Glucagon-like peptide-1 receptor mediates the beneficial effect of liraglutide in an acute lung injury mouse model involving the thioredoxin-interacting protein

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INTRODUCTION

Therapeutic agents can be developed via conventional drug-discovery pipelines or through the process of drug repurposing (33). Incretin is defined as a gut-produced hormone that is capable of stimulating insulin secretion by pancreatic β-cells postprandially, in a glucose concentration-dependent manner. The two known incretins are glucagon-like peptide-1 (GLP-1), produced by intestinal endocrine L cells, and gastric inhibitory polypeptide (also known as glucose-dependent insulino-tropic polypeptide, GIP), produced by intestinal endocrine K cells (6, 9). During the past two decades, functional explorations on these two incretins, especially GLP-1, have led to the development of two categories of novel type 2 diabetes (T2D) therapeutic agents, namely GLP-1 receptor (GLP-1R) agonists and dipeptidyl peptidase 4 (DPP-4) inhibitors (18). Members in the first category are exogenous GLP-1R agonists with the half-life longer than native GLP-1, while members in the second category can prevent the degradation of endogenous incretins. Active GLP-1 molecules include GLP-17–36amide and GLP-17–37 (30). The first FDA-approved GLP-1R agonist for treating diabetes is exenatide with the brand name Byetta. Exenatide is the synthetic version of exendin-4, a peptide isolated from Gila monster saliva gland (8, 10, 35). Liraglutide, commercially known as Victoza, is a modified human GLP-17–37 with a longer half-life, administrated daily for T2D subjects.

GLP-1R is expressed in pancreatic islets, as well as in a number of extrapancreatic organs or cell lineages, indicating that GLP-1 and GLP-1-based drugs can exert extra-pancreatic functions. Even for pancreatic β-cells, GLP-1 and GLP-based drugs exhibit functions beyond as a simple incretin hormone. They can protect pancreatic β-cells from high glucose or oxidative stress-induced cell apoptosis, and this feature can be shared by other cell lineages (27, 44). Intensive studies have shown various beneficial effects of GLP-1 and GLP-based drugs on the central nervous system, cardiovascular system, gastrointestinal system, liver, adipose tissues, as well as the immune system (25, 41). Not all those extra-pancreatic functions of GLP-1 can be clearly attributed to GLP-1R expressed in the target organ. For example, it is unlikely that hepatocytes express GLP-1R (5, 31). Hepatic functions of GLP-1, especially those on glucose and lipid homeostasis, can be attributed to both the gut-pancreatic-liver axis and yet to be further identified mechanisms (16, 18, 40). Furthermore, not all pharmacological functions of GLP-1-based drugs rely on the presence of GLP-1R (4, 13, 56). The function of GLP-1 and GLP-based drugs in the lung and respiratory system, however, needs to be further explored, considering that GLP-1R is most abundantly expressed in the lung, in rodents, monkey and human (5, 7, 34).

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An early in vitro study demonstrated that GLP-1 can stimulate surfactant secretion in human type II pneumocytes isolated from cadaveric organ donors (53). The anti-inflammatory and anti-oxidative stress effect of GLP-1 and GLP-based drugs were then demonstrated by several preclinical investigations (2, 22, 32, 41). A few studies have shown the beneficial effects of Liraglutide or exenatide in a mouse chronic obstructive pulmonary disease (COPD) models (3, 54). Others have demonstrated that administration of liraglutide reduced LPS-induced acute lung injury in mice, involving the restoration of LPS-reduced surfactant protein-A (SP-A) and Thyroid transcription factor-1 expression (58), or the prevention of LPS-induced polymorphonuclear neutrophil (PMN) extravasation, lung injury, and alveolar-capillary barrier dysfunction (55). The involvement of GLP-1R in those pulmonary beneficial effects, however, needs to be investigated.

Here, we aimed to test the potential “preventative” effect of liraglutide in LPS-induced acute lung injury, focusing on the involvement of GLP-1R. We have also assessed the effect of LPS challenge and liraglutide treatment on lung thio-redoxin-interacting protein (TxNIP) expression. As a key inflammasome component, TxNIP is the major mediator of glucotoxicity and a therapeutic target of GLP-1/cAMP signaling cascade in pancreatic islet β-cells, demonstrated by our team and by others (46, 50).

