DAPAGLIFLOZIN: A NOVEL SODIUM-GLUCOSE COTRANSPORTER TYPE 2 INHIBITOR FOR THE TREATMENT OF TYPE 2 DIABETES MELLITUS

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ABSTRACT
Dapagliflozin is the lead agent in a new class of oral antidiabetic agents known as sodium-glucose cotransporter type 2 (SGLT2) inhibitors, which represent a novel approach to the management of type 2 diabetes mellitus. By selectively and reversibly blocking the SGLT2 receptor, dapagliflozin prevents the reabsorption of glucose at the renal proximal tubule. Dapagliflozin produces a sustained, dose-dependent reduction in plasma glucose levels while simultaneously improving insulin secretion and sensitivity. Dapagliflozin has a favourable safety profile, with the rates of hypoglycaemia similar to those of placebo. Genital and urinary tract infections were more commonly reported in patients taking dapagliflozin (2–13%) than those taking placebo (0–8%). Dapagliflozin does not appear to cause electrolyte disturbances, hepatotoxicity, or nephrotoxicity. Results from clinical trials have been promising, and well-designed clinical programs that address the long-term safety and efficacy of dapagliflozin are under way.

KEYWORDS: Hypoglycaemia, SGLT-2 inhibitors, Dapagliflozin, diabetes, glycosuria.

INTRODUCTION
Diabetes is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels.
Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycaemia.[1,2]

Symptoms of marked hyperglycaemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision.

Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycaemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycaemia with ketoacidosis hyperosmolar syndrome.

Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes.[2,3]

**Classification of diabetes mellitus**

I. Type 1 diabetes (insulin-dependent diabetes mellitus or IDDM)
   a) Autoimmune
   b) Idiopathic

II. Type 2 diabetes (noninsulin-dependent diabetes mellitus or NIDDM)

III. Secondary diabetes
   a) Genetic defects of b cell function (e.g., maturity onset diabetes of the young)
   b) Genetic defects of insulin action pathway
   c) Exocrine pancreatic disease
d) Endocrinopathies (e.g., Cushing’s syndrome, acromegaly)
e) Drugs or chemicals
f) Infections (e.g., congenital rubella)
g) Other genetic syndromes (e.g., Down, Klinefelter syndromes)

IV. Gestational diabetes
(Classification proposed by the Expert Committee on the Diagnosis and the Classification of Diabetes Mellitus under the sponsorship of the American Diabetes Association (Diabetes Care)

Type 2 Diabetes Mellitus

**TYPES OF DIABETES MELLITUS**

**TYPE -1**
The cause is an absolute deficiency of insulin secretion. Individuals at increased risk of developing this type of diabetes can often be identified by serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers. In the other, much more prevalent category and accounts for only 5–10% of all diabetes, is a juvenile-onset diabetes; it results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas by CD4 and CD8 T cells and macrophages infiltrating the islets. In this case insulin therapy is required for survival, to prevent the development of ketoacidosis, coma and death.\(^{[3,4]}\)

**TYPE -2**
Type 2 Diabetes Mellitus (T2D) is a complex heterogeneous group of metabolic condition characterized by elevated levels of serum glucose; according to WHO, it is defined as resulting from a defect in both insulin secretion and in insulin sensitivity. β-cell dysfunction includes abnormalities in pulsatility and in kinetics of insulin secretion, quantitative and qualitative abnormalities of insulin, β-cell loss and its progression.\(^{[4,5]}\)

**CLASSIFICATION OF ANTI-DIABETIC DRUGS**

1) Sulfonylurea
   (a) First generation
   • Tolbutamide
   • Chlorpropamide
(b) second generation
- glibenclamide
- glipizide (glucotrol)
- gliclazide
- glimepride (amaryl)

2) BIGUANIDES
a) Metformin (glucophage)

3) PHENYL ALANINE ANALOGUES
a) Repaglinide (starlix)
b) Nataglinide (Prondin)

4) THIAZOLIDINEDIONES
a) Rosiglitazone (Avandia)
b) Pioglitazone (Actos)

5) DIPEPTIDYL PEPTIDASE-4 (DPP-4) INHIBITORS
a) Alogliptin (Nesina)
b) Linagliptin (Tradjenta)
c) Saxagliptin (Onglyza)
d) Sitagliptin (Januvia)

6) SODIUM GLUCOSE CO-TRANSPORTER (SGLT-2) INHIBITORS
a) Canagliflozin (Invokana)
b) Dapagliflozin (Farxiga)
c) Empagliflozin (Jaradiance)

SGLT2 Inhibitors: A New Class of Diabetes Medications
Sodium-glucose co-transporter 2 (SGLT2) inhibitors are a new class of diabetic medications indicated only for the treatment of type 2 diabetes. In conjunction with exercise and a healthy diet, they can improve glycaemic control. They have been studied alone and with other medications including metformin, sulfonylureas, pioglitazone, and insulin.

