REVIEW ARTICLE

Drugs 1999; 58 Suppl. 1: 31-39
0012-6667/99/0001-0031/$04.50/0

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The Antihyperglycaemic Effect of Metformin

Therapeutic and Cellular Mechanisms

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Abstract

Metformin is regarded as an antihyperglycaemic agent because it lowers blood glucose concentrations in type 2 (non–insulin-dependent) diabetes without causing overt hypoglycaemia. Its clinical efficacy requires the presence of insulin and involves several therapeutic effects. Of these effects, some are mediated via increased insulin action, and some are not directly insulin dependent.

Metformin acts on the liver to suppress gluconeogenesis mainly by potentiating the effect of insulin, reducing hepatic extraction of certain substrates (e.g. lactate) and opposing the effects of glucagon. In addition, metformin can reduce the overall rate of glycogenolysis and decrease the activity of hepatic glucose-6-phosphatase. Insulin-stimulated glucose uptake into skeletal muscle is enhanced by metformin. This has been attributed in part to increased movement of insulin-sensitive glucose transporters into the cell membrane. Metformin also appears to increase the functional properties of insulin- and glucose-sensitive transporters. The increased cellular uptake of glucose is associated with increased glycogen synthase activity and glycogen storage. Other effects involved in the blood glucose-lowering effect of metformin include an insulin-independent suppression of fatty acid oxidation and a reduction in hypertriglyceridaemia. These effects reduce the energy supply for gluconeogenesis and serve to balance the glucose-fatty acid (Randle) cycle. Increased glucose turnover, particularly in the splanchnic bed, may also contribute to the blood glucose-lowering capability of metformin.

Metformin improves insulin sensitivity by increasing insulin-mediated insulin receptor tyrosine kinase activity, which activates post-receptor insulin signalling pathways. Some other effects of metformin may result from changes in membrane fluidity in hyperglycaemic states.

Metformin therefore improves hepatic and peripheral sensitivity to insulin, with both direct and indirect effects on liver and muscle. It also exerts effects that are independent of insulin but cannot substitute for this hormone. These effects collectively reduce insulin resistance and glucotoxicity in type 2 diabetes.

Since its introduction in 1957, metformin has become an established treatment for type 2 (non–insulin-dependent) diabetes mellitus.[1,2] It is used as monotherapy and in combination with other types of oral antidiabetic agent or insulin, thereby offering a unique profile of therapeutic effects. The blood glucose-lowering effect of metformin is complemented by potentially beneficial effects on

Table I. Major blood glucose-lowering effects of metformin

Parameter	Effect
Hepatic glucose output	Decreased
Peripheral glucose utilisation	Increased
Fatty acid oxidation	Decreased
Glucose turnover	Increased

blood lipid profiles and improvements in various micro- and macrovascular parameters. Metformin does not cause weight gain, and tends to reduce hyperinsulinaemia, which serves to counter insulin resistance and its clinical sequelae.

Typically, metformin reduces basal hyperglycaemia by 1 to 3 mmol/L and decreases haemoglobin A1c (HbA_{1c}) by 1 to 2%.[3-5] However, metformin alone does not precipitate overt hypoglycaemia; hence its designation as an antihyperglycaemic agent. Metformin appears to require the presence of insulin to lower blood glucose levels, although the drug does not stimulate insulin secretion. Metformin exerts a variety of insulin-dependent and insulin-independent actions, although the insulin-independent effects are not a substitute for insulin. Moreover, metformin has different actions in different tissues, which collectively account for the blood glucose-lowering effect.[6,7] This review considers the sites and mechanisms of action responsible for the antihyperglycaemic effect of metformin.

1. Antihyperglycaemic Effects

The main gluco-regulatory effects of metformin involve suppression of hepatic glucose output, increased peripheral glucose utilisation, reduced fatty acid utilisation and increased glucose turnover, particularly in the splanchnic bed (table I). In addition, metformin alters glucose handling by erythrocytes and reduces hypertriglyceridaemia. Thus, metformin can affect a variety of gluco-regulatory processes, both directly and indirectly via an improved metabolic environment.