**MATERIALS AND METHODS**

**Reagents and cell cultures.** Both LPS (Escherichia coli; serotype 0111:B4) and dexamethasone were purchased from Sigma-Aldrich (Oakville, Canada) while native GLP-1 was the product of Abcam Canada (Toronto, Canada). Liraglutide was kindly provided by Novo Nordisk (Novo Alle, Denmark).

**Experimental animals.** Eight-week-old male C57BL/6J mice, purchased from Princess Margaret Cancer Centre, University Health Network (Toronto, Canada), were used as wild-type (WT) controls. Male GLP-1R−/− mice also have a C57BL/6J background, as previously described (39). Mice were maintained at ambient room temperature and relative humidity of 50%, with free access to food and water under a 12:12-h light-dark cycle. Mice were divided into three groups (5–8 per group, detailed in related figure legend), designated as Control, LPS, and Lir/LPS for either WT mice or GLP-1R−/− (KO) mice. Briefly, mice were either subcutaneously injected with PBS (for Control and LPS groups) or liraglutide (800 μg/kg) (for Lir/LPS group).

**Two hours later, mice were anesthetized by inhaling isoflurane (5%), followed by oral intubation and intratracheal injection of LPS (10 mg/kg), 50 μl) (for LPS and Lir/LPS groups) or PBS (for Control group) with a 20-gauge catheter. Four hours later, mice were anesthetized by inhaling isoflurane followed by oral intubation and intratracheal injection of LPS (10 mg/kg, 50 μl) (for LPS and Lir/LPS groups) or PBS (for Control group). Two hours later, mice were anesthetized by inhaling isoflurane, followed by oral intubation and intratracheal injection of LPS (10 mg/kg, 50 μl) (for LPS and Lir/LPS groups) or PBS (for Control group), followed by cell counting with a hemocytometer.

**Lung tissue collection.** The five lung lobes were collected for various purposes, as documented by our group and by others (24, 28, 52). Briefly, the whole left lung was for bronchoalveolar lavage fluid (BALF) collection. The superior, the middle, and the inferior lobe of the right lung were for quantitative RT-PCR, wet/dry (W/D) ratio measurement, and histology and immunohistochemistry study, respectively. The postcaval lobe was collected for Western blot analyses. Samples for control and treatment groups were collected at the same time under the same conditions.

**Lung injury scoring.** For the histology study, lung tissues were fixed in 4% paraformaldehyde overnight, embedded in paraffin, and sectioned for H&E staining (45). To semiquantitatively evaluate lung injury, pathological categories, including neutrophils in the alveolar space, neutrophils in the interstitial space, hyaline membrane formation, proteinaceous debris filling in the airspaces, and alveolar septal thickening, were scored. The grading of the lung pathology was performed by a pathologist in a blinded fashion, using standard criteria, as we have described previously (23).

**Measurement of protein concentration and cell count in BALF.** A median thoracotomy was performed following the lung tissue collection. The right hilum was clamped, and BALF was collected after an intratracheal injection of 400 μl of PBS into the left lung. This procedure was repeated once. The fluid recovery rate was around 90%. The collected BALF was centrifuged at 400 g for 5 min at 4°C. The cell-free BAL supernatant was processed to measure the protein concentration using a BCA protein assay kit. The pelleted cells were resuspended in 100 μl of PBS followed by cell counting with a hemocytometer.

**Lung W/D weight ratio.** The degree of lung edema was quantitatively assessed by determining the lung W/D weight ratios, performed as we described previously (51). Briefly, the fresh middle lobe of the right lung was weighed first, followed by a drying procedure in an oven at 80°C for 48 h, and lung weight measurement again.

**RNA extraction, reverse transcription, and quantitative RT-PCR.** Total RNA was isolated using TRI reagent (Sigma Aldrich, Oakville, ON, Canada) or RNAeasy mini kit (Qiagen, Düsseldorf, Germany). cDNA was synthesized from RNA samples using a high fidelity cDNA synthesis kit (Applied Biosystems, Waltham, MA). Real-time PCR was performed using Sensfast SYBR (Bioline Scientific, Manchester, UK) or Advanced Universal SYBR Green Supermix (Bio-Rad, Mississauga, ON, Canada) with a 7900 fast real-time PCR system (Applied Biosystems). Quantification was calculated for cytokine, chemokine, and ANP genes against standard curves, as previously described (1). Relative quantification was calculated for TxNIP gene using 2−ΔΔCt method. All quantities are expressed as folds of differences relative to the PBS control. Quantitative RT-PCR primers used in this study are listed in Supplemental Table S1 (all Supplemental material is available at https://doi.org/10.6084/m9.figshare.12702569.v1).

