

척수 손상 백서에서 MK801 투여가 체성감각 유발전위 및 척수 조직에 미치는 효과

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= Abstract =

The Effect of MK801 on SSEP and Pathology in Chronic Spinal Cord Injured Rat

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Objectives : This study was undertaken to investigate the effect of noncompetitive NMDA receptor blocker, MK801 on motor recovery, SSEP and pathology in spinal cord injured rat.

Methods : The effects of MK801 on neuronal function protection, SSEP, and pathology were measured on spinal cord injury rats which were divided into 6 groups according to dose, time of drug delivery and magnitude of injury. Spinal cord injury was made with the magnitude of 25gm - cm and 50gm - cm on 42 rats. BBB locomotor function test was performed to evaluate the motor power recovery in hindlimb for 2 weeks after injury. After motor function test was completed, SSEP was measured. Amplitude and latency of the P1, N1 peak was measured and compared between groups. Finally rats were sacrificed, and pathologic findings including measurement of area of necrotic cord were studied and compared between groups.

Results : Motor recovery at 2 weeks was better in MK801 group comparing to saline control group. SSEP at 2 weeks showed no difference in N1, P1 latencies, but significantly greater amplitude in MK801 group, compared to saline control group. On light microscope, there was no specific histologic differences between experimental groups. The cystic necrotic area in coronal plane was measured and compared in each group. The necrotic area was significantly smaller in MK801 1mg/kg group (delivered after injury) than vehicle group. The necrotic area in MK801 5mg/kg group and MK801 1mg/kg group (delivered before injury) was smaller than vehicle group even though it was not statistically significant.

Conclusion : From the above result, it is speculated that NMDA blocker, MK801 can improve impaired neuronal function in spinal cord injury.

KEY WORDS : Spinal cord injury · NMDA receptor · MK801 · SSEP.

서 론

glutamate
26)

NMDA

가

MK801 가

¹¹⁾¹³⁾

NMDA

가 ¹²⁾¹⁵⁾¹⁷⁾²⁰⁾²¹⁾²⁸⁾ NMDA

가

⁴⁾ NMDA

가 BBB(blood - brain - barrier)

NMDA MK801 (< + > - 5 - methyl - 10,11 - di - hydro - 5H - dibenzo<a, d> cycloheptene - 5, 10 - immi - ne maleate, Sigma, Saint Louis, MO, U.S.A.) CNS - 1102

MK801 가 NMDA BBB

⁹⁾¹¹⁾²¹⁾

NMDA

⁹⁾¹⁰⁾¹¹⁾¹⁶⁾²¹⁾

MK801

MK801

재료 및 방법

1. 실험 동물 및 실험 설계

300 350gm Sprague - Da - wley 42 6
A D 25gm - cm E, F
50gm - cm 가 A
15 MK801 saline vehicle
, B 15 MK801(1mg/kg)
, C 15 MK801(5mg/kg)
, D 15 MK801(1mg/kg)
, E 15 MK801 saline
vehicle , F 15
MK - 801(1mg/kg)

2. 약물투여

NMDA MK - 801
10 bolus

3. 척수손상을 위한 1차 수술

Sodium pentobarbital(30mg/kg) 8, 9
NYU spinal cord dropping device(NY, NY, U.S.A.) clamp moderate injury
1 4 25mm , severe injury
5, 6 50mm 2mm, 10gm rod cord
compression parameter , ,
(compression rate - dD/dT)

4. 동물 처리 및 행동 검사

2 1, 4, 7, 10, 14
5 BBB locomotor rat - ing scale(Basso, Beattie, Bresnahan)
³⁾ BBB test
21 scale limb
paw

5. 척수 체성감각 유발전위 검사를 위한 2차 수술

2 pentobarbital(50mg/kg) (Model 683, Harvard Apparatus, South Natick, MA, U.S.A.)

4mm, 7mm bregma

6. 척수 체성감각 유발전위의 유발 및 기록

1.4mm 0.2mm 1.0mm

bregma 2mm, 2mm (A365D stimulus isolator, World Precision Instruments, Inc. New Haven, Connecticut, U.S.A.) (Pulsemaster A300, Inc. New Haven, Connecticut, U.S.A.) 0.1mAmp 9mAmp 1 4Hz 0.2msec 5mAmp Spike2 30 3000Hz 1000 10,000 80~100

7. 척수 조직병리 검사
가 urethane (30%, 3cc/kg, Sigma, Saint Louis, MO, U.S.A.) 4% paraformaldehyde 2cm central canal (coronal section) 1µm
CCD image analysis software (Im-agepro plus version 3.0, Media Cybernetics, U.S.A.)

