

Imeglimin improves hyperglycemia and hypoglycemia-induced cell death and mitochondrial dysfunction in immortalized adult mouse Schwann IMS32 cells

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ABSTRACT

Aims/Introduction: Imeglimin, a novel oral antidiabetic drug, enhances glucose-stimulated insulin secretion, improves insulin sensitivity, and reduces mitochondrial reactive oxygen species (ROS) generation. Diabetic neuropathy is driven by oxidative stress caused by hyperglycemia, with mitochondrial ROS overproduction playing a central role. Hypoglycemia also contributes to oxidative stress. This study evaluates the effects of imeglimin on Schwann cells under high- and low-glucose conditions.

Materials and Methods: We used IMS32 cells, an immortalized mouse Schwann cell line, to investigate cell survival and mitochondrial function under normal, high-, and low-glucose conditions. Assessments included mitochondrial oxidative stress, cytochrome c release, mitochondrial membrane potential, oxygen consumption rate (OCR), Complex I activity, and ATP synthesis.

Results: High- and low-glucose conditions caused cell death, elevated mitochondrial ROS, triggered cytochrome c release, disrupted mitochondrial membrane potential, and increased OCR and Complex I activity, while suppressing ATP synthesis. Imeglimin treatment mitigated cell death, reduced oxidative stress, restored mitochondrial membrane potential, normalized OCR and Complex I activity, and improved ATP synthesis under both glucose conditions.

Conclusions: Fluctuations in glucose levels impair mitochondrial function in Schwann cells, contributing to peripheral nerve damage in diabetic neuropathy. Imeglimin demonstrated protective effects by alleviating mitochondrial dysfunction and preventing apoptosis signaling. These findings suggest the potential application of imeglimin in preventing and treating diabetic neuropathy; however, the clinical implications require further investigation.

INTRODUCTION

Diabetic neuropathy is one of the most prevalent complications of diabetes and is characterized by limb pain and a significant

decline in the quality of life^{1, 2}. Multiple mechanisms have been implicated in the development of diabetic neuropathy, such as oxidative stress, the polyol pathway, the hexosamine pathway, protein kinase C activation, and the accumulation of advanced glycation end-products (AGEs). Oxidative stress is widely regarded as a key contributing factor in this process³. In our

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previous study, we demonstrated that both prolonged hyperglycemia and intermittent episodes of short-term hyperglycemia and hypoglycemia could trigger oxidative stress through endoplasmic reticulum stress and induce cell death in Schwann cells⁴. Reactive oxygen species (ROS) are predominantly generated during mitochondrial ATP synthesis^{5, 6}. In diabetes, elevated glucose levels lead to excessive mitochondrial ROS production, which exacerbates oxidative stress^{7, 8}, impairs mitochondrial function^{9, 10}, and diminishes ATP generation¹¹.

Imeglimin, a novel oral hypoglycemic agent classified under “glimins,” contains tetrahydrotriazines and specifically targets mitochondrial dysfunction¹². Imeglimin exerts its glucose-lowering effects by enhancing insulin secretion from pancreatic beta cells and ameliorating insulin resistance in skeletal muscle and hepatic tissues. Furthermore, it is suggested to mitigate mitochondrial dysfunction by inhibiting the mitochondrial electron transport chain Complex I^{12–17}. Imeglimin reduces excessive ROS production and protects endothelial cells from hyperglycemia-induced apoptosis by preventing cytochrome c release¹⁸. However, its potential therapeutic effects on diabetic neuropathy remain unclear.

Schwann cells, which are glial cells of the peripheral nervous system, play a critical role in preserving the structural integrity of nerves and providing neuronal support. Under diabetic conditions, Schwann cells experience glucose-induced damage, and their supportive functions are impaired, resulting in peripheral nerve injury^{19–21}. Previous studies have indicated that mitochondrial DNA (mtDNA) abnormalities and dysfunction in Schwann cells are key drivers of peripheral neuropathy²¹. Therefore, Schwann cell lines, such as IMS32 cells, have been extensively utilized as *in vitro* models for studying diabetic neuropathy^{22, 23}.

In this study, we assessed the effects of hyperglycemia and hypoglycemia on Schwann cell viability and mitochondrial function, including oxidative stress, cytochrome c release, mitochondrial membrane potential, oxygen consumption rate (OCR), mitochondrial Complex I activity, and ATP production. In addition, we evaluated the therapeutic potential of imeglimin in alleviating these mitochondrial abnormalities.

MATERIALS AND METHODS

Reagents

Dulbecco's modified Eagle medium (DMEM) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were sourced from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was obtained from Gibco (Paisley, UK). Imeglimin hydrochloride was acquired from MedChem Express (Monmouth Junction, NJ, USA).

Cell culture

Immortalized adult mouse Schwann (IMS32) cells²³ were kindly gifted by Prof. Kazuhiko Watabe (Kyorin University, Tokyo, Japan). Cells were cultured in DMEM-low glucose containing 5.6 mM glucose (normal glucose), supplemented with 5% FBS

and maintained at 37°C in a humidified atmosphere of 5% CO₂/95% air. Culture medium was refreshed every 2–3 days, and cells were subcultured weekly using 0.05% trypsin–EDTA. For experiments, IMS32 cells were subjected to different glucose concentrations (5.5 mM as normal glucose, 2.5 mM as low glucose, and 25 mM as high glucose) for 1 h. To control for the osmolality differences, mannitol was added to the 5.5 mM glucose medium to match the osmolality of the 5.5 mM glucose medium. Imeglimin was initially dissolved in dimethyl sulfoxide (DMSO) to prepare a 100 mM stock solution and then further diluted with culture medium to the working concentration of 100 µM. Vehicle control groups received an equivalent volume of DMSO. The 100 µM concentration was selected based on previous *in vitro* studies that used 100 µM¹⁸ and 500 µM²⁴, while considering that the maximum plasma concentration (*C*_{max}) of imeglimin after a single 1,000 mg dose is reported to be 1,285 ng/mL (~6.7 µM) in a clinical pharmacokinetic study²⁵.