SGLT2 is a protein in humans that facilitates glucose reabsorption in the kidney. SGLT2 inhibitors block the reabsorption of glucose in the kidney, increase glucose excretion, and lower blood glucose levels. [5,6]
How they work

SGLT2 is a low-affinity, high capacity glucose transporter located in the proximal tubule in the kidneys. It is responsible for 90% of glucose reabsorption. Inhibition of SGLT2 leads to the decrease in blood glucose due to the increase in renal glucose excretion. The mechanism of action of this new class of drugs also offers further glucose control by allowing increased insulin sensitivity and uptake of glucose in the muscle cells, decreased gluconeogenesis and improved first phase insulin release from the beta cells.

MECHANISM OF ACTION

Sodium-glucose co-transporter-2 inhibitors work by inhibiting SGLT2 in the Proximal convulated tubule, to prevent reabsorption of glucose and facilitate its excretion in urine. As glucose is excreted, its plasma levels fall leading to an improvement in all glycaemic parameters.
This mechanism of action is dependent on blood glucose levels and, unlike the actions of thiazolidinedione (mediated through GLUTs), is independent of the actions of insulin. Thus, there is minimal potential for hypoglycaemia, and no risk of overstimulation or fatigue of the beta cells. Because their mode of action relies upon normal renal glomerular-tubular function, SGLT2i efficacy is reduced in persons with renal impairment.\textsuperscript{[5,6]}

**What are the benefits of taking an SGLT2 inhibitor?**

**SGLT2 inhibitors**
- lower blood glucose levels- SGLT-2 works with an insulin independent mechanism of action it lowers blood glucose level by increasing the excretion of blood glucose through urine.
- can cause weight loss in some people-Weight loss during SGLT2 therapy is much lower than expected, given the amount of energy lost through glycosuria. Glycosuria may signal the CNS to change appetite regulation, though this mechanism requires more research. Patients with T2DM might experience more weight loss during SGLT2 inhibitor therapy if they followed stricter dietary recommendations to decrease appetite and overeating
- can reduce systolic blood pressure in some people- SGLT2 inhibitors significantly decreased SBP after 1 month and DBP after 6 months in obese patients with type 2 diabetes. The main mechanism of the BP-lowering effect may be plasma volume reduction by osmotic diuresis at 2 weeks and after SGLT2 inhibitor administration.
- can reduce heart complications in some people- SGLT-2 inhibitors likely reduce arterial stiffness via a multifactorial mechanism which incorporates weight loss, decreased fasting insulin levels, vascular smooth muscle relaxation, and multiple beneficial anti-inflammatory effects.

**DAPAGLIFLOZIN**

Dapagliflozin is a novel inhibitor of renal sodium glucose co transporter 2, which allows an insulin independent approach to improve type 2 diabetes hyperglycaemia. It is a C-aryl glucoside derivative and is chemically known as (1s)-1, 5-anhydro-1-C-[4chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D- glucitol.\textsuperscript{[8,9]}
Pharmacokinetics

