Evaluation of stress response using saliva sample in adult male mouse

Abstract of the Doctoral Thesis

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Saliva is recognized as low-invasive sample for detection of physiologically active substances instead of plasma or serum. In order to clarify the whether salivary corticosterone is available to evaluate stress response in mice, first of all, we confirmed that salivary corticosterone was detected by enzyme immunoassay and that glucocorticoids transferred from blood to saliva in mice treated with exogenous cortisol (2.0 mg/kg intraperitoneally injection) in chapter 2. Next, we compared the effects of two different types of anesthetic agents (mixed anesthetic agents and pentobarbital) on salivary secretion, and compared corticosterone levels between plasma and saliva in response to restraint stress (60 min) with or without anesthesia. Additionally, we examined effect of anticancer drug, 50 mg/kg intraperitoneally injection of cyclophosphamide, on salivary secretion in chapter 3. Finally, we evaluated the adequate recovery period (1, 3, 5 and 7 days) from anesthesia for saliva collection between pre-stress and post-stress in the mouse of same individual and also examine salivary corticosterone and amylase activity in chapter 4. As a results of this investigation, corticosterone was detected in saliva by enzyme immunoassay and the cortisol was detected in plasma and saliva in the mouse treated with cortisol (chapter 2). Salivary corticosterone levels and volume of saliva were not affected by two different type of anesthetic agents (mixed anesthetic agents and pentobarbital). However, salivary protein levels in mouse treated with mixed anesthetic agents were significantly lower than the levels in mouse treated with pentobarbital. The plasma corticosterone levels of restraint (60 min) group were significantly higher than the levels of the non-stress control group with or without anesthesia. The salivary corticosterone levels of the restraint group were also significantly higher than the levels of the control group. Additionally, cyclophosphamide treated group did not show the significant increase of salivary corticosterone levels and did not show the significant differences in the volume of secreted saliva and in salivary protein levels compared to the vehicle control group (chapter 3). In the investigation for the adequate recovery period (1, 3, 5 and 7 days) from anesthesia for saliva collection, restraint stress significantly increased salivary

corticosterone levels in all four groups. On the other hand, the statistical evidence of corticosterone increase is more rigorous in a 7-day recovery group ($p < 0.001$) than the others ($p < 0.05$). Moreover, salivary amylase activities were shown significant increase in 3- and 7-day recovery groups by restraint stress, but not in 1- and 5-day recovery groups. In conclusion, we suggest that the collected saliva is available for enzyme immunoassay of corticosterone and that the salivary corticosterone levels reflect the plasma corticosterone levels, and it will be a useful less-invasive biomarker of physical stress in mice. Moreover, to evaluate restraint stress by the salivary corticosterone level and amylase activity in mice under anesthesia, adequate recovery period from anesthesia is preferable for a week. Additionally, the present study may contribute to concepts of Reduction and Refinement of the 3Rs in small animal experiments.