovered twice n the century. Today, it is one of the most popular oral hypoglycemic agent with about 25% of the market if oral hypoglycemic agents. Chemically, it is a biguanide.

Mode of Action

It has multiple sites of action on the liver, muscles, fat and gut, it reduces the insulin resistance, body weight and blood lipids. It decreases glucose absorption from the gut (6). It increases insulin receptor binding and augments post receptor effects. It increases peripheral glucose utilization and insulin mediated suppression of hepatic neogluconesis. Lipolysis, free fatty acid concentration and lipid

oxidation are also reduced by metformin. Metformin increase lactate production from glycolysis in the intestine and reutilization of lactate occurs for gluconegenesis. This is the mechanisms by which it protects against hypoglycemia. It decreases triglycerides. LDL-c and increases fibronolytic activity.

Clinical use

It is an important tool for management of obese diabetics, but is ineffective in type 1 diabetes and when blood sugar level are above 300mg/dl. Maximum dose that can be given is 3g/day. It is associated with certain gastrointestinal side effect like nausea, metallic taste in the mouth, increased bowel frequently and macrocytic anemia (noted in few patients). Lactic acidosis is rare, but deadly, side effect in patients who are receiving full dose of metformin and are suffering from severe cardiovascular and or renal failure. It should be avoided when serum creatinine level is more than 2mg/dl or in anoxemic states.

Thiazolidinediones

The discovery of perioxisome proliferator activated receptor (PPAR) and their subtypes has led to the discovery of a new generation of drugs. The two major PPAR receptors are α and γ, and both are expressed by obligate heterodimerisation with retinoic acid x receptor (RX Rα and Rα Rγ). The PPAR(α) is primarily responsible for lipolysis by activation lipase malic enzyme, bifunctional enzyme and medium chain acyl CoA dehydrogenase. On the other hand PPAR(γ) is primarly responsible for the adipocyte differentiation and at the metabolic level, in free fatty and lipid anabolism and storage. The pronounced hypoglycemic effect seen by PPAR(γ) agonists is attributes primarily to adipocycle differentiation and/or activation.

The PPAR(γ) agonists, ciglitazone, englitazone, troglitazone, pioglitazone and resiglitazone are some of the member of this class. They decrease hepatic glucose output and increase peripheral glucose utilization by improving insulin sensitivity at hepatic and muscle sites. They restore the sensitivity of phosphonel pyruvate carboxy kinase (PEPCK) to insulin, thereby decreasing glycogenolysis. They also increase peripheral triglycerida clearance and decreaed hepatic triglycerida synthsis, independent of insulin (7). At the cellular level, they increase the binding and tyrosine kinase activity of insulin receptors, activate post receptor signaling proteins and enhance insulin induced traslocation of GLUT-4 on the the plasma membranes (7). All these effects are dependent on insulin. These agents do not stimulate insulin secretion from β-cells and are therefore not effective in insulinopenic subjects (8). Troglitazone was marketed in USA, but was subsequently withdrawn due to hepatoxicity. Resiglitazone and pioglitazone are devoid of hepatotoxity and are currently available.

PPAR (α) agonists such as fibrates are not effective hypoglycemic agents, but they lower LDL cholesterol and triglycerida and raise HDL, thus offering protection against increased coronary morbidity and mortality, which is seen in type 2 diabetes (7). Retionoids, which activate RXR receptors are being developed to control diabetes. One such product LG 100268 has shown significant promise, in that, in addition to being an insulin sensitizer it cause weight reduction in contrast to PPAR (γ) againsts (8).

A new class of drugs which are plant extracts and act through inhibition of protein tyrosine kinase are being investigated. In addition to hypoglycemic effect, these block the formation of proinflammatory cytokines such as TNF α. Compounds in this class includes CLX 0301, CLX 0302, CLX 0900 and CLX 0901. This group of drugs also lower cholesterol and triglycerides. Again these are sensitizers and are not effective in type 1 diabetes (9,10)

β_3 Adrenergic Receptor Agonists

β_3 adrenergic receptors are present in brown and white adipose tissues and mediate catecholamine stimulated thermogenesis and lipolysis. A polymorphism in β_3 adrenergic receptor due to missense mutation in the gene coding for it, has been identified in Finns and Pima Indians. This has been linked to lower basal metabolic rate, greater visceral adiposity and early onset of type 2 diabetes, in these ethnic groups. This observation has stimulated the use of selective β_3 adrenoceptor agonists such as CL 316, 2443, which do not cross react with other β- adreneceptors, for treating obesity and improving insulin sensitivity (11). In obese diabetic animals models, β_3 adrenergic receptor agonists reduce body weight by increasing energy expenditure and reduce fat depots without inducing a decrease in food intake. Reduction in blood glucose along with triglycerida concentration are observered within a week of their usage (10). Preliminary results in type 2 diabetes patients with

these drugs have confirmed these benefical effects (11).

