Neuroprotective effect of minocycline & estrogen after Spinal Cord Injury

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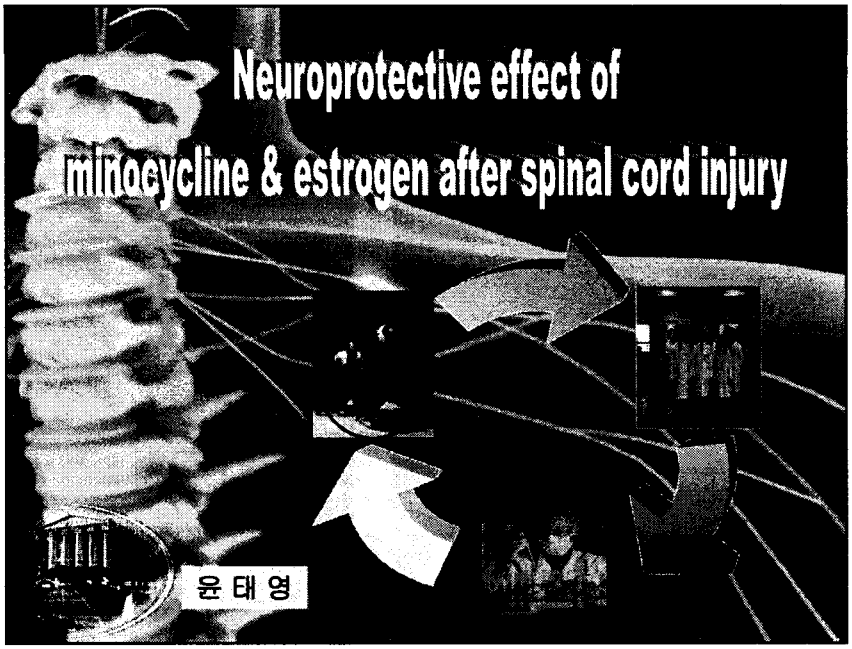
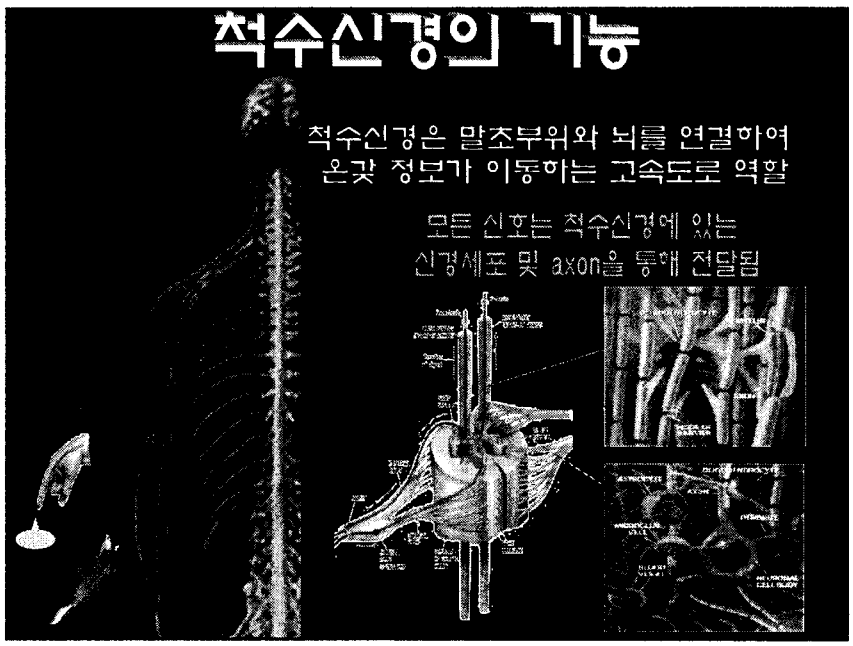
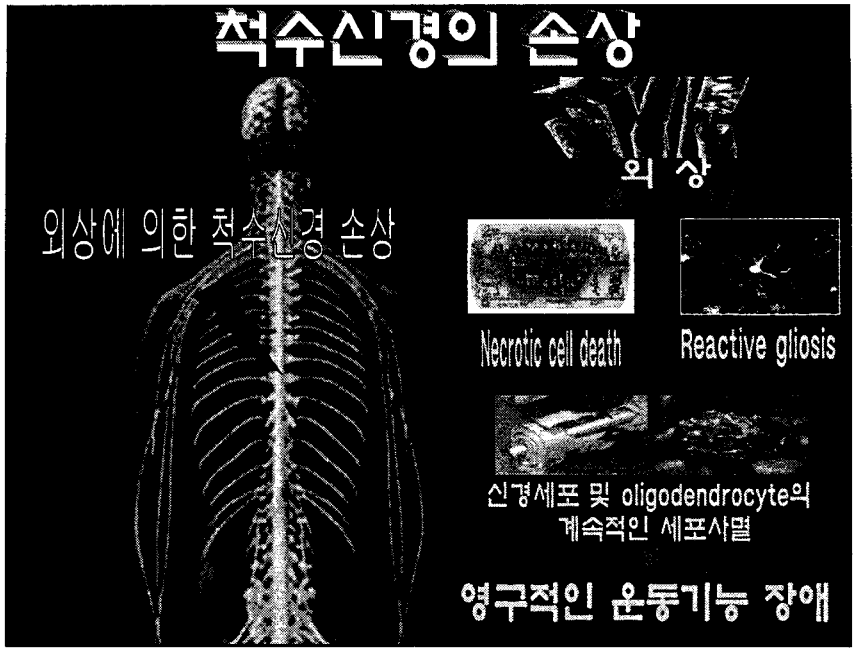
Traumatic spinal cord injury (SCI) initiates a complex series of cellular and molecular events that induce massive apoptotic cell death leading to permanent neurological deficits in human. However, the external apoptotic death signal(s) generated after SCI has not been identified. Furthermore, there is still no therapeutic agent effective for recovering motor functions after SCI in humans. Only one agent, methylprednisolone (MP), is currently being applied clinically to treat SCI. However, the clinical significance of recovery after MP treatment is unclear and must be considered in the light of potential adverse effects of its high-dose treatment. Although a number of potential pharmacological treatments have been investigated, other therapeutic agents designed to reduce cell death after SCI must be investigated.