Cell viability and cytotoxicity assessment

The MTT assay was employed to assess cell viability²⁶. The IMS32 cells were seeded at a density of 1×10^4 cells per well in 96-well plates under various conditions. Following a 1 h treatment period, cells were incubated with MTT (0.5 mg/mL) for 3 h at 37°C. After incubation, the medium was discarded, and the resulting formazan crystals were dissolved in DMSO. Absorbance at 535 nm was measured using a microplate reader. Viability data were normalized to the normal glucose condition and presented as a percentage.

Cell cytotoxicity was assessed by measuring lactate dehydrogenase (LDH) release into culture supernatants using the CytoTox 96 Non-Radioactive Cytotoxicity Assay Kit (Promega, Madison, WI, USA) following the manufacturer's protocol. Absorbance was measured at 420 nm using a microplate reader for LDH quantification.

For cell enumeration, IMS32 cells were plated at a density of 5×10^4 cells per well in 6-well plates and cultured for 24 h. The cells were treated with 100 µM imeglimin for 24 h, after which the medium was replaced with fresh medium containing the specified glucose concentrations. After two washes with phosphate-buffered saline (PBS), the cells were detached using 0.25% trypsin, stained with trypan blue, and the viable cell count was performed using a hemocytometer under an inverted microscope.

Mitochondrial oxidative stress

Reactive oxygen species (ROS) were detected using MitoSOX Red (M36008; Thermo Fisher Scientific, Waltham, MA, USA). Samples were treated with 5 µM MitoSOX Red and incubated for 30 min. MitoSOX-stained cells were visualized under a confocal microscope (Carl Zeiss, Oberkochen, Germany). Images obtained using the InCell Analyzer 2,200 were analyzed with IN Cell Investigator software (GE Healthcare, Chicago, IL, USA).

Mitochondrial membrane potential

Mitochondrial membrane potential was assessed using a JC-10 Mitochondrial Membrane Potential Assay Kit (ab112134; Abcam, Cambridge, MA, USA) according to the manufacturer's instructions. Cells were treated with JC-10 dye in the provided assay buffer, and fluorescence signals were measured with a spectrofluorometer at excitation/emission wavelengths of 540/590 nm (orange fluorescence) and 488/527 nm (green fluorescence). Data were recorded in units of relative fluorescence. Higher orange-to-green fluorescence ratios indicate an elevated mitochondrial membrane potential.

Immunohistochemistry of cytochrome c

To detect cytochrome c by immunohistochemistry, the cells were immobilized with 4% paraformaldehyde in PBS for 15 min, permeabilized using 0.2% Triton X-100 for 10 min, and blocked with 2% goat serum in PBS for 1 h. After blocking, the cells were incubated with a monoclonal anti-cytochrome c antibody (1:300 dilution; # 12963; Cell Signaling Technology, Danvers, MA, USA) for 1 h. Afterward, they were treated with an Anti-Mouse IgG Alexa Fluor® 488 Conjugate (1:1000 dilution; # 4408S; Cell Signaling, Danvers, MA, USA) for 1 h at room temperature in the dark. After final washes, the cells were covered with Fluoroshield containing 4',6-diamidino-2-phenylindole (DAPI) and sealed under a coverslip.

Western blotting analysis

Cytosolic and mitochondrial fractions were prepared using a Mitochondria Isolation Kit (Thermo Scientific, Waltham, MA, USA) and resuspended in RIPA buffer containing protease inhibitors (Sigma-Aldrich). Proteins were separated on gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Hercules, CA, USA), and blocked with 5% non-fat milk in TBS-T for 1 h at 25°C. Membranes were incubated overnight at 4°C with primary antibodies (Cytochrome c, 1:1000; Cox IV, 1:1000; # 4280S, 4844S; Cell Signaling; β -actin, 1:5000; sc-69879; Santa Cruz Biotechnology, Dallas, TX, USA) in Can Get Signal solution 1 (TOYOBO, Japan), washed with TBS-T, and treated with HRP-conjugated secondary antibodies (# 7074S, Cell Signaling Technology for 1 h). Immunoreactive bands were detected using Clarity MAX Western ECL substrate (Bio-Rad), recorded with an ImageQuant LAS-4000 (GE Healthcare, Chicago, IL, USA), evaluated with ImageQuantTL software, and adjusted relative to Complex I and β -actin.

OCR

The oxygen consumption rate (OCR) was measured using MitoXpress (Agilent, Santa Clara, CA, USA) following the manufacturer's protocol. Cells were plated at 1×10^5 cells/well in black, clear-bottom 96-well plates and incubated overnight. The MitoXpress Xtra probe was added to each well, and mineral oil was applied to seal the plate. OCR readings were

recorded at an excitation wavelength of 380 nm and an emission wavelength of 670 nm for 60 min at 37°C.

Complex I activity

Cytosolic and mitochondrial fractions were separated using a Mitochondria Isolation Kit for Cultured Cells (Thermo Scientific) according to the manufacturer's instructions. The enzymatic activity of mitochondrial Complex I was quantified using a Mitochondrial Complex I Activity Colorimetric Assay Kit (ab287847; Abcam). The absorbance was measured at 600 nm using a plate reader (TECAN, Männedorf, Switzerland).

