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L-Arginine improves DNA synthesis in LPS-challenged enterocytes

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1. ABSTRACT

The neonatal small intestine is susceptible to damage by endotoxin, and this cytotoxicity may involve intracellular generation of reactive oxygen species (ROS), resulting in DNA damage and mitochondrial dysfunction. L-Arginine (Arg) confers a cytoprotective effect on lipopolysaccharide (LPS)-treated enterocytes through activation of the mammalian target of the rapamycin (mTOR) signaling pathway. Arg improves DNA synthesis and mitochondrial bioenergetics, which may also be responsible for beneficial effects of Arg on intestinal mucosal cells. In support of this notion, results of recent studies indicate that elevated Arg concentrations enhances DNA synthesis, cell-cycle progression, and mitochondrial bioenergetics in LPS-treated intestinal epithelial cells through mechanisms involving activation of the PI3K-Akt pathway. These findings provide a biochemical basis for dietary Arg supplementation to improve the regeneration and repair of the small-intestinal mucosa in both animals and humans.

2. INTRODUCTION

The intestinal epithelium constitutes the largest and most important internal barrier against the external environment, which is continuously exposed to potentially harmful antigens, pathogens, toxins and air pollutants (1,2). Enterocytes, goblet cells, enteroendocrine cells, crypt stem cells, and other cell types account for approximately 90%, 8%, 0.5%, 0.5%, and 1% of the mucosal epithelial cell population in the small intestine (3, 4). It is known that stressful conditions (e.g., weaning) and bacterial infection cause intestinal mucosal injury involving disruption of tight junctions and elevation of cellular permeability (5-9).

Enterocytes are particularly vulnerable to chemo agents that interfere with DNA synthesis (10,11). This cytotoxicity may involve intracellular generation of reactive oxygen species (ROS) from the xanthine oxidase pathway, resulting in generation of hydroxyl radicals, which subsequently can cause DNA damage (12). Mitochondria are considered as the main source for endogenous ROS and also the major suppliers of ATP to maintain biological function; therefore, bioenergetics failure induced by mitochondrial dysfunction may play a role in intestinal injury (12-14). Furthermore, mitochondria participate in the regulation of both cell proliferation and death, and are thus potential mediators of the Phosphatidylinositol 3-kinase/Protein kinase B (PI3K/Akt) signaling pathway (15). PI3K/Akt is an important

pathway implicated in the proliferation and survival of cells and inhibition of the PI3K/Akt pathway impair G2/M transition of the cell cycle (16).

L-Arginine (Arg) has recently been recognized as a functional amino acid to regulate key metabolic pathways beyond protein synthesis (17-18). Of particular interest, Arg has been demonstrated to confer a cytoprotective effect on LPS-treated enterocytes and to play a role in intestinal villous recovery after injury and cell migration (6, 7, 19). To date, the underlying biochemical mechanisms are largely unknown. Increased synthesis of DNA and protein in enterocytes may be responsible for this beneficial effect of Arg. We have demonstrated Arg increased protein synthesis involving mammalian target of the rapamycin (mTOR) signaling pathway in LPS-treated enterocytes (7). In the present article, we review the results of recent studies regarding the effects of Arg on DNA synthesis and mitochondrial respiration, as well as signaling pathways involving PI3K/Akt.

3. EFFECTS OF ARG ON DNA SYNTHESIS AND CELL-CYCLE PROGRESSION OF LPS-TREATED PORCINE ENTEROCYTES

3.1. General study protocols

Intestinal porcine epithelial cells (IPEC-1) were obtained from the jejunum of newborn pigs, as described previously (20). Cells were cultured with Dulbecco's modified Eagle's F12 Ham medium (DMEM-F12) medium (Hyclone, USA) containing 5% fetal bovine serum (FBS) (Gibico, USA), 1% an antibiotic solution (P/S; Sigma, USA), 2 mM L-glutamine (Sigma, USA), 0.1 % ITS (ScienCell, USA), and 5 μg/L mEGF (BD Biosciences, USA) at 37 ^{o}C in a 5% CO_2 incubator. After an overnight incubation, the cells were starved for 6 h in Arg-free DMEM. The 5% FBS in the Arg-free DMEM provided 10 μM Arg. The cells were then cultured in medium containing 10, 100 or 350 μM Arg (Sigma, USA) and 0 or 20 ng/ml LPS (Sigma, USA) The cells cultured in the presence of 10 μM Arg virtually did not proliferate. The culture medium was changed every 2 days.

After a 4-day period of culture, DNA synthesis during cell proliferation in all treatment groups was quantified using 5-ethynyl-2'-deoxyuridine (EdU) incorporation using Cell-Light^{TM} EdU Kit (Rui Bo Biotechnology Limited Company, Guangzhou, China) (21,22). Briefly, cells were cultured in DMEM medium containing 50 μM Edu for 2 h. Following incorporation, cells were washed with PBS twice and fixed for 30 min in 4% parafoemaldehyde at room temperature. After washing with a 2 mg/ml glycine solution, the cells were permeablized with 0.5% Triton X-100 in PBS for 10 min and washed with PBS for 5 min. Thereafter, cells were incubated in Apollo® staining reaction solution for 30 min without light. The cells were washed three times with PBS containing 0.5% Triton X-100 for 10 min in each rinse. For subsequent DNA staining, cells were counterstained with the Hoechst 33342 reaction solution for 30 min in the dark. Subsequently, the cells were washed twice with PBS for 3 min in each rinse, and examined immediately under a fluorescent microscope (magnification × 400). An Olympus BX51 microscope (Olympus, Japan) was used to observe EdU-positive cells. Images of the Apoll® 567 Hoechst 33342 were captured. The percentage of EdU-positive cells was expressed as the ration of red nuclei cells to blue nuclei cells in at least five different microscopic fields randomly selected for counting at 200-fold magnification.