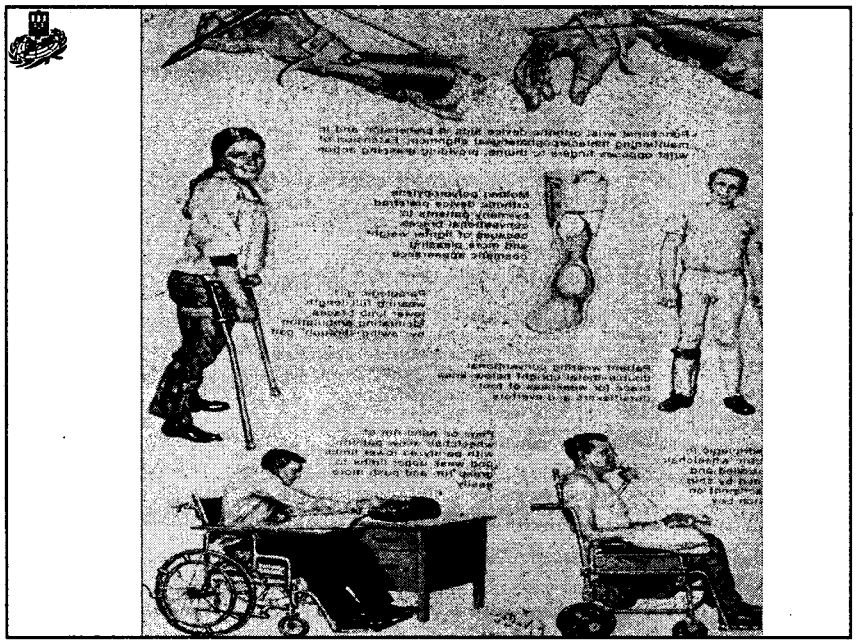
Previously we reported that TNF-α is served as an external signal initiating apoptosis in neurons and oligodendrocytes after SCI and the apoptotic cascade initiated by TNF-α may be mediated in part by NO via up-regulation of iNOS induced in response to TNF-α. We also found that anti-inflammatory drug, minocycline and 17β-estradiol was neuroprotective after SCI. Recently we also found that minocycline improves functional recovery after SCI in part by reducing apoptosis of oligodendrocytes via inhibition of proNGF production in microglia. Especially, p38MAPK was only activated in microglia, and minocycline treatment inhibited proNGF production by inhibition p38MAPK activation after SCI. Furthermore, minocycline treatment significantly inhibited $p75^{NTR}$ expression and $p75^{NTR}$-mediated apoptosis of oligodendrocytes, leading to inhibition of demyelination and axon degeneration as compared with vehicle control. We also found that 17β-estradiol significantly inhibits oligodendrocyte cell death after SCI. In addition, 17β-estradiol treatment attenuated JNK3 activation at delayed time after injury, which is involved in apoptosis of oligodendrocytes, leading to functional improvement. These data suggest that after SCI, minocycline and/or 17β-

estradiol treatment improved functional recovery in injured rat in part by regulating expression of proNGF, p75NTR or by inhibiting Rho-GTPase, JNK3 activation, which might be involved in apoptotic cell death of oligodendrocytes after SCI. The significance of the proposed research is that it could lead us to therapeutic interventions for preventing cell death thereby improving functional recovery after SCI. This research was supported in part by grants from the Korea MOST Neurobiology Research Program and Seoul City Research Fund (Creation of Geriatric Natural-MediCluster).