The variety of effects exerted by metformin appears to be related, at least in part, to the markedly different concentrations of the drug in different tissues seen at different times after administration. Metformin is rapidly absorbed and rapidly excreted unchanged in the urine.[8] In clinical use an oral dose of 500 to 1000mg results in a maximum concentration of 1 to 3 mg/L (about 1 to 2×10^{-5} mol/L) in venous plasma after about 2 hours.

When 50 mg/kg metformin was given orally to normal and streptozotocin diabetic mice (equivalent to 3000mg in a 60kg person on a weight-for-weight basis), the maximum concentration of metformin was about 3×10^{-5} mol/L in peripheral venous plasma and 5 to 6×10^{-5} mol/L in the hepatic portal vein.[9] Extremely high concentrations of metformin accumulated in the walls of the jejunum and ileum (up to 3×10^{-3} mol/L) compared with the liver (up to 3×10^{-4} mol/L) or muscle and fat (up to approximately 5×10^{-5} mol/L).

1.1 Hepatic Glucose Output

There is substantial evidence that metformin reduces hepatic gluconeogenesis. Studies in isolated perfused livers and hepatocytes from animals have shown that metformin acts directly on the liver to reduce gluconeogenesis from a range of substrates including lactate, pyruvate, alanine, glutamine and glycerol.[10-12] In the absence of added insulin there is little effect until the metformin concentration is above that normally found within the hepatic portal vein. However, in the presence of insulin, therapeutic concentrations of metformin have been reported to suppress gluconeogenesis, thus showing a synergistic effect with insulin (fig. 1).[11,12] This effect was increased in the presence of raised glucose concentrations,[13] and metformin also suppressed the gluconeogenic effect of glucagon.[13,14] While low concentrations of metformin promote the antigluconeogenic action of insulin, higher concentrations can exert several non–insulin-dependent effects that contribute to a reduction in hepatic glucose production. These include decreased hepatic uptake of gluconeogenic precursors such as lactate and possibly amino acids.[15,16] High concentrations of metformin might also reduce the mitochondrial NAD to NADH ratio,[11] causing a slight lowering of cellular ATP that is sufficient to enhance the flux through pyruvate kinase.[17]

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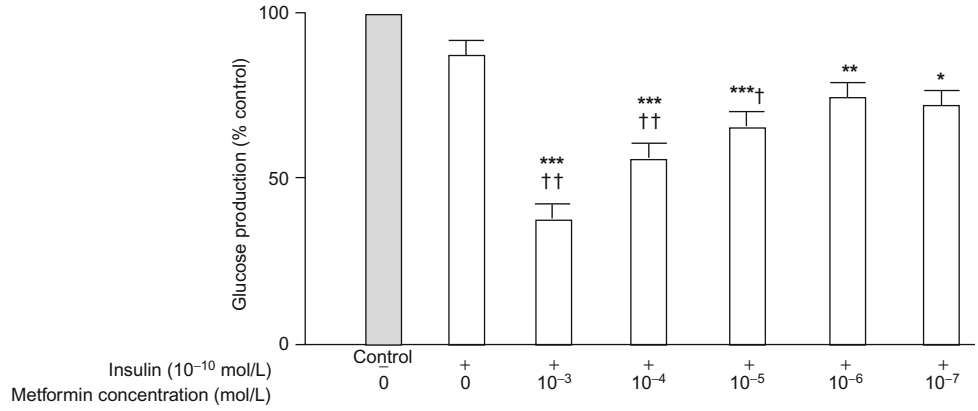

Fig. 1. Interaction of insulin and metformin to suppress gluconeogenesis from lactate (10 mmol/L) and pyruvate (1 mmol/L) by isolated hepatocytes from 48-hour starved rats after incubation for 1 hour.[12] Values are mean ± standard error; n = 6. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs* control; † $p < 0.05$, †† $p < 0.01$ *vs* insulin alone.