INSULIN MIMETICS

Mimetics are agents, which though chemically and structurally not related to insulin , have insulin like action. Most of these are under trial, e.g. vanadium. Some trace elements like chromium and zinc are also used clinically.

Vanadium slats

Vanadium is a ultra trace element. It compounds such as vanadyle orthovandate, metavandate and peroxovandate, have been also shown to have insulinomimetic effects on adipocytes, hepatocytes and the skeletal muscles, as well as in hyperinsulinemic and hypoinsulinemic animals models of the diabetes. They act by a mechanism independent of insulin and near euglycemia is achieved in animal models with in 1-2 weeks. In animal models, vanadium salts induce decrease in body weight, attributed to its central anorectic effects (12). These salts act by increasing the phosphorylation of insulin receptor either by activation of the tyrosine kinase activity or by inhibition of the phosphotyrosyl phosphatase that dephosphorylates the receptor (12) and may also act on post receptor sites (mitogen activated protein kinase and cytosolic insulin independent tyrosine kinase) (12). Importantly these compounds are effective even in situations where the insulin signal transduction pathway is defective. Usual dosage is 100mg/day and the effects lasts for upto two weeks after discontinuation. Major side effects are gastrointestinal, however, there are fears of its mitogenic potential, as it stimulates tyrosine kinase. A synethetic organic complex of vanadyl (bis maltatato) oxovanadium with high lipophilicity and peroxovandium compounds appear promising (12).

IGF –1 receptor agonists are also being developed for selective hypoglycaemic action, prolonged duration of effects and perhaps for use by the oral route.

MISCELLANEOUS INSULIN MODULATORS

Various other factors modulate the action of insulin after its administration (Table 3).

Table 3. Factors Affecting the Biovailibility and Absorption rate of Subscutaneously injected Regular Human Insulin.

Factor	Effect
Origin of Insulin	Human insulin results in more rapidly absorbed than animals insulin
Depth of injection	Intramuscular injections are absorbed more rapidly than subcutaeneous injections
Insulin concentration	Dilute solution (U40) are more rapidly absorbed than concentration preparation (U100)
Dose of insulin	Higher doses of injected regular insulin have a prolonged duration of action in comparison with lower doses.
Mixing insulins	Regular insulin maintains its potency and time action profile when mixed NPH insulin, but mixing with Lente or Ultralentre significantly slows the absorption and blunts the activity of regular insulin
Exercise	Exercising a muscle group prior to injection into that area increase the rate of insulin absorption
Local heat, massage	Local heat and message following injection increase the absorption rate of regular insulin

Site and Depth of Insulin Injection

It is one of the important factors. It was recently established that the anterior abdominal wall is preffered for regular insulin and thigh is considered a better site for long acting insulin absorption. Insulin given intramuscularity is rapidly absorbed, as compared to insulin given subcutaneously (13,14). The site of insulin injection plays an important role in determining the absorption kinetics, injection in an exercising limb, leads to a more rapid absorption.

Dose of Insulin

Higher dose of insulin is absorbed slower as compared to a lower dose. When the requirement is very high, it may be preferable to change over to a higher concentration or splitting to multiple doses.

Temperature

Denaturation of insulin occurs at a temperature above 25^0C. If insulin is exposed to above 25^0C for months, significant amount of insulin is denatured. Increased local temperature leads to rapid absorption of insulin from local site.

Insulin Antibodies

Insulin antibodies can be present at the local site or systemically. Antibodies present at the local site interfere in adsorption of insulin, while systemic antibodies release insulin, while systemic antibodies release insulin erratically and worsen the glycemic control.

Insulin Species

The species of insulin also modulates its action. The animals insulins (porcine and bovine) have slower absorption and longer duration of action than purfied preparation and human insulins.

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