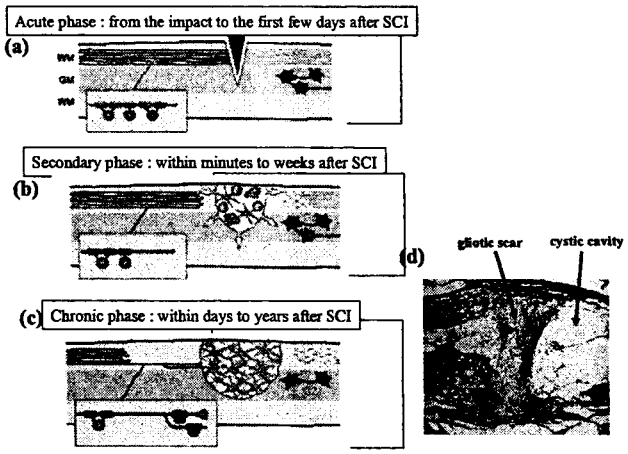

Neuroprotective effect of
minocycline & estrogen after spinal cord injury
윤 태 영


척수신경의 기능
척수신경은 말초부위와 뇌를 연결하여
온갖 정보가 이동하는 고속도로 역할
모든 신호는 척수신경에 있는
신경세포 및 axon을 통해 전달됨

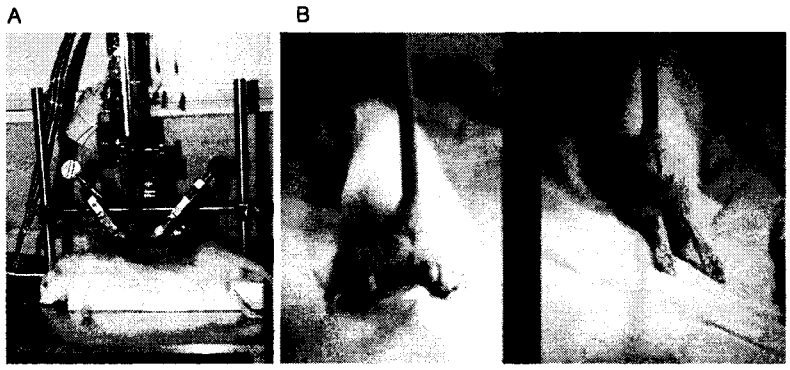

척수신경의 손상
외 상
외상에 의한 척수신경 손상
Necrotic cell death
Reactive gliosis
신경세포 및 oligodendrocyte의
계속적인 세포사멸
영구적인 운동기능 장애