Therapeutic and supratherapeutic concentrations of metformin have been consistently shown to cause only partial suppression of gluconeogenesis.[1,7] This is consistent with clinical experience to show that metformin is rarely associated with hypoglycaemia to any degree, and that the drug does not cause serious or fatal hypoglycaemia, even after overdosage.[1]

In addition to partial suppression of gluconeogenesis, metformin appears also to reduce hepatic glucose output by decreasing the overall rate of glycogenolysis.[7] No consistent effect of metformin has been demonstrated on total hepatic glycogen content, but there are reports that metformin can increase both glycogenesis and glycogenolysis.[7] In the livers of diabetic mice, metformin increased levels of the active forms of both glycogen synthase and glycogen phosphorylase, indicating increased glycogen turnover.[18] Patients with type 2 diabetes treated with metformin showed a reduction in overall glycogenolysis that was associated with decreased cycling of glucose-6-phosphate.[19]

Reductions in both gluconeogenesis and glycogenolysis by metformin probably reflect in part the drug's suppressive effect on hepatic glucagon activity.[13,14] Glucagon activates hepatic adenylate cyclase, and the ability of insulin to inhibit adenylate cyclase is reduced in insulin-resistant diabetic states. Metformin restores the capacity of insulin to inhibit hepatic adenylate cyclase, thus providing an explanation for the improvement in hepatic insulin action.[20-22] Metformin also decreases the activity of glucose-6-phosphatase in the livers of diabetic animals and insulin-resistant liver cells, which indicates a further means of reducing hepatic glucose output.[7,21,23-25]

1.2 Peripheral Glucose Utilisation

Although metformin exerts little glucose-lowering activity in normoglycaemic individuals with normal insulin sensitivity, it increases insulin-stimulated glucose utilisation under conditions of hyperglycaemia and/or insulin resistance.[6,7] For example, metformin increased glucose utilisation during a hyperglycaemic-hyperinsulinaemic infusion in normal rats and during hyperinsulinaemic glucose clamp studies in mildly hyperglycaemic diabetic rats.[26,27] The bulk of insulin-stimulated peripheral glucose utilisation takes place in skeletal muscle, and several studies have shown that administration of metformin enhances glucose uptake and glycogen synthesis in muscle of diabetic animals[18,26-28] (fig. 2) and patients with type 2

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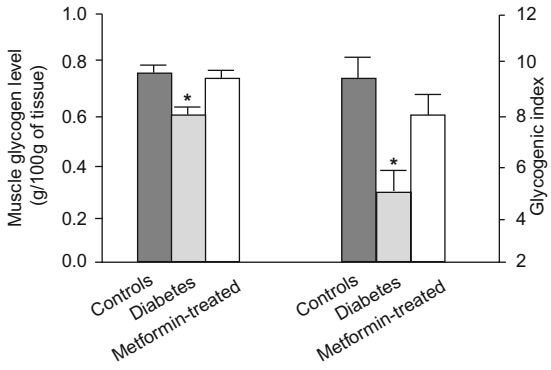

Fig. 2. Muscle glycogen level and muscle glycogenic index (^{3}H-3-glucose counts/gram of tissue × plasma ^{3}H-glucose specific activity × 100) of hind-limb muscle from n2 streptozotocin diabetic rats after a euglycaemic insulin clamp procedure.[26] Compared with that in nondiabetic (control) rats, glycogen accumulation was impaired in the diabetic state and normalised by metformin treatment (100 mg/kg/day for 5 weeks). Values are mean ± standard error; n = 8. * $p < 0.01$ *vs* control.

diabetes.[29] In some studies, metformin treatment also produced a small increase in glucose oxidation but no increase in lactate production by skeletal muscle.[6,7]

Addition of metformin *in vitro* enhanced insulin-stimulated glucose uptake by skeletal muscle biopsies from diabetic and nondiabetic insulin-resistant individuals.[30,31] However, short term *in vitro* studies in normal muscle have been inconclusive, suggesting that a direct effect of metformin on insulin-stimulated glucose uptake is usually evident only in insulin-resistant states.[7] It is appreciated that metformin will also increase insulin sensitivity indirectly through a general improvement in the metabolic environment that results from the drug's other antihyperglycaemic mechanisms.[6,7] There is also a possibility that metformin might enhance the mass action effect of glucose, in which hyperglycaemia itself enhances glucose disposal.[32,33]

Metformin has been reported to increase insulin-stimulated glucose uptake by adipose tissue in most, but not all, studies with this tissue.[7] However, other actions of metformin that increase glucose turnover and utilisation usually cause the expenditure of sufficient energy for adipose depots not to be increased. Indeed weight stabilisation or slight weight loss is commonly observed during metformin therapy.[1]

The mechanism through which metformin enhances insulin-stimulated glucose uptake in insulin-resistant states has been attributed mainly to increased translocation to and activity of glucose transporters in the plasma membrane (discussed below).