ATP

Intracellular ATP levels were measured using the CellTiter-Glo 2.0 Cell Viability Assay (G9241; Promega) in accordance with the manufacturer's instructions. Luminescence was detected with a plate reader, and ATP levels were standardized to the protein content of each sample.

Statistical analysis

Data are presented as means $\pm$ standard error (SE) based on the specified number of experiments. Statistical comparisons were performed using one-way ANOVA, followed by Tukey–Kramer or Dunnett's post-hoc tests. Statistical significance was set at less than 0.05.

RESULTS

Imeglimin ameliorates IMS32 cell death induced by high and low glucose levels

We investigated the protective effects of imeglimin on Schwann cells exposed to abnormal glucose conditions. IMS32 cells were subjected to high (25 mM) or low (2.5 mM) glucose concentrations for 1 h, with cells maintained in a normal glucose (5.5 mM) serving as controls. Both high and low glucose conditions significantly compromised cell health, as demonstrated by reduced cell counts and decreased variability measured by the MTT assay (Figure 1a,b). Concurrently, elevated LDH release in both conditions confirmed increased cell death (Figure 1c). Treatment with 100 μ M imeglimin effectively preserved cell viability and numbers while significantly reducing LDH release under both stress conditions. To distinguish between osmotic and glucose-specific effects, we conducted osmolality control experiments using mannitol to normal glucose (5.5 mM) to match the osmolality of high glucose (25 mM) did not induce cell death, indicating that hyperglycemic toxicity was not due to osmotic effects. However, when mannitol was added to low glucose (2.5 mM) to match the osmolality of normal glucose (5.5 mM), cell death still occurred, confirming that hypoglycemic damage was independent of osmotic changes (Figure 1d).

These findings demonstrate that both hyperglycemia and hypoglycemia induce Schwann cell death through mechanisms independent of osmolality, and that imeglimin effectively protects against these glucose fluctuation-induced cellular injuries.

