学位論文の要旨

論文題目Studies on the Utilization of Melatonin for Livestock Production(メラトニンの家畜生産への利用に関する研究)

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1. General introduction

The overuse of antibiotics threatens both the development of livestock industry and the public health. The use of antibiotics in animal food production will become even more severely restricted in the future. Provision of appropriate pharmaceutical, such as melatonin can have some anti-inflammatory effects in the experimental animals. The study aimed to investigate the protective effects of melatonin on lipopolysaccharide (LPS)-stimulated bovine mammary epithelial cells (bMECs) and quail ovary granulosa cells *in vitro*, and the potential effects of melatonin on progesterone secretion in granulosa cells of the Japanese quail.

2. The anti-inflammatory and antioxidant effects of melatonin on LPS-stimulated bMECs

To evaluate the therapeutic potential of melatonin in mastitis, the ability of melatonin to protect bMECs from the harmful effects of LPS was examined. bMECs isolated from fresh milk were pretreated with or without melatonin (10 or 100 µg/mL) for 12 h and then incubated for 12 h in the absence or presence of 100 ng/mL LPS. The result shows that melatonin inhibited the LPS-binding protein–CD14–TLR4 signaling pathway in bMECs, which had opposing effects on pro-inflammatory and anti-inflammatory mediators. Melatonin decreased LPS-induced expression of pro-inflammatory cytokines, chemokines, and positive acute-phase proteins (APPs), including *tumor necrosis factor-a, interleukin (IL)-1β, IL-6, granulocyte-monocyte colony-stimulating factor, chemokine CC motif ligand (CCL)2, CCL5, serum amyloid A, haptoglobin, C-reactive protein, ceruloplasmin, and <i>a-1 antitrypsin*, and increased expression of the anti-inflammatory cytokine *IL-1Ra* and the negative APP *fibrinogen*. In addition, melatonin increased dityrosine levels but suppressed nitrite levels by upregulating the expression of *nuclear factor E2-related factor (Nrf2)* and *heme oxygenase-1* in the Nrf2 antioxidant defense pathway. Finally, melatonin administration increased the viability of LPS-stimulated bMECs. The results confirm the hypothesis that melatonin can protect the bMECs from the LPS-induced cell damage.

3. Protective effect of melatonin on LPS-stimulated granulosa cells in the Japanese quail

To evaluate the potential of melatonin to protect cultured granulosa cells from the harmful effects of LPS in the Japanese quail. Granulosa cells isolated from the Japanese quails were pretreated with or without melatonin (10 or 100 μ g/mL) for 12 h and then incubated for 12 h in the absence or presence of 100 ng/mL LPS. Beneficial effects were observed when melatonin was administered to LPS-stimulated

cultured granulosa cells of the Japanese quail. Melatonin decreased LPS-induced expression of *IL-1\beta*, *IL-6*, *IL-8*, and suppressed the nitrite level. On the contrary, melatonin increased the dityrosine level. In addition, melatonin administration increased the viability of LPS-stimulated granulosa cells *in vitro*. These results suggest that melatonin protects cultured granulosa cells from LPS-induced inflammatory and oxidative stress damage and provide evidence that melatonin might have therapeutic utility in ovarian follicle infection in the Japanese quail.

4. Melatonin does not affect progesterone basal secretion but suppresses the luteinizing hormone receptor expression in granulosa cells of the Japanese quail

Whether exposure of granulosa cells of the Japanese quail to melatonin would create changes in progesterone production was determined. For in vitro experiments, granulosa cells were isolated from pre-ovulatory follicles (F1–F3) when the F1 follicles were predicted to be either immature or mature (at 3-6 or 18-21 h after oviposition, respectively). Granulosa cells were cultured for 12 h with or without melatonin concentration gradients of 0.0001-100 µg/mL, thereby averting luteinizing hormone (LH) stimulation. It was found that melatonin receptor subtypes (Mel-1a, 1b, and 1c) were expressed in the granulosa cells of the pre-ovulatory F1 follicles. Melatonin suppresses the LHCGR mRNA expression at low concentrations in granulosa cells of F1 follicles but does not affect the basal secretion of progesterone in cultured granulosa cells of the F1-F3 follicles. For the in vivo experiment, quails received intraperitoneal injection of melatonin (0.67 mg/kg body weight) or mock-vehicle at 3 or 18 h after oviposition, respectively. The birds were decapitated to collect serum 3 h later (at 6 or 21 h after oviposition, respectively). Results shows that melatonin treatment at a low concentration has no influence on the serum progesterone concentration at 6 h post-oviposition, but suppresses progesterone level 21 h after oviposition in the Japanese quail. These results demonstrated that only the low melatonin concentrations had negative effects on progesterone production of the Japanese quail; with the drastically exceeding physiological melatonin used in the anti-inflammatory experiment, no harmful effects were detected under the present situation.

5. General summary

Hypertonic melatonin concentrations protected the bMECs and granulosa cells from the LPS-induced cell damage, and had no harmful effects on progesterone production of the Japanese quail. These findings add some new information to develop succedanea of antibiotics, and therefore may contribute to the improvement of livestock industry and the public health.