The cell cycle was analyzed using propidium iodide DNA staining (23). Briefly, after a 4-day period of culture in DMEM medium containing 10, 100 or 350 μM Arg and 0 or 20 ng/ml LPS, medium and cells were collected separately. Cells were washed with PBS and filtered by a 200-mesh screen. Cells were then suspended in PBS and fixed them in 70% ethanol and stored at 4^{o}C for 24 h. After two washes with PBS, the fixed cells were incubated in 100 μl RNaseA at 37 ^{o}C for 30 min, followed by staining of the DNA with 100 μl propidium iodide (PI) at 4 ^{o}C for 30 min in the dark. Before flow cytometry analysis, each sample was incubated at room temperature for 1 h. The PI-DNA complex was measured using FACSort flow cytometry (Becton Dickinson, CA, USA) at 488 nm excitation and 617 nm emission. The percentage of cells at G1, S and G2 phases of the cell cycle and apoptosis were determined by analysis with the CellQuest Pro® software (Becton Dickinson, CA, USA).

3.2 Arg improved DNA synthesis and cell-cycle progression in LPS-treated porcine enterocytes

The neonatal small intestine is susceptible to damage by endotoxin, and there is growing interest in developing effective methods for prevention and treatment in both clinical medicine and animal production (19,24,25). We previously reported that LPS inhibited cell proliferation and protein synthesis in IPEC-1 cells but Arg conferred a cytoprotective effect on these cells through the activation of the mTOR signaling pathway (7). Here, we focused on the roles of their DNA synthesis in cytoprotective effect of Arg in LPS-induced intestinal cells.

Bacterial LPS, which is often used to provide a model of microbial infection, may induce cell apoptosis, increase epithelial paracellular permeability, DNA damage and mitochondrial dysfunction (10,12,26). LPS induces mitochondrial ROS generation and then causes DNA damage. Our results showed that the percentages of EdU-positive cells (Figure 1) and cells in the S-phase (Figure 2) were decreased in response to LPS treatment. However, an increase in EdU incorporation was observed when the extracellular concentration of Arg was increased from 10 μM to 100 or 350 μM (Figure 1). The synthesis of polyamines and nitric oxide (NO) from Arg may be