Dapagliflozin is rapidly and well absorbed after oral administration. Maximum Dapagliflozin plasma concentrations occur within 2 hours of administration (in the fasted state). Bioavailability is 78% with the 10 mg once daily (OD) dosing. It can be taken with or without food. It is 91% protein-bound, and this is not affected by hepatic or renal disease. Dapagliflozin is metabolized to its inactive metabolite – Dapagliflozin 3-O-glucuronide – in the liver and kidney by the enzyme uridine diphosphate-glucuronosyltransferase 1A9. The mean plasma terminal half-life for Dapagliflozin is 13 hours (10 mg dosing). Dapagliflozin and its metabolites are mainly excreted via the urine, and the excretion is impaired in the presence of renal disease; 15% is excreted unaltered via the faeces and 2% excreted unaltered via the urine.\(^\text{[10]}\)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Dapagliflozin</th>
<th>Canagliflozin</th>
<th>Empagliflozin</th>
<th>Ipragliflozin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trade name</td>
<td>Forxiga</td>
<td>Invokana</td>
<td>Jaradiance</td>
<td>Suglat(Japan)</td>
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<tr>
<td>Tablets (mg)</td>
<td>5mg,10mg</td>
<td>100mg,300mg</td>
<td>10mg, 25mg</td>
<td>25mg,50mg</td>
</tr>
<tr>
<td>Dosage(daily)</td>
<td>5mg</td>
<td>100mg</td>
<td>10mg</td>
<td>25mg</td>
</tr>
<tr>
<td>Cost (daily)INR</td>
<td>771.19</td>
<td>584.5</td>
<td>174.5</td>
<td>NA</td>
</tr>
<tr>
<td>Oral bioavailability (%)</td>
<td>78</td>
<td>65</td>
<td>60</td>
<td>NA</td>
</tr>
<tr>
<td>Time of maximum peak (t\text{max}) (hr)</td>
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<td>1-2</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td>Plasma protein binding(%)</td>
<td>118</td>
<td>119</td>
<td>74</td>
<td>1-2</td>
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<tr>
<td>Volume of distribution (l)</td>
<td>91</td>
<td>98</td>
<td>86</td>
<td>NA</td>
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<tr>
<td>Half time (t-) (hr)</td>
<td>12.2</td>
<td>11-13</td>
<td>12.4</td>
<td>NA</td>
</tr>
</tbody>
</table>
Metabolism

| Extensive glucuronidation to inactive conjugates (primarily dapagliflozin3-O-glucuronide) | Extensively metabolised by O-glucuronidation to two major inactive metabolites | Extensively metabolised by glucuronidation and to a lesser extent, oxidation to 6 inactive metabolites | Extensively metabolised by glucuronidation to two major inactive metabolites |

Elimination

| Primarily in urines as inactive metabolites: 2 % eliminated as unchanged drug in urine | Elimination in urines and faeces: 1 % eliminated as unchanged drug in urine | Eliminated in urine and faeces – 28.6% excreted unchanged in urine. | Primarily in urine as inactive metabolites: 31 % eliminated as unchanged drug in urine |

**Pharmacodynamics**

Dapagliflozin is associated with dose-dependent glycosuria and increased diuresis averaging 375 mL/day. There is a transient increase in urinary sodium excretion, but this does not appear to affect serum sodium. There is a transient increase in urinary uric acid excretion, but a sustained reduction in serum uric acid levels. The mechanism of reduced serum uric acid levels is unclear, but was recently proposed to be due to glycosuria-induced uric acid secretion via GLUT9 isoform 2 in the proximal tubule or inhibition of uric acid uptake at the collecting duct of the renal tubule. Additionally, this could be due to the weight reduction associated with Dapagliflozin.\(^{[12,13]}\)

**Monotherapy**

Dapagliflozin has been studied as a monotherapy against placebo and versus metformin or placebo. In these studies, the mean HbA\(_1\text{c}\) reduction compared to placebo at 24 weeks was 0.66–1.45%, and weight reduction was 1.0–2.73 kg. There was a reduction in fasting glucose, and more patients achieved an HbA\(_1\text{c}\) <7% in at least one study. Genital and urinary infections are more common (3.7% and 2.3% difference, respectively) compared to placebo.\(^{[14,15]}\)

**Dual therapy**

In dual therapy, dapagliflozin has been studied (against placebo) with metformin, metformin slow/extended-release, glimepiride, pioglitazone, sitagliptin and exenatide. The highest reduction in HbA\(_1\text{c}\) was seen when dapagliflozin was combined with metformin. Here, HbA\(_1\text{c}\) reduced by 0.8% following 102 weeks of therapy, compared to 0.5%–0.68% when combined with the other agents. The highest reduction in weight was seen when combined with sulfonylureas (SUs). When combined with glipizide, an average 4.4 kg in weight was
lost compared to glipizide alone. In comparison, weight loss of 1.74 kg with metformin and 1.8 kg with sitagliptin has been reported. Across all studies, a 5%–10% increase in genital infections was reported. There are no human studies of dapagliflozin with glucagon-like peptide-1 analog therapy.\cite{16}

**Triple therapy**

Dapagliflozin has been used with metformin and sitagliptin, metformin and saxagliptin, and metformin and an SU, in triple combinations. HbA$_{1c}$ reduction of up to 0.6% and body weight reductions of 2.2 kg were reported. Genital and urinary infections were higher than in control groups.

