THE POTENTIAL ROLE OF CALPAIN IN THE ZYMOSAN-INDUCED PAW INFLAMMATORY PAIN RATS

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Objective: To investigate the potential role of calpain in the spinal cord in the zymosan-induced paw inflammatory pain rats model.

Methods: (PartI): Forty-eight Sprague-Dawley rats were divided into three groups: 1) control rats (n=8), 2) sham surgery rats (n=16), 3) zymosan-induced paw inflammation rats (n=24). The group 3) is divided into three sub-groups according to killing time points. Mechanical withdrawal threshold was tested with von Frey filament and maximum thickness of paw was measured with calibrated micrometer at 0.5h,1h,2h,4h,8h,24h and 48h after zymosan injection, respectively. All rats were killed at different occasions following surgery to examine calpain activity, IkBα and nuclear NFkB express in the spinal dorsal horn with the means of western blot analysis. (Part II): Sixty-four Sprague-Dawley rats were divided into three groups: 1) sham surgery rats (n=16), 2) zymosan-induced paw inflammation rats with intraperitoneal DMSO treatment 30min before zymosan injection (n=24), and 3) zymosan-induced paw inflammation rats with intraperitoneal calpain inhibitor ALLN treatment 30min before zymosan injection (n=24). The group 2) and group 3) were divided into three sub-groups according to killing time points, respectively. Mechanical withdrawal threshold was tested with von Frey filament and maximum thickness of paw was measured with calibrated micrometer at 0.5h, 1h, 2h, 4h, 8h, 24h and 48h after zymosan injection, respectively. All rats were killed at different occasions following surgery to examine the COX-2 express in the spinal dorsal horn with western blot analysis.

Results: (PartI): Mechanical withdrawal threshold and maximum thickness of paw in the zymosan-induced paw imflammation rats significantly increased compared with control and sham rats (P<0.05, P<0.01). Calpain in the ipsilateral spinal dorsal horn was dramatically activated after zymosan injection, together with significant decreased express of IkBα and increased express of nuclear NFkB.(Fig 1-1 and Fig 1-2)

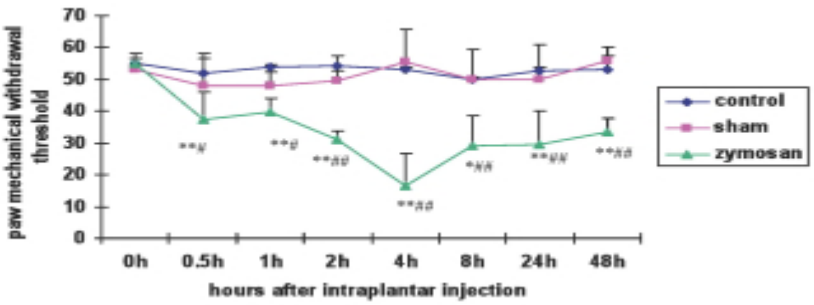

*Figure 1-1: Mechanical withdrawal threshold in response to the mechanical stimuli of the left hind paws of control, sham and zymosan group rats. Data presented as mean±SD, one-way ANOVA with Post Hoc tests. *P<0.05, **P<0.01, compared with control group. #P<0.05, ##P<0.01, compared with sham group.*