8. 통계 분석

BBB ANOVA test Post Hoc Test (Turkey HSD)

결 과

1. 운동 검사 결과

Moderate injury BBB 가
7 MK801 1mg/kg vehicle

가 MK801 (2, 3, 4) vehicle MK801 1mg/kg (2, 4) MK801 5mg/kg (3) (Table 1, 2). Severe injury BBB 가 MK801 1mg/kg vehicle (Table 3, 4).

2. 체성 감각유발전위 변화

1) 정상 체성 감각 유발전위

positive deflection negative peak(N1) positive deflection (P1) N1 (peak latency) 28.8 ± 1.2msec, P1 45.8 ± 1.7msec N1 (peak amplitude) 44.9 ± 17.7 µv, P1 91.0 ± 34.9 µv (Fig. 1).

2) 척수 손상 및 약물 투여후 체성감각 유발전위의 변화

Moderate injury 2 9

Table 1. BBB test score in left lower extremity during behavioral recovery after moderate injury

	D1	D4	D7	D10	D14
Group A	0.8 ± 0.9	3.0 ± 1.6	5.2 ± 1.1	6.8 ± 1.2	8.6 ± 1.9
Group B	1.0 ± 0.9	3.8 ± 1.6	6.9 ± 1.8	9.6 ± 2.1*	13.1 ± 2.4*
Group C	0.7 ± 0.7	3.4 ± 1.1	6.4 ± 1.7	9.2 ± 1.6*	12.8 ± 2.3*
Group D	1.4 ± 0.9	3.4 ± 2.1	7.8 ± 2.2*	10.8 ± 2.0*	14.7 ± 2.2*

D : day BBB : Basso Beattie and Bresnahan Values are means ± S.D. Group A : control group Group B : MK801 1mg/kg, i.v., 15min after injury Group C : MK801 5mg/kg, i.v., 15min after injury Group D : MK801 1mg/kg, i.v. 15min before injury * : p < 0.05 vs Group A

Table 2. BBB test score in right lower extremity during behavioral recovery after moderate injury

	D1	D4	D7	D10	D14
Group A	0.8 ± 0.7	3.2 ± 1.4	5.2 ± 1.1	6.7 ± 1.6	9.4 ± 1.6
Group B	1.1 ± 0.7	3.4 ± 1.1	7.6 ± 1.7	10.3 ± 2.6*	14.0 ± 1.7*
Group C	0.8 ± 0.8	3.0 ± 1.4	7.0 ± 1.8	9.3 ± 2.0*	13.3 ± 1.9*
Group D	1.0 ± 0.8	3.6 ± 1.3	8.0 ± 1.4*	10.6 ± 1.7*	14.9 ± 1.7*

Legends are the same as in Table 1.

Table 3. BBB test score in left lower extremity during behavioral recovery after severe injury

	D1	D4	D7	D10	D14
Group E	0.3±0.5	1.5±1.3	3.0±0.8	4.3±0.9	5.5±0.6
Group F	0.6±0.6	1.8±0.9	3.8±1.0	5.3±1.0	7.3±0.5

D : day BBB : Basso Beattie and Bresnahan
 Values are means ± S.D.
 Group E : control group
 Group F : MK801 1mg/kg, i.v., 15min after injury

Table 4. BBB test score in right lower extremity during behavioral recovery after moderate injury

	D1	D4	D7	D10	D14
Group E	0.3±0.5	1.5±0.6	3.0±0.8	4.7±1.0	6.0±0.8
Group F	0.3±0.5	1.5±0.6	4.3±1.0	6.8±1.3	8.5±1.0

Legends are the same as in Table 3

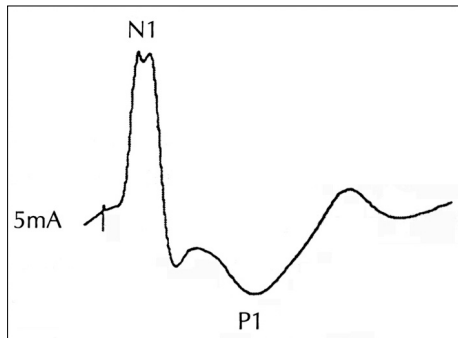


Fig. 1. Somatosensory evoked potentials(SSEP's) after laminectomy without spinal cord injury.