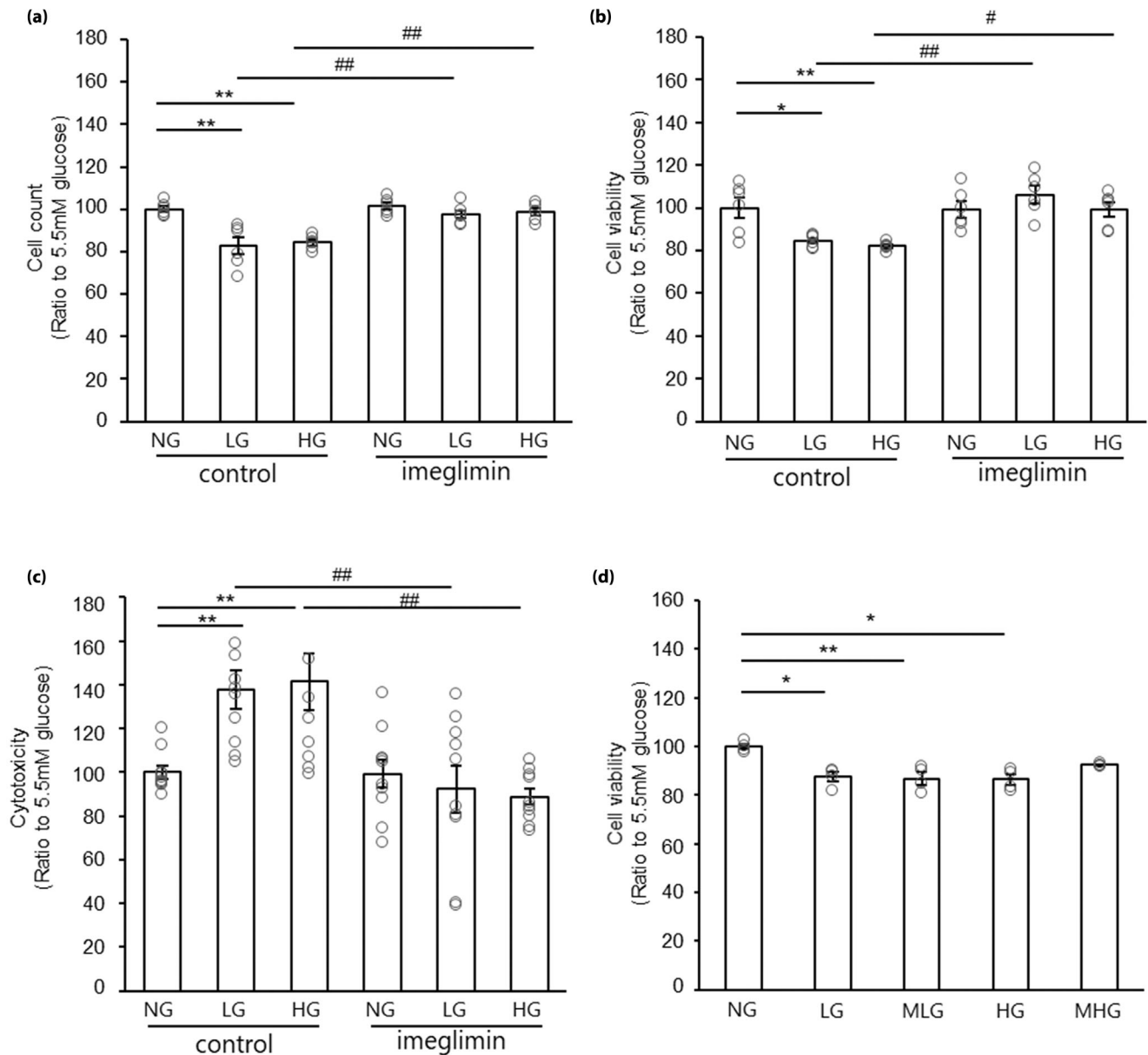


Figure 1 | Cell viability in IMS32 cells treated with or without 100 μ M imeglimin under various glucose conditions. Cell viability was determined by (a) cell count, and (b) MTT assay. Cytotoxicity was determined by (c) the released LDH. (d) To evaluate the effect of osmotic changes on cell viability, MTT assay was performed using 2.5 mM glucose (LG) supplemented with 3 mM mannitol to match the osmolarity of NG (MLG), and 5.5 mM glucose (NG) supplemented with 19.5 mM mannitol to match the osmolarity of HG (MHG). Cells were exposed to NG (5.5 mM glucose), LG (2.5 mM glucose), and HG (25 mM glucose) medium conditions for 1 h. Imeglimin was added to the culture medium for 24 h. Values are presented as mean $\pm$ SE ($n = 4$ –10). * $P < 0.05$, ** $P < 0.01$ compared with NG. # $P < 0.05$, ## $P < 0.01$ compared with IME (–) control.

Imeglimin prevents cytochrome c release induced by high and low glucose

Cytochrome c release from mitochondria into the cytosol was analyzed under glucose stress, using normal glucose conditions as the control. Immunohistochemistry revealed that under normal glucose conditions, cytochrome c was retained in mitochondria. In contrast, high- and low-glucose conditions

induced its release into the cytosol, as indicated by increased cytoplasmic fluorescence (Figure 2a). Western blotting confirmed a significant increase in the cytosolic cytochrome c levels under both conditions relative to normal glucose controls, and this elevation was suppressed by imeglimin. These results indicate that imeglimin inhibits cytochrome c release, thereby reducing cell death pathways in Schwann cells.