**With insulin**

A number of studies of dapagliflozin in combination with insulin have been undertaken, and a further study is ongoing (ClinicalTrials.gov identifier: NCT02096705). Studies to date demonstrate benefits in HbA$_{1c}$ (-0.4%), weight (-3.33 kg), along with a stable insulin dose (-19.2 units when compared to increased requirements in the placebo group) at 104 weeks. Increased hypoglycemia rates were noted at 48 weeks, which levelled out by 104 weeks. There were higher rates of genital and urinary infections.\cite{16,17}

**Benefits other than glucose**

**Weight**

Weight loss is a benefit of therapy with dapagliflozin. It is thought to be through fluid loss in the initial phase of treatment, and then a more gradual loss through the net calorie deficit (typically 200–300 kcal per day). More recently, it has been shown that dapagliflozin therapy also reduces fat mass. Meta-analyses (Table 2) show about a 1–2 kg weight reduction with this agent, and up to 5 kg when used in combination with SUs.

**Hypertension**

Investigators have noted a lowering of both systolic and diastolic BP in patients, and this is thought to be due to a combination of the diuretic effect of glycosuria, a natriuretic effect, as well as via weight loss. The BP lowering is noted as early as 1–2 weeks of treatment initiation, and averages 4/2 mmHg. The BP-lowering effects of dapagliflozin also manifest in patients on established antihypertensive therapy.
Sjöström et al pooled safety data from 13 placebo-controlled Phase IIIB/III studies, and found a slightly higher cumulative frequency of orthostatic reactions over 24 weeks with dapagliflozin (13.1% versus 11.3% with placebo), although adverse events due to orthostatic hypotension were rare (0.1%) and not serious.[18]

**Lipids**

Dapagliflozin 10 mg OD has shown a mean change compared to placebo in total cholesterol of 2.5%, high-density lipoprotein of 3.3%, low-density lipoprotein of 3.9%, and triglycerides of -2.0%. Meaningful long-term data are required to understand if this has any clinical impact.[19]

**Quality of life**

Dapagliflozin-treated patients have been shown to have a high health-related quality-of-life (HRQOL, EQ-5D) score in several domains. This persisted for 102 weeks. In a triple-therapy regimen, dapagliflozin showed greater improvement over placebo in weight change-related QOL, similar obesity-specific QOL, and greater treatment satisfaction.

**Side effects of Dapagliflozin may include:**

- **Hypoglycaemia** - This adverse event is seen with some glucose-lowering therapies and is occasionally a limitation factor in achieving good glycaemic control. Hypoglycaemia is also associated with negative effects such as undesirable symptoms like weight gain (through increased appetite), poor adherence to therapy, uncontrolled glycaemia because of the fear of new hypoglycaemic episodes, poor quality of life and, in some patients, accidents such as falls and cognitive disorders, mainly in elderly and frail patients. A link between severe hypoglycaemia and increased cardiovascular risk and total mortality has recently been described. Because of their mechanism of action – which is not dependent on insulin secretion and includes lowering the renal threshold for glucose from 200mg/dl to around 100mg/dl while maintaining glucose levels above the hypoglycaemic range – the SGLT-2-i are less likely to cause hypoglycaemia by themselves. This means that as a monotherapy add-on to metformin (Met), there is no real (or only marginal) increase in hypoglycaemia compared with a placebo or when added to other drugs that can cause hypoglycaemia, such as sulphonylurea (SU), Met + SU, pioglitazone (Pio) with or without SU and especially insulin. Thus, the incidence of hypoglycaemia is low except in patients taking SUs or insulin as either their allocated treatment or in addition.
• **Genital infection**- By their very nature, the most common major safety concern of SGLT-2 inhibition is that the drugs cause glucose levels to rise in urine, which can lead to urinary tract and genital infections, increased urinary frequency and electrolyte imbalances. An increase in urinary glucose excretion caused by SGLT-2 treatment also has the potential to increase fungal growth in the perineum and genitourinary tract. Non-sexually transmitted perineal and genitourinary tract mycotic infections are considered adverse events of particular interest. In fact, use of the three drugs (canagliflozin, dapagliflozin and empagliflozin) is accompanied by an increase in genital infections compared with a placebo and affects more women than men (by four to five times), mostly as vulvitis. However, the vast majority of cases are diagnosed with typical symptoms and require only a single standard antibiotic treatment. In women, the diagnosis is mostly mycotic vulvo vaginitis and, in men, mycotic balanitis. Rates of genital infection in men are several-fold lower than rates of vulvo vaginitis in women. Genital infections are more frequent in premenopausal women and much more common in those with a history of genital infection and/or obesity, and not influenced by baseline HbA1c levels.