1.3 Fatty Acid Oxidation

In vitro studies in muscle and liver have shown that biguanides can reduce the rate of fatty acid oxidation.[34] The oxidation of palmitate by rat diaphragm homogenates was reduced by 30% with metformin 10^{-4} mol/L, with no direct effect on glucose oxidation[35] (S. Muntoni, personal communication). This provides a means whereby metformin can correct any imbalance of the glucose-fatty acid (Randle) cycle in the diabetic state.

In indirect calorimetric studies in patients with type 2 diabetes, lipid oxidation was decreased by metformin, whereas glucose oxidation was slightly increased.[36,37] Suppression of fatty acid oxidation (about 15 to 25%) correlated approximately with suppression of hepatic glucose production, suggesting that reduced hepatic energy supply from fatty acid oxidation contributed to reduced gluconeogenesis.[37] The decrease in fatty acid oxidation occurred in the basal state and was not related to prevailing insulin levels. Metformin can decrease fatty acid turnover and reduce circulating free fatty acid (FFA) levels, probably via increased re-esterification rather than decreased lipolysis.[37] However, decreased FFA levels appear to be insufficient to account for the reduction in fatty acid oxidation, which indicates a direct action of metformin that is independent of insulin levels.[37]

Metformin often reduces circulating triglyceride levels in hypertriglyceridaemic patients; this is associated with decreased synthesis and in-

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creased clearance of very low density lipoproteins (VLDL).[1,7,38,39] Reductions in triglyceride levels reduce insulin resistance[40,41] and provide an indirect route through which metformin can improve the metabolic environment and reduce hyperglycaemia.

1.4 Glucose Turnover

Administration of metformin to insulin-resistant obese Zucker rats increased rates of glucose turnover and glucose recycling, which suggests potential mechanisms for increased glucose expenditure and a contribution to the lowering of blood glucose levels.[42] This appears to be achieved, at least in part, by high concentrations of metformin in the intestinal wall that stimulate anaerobic metabolism of glucose and cause the release of lactate into the portal vein.[43] Indeed, metformin causes a greater stimulation of glucose utilisation (per unit weight of tissue) by the intestine than the skeletal muscle.[27,42,43] The lactate is metabolised by the liver and peripheral tissues.[43]

Erythrocytes of patients with type 2 diabetes show impaired uptake of glucose and storage of glycogen under conditions of hyperglycaemia.[44] Interestingly, metformin corrects this by increasing glucose uptake and glycogen levels in these cells (fig. 3).[44] This substantiates the view that metformin can promote certain non–insulin-dependent (as well as insulin-dependent) glucose transport mechanisms.

2. Cellular Mechanisms

Metformin counters insulin resistance predominantly by enhancing insulin action. It also exerts effects on glucose transport that are not acutely sensitive to insulin, and it appears to reduce defects in cell membrane fluidity that are caused by glycosylation.

2.1 Increased Glucose Transport

Metformin increased translocation of the insulin-sensitive glucose transporter isoform GLUT4 into the plasma membrane in several *in vitro* studies in adipocytes.[45,46] Increased translocation of GLUT1 was also noted in some studies in adipocytes and L6 muscle cells, but there was no change in the total pool of transporters, which implied a lack of effect on transporter synthesis.[45,47] Although these studies involved chiefly supratherapeutic concentrations of metformin, administration of the drug to insulin-resistant fatty rats increased insulin-stimulated translocation of GLUT4 and GLUT1 into the plasma membrane of ad-

