7-2.

Usefulness of human factor-based chromogenic substrate assay for evaluation of FVIII-equivalent activity of emicizumab

（大学院博士課程 3 年臨床検査医学科）
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【Background】 Emicizumab is a bispecific antibody mimicking activated factor VIII（FVIII）and increasingly used in prophylaxis against bleeding in hemophilia A. The activated partial thromboplastin time（aPTT）is markedly shortened in patients treated with emicizumab, highlighting the need for an appropriate and easy-to-access test for emicizumab.

【Objectives】 To assess the usefulness of the human-factor based chromogenic substrate assay（hCSA）with reference to the thrombin generation assay（TGA）, the standard coagulation assay, for measurement of emicizumab.

【Methods】 The coagulability of spiked emicizumab and recombinant FVIII（rFVIII）was measured and compared with TGA using extrinsic and intrinsic pathway triggers, hCSA, one-stage clotting assay（OSA）, and clot waveform analysis（CWA）. We also investigated the combined effect of 340 nM emicizumab and rFVIII using the same assays.

【Results】 The peak thrombin by TGA, FXa generation by hCSA and ad|min1| by CWA consistently showed linear relationship between emicizumab and FVIII at wide range of emicizumab concentrations. FVIII-equivalent activity of emicizumab in hCSA can be approximated by halving that in TGA triggered by the extrinsic pathway reagent（27.3 IU/dL vs. 13.9 IU/dL）under steady-state（340 nM）of emicizumab. Both in TGA and hCSA, the additive effect of added FVIII on therapeutic emicizumab concentration（340 nM）was maintained at low levels of FVIII but gradually decreased at higher levels.

【Conclusions】 FVIII-equivalent activity of emicizumab in hCSA can be easily approximated to TGA, and the additive effect of FVIII on emicizumab is diminished at high concentrations. Further studies are needed to determine the clinical applicability of hCSA.

7-3.

Circadian clock gene BMAL1 positively correlates with genes regulating steroid biosynthesis in human granulosa cells

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【Introduction】 Circadian clocks have an essential role in the daily physiological regulation. The transcription factor brain and muscle arnt-like protein-1（BMAL1）is a principal driver of a molecular clock in mammals. Importantly, disruption in circadian clock function have been implicated in reproductive physiology, including ovulation, embryonic implantation and parturition. Bmal1 knockout mice were found to have significantly reduced ovulation and be infertile. Furthermore, granulosa cells proliferation, luteinization and steroid biosynthesis are tightly related to oocyte maturation and follicular development. In this study, we analyzed the circadian clock gene expression and its effect on genes regulating steroid biosynthesis in human granulosa cells.

【Methods】 We analyzed BMAL1 and genes regulating steroid biosynthesis in human granulosa cells using RT-qPCR. Small interfering RNA was used to elucidate the activity and function of BMAL1 in KGN, a steroidogenic human ovarian granulosa cell line.

【Results】 We demonstrated that expression of BMAL1 positively correlates genes regulating steroid biosynthesis in human granulosa cells. We found that circadian clock genes exhibited rhythmic change and were further enhanced by dexamethasone synchronization in granulosa cells.