• **Urinary tract infections**- Urinary tract infections (UTIs) are common in patients with T2DM. Johnsson et al. pooled the safety data of 12 randomized placebo-controlled trials with dapagliflozin to evaluate the relationship between glycosuria and UTIs in patients with inadequately controlled diabetes (HbA1c > 6.5-12%). Patients were treated with dapagliflozin (2.5mg, 5mg or 10mg) or placebo once daily either as monotherapy or as an add-on to Met, insulin, SU or thiazolidinedione for 12-24 weeks.

• **Blood pressure**-Chronic osmotic diuresis caused by glycosuria would be anticipated to reduce blood pressure, and dose-related increases in 24-h urinary volumes of between 100ml and 470ml have been reported. Reductions in systolic blood pressure (SBP) of up to 5mmHg have been described in trials of dapagliflozin, whether used as an add-on therapy or on its own. Canagliflozin has similarly been shown to significantly reduce SBP. It is well known that SGLT-2-i have diuretic-like effects, lowering SBP by 3-5mmHg, which could benefit the majority of patients with T2DM. The precise mechanism behind the BP-lowering action of SGLT-2-i, however, is still unclear and does not appear to be based on natriuretic effects. Indeed, although these agents have mildly natriuretic effects, they are nothing like diuretics. Part of their BP-lowering effect is presumed to be due to osmotic diuresis. However, they might also reduce BP too much.
in some patients, with consequences including hypotension (mainly postural), dizziness and dehydration.

- **Renal effects**: Chronic kidney disease (CKD) is a major health problem in patients with T2DM. Stage 3-5 CKD (GFR < 60ml/min) affects around 25% of such patients and represents an under-recognized problem in clinical practice. Most OADs have limitations in cases of renal impairment because they require dose adjustments or are contraindicated for safety reasons. In fact, the activity of SGLT-2 depends on the number of nephrons, which means that the first consequence of renal impairment on SGLT-2 action is reduced efficacy. For this reason, the use of SGLT-2 is neither recommended nor allowed in those with an estimated GFR (eGFR) < 40ml/min because of the lack of effectiveness rather than risks.

**METHOD OF DETERMINATION OF DAPAGLIFLOZIN**

*Dapagliflozin API by UV Spectroscopy*

Dapagliflozin was developed and assessed by UV- Spectrophotometric method. The developed method was validated as per ICH guidelines. The drug showed two different wavelengths of maximum absorption, at 203nm and 237nm. This method can be successfully applied for the estimation of Dapagliflozin in bulk for routine analysis with UV detection at 237nm. A Lab India UV-Visible spectrophotometer with 1cm matched quartz cells and ethanol solvent were employed in this method.[21] The Developed method obeyed Beer’s-Lambert’s law in the concentration range of 0.5-0.9µg/ml, having correlation coefficient of 0.994. Different validation parameters like, precision (intra-day and inter-day studies), limit of detection, limit of quantitation, ruggedness and robustness were studied and were found to be within the limits.

*DAPAGLIFLOZIN API BY RP-HPLC*

Dapagliflozin developed and validated by RPHPLC method. The methods were validated as per ICH guidelines. The linearity was found to be 25-150µg/ml and 1-5µg/ml for RP-HPLC. The RP-HPLC method was validated for accuracy, linearity, precision, LOD, LOQ, system suitability and robustness.[22] All the results were found to be within the limits as per ICH guidelines and hence the proposed two methods can be successfully employed for the determination of Dapagliflozin in its API for regular and routine analysis.
CONCLUSION
SGLTs inhibitors represents a highly promising noble class of oral agents for the treatment of T2DM. As SGLTs inhibitors may occupied a unique position as anti-diabetic drugs. Their unique mechanism of action provides an important in both fasting and postprandial hyperglycaemia without increasing insulin secretion or causing weight gain, hypoglycaemic, gastrointestinal side effects or fluid retention. SGLTs Inhibitors would be expected to be beneficial in the treatment of type 2 diabetes as monotherapy or in combination with other hypoglycaemic agent or insulin. Furthermore because of their potential role in weight loss they would be very appealing to patient and medically useful in the treatment of type 2 diabetes and associated comorbid conditions such as hypertension and dyslipidaemia in effective and highly specific inhibition of SGLTs of Dapagliflozin making one daily treatment feasible effective and safe. Hence these agent should be considered as alternative type 2 diabetic therapy in patient with inadequately controlled glycaemic treated with monotherapy.

REFERENCES
1. DrugBank: Dapagliflozin (DB06292).