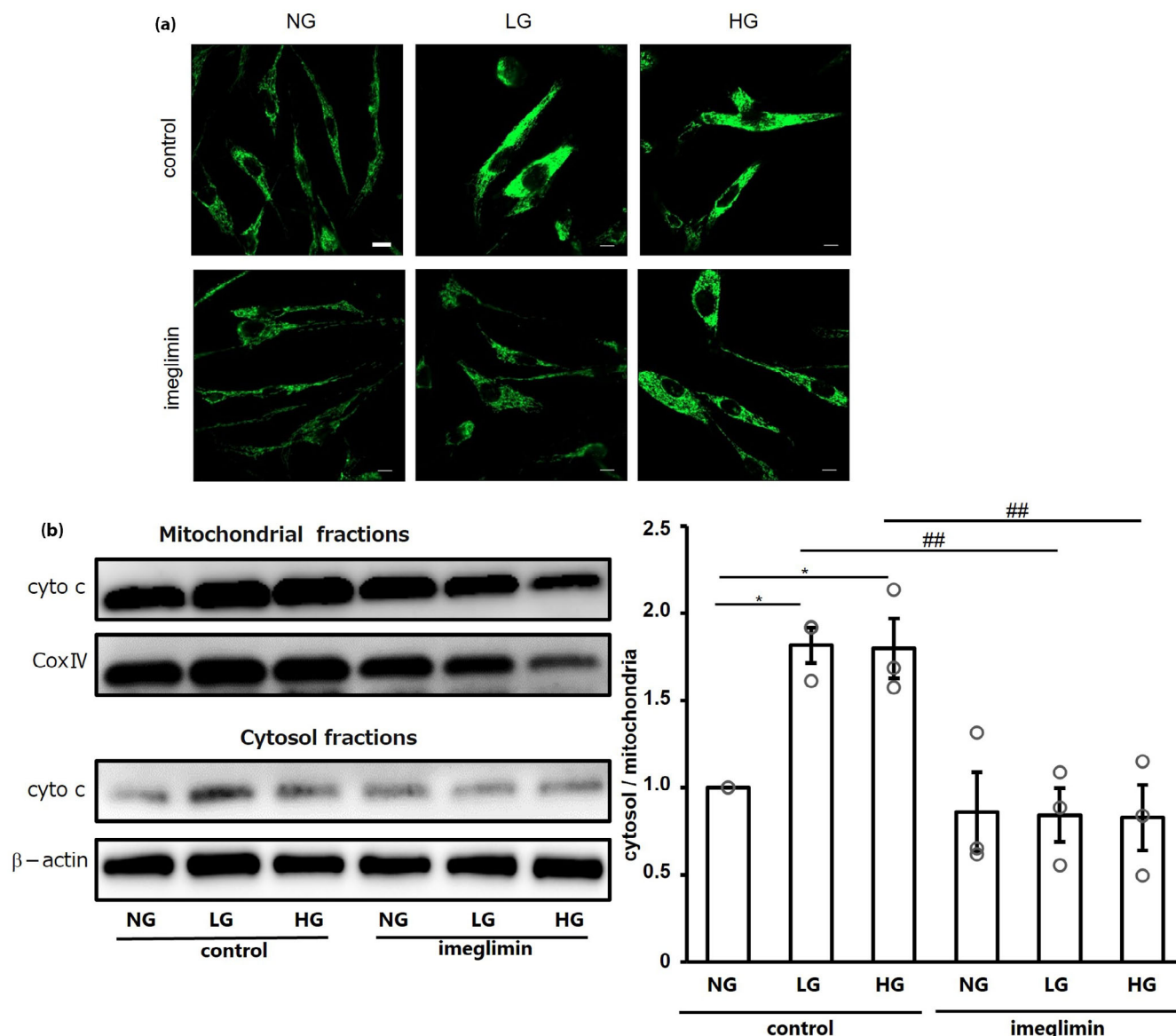


Figure 2 | Cytochrome c distribution in IMS32 cells treated with or without 100 μ M imeglimin under high- and low-glucose conditions. (a) Immunostaining was performed with specific anti-cytochrome c antibody. (b) Protein expression of cytochrome c in mitochondrial and cytosol fractions under various glucose conditions. Normalization was carried out using Cox IV and β -actin as a loading control. The density of cytochrome c bands in mitochondrial fractions and c cytosolic fractions were measured and the ratio was calculated. Cells were exposed to NG (5.5 mM glucose), LG (2.5 mM glucose), and HG (25 mM glucose) medium conditions for 1 h before measurement. Imeglimin was added to the culture medium for 24 h. Value are presented as mean $\pm$ SE ($n = 3$). * $P < 0.05$ compared to 5.5 mM glucose. ** $P < 0.01$ compared with IME (–) control.

Imeglimin reduces mitochondrial oxidative stress caused by high and low glucose levels

Mitochondrial oxidative stress was assessed by MitoSOX fluorescence, with cells maintained in normal glucose serving as the baseline control. Relative to these control conditions, both high- and low-glucose conditions significantly increase mitochondrial ROS production. Treatment with imeglimin reduced the elevated ROS levels (Figure 3). These findings suggested that

imeglimin mitigated glucose-induced mitochondrial oxidative stress induced by glucose fluctuations.

Imeglimin stabilizes mitochondrial membrane potential under glucose stress

The mitochondrial membrane potential was analyzed under high- and low-glucose conditions, using cells maintained in normal glucose as the reference group. Both conditions

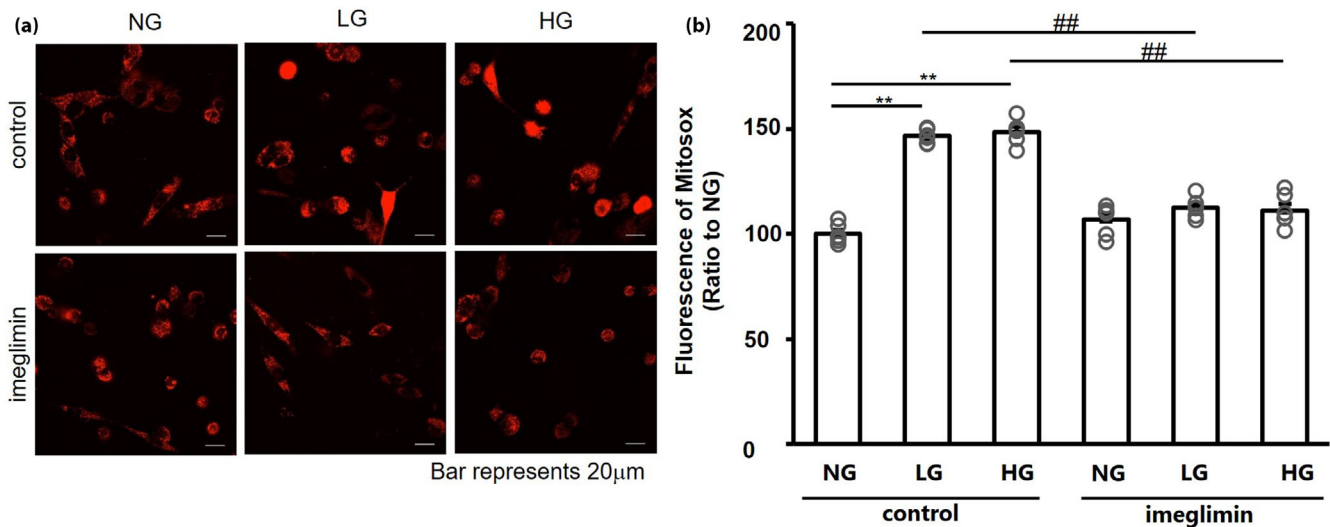


Figure 3 | Mitochondrial oxidative stress in IMS32 cells treated with or without 100 μM imeglimin under high- and low-glucose conditions. (a) Representative images of MitoSOX Red-stained cells were obtained using a confocal microscope. (b) Quantitative analysis of mitochondrial ROS levels under various glucose conditions. Cells were exposed to NG (5.5 mM glucose), LG (2.5 mM glucose), and HG (25 mM glucose) medium conditions for 1 h before measurement. Imeglimin was added to the culture medium for 24 h. Values are presented as mean ± SE (n = 6). **P* < 0.05 compared with NG, ##*P* < 0.01 compared to IME (–) control. ***P* < 0.01 compared with NG, ##*P* < 0.01 compared to IME (–) control.

significantly reduced the mitochondrial membrane potential compared with normal glucose conditions, indicating mitochondrial dysfunction (Figure 4a). Imeglimin effectively restored the mitochondrial membrane potential, suggesting its role in stabilizing mitochondrial function during glucose fluctuations.

Imeglimin normalizes oxygen consumption rate (OCR) in glucose-stressed Schwann cells

Mitochondrial respiration was assessed by measuring OCR. Both high- and low-glucose conditions caused an increase in the OCR relative to normal glucose, suggesting overactivation of the electron transport chain. Imeglimin treatment significantly reduced OCR to normal levels (Figure 4b). These results indicate that imeglimin reverses mitochondrial respiratory abnormalities caused by glucose stress.