**Immunoblotting.** Western blot analysis was performed to evaluate the protein expression of TxNIP. The membranes were incubated with anti-TxNIP (1:1,000, no. 14715, Cell Signaling Technology, Danvers, MA) and anti-GAPDH (1:1,000, no. 5174, Cell Signaling Technology) (as a housekeeping control) overnight at 4°C, respectively. The horseradish peroxidase-labeled secondary antibodies (1:2,000) were added and incubated for 1 h at room temperature. Protein bands were detected with the enhanced chemiluminescence Western blot detection system.

**Immunohistochemistry staining.** To examine apoptosis, immunohistochemical staining for cleaved-caspase-3 in lung tissue slides was performed (29). Sections were deparaffinized and incubated for 5 min at 120°C. Primary anti-cleaved-caspase-3 antibody (1:200, no. 9661, Cell Signaling Technology) was incubated overnight at 4°C. Antibody dep-ositions were visualized using diaminobenzidine. Nuclei were counterstained with hematoxylin. The percentage of cleaved-caspase-3-positive cells were calculated for assessing lung cell apoptosis. Briefly, 10 nonoverlapping visual fields (×400) for each slide were randomly selected, and the Trainable Weka Segmentation plug-in was loaded with ImageJ software to calculate the proportion of cleaved-caspase-3-positive cells in the lung. For GLP-1R detection in pancreatic islet and in mouse lung, a monoclonal antibody (Mab 7F38) described by Jensen et al. was used (17). Antibodies used in this study are listed in Supplemental Table S2.

**Statistical analysis.** Continuous variables were expressed as means ± SD. Comparisons between groups were made with the Student’s t test. Differences among multiple groups were analyzed with one-way ANOVA followed by the Tukey test. A P value of less than 0.05 is considered as significantly different. The statistics were performed using the SPSS 22.0 Statistics Software Package (IBM, Armonk, NY).
RESULTS

Liraglutide pretreatment attenuates LPS-induced acute lung injury in WT mice. To determine whether liraglutide pretreatment can provide a preventative effect on acute lung injury induced by LPS challenge, we injected liraglutide (800 μg/kg) subcutaneously 2 h before the intratracheal LPS (10 mg/kg) delivery (Fig. 1A). Mice were euthanized for lung tissue collection 4 h after LPS challenge. Figure 1B shows that 4 h after LPS challenge, mouse lung tissue developed severe degrees of inflammatory cell infiltration, interstitial and intra-alveolar edema, and patchy hemorrhage, interalveolar septal thickening, and hyaline membrane formation. Such damages were associated with collapsed pulmonary alveoli. Liraglutide pretreatment significantly reduced mononuclear inflammation and other pathological lesions caused by LPS challenge. We then conducted lung injury score assessment in a double-blind manner. When we compared the lung injury score with that of the PBS control group, the lung injury score in LPS-challenged group was 4 times higher, while liraglutide pretreatment effectively attenuated the lung injury score elevation (Fig. 1C). Figure 1, D–F show that the lung tissue W/D weight ratio, BALF inflammatory cell counts and BALF protein concentration were significantly increased 4 h after LPS challenge. These elevations, except for elevated BALF inflammatory cell counts (Fig. 1E), were effectively attenuated by liraglutide pretreatment.

GLP-1R−/− mice show the lack of protection by liraglutide pretreatment in LPS-induced acute lung injury. Using the novel monoclonal antibody against mouse GLP-1R (Mab 7F38) to conduct immunostaining, we demonstrated GLP-1R expression in wild-type, but not GLP-1R−/− mouse lung, with GLP-1R expressed in mouse pancreatic islets as a positive control. It appears that GLP-1R is mainly expressed on the alveolar wall (Fig. 2A). The above liraglutide pretreatment and LPS challenge, as well as the postmortem lung tissue assessments were then repeated in age-matched male GLP-1R−/− mice, with the same C57BL/6J genetic background. Figure 2, B and C shows that in GLP-1R−/− mice, LPS challenge induced a similar degree of lung injury as that observed in WT mice. Evidently, LPS-induced lung injury in GLP-1R−/− mice cannot be attenuated by liraglutide pretreatment. Comparing with WT mice, LPS challenge generated a similar elevation on lung tissue W/D ratio, BALF cell counts and BALF protein concentration in the sex- and age-matched GLP-1R−/− mice, while none of these three parameters were corrected or partially corrected by liraglutide pretreatment (Fig. 2, D–F). Hence, we conclude that the protective effect against LPS challenge induced acute lung injury in C57BL/6J mice by liraglutide pretreatment is a GLP-1R-dependent event.