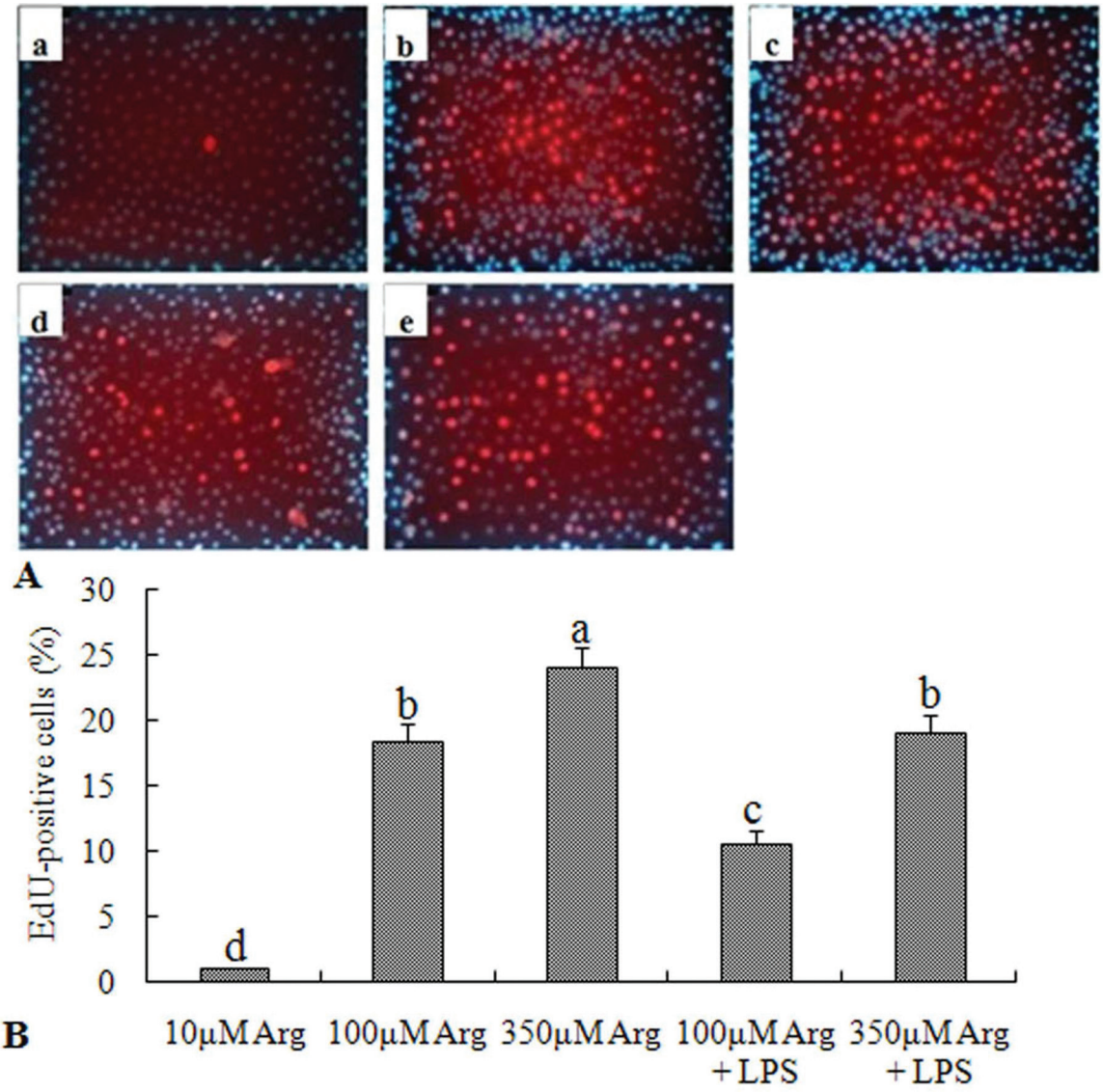

Figure 1. DNA synthesis in IPEC-1 cells. DNA synthesis during the proliferation of IPEC-1 cells was quantified by EdU incorporation (red color) using Cell-LightTM EdU Kit (Rui Bo Biotechnology Limited Company, Guangzhou, China). Nuclei are shown in blue color. (A) Representative images of EdU staining (magnification ×200) of cells treated with 10 μM Arginine (a), 100 μM Arginine (b), 350 μM Arginine (c), 100 μM Arginine + 10 ng/ml LPS (d), or 350 μM Arginine + 10 ng/ml LPS (e) for a 4-day period. (B) The percentage of EdU-positive cells (the number of red nuclei versus the number of blue nuclei in at least five different microsopic fields of vision). Data are expressed as means ± SEM, n = 4 independent experiments. a–d Means sharing different letters differ ($P < 0.05$), as analyzed by one-way analysis of variance and the Student-Newman-Keuls multiple comparison test (89, 90).

responsible for its DNA repair effect (27). Polyamines can stabilize DNA and promote protein synthesis, whereas physiological levels of NO enhance intracellular cyclic guanosine monophosphate (cGMP) content and also stimulate DNA synthesis in cells, including endothelial cells and tumors (28-30). An increase in DNA synthesis plays an important role in increasing proliferation of enterocytes for repair of the mucosal barrier (7, 31). In addition, flow cytometry analysis showed that the percentage of cells in the GI phase increased, while the percentage of cells in the S phase decreased in the 100 μM Arg + LPS group, compared with the 100 μM Arg group (Figure 2). Polyaminew and NO also have a positive effect on progression through the cell cycle (32). In the presence of DNA damage induced by LPS, the G1/S checkpoint prevents cells from entering the S phase by inhibiting the initiation of replication (33). Also, amino acid deficiencies potently arrest cell-cycle progression and down-regulate expression of proliferation-control proteins (34). In support of this notion, reducing Arg concentration from 100 μM (a physiological level in the plasma of mammals (35) to 10 μM impeded the cell cycle, and increasing Arg concentration from 100 to 350 μM greatly increased DNA synthesis.

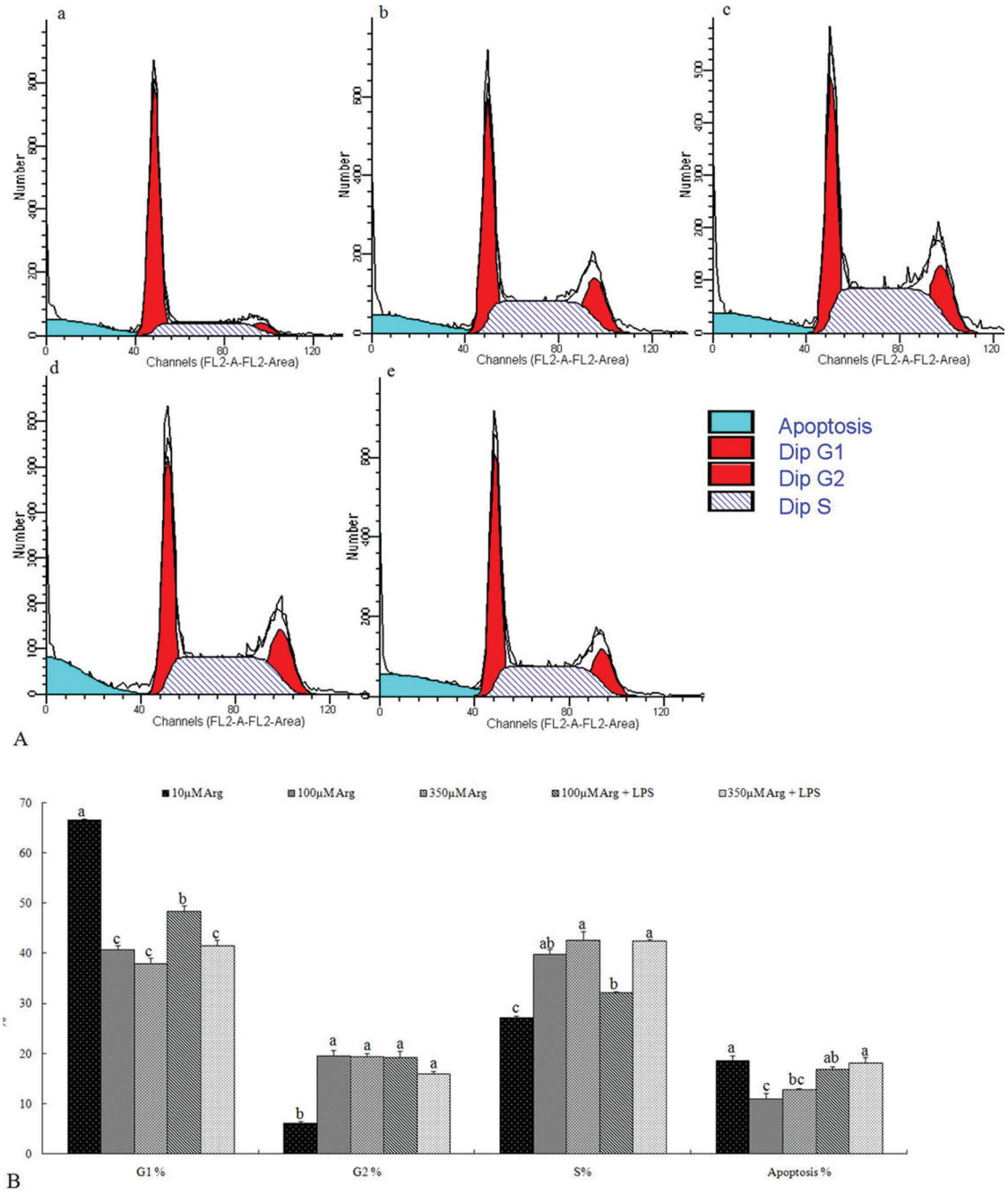

Figure 2. Cell cycle of IPEC-1 cells analyzed using propidium iodide DNA staining and flow cytometry. (A) Representative flow-cytometry diagrams of cells treated with 10 μM Arginine (a), 100 μM Arginine (b), 350 μM Arginine (c), 100 μM Arginine + 10 ng/ml LPS (d), or 350 μM Arginine + 10 ng/ml LPS (e) for a 4-day period. (B) The percentage of cell population in each phase of the cell cycle. Data are expressed as means ± SEM, n = 4 independent experiments. a–c Means sharing different letters differ ($P < 0.05$), as analyzed by one-way analysis of variance and the Student-Newman-Keuls multiple comparison test (89, 90).