Imeglimin suppresses mitochondrial Complex I overactivation

In comparison with normal glucose conditions, high- and low-glucose conditions significantly increase Complex I activity, which is a key contributor to ROS production. Imeglimin treatment reduced Complex I activity under both conditions (Figure 4c). These findings suggested that imeglimin helps maintain proper mitochondrial function by suppressing Complex I overactivation during glucose fluctuations.

Imeglimin restores ATP production impaired by high and low glucose

ATP production was slightly but significantly reduced under both high- and low-glucose conditions compared with normal

glucose levels (Figure 4d). Imeglimin treatment restored the ATP levels to near-normal levels. These results indicate that imeglimin facilitates the recovery of energy production, which is impaired by hyperglycemia and hypoglycemia.

DISCUSSION

This study provides novel evidence that both hyperglycemia and hypoglycemia disrupt mitochondrial function in Schwann cells. These disruptions include increased oxidative stress within the mitochondria, hyperactivation of Complex I, reduced membrane potential, and decreased ATP synthesis, ultimately leading to cell death.

Our experimental model was specifically developed to examine acute cellular responses to glucose fluctuations, offering insights into the early molecular mechanisms that may trigger or worsen neuropathy. While this approach does not completely mirror the intricate *in vivo* conditions associated with chronic hyperglycemia, it provides a well-controlled system for investigating specific pathways affected by glucose variability.

Imeglimin counteracted these mitochondrial impairments, suggesting its potential utility as a therapeutic agent for managing diabetic neuropathy (Figure 5). Imeglimin is known to exert diverse biological effects, such as protecting pancreatic β-cells, enhancing NAD⁺ synthesis, and improving glucose metabolism in the liver and skeletal muscles²⁷. Previous studies have highlighted its ability to reduce oxidative stress, as observed in human endothelial cells and the livers of mice fed a high-fat, high-sucrose diet^{13, 18}. These antioxidant properties are