Pathophysiology of SCI



NYU impactor



Minocycline

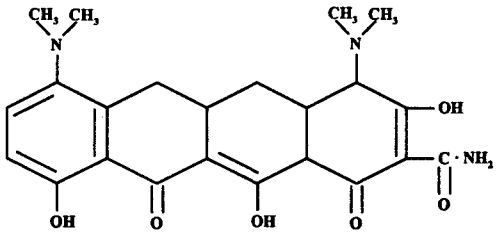


FIG. 2

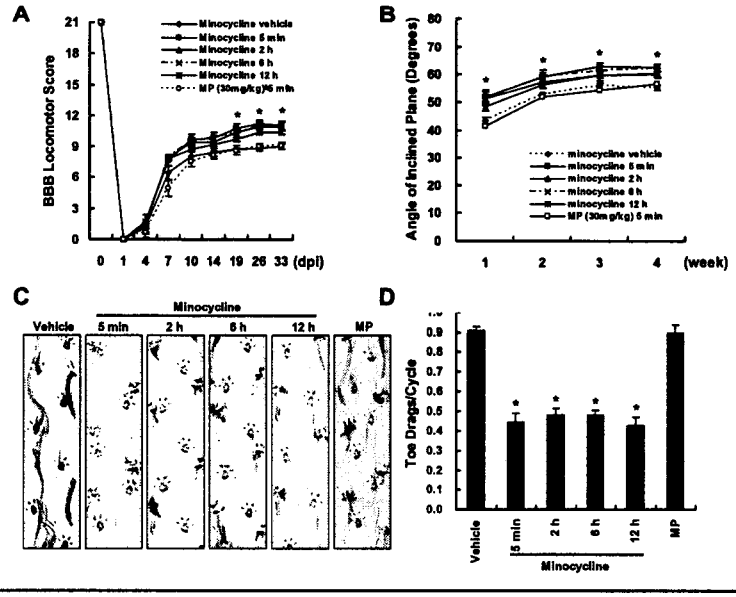
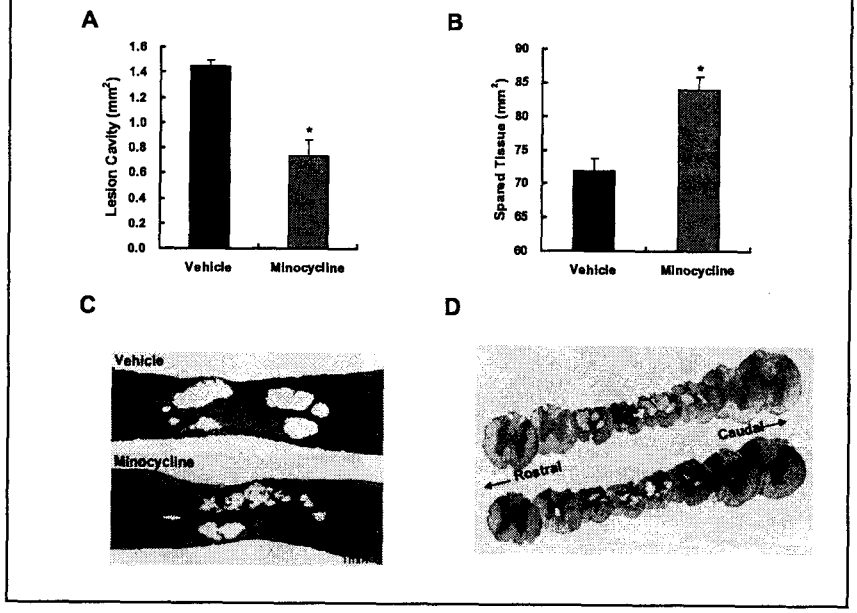
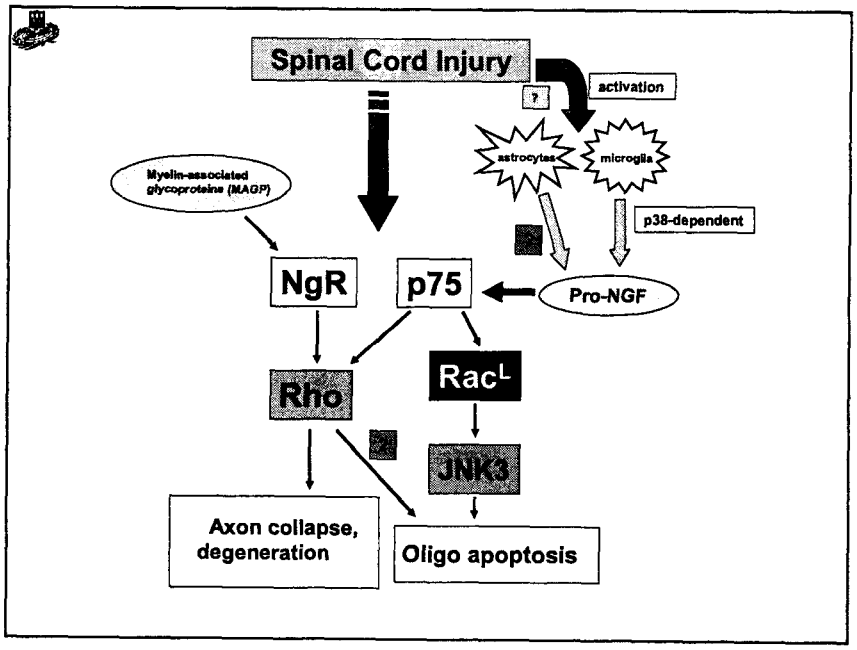
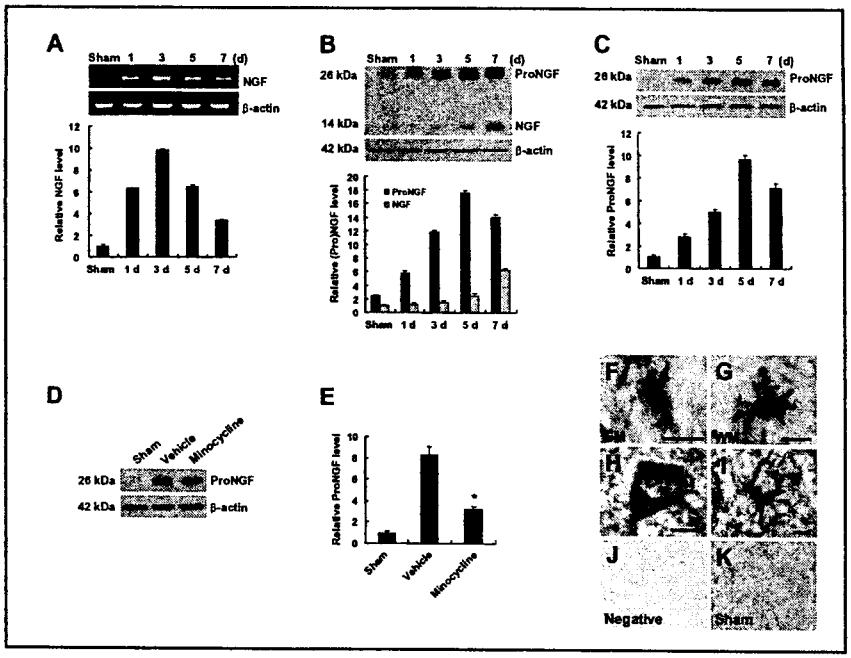
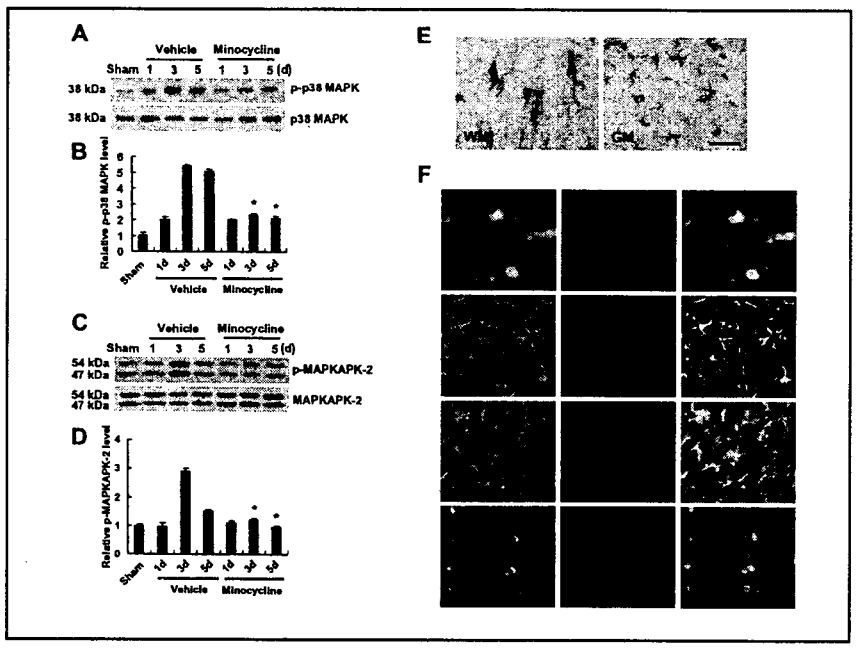