Liraglutide pretreatment attenuates LPS-induced lung epithelial cell apoptosis. Immunohistochemistry staining for cleaved caspase-3 was then conducted for determining the effect of 4-h LPS challenge on apoptosis in the lung and the potential preventative effect of liraglutide treatment. Figure 3A shows the representative lung images in WT mice without and with LPS challenge, and in LPS-challenged mice with liraglutide pretreatment. Obviously, the attenuation effect of liraglutide on lung inflammation and collapses of lung tissue is associated with reduced number of cleaved caspase 3-positive cells in the lung. Quantitative analyses show that LPS challenge elevated cleaved caspase 3-positive cell number more than 10-fold, while liraglutide pretreatment significantly reduced such elevation (Fig. 3B). In GLP-1R−/− mice, LPS challenge generated a similar elevation on cleaved caspase 3-positive cells as compared with the WT mice, while liraglutide pretreatment generated no appreciable attenuation (Fig. 3, C and D).

Liraglutide pretreatment attenuates LPS-induced pulmonary cytokine and chemokine gene overexpression in a GLP-1R-dependent manner. Endotoxin- or LPS-induced acute lung injury can provoke elevated expression of certain proinflammatory cytokine and chemokine genes. Elevated proinflammatory cytokine or chemokine gene expression in the lung also reflects the degree of inflammation. Here, we assessed expression of two representative chemokine genes (MIP-1α or CCL3, and MIP-2 or CXCL8) and four cytokine genes (IL-6, IL-1β, IL-18, and TNF-α) in the mouse lung, with or without LPS challenge, and in the presence or absence of liraglutide pretreatment. We observed that in WT mice, expression levels of these six genes were increased significantly following a 4-h LPS challenge (Fig. 4). The elevation levels vary from 2.2-fold to >100-fold among these six genes. Among them, MIP-2 showed the highest fold of change (Fig. 4B), while IL-18 showed the modest change (Fig. 4F). Liraglutide pretreatment attenuated the elevation of all these six genes efficiently. Specifically, liraglutide pretreatment restored IL-18 mRNA level to that comparable with the PBS control group (Fig. 4F). For the other five genes, their expression levels in the LPS/liraglutide group were also significantly reduced.

In GLP-1R−/− mice, LPS challenge generated similar stimulatory effects on expression of these six chemokine or cytokine genes in the lung (Fig. 5), compared with effects of LPS in the WT mice (Fig. 4). Thus, responses to acute LPS challenge on proinflammatory cytokine and chemokine gene expression were not dependent on the presence of GLP-1R. By contrast, the protective effects of liraglutide pretreatment on attenuating expression of these six genes were completely lost in GLP-1R−/− mice (Fig. 5). These observations indicate that the protective effect of liraglutide during LPS-induced acute lung inflammatory injury is at least partially mediated through lung GLP-1R in blocking LPS-induced proinflammatory cytokine and chemokine gene expression.

Liraglutide pretreatment blocks LPS challenge-induced TxNIP expression in WT but not in GLP-1R−/− mouse lung. To understand mechanistically how the GLP-1 based drug liraglutide protects the lung from LPS-induced acute inflammatory injury, we then assessed two potential therapeutic targets of GLP-1-based drugs in the lung.

Atrial natriuretic peptide (ANP) is a potent pulmonary vasodilator (19, 20). In an ovalbumin induced COPD mouse model, long-term liraglutide treatment led to increased pulmonary expression of nppa, a gene that encodes ANP (3), which is a vascular dilator. Hence, we assessed nppa expression with quantitative RT-PCR in the mouse lung tissues. In both WT mice and GLP-1R−/− mice, LPS treatment increased nppa gene expression significantly, while liraglutide pretreatment had no appreciable effects on its expression (Supplemental Fig. S1).