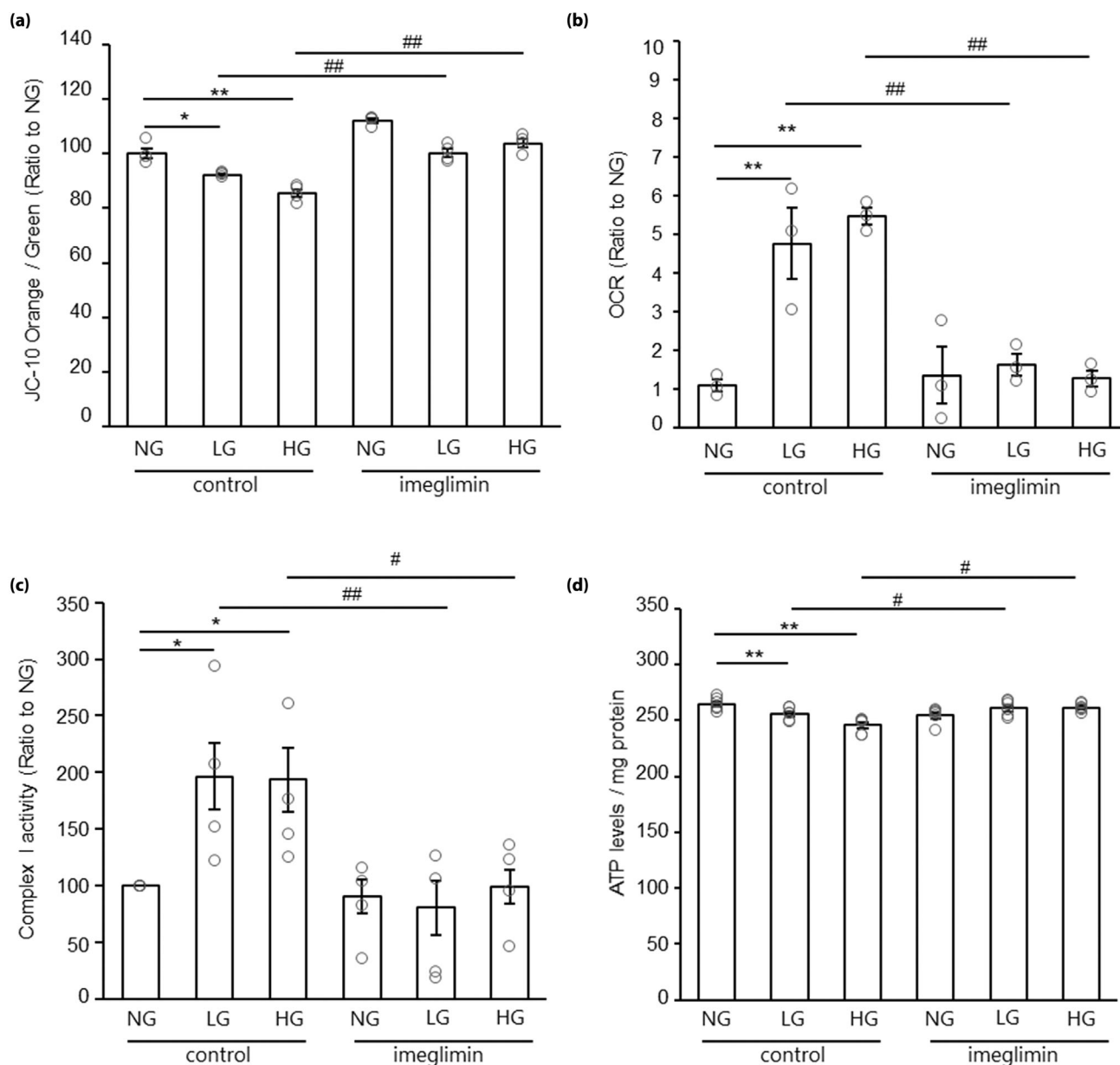


Figure 4 | Mitochondrial function in IMS32 cells treated with or without 100 μ M imeglimin under high- and low-glucose conditions. (a) Mitochondrial membrane potential, (b) Oxygen consumption rate (OCR), (c) Complex I activity, (d) ATP levels. Cells were exposed to NG (5.5 mM glucose), LG (2.5 mM glucose), and HG (25 mM glucose) medium conditions for 1 h before measurement. Imeglimin was added to the culture medium for 24 h. Values are presented as mean $\pm$ SE ($n = 3-6$). * $P < 0.05$, ** $P < 0.01$ compared with NG. # $P < 0.05$, ## $P < 0.01$ compared with IME (-) control.

associated with their capacity to mitigate cardiac steatosis²⁸, alleviate renal fibrosis²⁹, and suppress inflammation in microglia²⁴.

Consistent with these findings, the present study demonstrated that imeglimin alleviated mitochondrial dysfunction in Schwann cells under glucose stress. Specifically, it mitigates

excessive Complex I activity, reduces the OCR, suppresses mitochondrial ROS production, restores mitochondrial membrane potential, and recovers ATP levels. By stabilizing mitochondrial bioenergetics and preventing cytochrome c translocation to the cytosol, imeglimin effectively interrupts cell death pathways and preserves Schwann cell viability despite

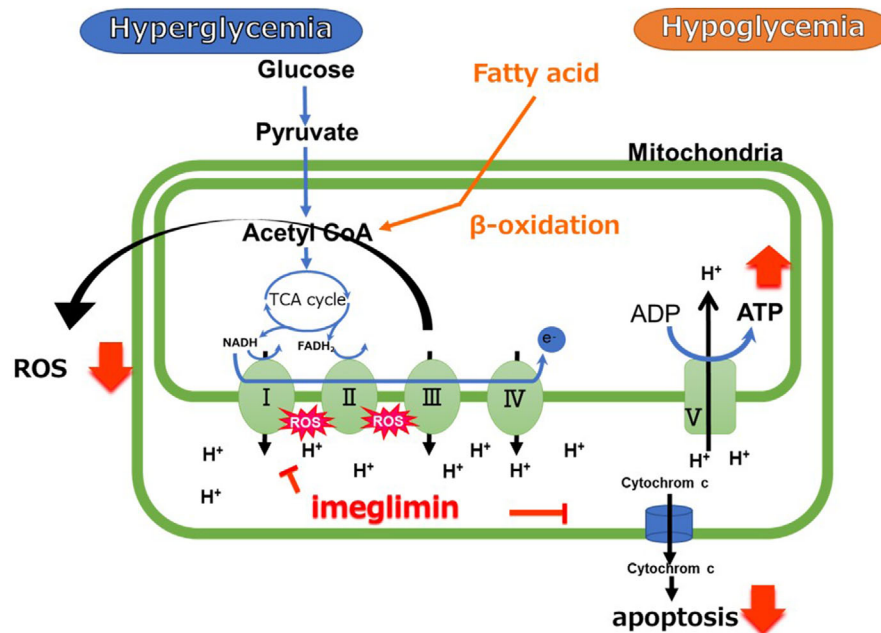


Figure 5 | Schematic representation of mitochondrial dysfunction under hyperglycemic and hypoglycemic conditions and the protective effects of imeglimin. Hyperglycemia and hypoglycemia impair mitochondrial function by increasing ROS production, disrupting the mitochondrial membrane potential, reducing ATP synthesis, and inducing apoptosis. Imeglimin alleviates these dysfunctions by suppressing excessive Complex I activity, reducing ROS production, stabilizing mitochondrial membrane potential, and mitigating ATP depletion and apoptosis.

glucose fluctuations. These protective mechanisms highlight imeglimin as a promising therapeutic candidate for preventing or treating diabetic neuropathy and other glucose-related complications.

Under hyperglycemic conditions, an excessive supply of electrons to the mitochondrial electron transport chain leads to increased ROS production, which disrupts the mitochondrial membrane potential^{9, 30–32}. Prior research has indicated that imeglimin suppresses Complex I hyperactivation in the liver of high-fat, high-sucrose (HFHS) rats¹³. These findings indicate that imeglimin reduces mitochondrial ROS overproduction and improves mitochondrial function by downregulating Complex I activity under hyperglycemic and hypoglycemic conditions. This regulation of Complex I may represent a critical mechanism of action of imeglimin. Furthermore, previous *in vivo* studies have shown that imeglimin increases the expression of the antioxidant enzyme Gpx1 and suppresses NADPH oxidase subunits such as Cybb and Ncf4, suggesting its potential to reduce oxidative stress through both antioxidant and antioxidative mechanisms¹³. While our current study demonstrates imeglimin's protective effects on mitochondrial function, we did not specifically evaluate changes in antioxidant enzyme expression or activity. Future investigation using this *in vitro* should examine these antioxidant pathways to provide a more comprehensive understanding of imeglimin's protective mechanisms in Schwann cells exposed to glucose stress.