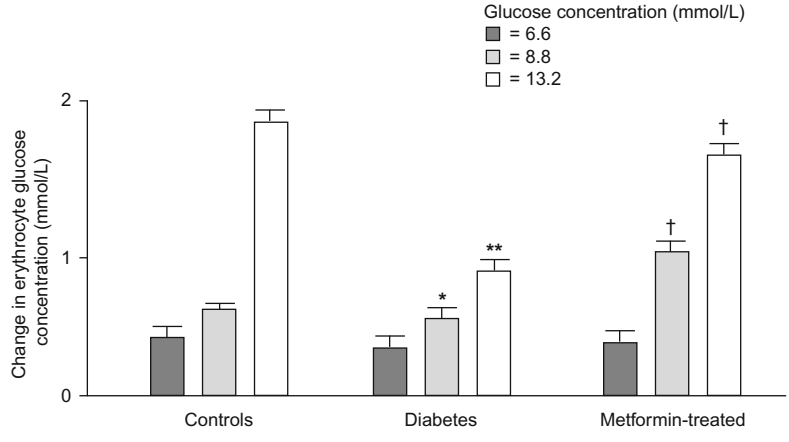

Fig. 3. Glucose uptake by erythrocytes from nondiabetic individuals (controls) and patients with type 2 diabetes before and after metformin treatment (850mg 3 times daily for 4 weeks). Erythrocytes were incubated at different glucose concentrations. Values are mean ± standard error; n = 10. * $p < 0.05$, ** $p < 0.001$ *vs* control; † $p < 0.001$ *vs* before treatment.

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ipocytes without causing *de novo* transporter synthesis.[48] To account for the accompanying increase in glucose transport, it was suggested that metformin also increased the functional activity of glucose transporters.[48] This is corroborated by studies in skeletal muscle, vascular smooth muscle cells and erythrocytes, which suggests that metformin can enhance the glucose transport capacity of GLUT4 and (probably to a greater extent) GLUT1 transporters that are present in the plasma membrane.[48-51] Interestingly, metformin increased insulin-stimulated glucose transport by *Xenopus laevis* oocytes, which are devoid of GLUT4, but no such effect was seen in the absence of insulin.[52] Glucose transport by GLUT3 into monocytes of patients with type 1 (insulin-dependent) diabetes was increased by metformin,[53] which further supports the view that metformin can enhance some insulin-insensitive glucose transport mechanisms as well as those that are acutely insulin sensitive.[7] Indeed, metformin enhances the mass action effect of glucose on glucose uptake (e.g. by erythrocytes) in patients with type 2 diabetes.[32,44,51]

Thus, metformin appears to improve the transmembrane transfer capacity of glucose transporters and to increase the translocation of transporters into the cell membrane. These effects require the presence of insulin or high levels of glucose, which suggests that metformin enhances the function of transporters preactivated by their natural stimulants.

2.2 Increased Insulin Action

Since the therapeutic efficacy of metformin is dependent on the presence of insulin (albeit not necessarily at high levels), the relationship between metformin and insulin has been the subject of much recent research.[7,54] Although metformin has been shown to increase the number of insulin receptors in various diabetic states, this does not appear to be related to its blood glucose-lowering effect.[1,54] Nevertheless, metformin increases most biological functions of insulin (at least in insulin-resistant states), including glucose transport, and glycogen and lipid synthesis.[7] This suggests that metformin might influence insulin receptor signalling or an early event in the postreceptor signalling process that is common to the diversity of insulin-mediated events known to be augmented by metformin. Particular attention has therefore focused upon insulin receptor phosphorylation, tyrosine kinase activity and the activation of insulin receptor substrate (IRS) proteins. *In vitro* studies in *Xenopus* oocytes and studies in erythrocytes from metformin-treated patients with type 2 diabetes showed that metformin increased insulin receptor tyrosine kinase activity (fig. 4).[55,56] Other studies have also indicated that metformin enhances tyrosine kinase pathways.[57-60] In *Xenopus* oocytes, metformin stimulated insulin receptor tyrosine kinase[55] and glycogen synthase[52] activity (fig. 5) in a concentration-dependent manner within the therapeutic range. Further studies in oocytes have indicated that increased insulin receptor tyrosine kinase activity in the presence of metformin is associated with the activation of phospholipase C,