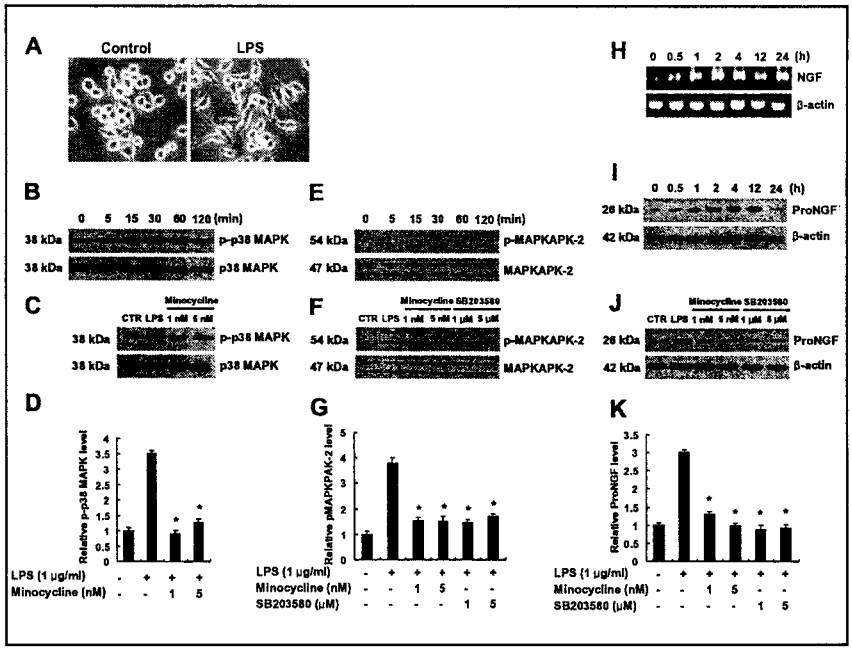
FIG. 3

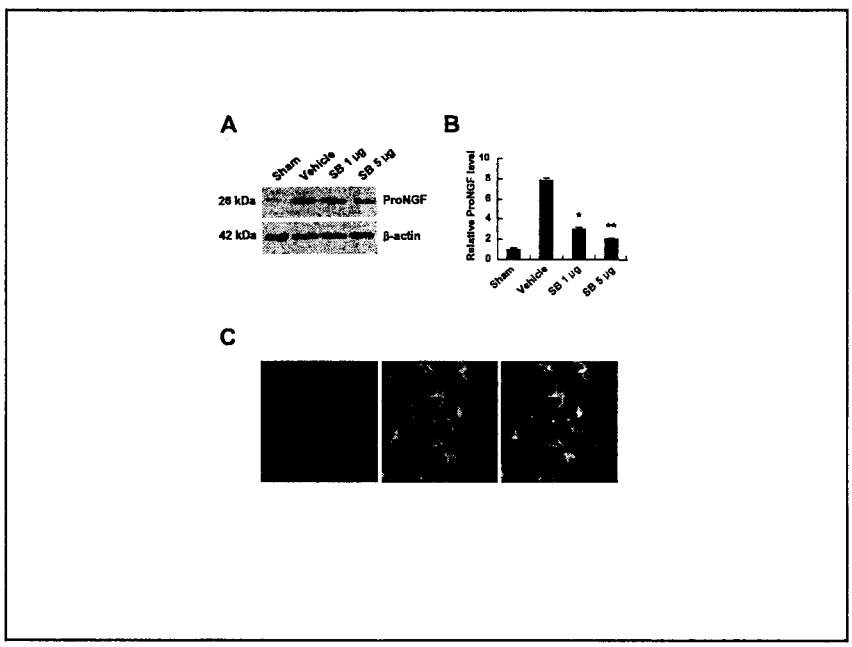

A
Lesion Cavity (mm²)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Vehicle
Minocycline
B
Spared Tissue (mm²)
90
85
80
75
70
65
60
*
Vehicle
Minocycline
C
Vehicle
Minocycline
D
Caudal
Rostral