Additionally, imeglimin has been reported to stimulate NAD^+ production³³, which can activate sirtuin 1 (SIRT1) and enhance mitochondrial function by promoting the expression of PGC-1 α ³⁴. Further studies are warranted to investigate these downstream pathways, including the roles of SIRT1, PGC-1 α , and transcription factor A, mitochondrial (Tfam), in promoting mitochondrial biogenesis in Schwann cells.

A prior study by Fernyhough *et al.* demonstrated that prolonged diabetic conditions result in reduced mitochondrial oxidative stress and downregulation of PGC-1 α in the nerves of diabetic rodents^{35, 36}. Using sciatic nerve samples from STZ-induced diabetic mice with 8 weeks of hyperglycemia, they proposed that chronic diabetes leads to a decline in mitochondrial content and function. In contrast, the present study focused on short-term glucose fluctuations and revealed that mitochondrial oxidative stress was acutely elevated after just 1 h of high or low glucose exposure. These findings highlight the importance of investigating both the acute and chronic effects of glucose variability on mitochondrial function in Schwann cells.

Imeglimin, a novel oral antidiabetic drug, enhances both insulin secretion and sensitivity, making it a promising therapy for type 2 diabetes^{37–41}. Although its glucose-lowering effects have been demonstrated, imeglimin also enhances mitochondrial function under diabetic conditions. The dual actions of glucose regulation and mitochondrial protection indicate its

potential efficacy in addressing diabetic complications⁴². While imeglimin is not recognized as a direct treatment for hypoglycemia, our findings reveal its unexpected protective capacity against cellular damage during low glucose conditions, particularly through preservation of mitochondrial function. Therefore, the potential of imeglimin to protect cells against damage associated with low glucose levels warrants further investigation.

Beyond its glucose-regulating effects, imeglimin preserves mitochondrial function under diabetic conditions. Its dual role in improving glycemic control and protecting mitochondria suggests its potential efficacy in preventing diabetic complications⁴². Imeglimin improves endothelial function, suggesting its potential benefits in atherosclerotic cardiovascular diseases¹⁸. Moreover, its anti-inflammatory effects on microglial cells indicate its potential role in mitigating central nervous system disorders such as dementia²⁴.

The findings of this study emphasized its protective effects on Schwann cell mitochondrial function, underscoring its potential as a therapeutic option for diabetic neuropathy. Furthermore, our previous investigations demonstrated that activation of the polyol pathway and ER stress are involved in both high- and low-glucose conditions. We found that the ER stress inhibitor 4-phenylbutyric acid (4-PBA)⁴ effectively restored decreased cell viability and reduced oxidative stress caused by both high and low glucose exposure. Additionally, the aldose reductase inhibitor, ranirestat⁴³ partially suppressed cell death under high-glucose conditions. The findings suggest that both ER stress and polyol pathway activation may play crucial roles in the pathogenesis of diabetic neuropathy, potentially serving as complementary therapeutic targets alongside mitochondrial protection strategies. However, clinical studies evaluating the effects of imeglimin on diabetic complications in patients with diabetes have not been performed, and further trials are necessary to confirm its efficacy in these patients.

Notably, clinical evidence shows that neuropathy can develop not only in patients with diabetes but also in individuals with impaired glucose tolerance (IGT) during episodes of hypoglycemia^{44, 45}. These observations align with our finding that even transient glucose fluctuations can trigger neuronal damage. This repetitive nature of these short-term glucose variations likely induces cumulative oxidative stress and progressive mitochondrial dysfunction, potentially accelerating the development and progression of diabetic neuropathy. Our results highlight the importance of minimizing glucose variability, beyond just controlling chronic hyperglycemia, in comprehensive management strategies for preventing and treating diabetic complications.

The present study has some limitations. (1) The experiments focused on the short-term effects of glucose fluctuations (1 h of exposure). Further studies are required to investigate the effects of long-term glucose variability. (2) We observed a reduction in total ATP levels but did not separately assess ATP concentrations in the cytosolic and mitochondrial compartments. Future studies should measure ATP production in these distinct cellular fractions to better understand the specific impact of glucose

fluctuations on mitochondrial bioenergetic versus glycolytic ATP production and to clarify which pathway is predominantly protected by imeglimin treatment. (3) Although imeglimin is known to induce the expression of antioxidant enzymes, this mechanism was not examined in this study. Therefore, a detailed analysis of these pathways is required. (4) The role of imeglimin in NAD⁺ synthesis and its downstream effects, such as SIRT1 activation and PGC-1 α /Tfam pathways, was not fully elucidated. Future studies should address these mechanisms to better understand their effects on mitochondrial biogenesis. (5) While our study provides a potential link between glucose fluctuations, Schwann cell mitochondrial dysfunction, and the protective role of imeglimin, further *in vivo* and clinical investigations are needed to confirm these mechanisms in the pathogenesis of diabetic neuropathy. (6) Although previous studies have documented the effects of imeglimin on mitochondrial morphology in pancreatic islets⁴⁶, we did not examine changes in mitochondrial morphology in Schwann cells exposed to glucose stress or imeglimin treatment. Future investigations should incorporate advanced microscopy techniques to analyze mitochondrial network dynamics, fusion/fission processes, and ultrastructural changes in Schwann cells under these conditions to provide additional mechanistic insights.

Imeglimin effectively reduces Schwann cell death induced by high and low glucose levels by suppressing Complex I activity and decreasing mitochondrial oxidative stress. These findings suggest that the potential therapeutic benefits of imeglimin extend beyond glycemic control and potentially prevent the onset and progression of diabetic neuropathy through its protective effects on mitochondrial function. Further investigation is needed to clarify its mechanisms of action and validate its efficacy in mitigating diabetic complications.

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Informed consent: N/A.

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