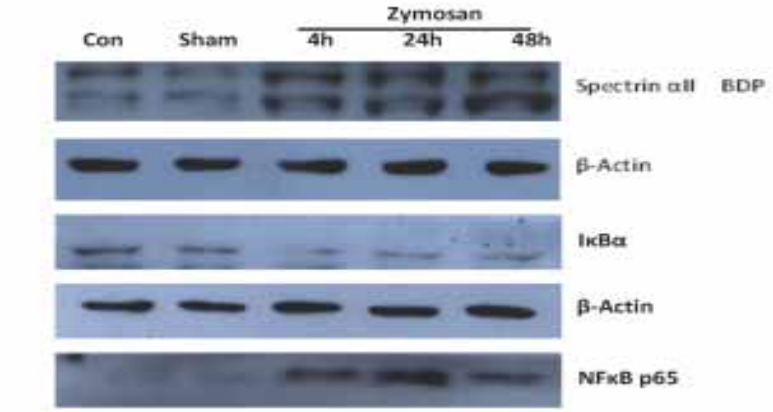

Figure 1-2: Western blot analysis of Spectrin all BDP, IkBα and nuclear NFkB p65 level in the ipsilateral lumbar spinal dorsal horn of rats after surgery. The Spectrin all BDP level significantly increased at 4h, 24h, 48h after zymosan injection. IkBα express significantly decreased and nuclear NFkB p65 express significantly increased at 4h, 24h, and 48h after zymosan injection.

(PartII): Intraperitoneal ALLN injection 30min before zymosan injection significantly reduced zymosan-induced mechanical withdrawal threshold and paw edema at 4h, 8h, 24h and 48h after zymosan injection compared with DMSO treatment. Meanwhile, calpain inhibitor ALLN treatment significantly decreased the COX-2 express in the spinal dorsal horn compared with DMSO treatment.(Fig 2-1 and Fig 2-2)

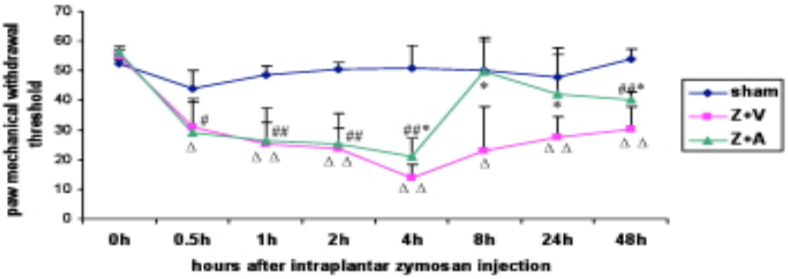

*Figure 2-1: Mechanical hyperalgesia induced by zymosan intraplantar injection is attenuated by the intraperitoneal inhibition of calpain. Treatment with calpain inhibitor ALLN starting 30min before zymosan injection significantly attenuates mechanical hyperalgesia induced by zymosan intraplantar injection at 4h, 8h, 24h and 48h. ΔP<0.05, ΔΔP<0.01, DMSO treated rats versus sham rats at the same time point. #P<0.05, ##P<0.01, ALLN treated rats versus sham rats at the same time point. *P<0.05, ALLN treated rats versus DMSO treated rats at the same time point.*

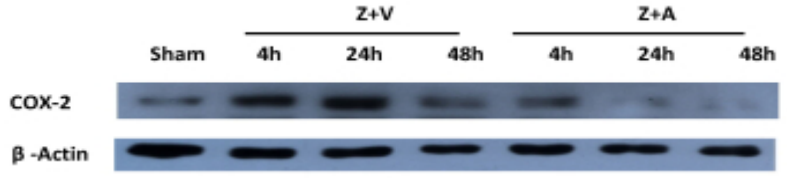

Figure 2-2: Western blot analysis of COX-s. Treatment with ALLN starting 30min before intraplantar zymosan injection decreases COX-2 expression at 4h, 24h and 48h in the spinal dorsal horn compared with treatment with DMSO at the same time point. Z+V: zymosan injection + DMSO treatment; Z+A: zymosan injection + ALLN treatment.

Conclusion: Calpain activation in the spinal dorsal horn maybe play a major role in the development of zymosan-induced inflammatory pain through degrading IkBα and activating NFkB. Administration of ALLN was effective to attenuate zymosan-induced paw inflammatory pain and decrease the COX-2 express in the spinal cord.

Summary: Calpain activation in the spinal dorsal horn maybe play a major role in the development of zymosan-induced inflammatory pain.