A well-known downstream target of GLP-1 and GLP-based drugs in pancreatic islet β-cells is TxNIP, a major mediator of glucotoxicity (42, 43, 50). Hence, we assessed both TxNIP mRNA and TxNIP protein levels in the mouse lung tissue. LPS challenge caused ~2.5-fold elevation in lung TxNIP levels in WT mice (Fig. 6A). In GLP-1R−/− mice, TxNIP elevation in response to LPS challenge was as high as seven-fold (Fig. 6B).

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Importantly, in WT mice, liraglutide pretreatment virtually blocked LPS-stimulated lung TxNIP elevation (Fig. 6A). In contrast, a further elevation in lung TxNIP levels was observed in LPS-challenged $GLP-1R^{-/-}$ mice with liraglutide pretreatment (Fig. 6B). Increased lung TxNIP expression in response to LPS challenge was also observed at the protein level by Western blotting in both WT mice and $GLP-1R^{-/-}$ mice (Fig. 5, C and D). The elevation was effectively attenuated with liraglutide pretreatment in WT mice but further increased in $GLP-1R^{-/-}$ mice (Fig. 6, C and D). Thus, we have not only revealed for the first time that lung TxNIP at both mRNA and protein levels can be increased in LPS-induced acute lung injury, but also identified that lung TxNIP, a member of inflammasome, is likely among the potential therapeutic targets of GLP-1 based drugs, involving the participation of GLP-1R.
**DISCUSSION**

Although it is well known that in mice, monkeys, or humans, GLP-1R mRNA is very abundantly expressed in the lung (5, 7, 34), functions of GLP-1R in the lung remain to be further assessed, partially because of the lack of reliable GLP-1R antibodies for either Western blot analysis or immunohistochemistry staining (31). Here, we used a novel GLP-1R antibody generated by Knudsen and colleagues (17) and
proved its expression in the lung. Further investigations should be conducted to determine the cell lineage distribution of GLP-1R in the lung. It is also important to determine whether GLP-1R expression in the lung and elsewhere can be influenced by inflammatory challenges, it represents a novel defense system.

To date, we have learned the overall beneficial effect of GLP-1 and GLP-based drugs in acute lung injury rodent models and in a mouse COPD model (3, 54, 55, 58), indicating the possibility for “repurposing” GLP-1-based drugs for lung injury treatment. Mechanistically, we learned that in the lung, GLP-1-based drugs can exert multiple functions, including the promotion of vasorelaxant expression, the stimulation of endothelial nitric oxide synthase/sGC/PKG signaling cascade, and the inactivation of the NF-κB inflammatory signaling (11, 12, 21). Whether these and other pulmonary effects are mediated through GLP-1R should be investigated.

In the current study, with the use of GLP-1R−/− mice (39), we aimed to determine the existence of the “preventative” effect of a commonly used GLP-1-based drug, liraglutide, in an acute lung injury model. Data obtained in this study allowed us to attribute our observed “preventative” beneficial effects of liraglutide to GLP-1R. Furthermore, our current study revealed a potential key therapeutic target, TxNIP, which is also a well-known target of GLP-1 and GLP-based drugs in pancreatic islets β-cells in diabetes subjects (50).

An early investigation on ovalbumin-induced mouse COPD model by Viby et al. (54) demonstrated the effect of liraglutide on improving lung function. Viby et al. (54), however, could not identify a clear underlying mechanism for the association between improved lung function in their COPD model and liraglutide administration. They did observe reduced expression of surfactant proteins in the COPD mouse model, along with increased expression of proinflammatory cytokines. However,
levels of surfactants and proinflammatory cytokines in that model were largely unaffected by liraglutide treatment. Using human type II pneumocytes isolated from cadaveric organ donors, Vara et al. (53), however, found that native GLP-1 or exendin-4-stimulated cAMP formation and phosphatidylcholine secretion in a drug dosage-dependent manner, and such effects were reversed by the GLP-1R antagonist, exendin (9–39). Thus, not only is gene expression differentially expressed in the

Fig. 4. Liraglutide pretreatment attenuates LPS-induced cytokine and chemokine gene overexpression in wild-type (WT) mice. Comparison of MIP-1a (CCL3; A) and MIP-2 (CXCL2; B) levels in the three designated WT mouse groups. Comparison of IL-1β (C), IL-6 (D), TNF-α (E), and IL-18 (F) levels in the three designated WT mouse groups. The results were normalized to the β-actin levels. One-way ANOVA was used. *Significant difference, \( P < 0.05 \) or **\( P < 0.01 \), compared with the control group. #Significant difference, \( P < 0.05 \) or ##\( P < 0.01 \), compared with the LPS group. n = 5 per group of mice.