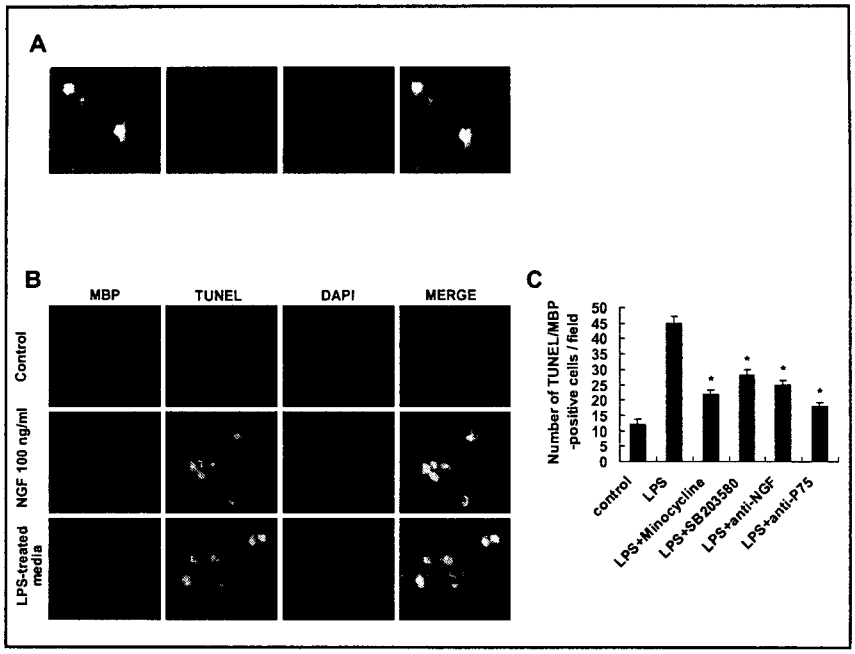

Spinal Cord Injury
activation
?
astrocytes
microglia
Myelin-associated glycoprotein (MAGP)
p38-dependent
NgR
p75
Pro-NGF
Rho
Rac^L
JNK3
Axon collapse, degeneration
Oligo apoptosis

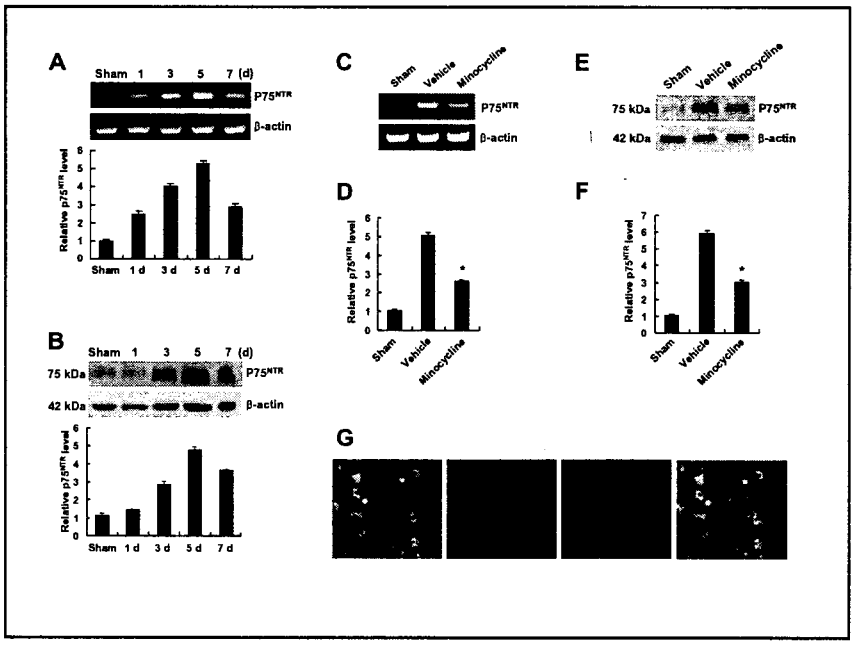

A
Sham 1 3 5 7 (d)
NGF
β-actin
Relative NGF level
Sham 1 d 3 d 5 d 7 d
B
Sham 1 3 5 7 (d)
26 kDa
ProNGF
14 kDa
NGF
42 kDa
β-actin
ProNGF
NGF
Relative (Pro)NGF level
Sham 1 d 3 d 5 d 7 d
C
Sham 1 3 5 7 (d)
26 kDa
ProNGF
42 kDa
β-actin
Relative ProNGF level
Sham 1 d 3 d 5 d 7 d
D
Sham
Vehicle
Minocycline
26 kDa
ProNGF
42 kDa
β-actin
E
Relative ProNGF level
Sham
Vehicle
Minocycline
F
G
H
I
J
Negative
K
Sham

