博 士 論 文 の 内 容 の 要 旨

氏名	Islam Md Aminul
学位名	博士（医工学）
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論文題目	Study on the effect of heat stress on mammary epithelial cells and its prevention （暑熱ストレスが乳腺上皮細胞に及ぼす影響とその予防に関する研究）

（博士論文の内容の要旨）

Global warming negatively influences productive parameters of dairy cattle including milk yield, milk composition, growth, and reproduction. Heat stress (HS) is a major environmental factor raising dairy cows in the Tropical and Subtropical countries of the world. It is traditionally believed that HS results in lower dry matter intake (DMI), which reduces the production of milk yield and protein contents of milk in dairy cows. However, several studies have reported that the decrease in milk production in HS accounts for 35% only by reduced feed intake. These results suggest that factors other than a reduction in energy intake may be responsible for reduced milk production, but the other factors and their mechanisms are unknown. The number of mammary epithelial cells (MECs) and their secretory activity determine the amount of milk produced during a lactation period. The secretory capacity of MECs also depends on the formation of tight junction (TJ) in mammary gland. However, the effect of HS on the number of MECs and TJ formation is still unknown. To solve this question, this study focused on unfolded protein response (UPR) activated under the endoplasmic reticulum (ER) stress condition, since accumulating evidence indicated that ER stress-mediated apoptotic cell death plays a critical role in HS-induced cellular damage. Therefore, the purpose of present study was to find out how different level of temperature affects TJ formation and viability of MEC based on the UPR signaling and its prevention.

To achieve the above objectives, MECs were culture at 39℃ temperatures (considered as mild-HS) or at ≥ 41º C (considered as severe-HS). Severe HS (41℃), but not 39℃, significantly increased the transcript levels of C/EBP homologous protein (*Chop*), activating transcription factor 4 (*Atf4*), activating transcription factor 6 (*Atf6*) and glucose regulated protein 78 (*Grp78*). Moreover, severe HS significantly down regulated the TJ protein encoding gene, *Cldn3* mRNA levels compared with the control. To measure the effect of different level of temperature on the viability of MECs, I performed 3-(4, 5-Dimethylthiazol 2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Only 41℃temperature significantly reduced the viability of MEC. Therefore, severe HS (41℃) induces all UPR component genes to cause severe ER stress, subsequently reduces MEC viability and decreased the TJ protein encoding gene expression. On the other hand, mild HS (39℃) increased only *Xbp1s* expression among all UPR component genes. Moreover, the *β-casein* (a representative milk protein) and TJ protein encoding gene (*Zo-1*, *Ocln* and *Cldn3*) expression were significantly increased in 39 °C heat treatment cells. To explore the mechanism of TJ formation by mild HS (39℃), I examined the expression level of temperature-sensitive transient receptor potential vanilloid 4 (*Trpv4*). The *Trpv4* mRNA level was significantly higher in mild HS (39℃) treatment cells. Moreover, the mRNA levels of *Zo-1*, *Ocln*, *Cldn3* and *β-casein* were significantly increased in response to the addition of GSK1016790A, which is a selective *TRPV4* channel agonist. Therefore, mild HS (39℃) regulates the TJ formation in MECs via cell membrane receptor TRPV4. In conclusion, the results indicate that mild heat stress induces the transcriptional level of *Xbp1s* via TRPV4 activity, which enhances the expression of the *β-casein* and TJ protein-encoding genes. On the other hand, severe heat stress induces the transcriptional level of *Chop*, which reduces the cell viability. It is speculated that the difference in UPR gene expression upon stimulation at 39 °C vs. 41 °C controls cell survival vs. cell death.

Based on the harmful effect of severe HS, an effective supplementation strategy is essential to maintain the cell population as well as adequate amount of milk production. 5-Aminolevulinic acid (5-ALA) is a natural non-alpha amino acid, which can reduce nephrotoxicity and apoptosis in murine

tubular epithelial cells. Therefore, I investigated the possible protective effects of 5-ALA against HS-induced damage to bovine MECs. Pretreatment with 5-ALA significantly increased the viability of MAC-T cells (bovine MECs line) and inhibited severe HS-induced ER stress-associated markers, *GRP78* and *CHOP* expression levels. These results indicate that 5-ALA can ameliorate the HS induced ER stress and raise the cell viability.

In summary, the present study indicates that ER stress level is determined by severity of HS. Severe HS induces the ER stress with upregulation of all UPR component genes. In particular, severe HS enhances the expression of proapoptotic marker CHOP, which plays important role in reducing MEC viability. On the other hand, mild HS enhances only the expression of XBP1, which regulates the TJ formation. Moreover, it is found that 5-ALA can ameliorate the ER stress in heat stressed bovine MECs. Although detailed examination using dairy cows is necessary in the future, one of the factors causing the decrease in milk production through heat stress could be MECs death arising from ER stress. Moreover, 5-ALA is expected as a feed additive to control the HS in dairy cows.