Fig. 5. Liraglutide pretreatment cannot attenuate LPS-induced cytokine and chemokine gene overexpression in GLP-1R\(^{−−}\) mice. Comparison of MIP-1a (CCL3; A) and MIP-2 (CCL8; B) levels in the three designated GLP-1R\(^{−−}\) mouse groups. Comparison of IL-1β (C), IL-6 (D), TNF-α (E), and IL-18 (F) levels in the three designated GLP-1R\(^{−−}\) mouse groups. The results were normalized to the β-actin levels. One-way ANOVA was used. **Significant difference, \( P < 0.01 \), compared with the control group; \( n = 7 \) for the control group; \( n = 8 \) for the LPS and LPS/Lir groups.
pulmonary defense system in acute inflammatory injury versus chronic inflammatory injury, responses of the lung to long-term versus short-term GLP-1-based drug administration are likely different as well. One may speculate that long-term liraglutide administration may exert more profound “metabolic” beneficial effects in addition to its anti-inflammatory effect observed in the acute injury model. To eventually resolve the puzzle, lung epithelial cell and pancreatic β-cell-specific GLP-1R knockout mouse lines are desired.

Kim et al. (19) have located mouse GLP-1R expression to cardiac atria and demonstrated that GLP-1R activation increased cardiac atria ANP secretion, leading to the reduction of blood pressure in mice. ANP is a natriuretic peptide hormone known as a potent pulmonary vasodilator (20). Although ANP is mainly produced in the heart, pulmonary ANP expression was reported and its pulmonary expression can be activated by long-term liraglutide treatment (3). In our LPS-induced acute lung injury model, we did not observe a stimulatory effect of liraglutide pretreatment on the expression of the ANP-encoding gene nppa in mouse lung, in contrast with the most recent observation made by Balk-Moller et al. in their COPD mouse model (3). Nevertheless, did not assess ANP protein level or its function in their COPD mouse model. The discrepancy observed by the two teams on pulmonary nppa expression can be attributed to the mouse models used, as well as the duration of liraglutide treatment. We, however, would like to suggest that nearly three-fold elevation on pulmonary nppa expression in response to LPS challenge observed in our current study represents a defensive response or protective mechanism. Although this elevation is not associated with GLP-1R, it deserves further investigation.

LPS is the key component of bacterial endotoxin, used in our current study for inducing acute lung injury. In a similar mouse model, Zhu et al. (58) found that LPS administration reduced SP-A and thyroid transcription factor-1 levels, while the reduction was reversed by simultaneous administration of liraglutide with LPS. In another study with the same mouse model, Xu et al. (55) showed that LPS-induced polymorphonuclear neutrophil (PMN) extravasation, lung injury, and alveolar-capillary barrier dysfunction, while simultaneous liraglutide administration prevented PMN-endothelial adhesion by inhibiting intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 expression. In the current study, liraglutide was not administered simultaneously with LPS, but as a “preventative agent,” administrated 2 h before intratracheal LPS delivery. The “preventative” effect observed in our current study suggests that

Fig. 6. Lung tissue thioredoxin-interacting protein (TxNIP) level is elevated following LPS challenge, and the elevation is attenuated by liraglutide pretreatment in a glucagon-like peptide-1 receptor (GLP-1R)-dependent manner. Comparison of lung TxNIP levels by quantitative RT-PCR in designated groups of wild-type (WT; A) and GLP-1R−/− mice (B). Comparison of lung TxNIP levels by Western blot analysis in designated groups of WT (C) and GLP-1R−/− mice (D). For A and C, n = 5. For B and D, n = 7 or 8. One-way ANOVA was applied. *Significant difference, P < 0.05 or **P < 0.01, compared with the control group. #Significant difference, P < 0.05 or ##P < 0.01, compared with the LPS group.
retrospective studies should be conducted in subjects with T2D who have received or have not received a GLP-1-based drug treatment, including liraglutide, as well as the weekly administered long-term active ones, such as semaglutide, Bydureon, and dulaglutide. Questions that can be addressed in such retrospective studies include whether T2D subjects under GLP-1-based drug treatment are less vulnerable to acute, as well as chronic lung inflammatory injury, and whether the treatment brings the beneficial effects for T2D subjects in coping with lung inflammation.