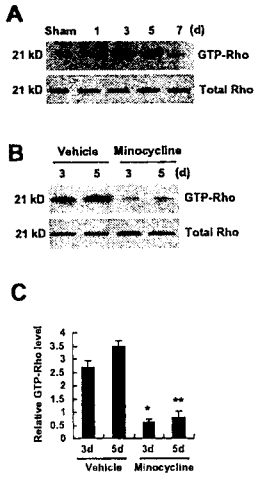

A
Vehicle
Minocycline
Sham 1 3 5 1 3 5 (d)
38 kDa
p-p38 MAPK
38 kDa
p38 MAPK
B
Relative p-p38 MAPK level
Sham
1d
3d
5d
Vehicle
Minocycline
C
Vehicle
Minocycline
Sham 1 3 5 1 3 5 (d)
54 kDa
47 kDa
p-MAPKAPK-2
54 kDa
47 kDa
MAPKAPK-2
D
Relative p-MAPKAPK-2 level
Sham
Vehicle
Minocycline
E
F

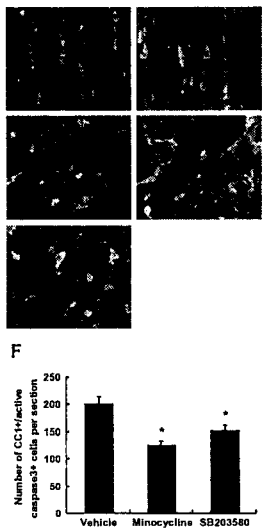

A
Control
LPS
H
0 0.5 1 2 4 12 24 (h)
NGF
β-actin
B
0 5 15 30 60 120 (min)
38 kDa
p-p38 MAPK
38 kDa
p38 MAPK
E
0 5 15 30 60 120 (min)
54 kDa
p-MAPKAPK-2
47 kDa
MAPKAPK-2
I
0 0.5 1 2 4 12 24 (h)
26 kDa
ProNGF
42 kDa
β-actin
C
Minocycline
CTR LPS 1 nM 5 nM
38 kDa
p-p38 MAPK
38 kDa
p38 MAPK
F
Minocycline SB203580
CTR LPS 1 nM 5 nM 1 μM 5 μM
54 kDa
p-MAPKAPK-2
47 kDa
MAPKAPK-2
J
Minocycline SB203580
CTR LPS 1 nM 5 nM 1 μM 5 μM
26 kDa
ProNGF
42 kDa
β-actin
D
Relative p-p38 MAPK level
LPS (1 μg/ml) - + + +
Minocycline (nM) - - 1 5
G
Relative pMAPKPAK-2 level
LPS (1 μg/ml) - + + + + +
Minocycline (nM) - - 1 5 - -
SB203580 (μM) - - - - 1 5
K
Relative ProNGF level
LPS (1 μg/ml) - + + + + +
Minocycline (nM) - - 1 5 - -
SB203580 (μM) - - - - 1 5

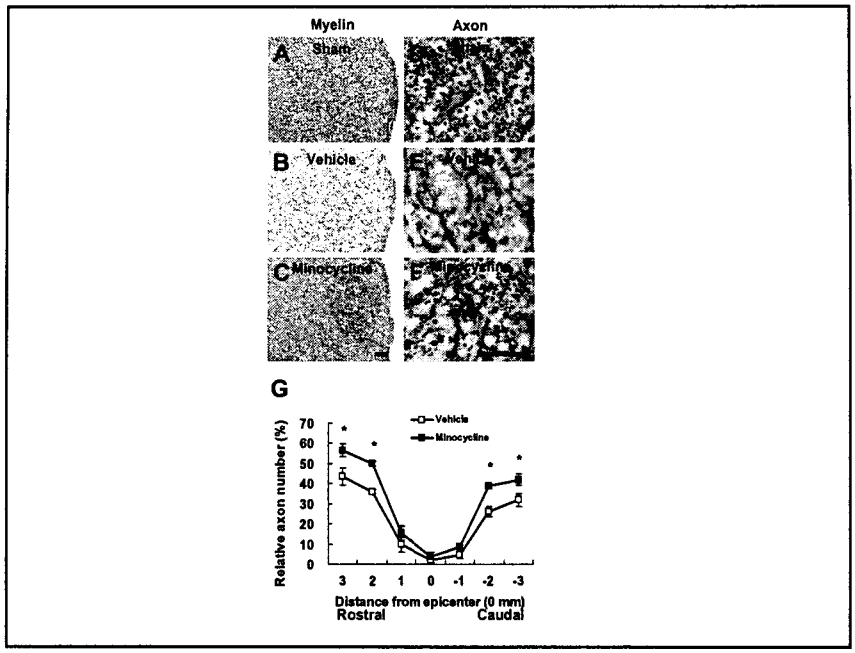

A
Sham
Vehicle
SB 1 μg
SB 5 μg
26 kDa
ProNGF
42 kDa
β-actin
B
Relative ProNGF level
Sham
Vehicle
SB 1 μg
SB 5 μg
C

