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QUALITATIVE AND QUANTITATIVE ANALYSES OF SMN 2 GENE EXPRESSION UPON EXPRESSION WITH HISTONE DE-ACETYLASE INHIBITORS AND POLYPHENOLS

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TAJUK: "QUALITATIVE AND QUANTITATIVE ANALYSES OF SMN2 GENE EXPRESSION UPON EXPOSURE WITH HISTONE DE-ACETYLASE INHIBITORS AND POLYPHENOLS"

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A	PROJECT DETAILS
i	Title of Research: **Qualitative and Quantitative Analyses Of SMN 2 Gene Expression Upon Exposure with Histone De-acetylase Inhibitors and Polyphenols**
ii	Account Number:
iii	Name of Research Leader: Dr. Teguh Haryo Sasongko
iv	Name of Co-Researcher: 1. Prof. Dr. Zabidi Azhar Mohd. Hussin 2. Assoc. Prof. Dr. Salmi Abd. Razak 3. Prof. Dr. Zilfalil Alwi
v	Duration of this research: a) Start Date : 1 October 2010 b) Completion Date : 30 September 2013 c) Duration : 36 months + extension of 6 months d) Revised Date (*if any*) : Extended to 30 June 2014
B	ABSTRACT OF RESEARCH
	(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English. This abstract will be included in the Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large) ***English*** Autosomal recessive SMA (Spinal Muscular Atrophy) is the second most common inherited disease, leading to early infancy death. The SMN1 mutations are leading cause of SMA. However, increasing expression of SMN2 has been exploited as therapeutic target for SMA. Several Histone Deacetylase Inhibitors (HDACIs) are known to increase SMN2 expression level. This study aimed to elucidate the effects of two hydroxamate-based HDACIs, SAHA and Dacinostat, and SRT1720, a synthetic SIRT1 activator with polyphenolic structure, on the SMN2 expression, CpG Islands (CGI) methylation and

SMN protein levels in fibroblasts taken from SMA Type I and Type II patients. It was found that the levels of overall SMN2 gene expression (Overall-SMN2), SMN2 exon 7 inclusion (E7-SMN2) and SMN protein levels were significantly increased in 10 μM SAHA-treated Type I and Type II cells. The mean methylation levels was significantly lower. Accordingly, the level of Overall-SMN2 and E7-SMN2 transcript increase were also significant. The transcript increase induced by Dacinostat led to more increase of SMN protein compared to SAHA in Type I cells (2.54 ± 1.57 fold). SMN2 gene expression (Overall-SMN2 and E7-SMN2) was also increased upon exposure to SRT1720. The mean CGIs methylation percentage and SMN protein level alteration were decreased modestly. SAHA-Dacinostat combination increased SMN2 expression in Type I, but not Type II cells. SRT1720-Dacinostat combination increase Overall-SMN2 and E7-SMN2 transcripts nearly double the increase induced by individual compounds. SRT1720-SAHA combination resulted in lower increase than that induced by SAHA alone but higher increase than that induced by SRT1720 alone. Furthermore, SMN protein was noted to be increased and CGIs was more demethylated in treated cells. In conclusion, SMN2 expression (Overall-SMN2 and E7-SMN2), SMN protein level and methylation level of CGIs were significantly altered upon exposure to SAHA, Dacinostat and SRT1720-Dacinostat combination in SMA fibroblast Type I and Type II. This is the first report about Dacinostat and SRT1720 effect on SMN2 modulation.

Bahasa Malaysia

Atrofi Muskular Spina (AMS) adalah penyakit keturunan kedua yang paling biasa berlaku. Ianya merupakan penyakit autosomal resesif dan boleh membawa kepada kematian awal bayi. Mutasi pada gen SMN1 merupakan faktor utama penyakit ini. Peningkatan ekspresi gen SMN2 telah dieksploitasikan sebagai sasaran rawatan terapeutik bagi AMS. Beberapa Histone Deacetylase Inhibitors (HDACIs) telah dikenalpasti dalam meningkatkan tahap ekspresi gen SMN2. Kajian ini bertujuan mejelaskan kesan hydroxamate-based HDACIs iaitu Dacinostat dan SAHA dan satu pengaktif SIRT1 yang memiliki struktur polifenolik iaitu SRT1720 ke atas tahap ekspressi SMN2, metilasi kepulauan CpG (CGI) dan paras protein SMN dalam fibroblas yang diambil daripada pesakit AMS jenis I dan jenis II. Tahap ekspresi keseluruhan SMN2 (Keseluruhan-SMN2), inklusi exon 7 gen SMN2 (E7-SMN2) dan paras protein SMN didapati telah meningkat dengan ketara dalam sel Jenis I dan II yang dirawat dengan 10 μM SAHA. Min tahap metilasi juga menunjukkan tahap yang jauh lebih rendah. Oleh itu, paras peningkatan transkripsi Keseluruhan-SMN2 dan E7-SMN2 juga signifikan. Peningkatan transkripsi yang disebabkan oleh Dacinostat telah membawa kepada peningkatan protein SMN yang lebih berbanding SAHA dalam sel Jenis I sel (2.54 ± 1.57 kali ganda). Ekspresi SMN2 (Keseluruhan-SMN2 dan E7-SMN2) juga meningkat apabila didedahkan kepada SRT1720. Walaubagaimanapun, min peratusan metilasi CGI dan pengubahan paras protein SMN telah menurun secara sederhana. Kombinasi SAHA-Dacinostat telah meningkatkan ekspresi SMN2 dalam sel Jenis I, tetapi tidak dalm sel Jenis II. Kombinasi SRT1720-Dacinostat telah meningkatkan transkripsi Keseluruhan-SMN2 dan E7-SMN2 hampir dua kali ganda disebabkan oleh komponen individu. Kombinasi SRT1720-SAHA mengakibatkan peningkatan yang lebih rendah berbanding peningkatan yang disebabkan oleh penggunaan SAHA semata-mata tetapi lebih tinggi berbanding peningkatan yang disebabkan oleh penggunaan SRT1720 semata-mata. Tambahan pula, protein SMN telah dikenalpasti meningkat dan CGI lebih banyak dinyahmetilasikan dalam sel-sel yang telah dirawat. Kesimpulannya, ekspresi SMN2 (Keseluruhan-SMN2 dan E7-SMN2), paras protein SMN dan tahap metilasi CGI telah diubah dengan ketara apabila didedahkan kepada SAHA, Dacinostat dan kombinasi SRT1720-Dacinostat dalam sel fibroblas Jenis I dan Jenis II dalam pesakit SMA. Ini adalah laporan pertama seumpamanya mengenai Dacinostat dan kesan SRT1720 pada modulasi SMN2.