4. EFFECTS OF ARG ON MITOCHONDRIAL FUNCTION OF LPS-TREATED PORCINE ENTEROCYTES

4.1. General study protocols

The XF-24 Extracellular Flux Analyzer and Cell Mito Stress Test Kit from Seahorse Biosciences were used to examine the effects of Arg treatment on mitochondrial respiration in LPS-induced cells (36). After a 2-day period of culture in DMEM medium containing 10, 100 or 350 μM Arg and 0 or 20 ng/ml LPS, the base medium was changed prior to the bioenergetic measurements to serum-free unbuffered (without sodium bicarbonate) DMEM medium base supplemented with 2 mM L-glutamine, 17.5 mM

D-glucose and 0.5 mM sodium pyruvate, at pH 7.4. To measure indices of mitochondrial function, oligomycin, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP), as well as rotenone and antimycin A were injected sequentially at the final concentrations of 0.5, 4, and 1 μM, respectively. This allowed for an estimation of the contribution of non-ATP–linked oxygen consumption (proton leak) and ATP–linked mitochondrial oxygen consumption (ATP production). The maximal respiration capacity was determined using the FCCP-stimulated rate. The spare respiratory capacity was represented by the maximal respiratory capacity subtracted from the baseline oxygen consumption rate (OCR). The residual oxygen consumption that occurred after addition of rotenone and antimycin A was ascribed to non-mitochondrial respiration and was subtracted from all measured values in the analysis. Owing to the effects of Arg on IPEC-1 proliferation, total cellular protein was determined and used to normalize mitochondrial respiration rates.

4.2. Arg improved mitochondrial bioenergetics of LPS-treated porcine enterocytes

Oxidation of energy substrates produce CO_2, water and ATP in cells (37, 38). Mitochondria are considered as the main source for endogenous ROS (13) and that resulted from the disruptions in the respiratory chain (39). Mitochondrial function damage induced by LPS was observed, showing with decrease in the basal respiration, maximal respiration and non-mitochondrial respiration (Figure 3). This is consistent with the results in mouse aortic endothelial cells and human tubular epithelial cells (40, 41). LPS induced alterations in oxygen consumption and radical generation and the indirect DNA damage induced by ROS involves the mitochondrial electron transport chain (40,42).

Mitochondria are also the major suppliers of ATP to maintain biological function and play a central role in satisfying higher demands for energy and anabolic needs during stress (13). A novel and important finding of our recent study is that Arg improved mitochondrial bioenergetics. To our knowledge, this is the first report demonstrating that Arg modulates mitochondrial oxygen consumption by cultured cells. Similarly, increasing Arg concentrations in blood through dietary supplementation with Arg enhances anti-oxidative capacity (43,44) and oxygen consumption and ATP production (45,46) in rats. Likewise, improving mitochondrial function in obese and diabetic rats through oral administration of interferon tau promotes oxidation of fatty acids and glucose, and, therefore, whole-body energy expenditure (47). Conversely, Arg deprivation affected mitochondrial bioenergetics (Figure 3). Consistent with this observation, Arg deficiency decreased the abundance of the mitochondrial inner membrane and matrix proteins, while impairing mitochondrial oxidative phosphorylation and ATP production (39). Although the mechanisms of positive effect of Arg on mitochondrial bioenergetics remains unclear, this regulation appears to be due, in part, to the stimulation of DNA synthesis and cell proliferation.

5. SIGNALING PATHWAY INVOLVED IN THE CYTOPROTECTIVE EFFECT OF ARG IN LPS-TREATED PORCINE ENTEROCYTES

5.1. General study protocols

We investigated the effects of Arg on the Growth arrest, the mRNA and protein abundances of DNA damage-45 (GADD45) alpha [an indicator of mucosal damage (48)], as well as the protein levels for PI3K, Akt and B-cell lymphoma/leukaemia-2 (Bcl2) (the cell-survival regulatory proteins). The rationale for our study was to explore the signaling pathways involved in the cytoprotective effect of Arg in LPS-treated porcine enterocytes. Our research protocol included the measurement of expression of GADD45 alpha mRNA using real-time quantitative RT-PCR (49-52). Briefly, after a 4-day period of culture in DMEM medium containing 10, 100 or 350 μM Arg and 0 or 20 ng/ml LPS, cells were collected using the Trizol-reagent (Invitrogen). Total RNA was extracted from cells according to the manufacturer's instructions and quantified by electrophoresis on 1% agarose gel and the measurement of optical density at 260 and 280 nm. The cDNA was reverse-transcribed from 0.2 mg of eluted RNA using a kit from Takara, according to the manufacturer's instructions. The real-time quantitative PCR for GADD45 alpha was performed in the Rotor-Gene Multi-filter system Rotor-Gene 3000 instrument (Corbett Research, Australia) with SYBR Premix Ex Taq kit (Takara), using the 18S house keeping gene as an internal control. The oligonucleotide sequences used to amplify genes were: GADD45 alpha (F) 5'-CGA GGA CGA CGA CAG GGA C-3' (R) 5'-AGC AAA ACG CTT GGA TCA GG-3'; 18S (F) 5'-AAT TCC GAT AAC GAA CGA GAC T-3' (R) 5'- GGA CAT CTA AGG GCA TCA CAG-3'. Cycle threshold (Ct) values are means of triplicate measurements. The comparative Ct value method was employed to quantitative expression levels for target genes relative to those for the 18S DNA. Data are expressed as the relative values to those of 10 μM Arg-treated cells.