As a key mediator of glucotoxicity and a member of the NLR family pyrin domain containing 3 (NLRP3) inflammasome component, TxNIP has been suggested as a therapeutic target for diabetes and other disorders (26, 38, 42). Studies by our team and by others have shown that GLP-1 or GLP-based drugs can inhibit TxNIP production in pancreatic β-cells, which is associated with the protection of β-cells from high glucose or oxidative stress-induced cell apoptosis (42–44, 46, 50). Our current study suggests that such a feature is shared by pancreatic β-cells and lung cells. TxNIP interacts with and inhibits thioredoxin, controlling redox status of the cell. Elevated TxNIP level leads to increased reactive oxygen species (ROS), which can trigger the apoptotic cascade in pancreatic β-cells. Lung cell apoptosis can be activated by a “cytokine storm”, with proinflammatory cytokines and chemokines mainly released by immune cells. It remains to be determined how important TxNIP elevation within the lung epithelia is in triggering their apoptosis in response to pathogen infections. Nevertheless, we show here for the first time that LPS challenge increases TxNIP elevation, associated with a broad spectrum of elevation in proinflammatory cytokine gene expression. Importantly, liraglutide treatment attenuated LPS challenge-induced TxNIP elevation, proinflammatory cytokine gene expression, as well as cell apoptosis. It is unlikely that all these effects are mediated by immune cells, as GLP-1R level in immune cells is relatively lower (31). In pancreatic β-cells, TxNIP elevation can be stimulated by high-glucose (46) or glucocorticoid (36) treatment. In response to a local infection in the lung or a systemic infection, including sepsis, blood glucose and glucocorticoid levels may increase at least temporarily as a response to stress. If high glucose is also responsible for lung TxNIP elevation, GLP-1-based drugs will be able to bring it down by its direct effect in the lung, as well as by its indirect metabolic effect on reducing blood glucose level via its function in stimulating insulin secretion. Considering these features and the lack of specific therapeutic agents for acute lung injury, GLP-1-based drugs may possess more advantages to be developed as repurposed drugs for lung inflammation, comparing with melanolin and vildaglaptin, suggested by recent studies (49, 57). We are currently facing the astonishing coronavirus disease 2019 (COVID-19) pandemic. Diabetes is one of the major comorbidities of COVID-19 patients who develop acute respiratory distress syndrome (ARDS) (15, 48). ARDS can be developed in COVID-19 pneumonia subjects, in which a “cytokine storm” attacks endothelial cells in multiple organs, including lung alveolar epithelial cells. The “cytokine storm” attack will eventually lead to damaged blood gas exchange, inactivated pulmonary surfactant, resulting in the formation of hyaline membrane in the alveolar space, severe pulmonary edema, and disruption of lung parenchyma structure and functions (37, 47). Since GLP-1-based drugs exert multiple beneficial effects in the lung, making them potential repurposed drug candidates for treating COVID-19 with or without T2D. This speculation is very exciting and could be tested with coronavirus-induced acute lung injury murine model that we have reported previously (14).

In summary, our current study revealed a novel extra-pancreatic and GLP-1R-dependent function of the GLP-1-based therapeutic agent liraglutide. In the lung, liraglutide can also target TxNIP, requiring the intact GLP-1R signaling. Further investigations are needed to determine how GLP-1R activation leads to ROS production in lung epithelia, lung immune cells, or both. We suggest that GLP-1-based drugs have the great potential to be repurposed drugs for lung injury.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

T.J. and M.L. conceived and designed research; W.Z., W.S., Y.Z., D.L. and T.J. performed experiments; W.S., Y.Z., D.L., M.L., and T.J. analyzed data; W.S., M.L., and T.J. interpreted results of experiments; W.Z. and W.S. prepared figures; W.Z. and T.J. drafted manuscript; W.S., M.L., and T.J. edited and revised manuscript; W.Z., W.S., Y.Z., D.L., M.L. and T.J. approved final version of manuscript.

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