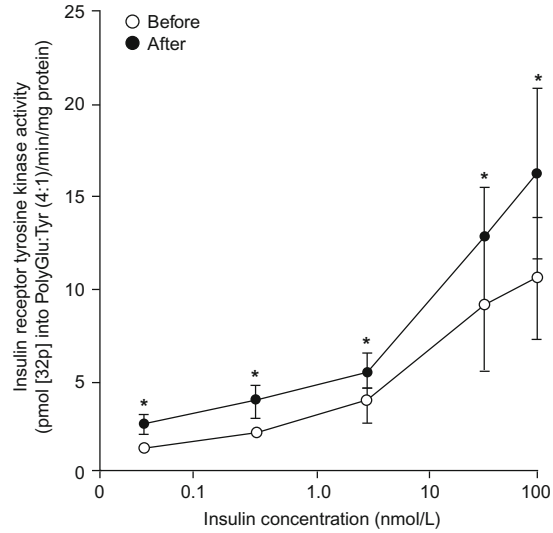

Fig. 4. Insulin-stimulated insulin receptor tyrosine kinase activity of insulin receptors solubilised from erythrocytes of patients with type 2 diabetes before and after metformin treatment (850mg twice daily for 10 weeks).[56] Values are mean ± standard error; n = 14. * $p < 0.0001$ *vs* before treatment.

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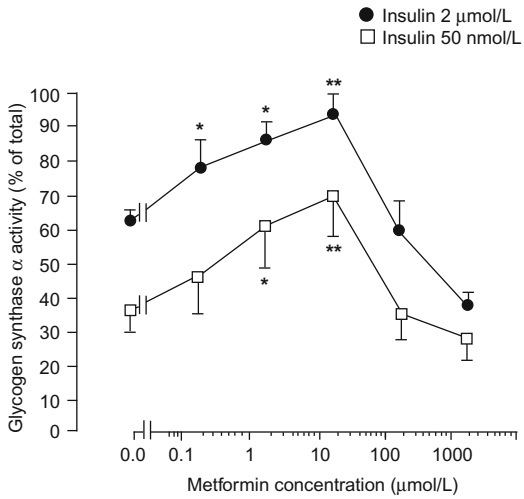

Fig. 5. Effect of metformin on glycogen synthase α-form activity induced by insulin in *Xenopus* oocytes incubated with insulin with and without the presence of metformin.[52] Values are mean ± standard error; n = 4. * $p < 0.01$, ** $p < 0.005$ *vs* absence of metformin.

elevated levels of inositol-1,4,5-trisphosphate (IP_3) and raised intracellular levels of free Ca^{++}.[55]

From these observations, metformin appears to bring about an increase in tyrosine kinase activity of the β subunit of the insulin receptor. This in turn facilitates the phosphorylation of receptor-associated IRS proteins.[57,58] Thereafter, key components of the postreceptor signalling cascades will be stimulated, e.g. phosphatidylinositol-3-kinase (PI3-kinase), a known intermediate in the insulin signalling pathway that leads to increased glucose uptake.[59,61] The mechanism through which metformin enhances insulin-mediated insulin receptor tyrosine kinase activity is not established. However, there is evidence that metformin alters the physicochemical properties of cell membranes, in particular reversing the detrimental effects on membrane fluidity that occur with non-enzymatic glycosylation in the presence of raised glucose levels.[62]

3. Conclusion

Metformin is an effective antihyperglycaemic agent with a unique portfolio of therapeutic effects and a variety of cellular actions. It lowers blood glucose levels predominantly by enhancing the action of insulin, but it also exerts some effects under hyperglycaemic conditions that are not acutely dependent on insulin. However, these latter effects cannot substitute for the action of insulin. Thus, metformin improves insulin sensitivity in insulin-resistant and hyperglycaemic states. The main therapeutic effects responsible for reducing blood glucose levels are reduced hepatic glucose output and increased peripheral glucose utilisation. In addition, metformin decreases fatty acid oxidation and increases glucose turnover. At the cellular level, metformin influences membrane events affecting tyrosine kinase activity and glucose transport processes. In particular, metformin increases insulin-mediated insulin receptor tyrosine kinase activity, which leads to the augmentation of a range of insulin signals. Metformin also increases the functional activity of glucose transporters, possibly via effects on the physicochemical properties of cell membranes.

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