Protein levels for GADD45 alpha, phosphorylated PI3K, Akt, phosphorylated Akt (Ser473), Bcl2 and phosphorylated Bcl2 were determined by western blot analysis (52, 53). Briefly, after a 4-day period of culture in DMEM medium containing 10, 100 or 350 μM Arg and 0 or 20 ng/ml LPS, cells were collected using RIPA Lysis Buffer (Beyotime Institute of Biotechnology, Shangshai, China) containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, sodium orthovanadate, sodium fluoride, EDTA, leupeptin and 0.1 mM PMSF. Cells were homogenized with a polytron homogenizer and centrifuged at 10, 000 × *g* for 10 min at 4 ^{o}C. Protein concentrations in cell homogenates were measured using the BCA method and bovine

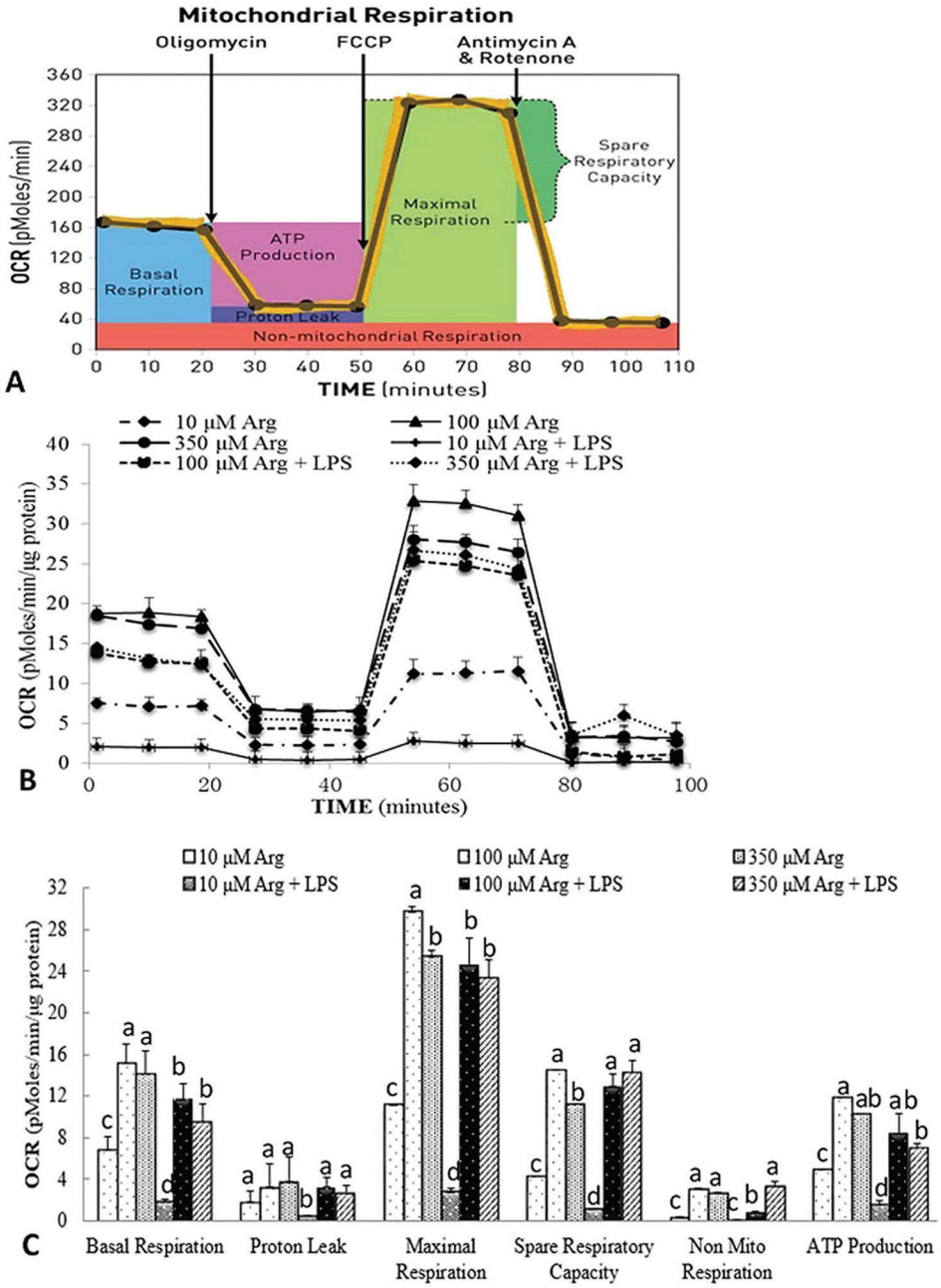

Figure 3. Mitochondrial respiration of IPEC-1 cells measured by the XF-24 Extracellular Flux Analyzer and Cell Mito Stress Test Kit from Seahorse Biosciences (North Billerica, MA, USA). Schematic (A) and oxygen consumption rate (OCR) (B) assessed by extracellular flux analysis. OCR was measured under basal conditions followed by the sequential addition of oligomycin (0.5 µM), FCCP (4 µM), rotenone (1 µM) or antimycin A (1 µM). Each data point represents an OCR measurement. (C) Individual parameters for basal respiration, proton leak, maximal respiration, spare respiratory capacity, non-mitochondrial respiration and ATP production. Cells were cultured in DMEM medium containing 10, 100 or 350 µM Arg and 0 or 20 ng/ml LPS for a 2-day period. The basal medium with 5% fetal bovine serum contained no putrescine, spermidine or spermine (detection limit = 0.5 µM), as analyzed by high-performance liquid chromatography (HPLC; 91). Arg concentrations in the final culture medium were verified by HPLC analysis (92). Data are expressed as means ± SEM, n = 4 independent experiments. a–d Means sharing different letters differ ($P < 0.05$), as analyzed by one-way analysis of variance and the Student-Newman-Keuls multiple comparison test (89, 90).

