USM SHORT-TERM PROJECT FINAL REPORT

FEBRUARY 2005

PROJECT TITLE:

ACUTE EFFECTS OF AMMONIA ON 'CITRULLINE - NO CYCLE ENZYMES', ARGINASE AND RELATED METABOLITES IN DIFFERENT REGIONS OF RAT BRAIN

NAME OF INVESTIGATOR:

Dr. Mummedy Swamy

CO-INVESTIGATOR:

En. Chandran Govindasamy

Department of Chemical pathology,

School of Medical Sciences,

USM

USM SHORT-TERM PROJECT FINAL REPORT

FEBRUARY 2005

PROJECT TITLE:

ACUTE EFFECTS OF AMMONIA ON 'CITRULLINE - NO CYCLE ENZYMES', ARGINASE AND RELATED METABOLITES IN DIFFERENT REGIONS OF RAT BRAIN

NAME OF INVESTIGATOR:

Dr. Mummedy Swamy

CO-INVESTIGATOR:

En. Chandran Govindasamy

Department of Chemical pathology,

School of Medical Sciences,

USM

Laporan Akhir Projek Peyelidikan [USM JP – 06]

Laporan Akhir Projek Peyelidikan [USM JP – 06]

BAHAGIAN PENYELIDIKAN & PEMBANGUNAN
CANSELORI
UNIVERSITI SAINS MALAYSIA

Laporan Akhir Projek Penyelidikan Jangka Pendek

1) Nama Penyelidik: **DR. MUMMEDY SWAMY**

.....

Nama Penyelidik-Penyelidik Lain (Jika berkaitan) : En. CHANDRAN GOVINDASAMY

.....

.....

.....

.....

.....

.....

.....

.....

.....

2) Pusat Pengajian/Pusat/Unit : Department of Chemical pathology,

School of Medical Sciences, Health Campus, USM

3) Tajuk Projek:

ACUTE EFFECTS OF AMMONIA ON "CITRULLINE - NO CYCLE ENZYMES ", ARGINASE AND RELATED METABOLITES IN DIFFERENT REGIONS OF RAT BRAIN.

4) (a) Penemuan Projek/Abstrak

(Perlu disediakan makluman di antara 100 – 200 perkataan di dalam Bahasa Malaysia dan Bahasa Inggeris. Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).

ACUTE EFFECTS OF AMMONIA ON 'CITRULLINE-NO CYCLE ENZYMES', ARGINASE AND RELATED METABOLITES IN DIFFERENT REGIONS OF RAT BRAIN

ABSTRACT

Nitric oxide (NO) is involved in many physiological and pathological processes in the brain. NO is synthesized from arginine by nitric oxide synthase (NOS) enzymes. Citrulline, which is formed as a by-product of the NOS reaction, can be recycled to arginine by successive actions of argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL) via the citrulline-NO cycle. Hyperammonemia is known to cause poorly understood perturbations of the citrulline-NO cycle. Both ASS and ASL genes are reported to be induced in astrocytes but not in neurons of aggregates exposed to 5 mM ammonium chloride, suggesting that hyperammonemic brain might increase its recycling of citrulline to arginine. To understand the role of citrulline-NO cycle in hyperammonemia, NOS, ASS, ASL and arginase activities, as well as nitrate/nitrite (NOx), the stable end products of NO, and other related metabolites were estimated in cerebral cortex (CC), cerebellum (CB) and brain stem (BS) of rats subjected to acute ammonia toxicity (0.8mmol of ammonium acetate per 100g body weight). NOx concentration and NOS activity were found to increase in all the regions of brain in acute ammonia toxicity. The activities of ASS (CC, CB and BS) and ASL (CC and CB) also showed an increase whereas the activity of arginase was not changed. The concentrations of arginine and ornithine were increased in all the regions of brain in acute ammonia

toxicity whereas citrulline concentration was not changed. Glutamine concentration was significantly increased in all regions of brain while glutamate and GABA concentrations were not changed. The results of this study clearly demonstrated the increased formation of NO, suggesting the involvement of NO in the pathophysiology of acute ammonia toxicity. The increased activities of ASS and ASL enzymes indicate the increased and effective recycling of citrulline to arginine in acute ammonia toxicity, making NO production more effective and contributing to its toxic effects.

AKUT KESAN AMMONIA TERHADAP 'ENZIM-ENZIM DALAM KITARAN CITRULLINE-NO', ARGINASE DAN LAIN-LAIN METABOLIT DALAM PELBAGAI BAHAGIAN OTAK TIKUS

ABSTRAK

Nitrik oksida (NO) terlibat dalam pelbagai proses fisiologi dan patologi di dalam otak. NO disintesiskan daripada arginine oleh enzim-enzim nitric oxide synthase (NOS). Citrulline yang terbentuk sebagai hasil sampingan daripada tindakbalas NOS, boleh dikitar semula kepada arginine oleh tindakbalas-tindakbalas argininosuccinate synthetase (ASS) dan argininosuccinate lyase (ASL) melalui kitaran citrulline-NO. Walau bagaimanapun, kesan keadaan ammonia yang tinggi terhadap kitaran citrulline-NO ini tidak dapat difahami dengan tepat. Dalam kajian yang lepas, kedua-dua gen ASS dan ASL didapati teruja dalam astrocytes, tetapi tidak dalam neurons, yang didedahkan kepada ammonium chloride pada kepekatan 5mM. Ini menunjukkan kandungan ammonia yang tinggi di dalam otak menyebabkan peningkatan pembentukan citrulline kepada arginine. Untuk memahami peranan kitaran citrulline-NO dalam keadaan ammonia yang tinggi, aktiviti-aktiviti NOS, ASS, ASL dan arginase serta kepekatan nitrat/nitrit (NOx) dan lain-lain metabolit dianggarkan di dalam korteks serebral, serebellum dan batang otak tikus yang telah dikenakan ketoksikan ammonia akut (0.8mmol ammonium acetate bagi setiap 100g berat badan). Kepekatan NOx dan aktiviti NOS didapati meningkat di dalam semua bahagian otak tikus dalam keadaan ketoksikan ammonia akut. Aktiviti-aktiviti ASS dan ASL juga menunjukkan peningkatan yang setara tetapi aktiviti arginase tidak berubah. Kepekatan arginine dan ornithine dalam pelbagai bahagian otak yang

mengalami ketoksikan ammonia akut menunjukkkan peningkatan tetapi tidak pada kepekatan citrulline. Kepekatan glutamine menunjukkan peningkatan yang ketara dalam semua bahagian otak tetapi kepekatan glutamate dan GABA tidak menunjukkan perubahan yang ketara. Keputusan yang didapati dari eksperimen ini jelas menunjukkan penghasilan NO yang tinggi ini menyumbangkan kepada penglibatan NO dalam patofisiologi ketoksikan ammonia akut. Peningkatan dalam aktiviti-aktiviti ASS dan ASL menunjukkan peningkatan dan keberkesanan kitaran semula citrulline kepada arginine dalam ketoksikan ammonia akut, juga menyebabkan penghasilan NO menjadi lebih berkesan dan seterusnya menyumbangkan kesan toksiknya.