Table 5. SSEP 2 weeks after moderate injury

	Latency(msec)		Amplitude(μv)	
	N1	P1	N1	P1
Group A	30.5±2.9	47.8±3.9	1.2±0.6	1.6±0.7
Group B	30.8±2.2	48.5±1.6	8.4±3.5*	10.9±6.8*
Group C	29.6±2.1	46.4±3.0	4.0±3.1	5.3±3.2
Group D	31.4±1.5	48.9±3.4	7.7±3.9*	9.3±4.9*

1. Values are means ± S.D.
 2. Values are means ± S.D.
 * : p < 0.05 vs group A

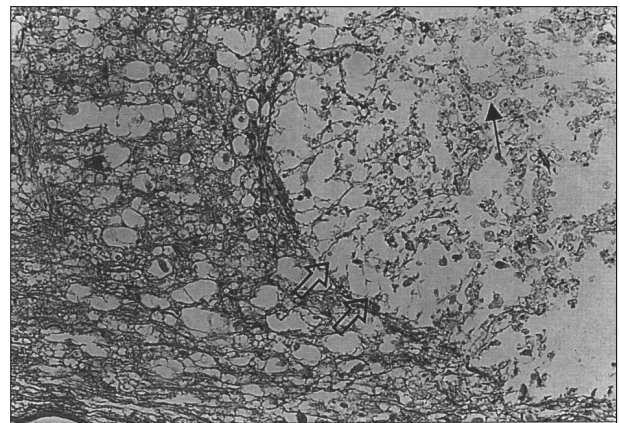


Fig. 2. Coronal section 2 weeks after moderate injury showing cystic necrosis() centered on impact area and there are capillary proliferations and infiltration of macrophages() around necrotic area(H-E stain, x40).

N1
 P1 가 가
 가 (Table 5).
 (1, 2, 3)
 vehicle
 1mg/kg vehicle
 가 가 (Table 5).
 Severe injury 2 3
 vehicle 3
 N1 P1 가
 MK801 1mg/kg 3 2
 가 N1 P1 가

3. 손상 척수의 조직 병리학적 변화

1) 병리 조직학적 관찰소견

Moderate injury

가
 가

가
 가 (Fig. 2).
 2) 종단면 척수에서의 손상 척수조직의 면적 비교
 vehicle 5.61 ± 2.27
 mm², MK801 1mg/kg 3.33 ± 1.43 mm²
 MK801 1mg/kg vehicle
 MK801 5mg/kg
 4.02 ± 1.45mm², MK801 1mg/kg
 4.25 ± 1.79mm² vehicle
 hicle MK801
 가 (Ta-
 ble 6).

고 찰

26)

Table 6. Necrotic cord area on coronal section 2weeks after moderate injury

	Necrotic cord area(mm ²)
Group A	5.61 ± 2.27
Group B	3.33 ± 1.43*
Group C	4.02 ± 1.45
Group D	4.25 ± 1.79

1. Values are Means ± S.D.

* : p < 0.05 vs Group A

Na, K, Ca, Mg

12) quisqualate, kainate NMDA, 3가 NMDA, kg

가 NMDA 가

Na Cl, K voltage dependent calcium conductance 5)26) NMDA (axon) (demyelination)

가 가 NMDA 23)

가 2)19)25) N1 P1 MK801

1933 averaging technique 가

14) 1947 8) fiber가 dorsal column medial le-spinocerebellar tract Severe injury moderate injury

sodium pump ATP 7)27) N1 P1 saline

0.1 9mAmp 0.1 0.5mAmp 가 가 MK801 1mg/kg (66%) N1 P1

가 가 5mAmp N1 (1, 2, 3) P1 saline 가 N1 P1 MK801 1mg/kg vehicle 가 가 MK801 1, 2, 3 MK801 1mg/kg 5mg/ kg 가 24) Pencalet 23) N1 P1 MK801 model 25gm - cm moderate degree 1933 MK801 가 MK801 saline 가 Severe injury moderate injury 2 MK801 N1 P1 saline 3 N1 P1 MK801 1mg/kg (66%) N1 P1 가 가

MK801 가
 MK801 가
 BBB saline MK801 가
 10 MK801 saline 가
 가 MK801 saline MK801 1mg
 가 MK801 5mg MK801 1mg
 Severe injury mod -
 erate injury saline MK801 MK801 1mg MK801
 MK801 가
 NMDA 가
 Olney NMDA 가
 chosis NMDA psy - NMDA MK801
 18) 가
 22) posterior cingulate cortex MK801 가
 retrosplenial cortex pyramidal neuron MK801 , MK801
 가
 1) pyramidal neuron vac -
 uole reaction reversible
 22) NMDA controversy가
 MK801 MK801 6
 5mg/kg 1mg/kg
 MK801 1) Moderate injury
 5mg/kg 가 vehicle
 가
 McIntosh 21) MK801 2) MK801 1mg/kg N1
 15 vehicle 가
 15 P1 가
 가 3) 2
 MK801 1mg/kg vehicle
 가 5mg/kg MK801 1mg/kg
 vehicle

4) Severe injury

가

MK801 1mg/kg

MK801

가

1mg/kg

5mg/kg

- : 1999 9 28
 - : 1999 11 10
 - :
138 - 736 388 - 1
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MK801 가

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