serum albumin as standard. All samples were adjusted to have an equal protein concentration. The supernatant fluid (containing cell proteins) was then diluted with 2× sodiumdodecyl sulfate (SDS) sample buffer (0.63 ml of 0.5 M Tris-HCl pH 6.8, 0.42 ml 75% glycerol, 0.125 g SDS, 0.25 ml β-mercaptoethanol, 0.2 ml 0.05 % solution of bromphenol blue, and 1 ml water to a final volume of 2.5 ml) and heated in boiling water for 5 min. After the

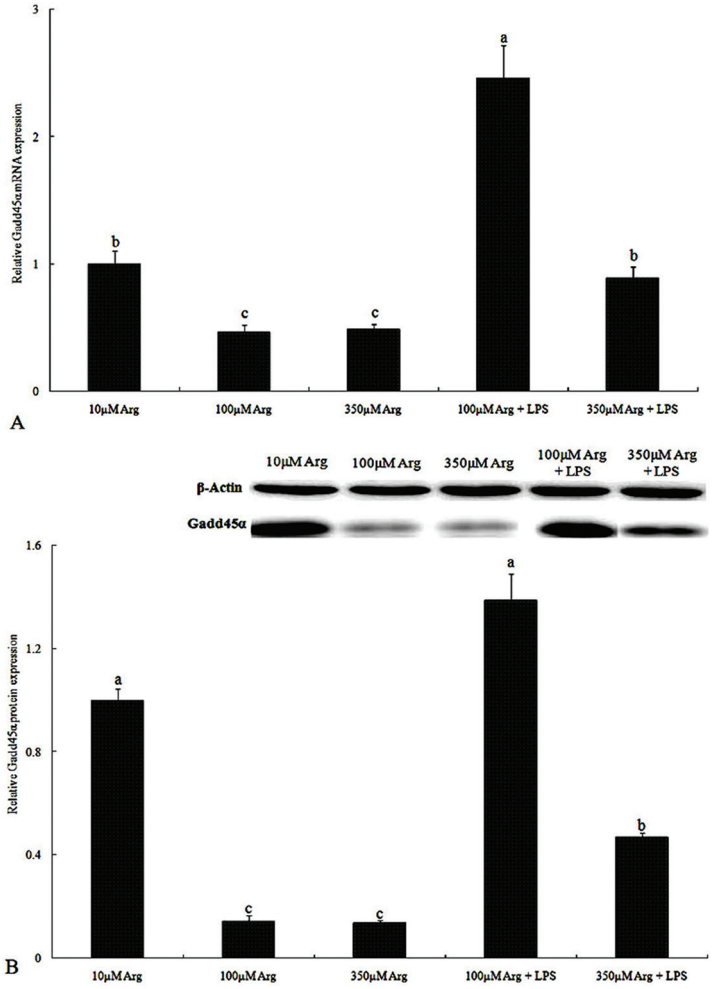

Figure 4. Growth arrest and DNA damage-45 alpha (GADD45 alpha) mRNA (A) and protein (B) expression in IPEC-1 cells determined by real-time quantitative RT-PCR and western blot analysis, respectively. Cells were cultured in DMEM medium containing 10, 100 or 350 μM Arg and 0 or 20 ng/ml LPS for a 4-day period. The comparative Ct value method was employed to quantitative expression levels for target genes relative to those for the 18S DNA and protein measurement were normalized to β-actin. Data are expressed as the relative values to those of 10 μM Arg-treated cells and as means ± SEM, n = 4 independent experiments. a–c Means sharing different letters differ (P < 0.05), as analyzed by one-way analysis of variance and the Student-Newman-Keuls multiple comparison test (89, 90).

solution was cooled on ice, it was used for western blot analysis (54). Aliquots of samples were loaded onto SDS-polyacrylamide gels. After separation on 4-12% gels, proteins were transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA) under 12 V overnight, using the Bio-Rad Transblot apparatus (Hercules, CA). Membranes were blocked in 5% fat-free dry milk in TTBS (20 mM Tris/150 mM NaCl, pH 7.5, and 0.1 % Tween-20) for 3 h and then were incubated with the following primary antibodies overnight at 4 ^{o}C with gentle rocking: GADD45 alpha (Cell Signaling, 1:1000), phosphorylated PI3K (Cell Signaling, 1:1000), Akt (Cell Signaling, 1:1000), phosphorylated Akt (Ser473) (Cell Signaling, 1:1000), Bcl2 (LifeSpan BioSciences, 1 μg/ml), phosphorylated Bcl2 (Ser70)(Cell Signaling, 1:1000) or β-actin (Cell Signaling, 1:1000). After washing three times with TTBS, the membranes were incubated at room temperature for 2-3 h with corresponding secondary antibodies at 1:10,000 (ZSGB-BIO, Beijing, China). Finally, the membranes were washed with TTBS, followed by development using Luminata Forte Western HRP Substrate according to the manufacturer's instructions (Millipore, MA, USA). The signals were detected on Fujifilm LAS-3000 (Tokyo, Japan). All protein measurements were normalized to β-actin and all data were expressed as the relative values to those of cells cultured with 10 μM Arg.

