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ORIGINAL

Urotensinergic system genes in experimental subarachnoid hemorrhage

M.Á. Muñoz-Sánchez[a], **A. Rodríguez-Rodríguez**[b,*], **J.J. Egea-Guerrero**[b], **E. Gordillo-Escobar**[b], **Á. Vilches-Arenas**[c], **A. Carrillo-Vico**[d,e], **J.M. Guerrero**[d,e], **F. Murillo-Cabezas**[b]

[a] *Servicio de Urgencias, Hospital Universitario Virgen del Rocío, IBIS/CSIC/Universidad de Sevilla, Spain*
[b] *Cuidados Críticos, Hospital Universitario Virgen del Rocío, IBIS/CSIC/Universidad de Sevilla, Spain*
[c] *Servicio de Medicina Preventiva y Salud Pública, Hospital Virgen Macarena, Universidad de Sevilla, Spain*
[d] *Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Spain*
[e] *Departamento de Bioquimica Médica, Biología molecular e Inmunología, Facultad de Medicina, Universidad de Sevilla, Spain*

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KEYWORDS
Subarachnoid hemorrhage;
Experimental model;
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Abstract
Objective: Cerebral vasospasm, one of the main complications of subarachnoid hemorrhage (SAH), is characterized by arterial constriction and mainly occurs from day 4 until the second week after the event. Urotensin-II (U-II) has been described as the most potent vasoconstrictor peptide in mammals. An analysis is made of the serum U-II concentrations and mRNA expression levels of U-II, urotensin related peptide (URP) and urotensin receptor (UT) genes in an experimental murine model of SAH.
Design: An experimental study was carried out.
Setting: Experimental operating room of the Biomedicine Institute of Seville (IBiS), Virgen del Rocío University Hospital (Seville, Spain).
Participants: 96 Wistar rats: 74 SAH and 22 sham intervention animals.
Interventions: Day 1: blood sampling, followed by the percutaneous injection of 100 μl saline (sham) or blood (SAH) into the subarachnoid space. Day 5: blood sampling, followed by sacrifice of the animals.
Main variables of interest: Weight, early mortality, serum U-II levels, mRNA values for U-II, URP and UT.
Results: Serum U-II levels increased in the SAH group from day 1 (0.62 pg/mL [IQR 0.36–1.08]) to day 5 (0.74 pg/mL [IQR 0.39–1.43]) ($p < 0.05$), though not in the sham group (0.56 pg/mL

* Corresponding author.
E-mail addresses: rodriguezana13m@gmail.com, swroder_an@hotmail.com (A. Rodríguez-Rodríguez).

day 1; 0.37 pg/mL [IQR 0.23–0.62] day 5; $p = 0.959$). Between-group differences were found on day 5 ($p < 0.05$). The ROC analysis showed that the day 5 serum U-II levels (AUC = 0.691), URP mRNA (AUC = 0.706) and UT mRNA (AUC = 0.713) could discriminate between sham and SAH rats. The normal serum U-II concentration range in rats was 0.56 pg/mL (IQR 0.06–0.83).
Conclusion: The urotensinergic system is upregulated on day 5 in an experimental model of SAH.

PALABRAS CLAVE
Hemorragia subaracnoidea;
Modelo experimental;
Biomarcadores;
Urotensina;
Sistema urotensinérgico

El sistema urotensinérgico en un modelo experimental de hemorragia subaracnoidea

Resumen
Objetivo: El vasoespasmo cerebral, una de las principales complicaciones secundarias a hemorragia subaracnoidea (HSA), se caracteriza por una constricción arterial que tiene lugar principalmente entre el día 4 y la segunda semana. La urotensina-II (U-II) ha sido definida como el péptido con mayor capacidad vasoconstrictora en mamíferos. Quisimos analizar los niveles séricos de U-II, así como los niveles de expresión de los genes de U-II, péptido relacionado con urotensina y receptor de urotensina, en un modelo murino experimental de HSA.
Diseño: Estudio experimental.
Ámbito: Quirófano experimental del Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío.
Participantes: Noventa y seis ratas Wistar: 74 con inyección percutánea de sangre (HSA), 22 con inyección percutánea de 100 μL de salino (Sham).
Intervenciones: Día 1: extracción de muestras de sangre. Posteriormente, inyección percutánea de 100 μL de salino (Sham) o de sangre (HSA) en el espacio subaracnoideo. Día 5: extracción de muestras de sangre y sacrificio del animal.
Principales variables de interés: Peso, mortalidad precoz, niveles séricos de U-II, valores de ARNm de U-II, péptido relacionado con urotensina y receptor de urotensina.
Resultados: Observamos un incremento en los niveles de U-II sérica en el grupo HSA desde el día 1 (0,62 pg/mL [RI 0,36-1,08]) al día 5 (0,74 pg/mL [RI 0,39-1,43]) ($p < 0,05$); pero no observamos tal diferencia en el grupo Sham (0,56 pg/mL [RI 0,06-0,83] día 1; 0,37 pg/mL [RI 0,23-0,62] día 5) ($p = 0,959$). Se encontraron diferencias en los niveles de U-II entre ambos grupos al quinto día ($p < 0,05$). El análisis de curvas ROC demostró que la U-II sérica al quinto día (AUC = 0,691), ARNm de péptido relacionado con urotensina (AUC = 0,706) y ARNm de receptor de urotensina (AUC = 0,713) podían discriminar entre ratas Sham y HSA. Además, definimos un rango de normalidad para los niveles de U-II séricos en ratas: 0,56 pg/mL (RI 0,06-0,83).
Conclusión: Este estudio demuestra por primera vez que el sistema urotensinérgico ve incrementada su expresión en el quinto día en un modelo de HSA.

Introduction

Spontaneous subarachnoid hemorrhage (SAH) has an annual incidence rate of 4–28 cases per 100,000 people.[1,2] SAH accounts for about 80% of all nontraumatic extravasated bleeding into the subarachnoid space, 5% of stroke deaths and over a quarter of potential life years lost due to stroke.[1,3,4] Approximately 15% of SAH patients die after aneurysmal rupture. Another 25–50% die within a month of the bleeding. Of those who survive, 40% present disabling sequelae.[5,6] It is estimated that cerebral vasospasm (CVS) is responsible for neurological deterioration, and even death, in 15–20% of patients with SAH.[5,7] CVS is characterized by diffuse and long-lasting arterial constriction. Several vasoconstrictor proteins have been shown to contribute to this narrowing process.[8–11] However, Urotensin-II (U-II), defined as the most potent vasoconstrictor peptide in mammals according to Ames et al. research study, is not among these biomarkers.[12]

U-II is an 11-amino-acid peptide with a cysteine disulfide bond, derived from the polypeptide precursor known as prepro-urotensin-II (preproU-II).[13] Proteolytic cleavage of the C-terminal fragment from this precursor is required for biological activity. The result of this proteolysis is an undecapeptide with a cyclic hexapeptide sequence, fundamental for this hormonal action.[14] Once the active form of the peptide is generated, U-II mediates its biological action by interacting with a specific plasma membrane G-protein coupled receptor identified as GRP14, or UT.[12] This receptor binds different U-II sequences, including the U-II