5.2. Arg stimulated the PI3K/Akt signaling pathway in LPS-treated porcine enterocytes

GADD45 alpha played an important role in the DNA damage response and could be an indicator of mucosal damage in the gut (48). LPS increased but Arg reduced expression of GADD45 alpha in IPEC-1 cells at both mRNA and protein levels (Figure 4), indicating

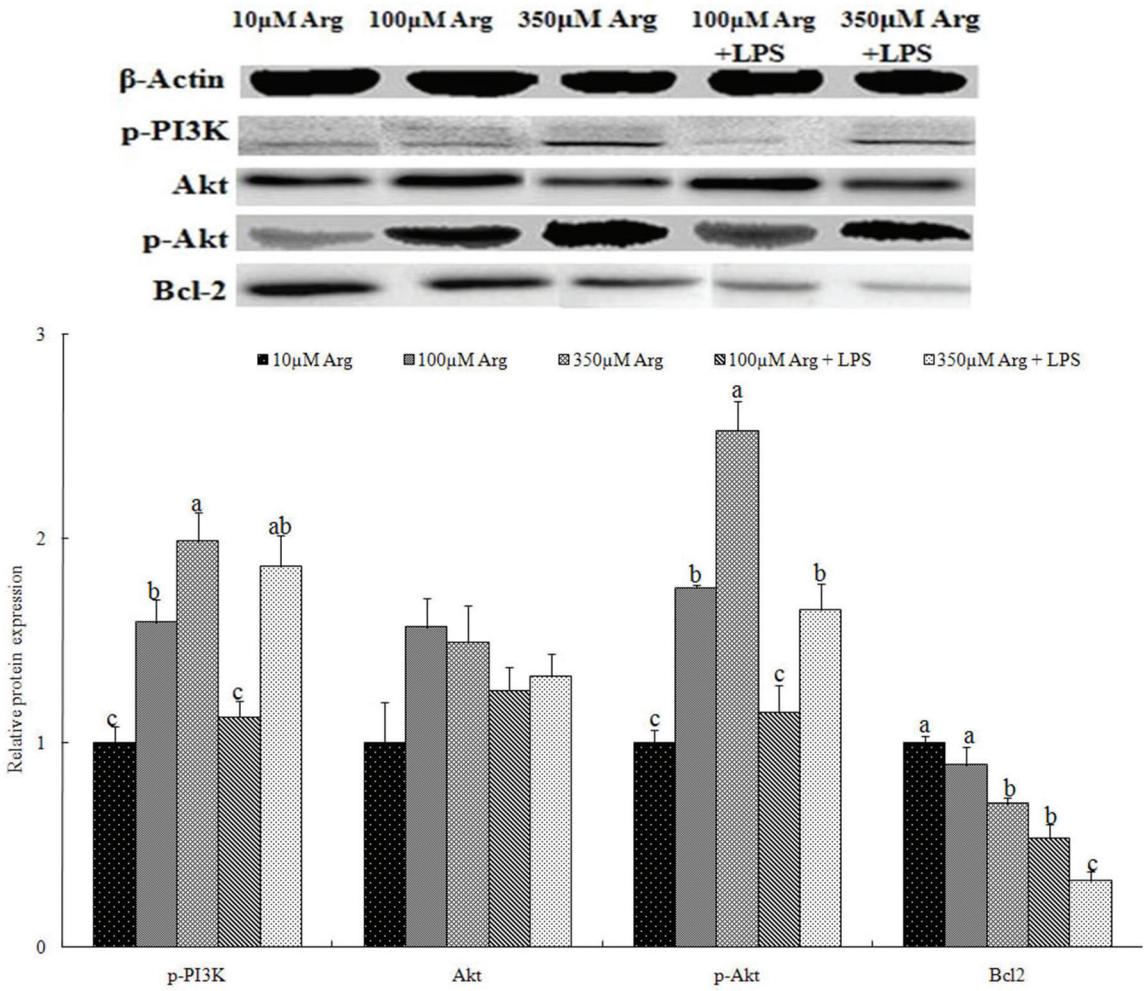

Figure 5. Abundances of proteins in the Akt-Bcl2 signaling pathway in IPEC-1 cells determined by western blot analysis. Cells were cultured in DMEM medium containing 10, 100 or 350 μM Arg and 0 or 20 ng/ml LPS for a 4-day period. All protein measurements were normalized to β-actin and all data were expressed as the relative values to those of cells cultured with 10 μM Arg.Data are expressed as means ± SEM, n = 4 independent experiments. a–c Means sharing different letters differ (P < 0.05), as analyzed by one-way analysis of variance and the Student-Newman-Keuls multiple comparison test (89, 90).

an important role for Arg in intestinal DNA repair under endotoxic and inflammatory conditions. GADD45 alpha has also been shown to be a potent cell-cycle regulator and can arrest cells in the G2/M phase of the cell cycle (55). GADD45 alpha expression is at the highest level in the G1 phase of the cell cycle and at the lowest level in the S phase (48). The finding that Arg decreased the expression of GADD45 alpha in IPEC-1 cells was consistent with the observation that physiological levels of Arg increased the number of IPEC-1 cells in the S phase.

Mitochondrial function may be regulated by the PI3K/Akt signaling pathway (17). This signaling pathway may also play a role in modulating the proliferation and survival of cells (16). Sheng *et al*. (56) have demonstrated that activation of PI3K/Akt is crucial for small-bowel and colon mucosal proliferation after food deprivation and subsequent re-feeding. Akt can be activated in response to DNA damage through PIKK family members (57). Expression of active Akt promotes cells to enter the S phase and DNA synthesis (56, 58), thereby enhancing DNA repair (57). Likewise, Arg increases DNA and protein synthesis by activating the PI3K/Akt signaling pathway in mammalian trophectoderm cells (59,60) and embryos (61). Conversely, inhibition of the PI3K/Akt pathway impairs G2/M transition of the cell cycle (16). Treatment with LPS reduced protein levels for phosphorylated Akt and Bcl2 in IPEC-1 cells in the present study (Figure 5). There is evidence that DNA damage triggers apoptosis through the down-regulation of the anti-apoptotic Bcl2 gene (62). Bcl2 is an oncogene that inhibits apoptosis, but paradoxically it also has an anti-proliferative effect (62,63). Thus, Bcl2 plays a regulatory role in cell

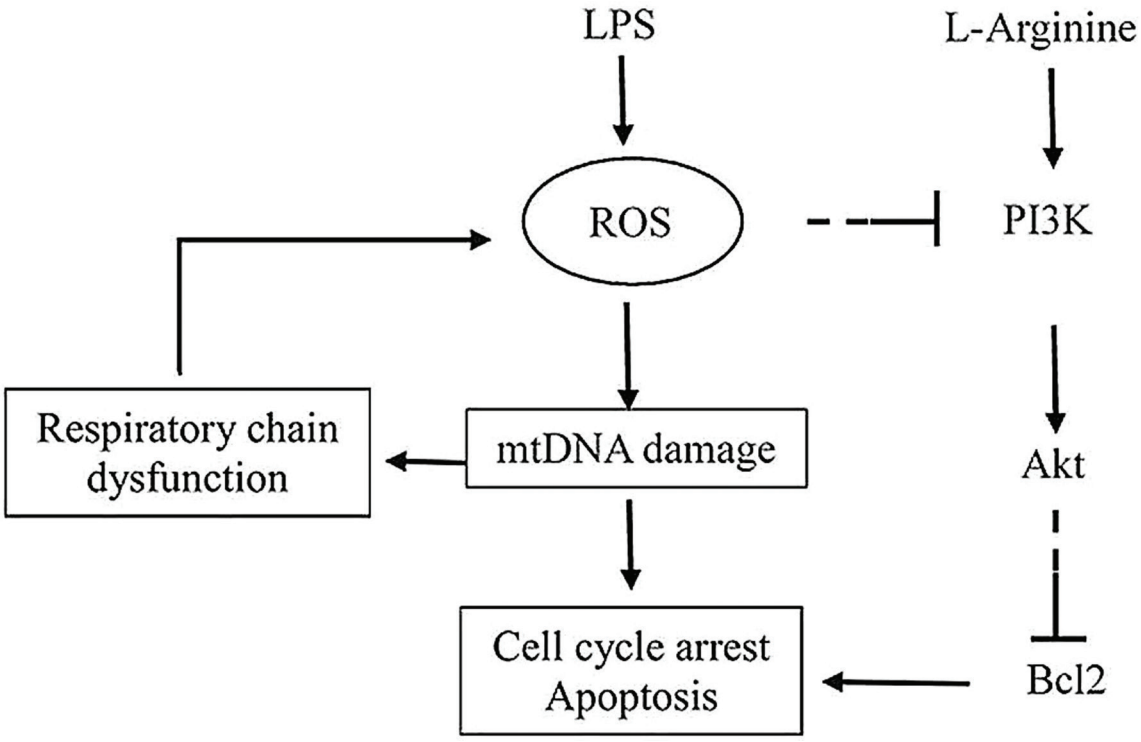

Figure 6. Possible mechanisms for the cytoprotective effect of arginine on mitochondrial dysfunction and cell-cycle impairment induced by LPS in in IPEC-1 cells. LPS, lipopolysaccharide; ROS, reactive oxygen species; PI3K, phosphatidylinositol 3-kinase; Akt, protein kinase B; Bcl2, B-cell lymphoma/leukaemia-2.

survival and proliferation. The role of PI3K/Akt pathway in the regulation of Arg on enterocyte proliferation was not be investigated by inhibiting this signaling pathway in this study. Nevertheless, it is noteworthy that Arg stimulated the PI3K/Akt pathway, while reducing Bcl2 protein levels both in normal and LPS-challenged IPEC-1 cells (Figure 5). L-Arg has also been demonstrated to increase the protein phosphorylation and, therefore, the activity of Akt-1 in ovine trophectoderm cells (64), which is likely through the action of polyamine and/or NO generation (65, 66). In support of this view, physiological levels of NO can stimulate the PI3K/Akt signaling pathway to promote cell survival (67, 68). Therefore, it is likely that Arg enhanced the proliferation and survival of IPEC-1 cells by activating the PI3K/Akt signaling pathway through the action of NO.

6. SUMMARY AND PERSPECTIVES

The intestinal mucosa has the highest rate of turnover among tissues in young pigs, which involves cell proliferation, migration, differentiation, and shedding, resulting in total renewal of the epithelial cell lining every 3 to 4 days. This rapid turnover makes enterocytes particularly vulnerable to chemo agents that interfere with DNA synthesis. Arg plays an important role in intestinal physiology and has been studied as a component of an oral rehydration solution to enhance intestinal absorption and villous recovery after injury and has been shown to be effective in protecting against gastrointestinal injury (69). The results of recent studies indicated that: (a) LPS induced mitochondrial dysfunction and cell-cycle impairment; and (b) Arg promoted DNA synthesis and mitochondrial bioenergetics in intestinal epithelial cells. Possible mechanisms for the cytoprotective effect of arginine on LPS-induced mitochondrial dysfunction and cell-cycle impairment are proposed in Figure 6. Arg stimulates PI3K and Akt phosphorylation, thereby inhibiting Bcl2 and ameliorating cell-cycle arrest and apoptosis brought about by an endotoxin. These findings provide a biochemical basis for beneficial effects of dietary Arg supplementation in improving the regeneration and repair of the small-intestinal mucosa. Like other functional amino acids (70-84), Arg plays vital roles in nutrition and metabolism. Animals and humans (young and adult, men and women) have dietary requirements of Arg for supporting optimal growth, development, and health in the small intestine and the while-body (85-88).

7. ACKNOWLEDGMENTS

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Abbreviations: Akt: Protein kinase B; Arg: Arginine; Bcl2: B-cell ymphoma/leukaemia-2; DMEM-F12: Dulbecco's modified Eagle's F12 Ham medium; ECAR: Extracellular acidification rate; EdU: 5-ethynyl-2'-deoxyuridine; FBS: Fetal bovine serum; FCCP: Carbonyl cyanide-p-trifluoromethoxyphen ylhydrazone; GADD45 alpha: Growth arrest and DNA damage-45 alpha; IPEC-1: Intestinal porcine epithelial cell line-1; mTOR: Mammalian target of the rapamycin; OCAR: Oxygen consumption rate; PI3K: Phosphatidylinositol 3-kinase; LPS: Lipopolysaccharide.

Key Words: Arginine, DNA synthesis, Cell Cycle, Mitochondrial Function, Intestine

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