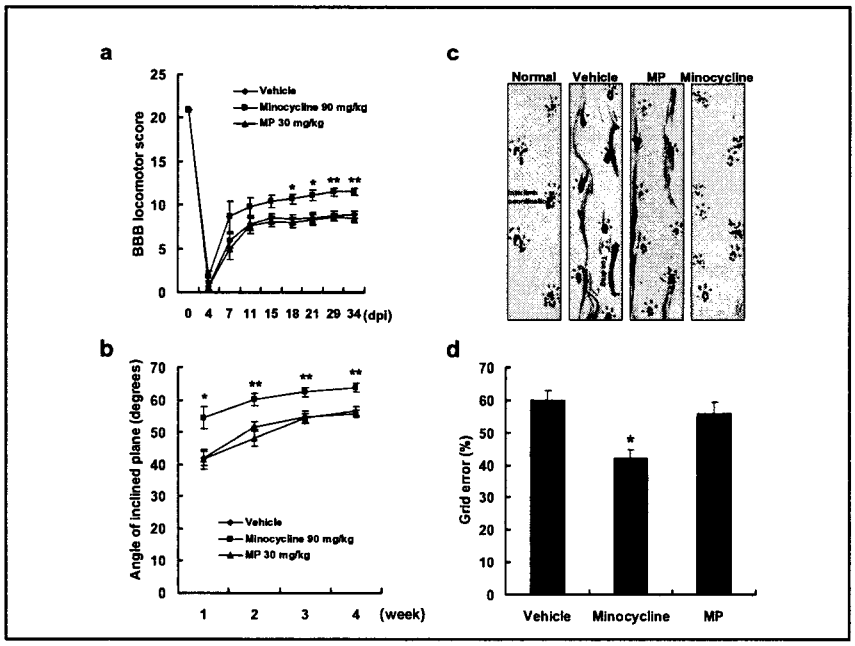

A
B
MBP
TUNEL
DAPI
MERGE
Control
NGF 100 ng/ml
LPS-treated media
C
Number of TUNEL/MBP -positive cells / field
control
LPS
LPS+Minocycline
LPS+SB203580
LPS+anti-NGF
LPS+anti-P75


A
Sham 1 3 5 7 (d)
P75NTR
β-actin
Relative p75NTR level
Sham 1 d 3 d 5 d 7 d
B
75 kDa
42 kDa
C
Sham
Vehicle
Minocycline
D
E
F
G

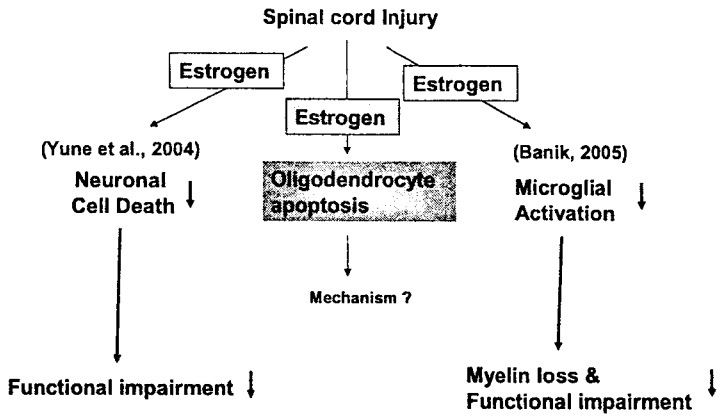

A
Sham 1 3 5 7 (d)
21 kD
GTP-Rho
21 kD
Total Rho
B
Vehicle Minocycline
3 5 3 5 (d)
21 kD
GTP-Rho
21 kD
Total Rho
C
Relative GTP-Rho level
4
3.5
3
2.5
2
1.5
1
0.5
0
*
**
3d 5d 3d 5d
Vehicle Minocycline


F
Number of CC1+/active caspase3+ cells per section
250
200
150
100
50
0
*
*
Vehicle Minocycline SB203580

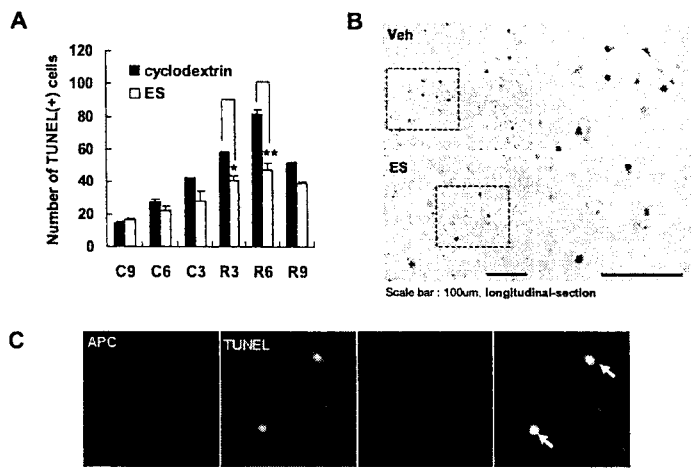

Myelin
Axon
A Sham
B Vehicle
C Minocycline
G
Relative axon number (%)
Vehicle
Minocycline
Distance from epicenter (0 mm)
Rostral
Caudal


a
BBB locomotor score
Vehicle
Minocycline 90 mg/kg
MP 30 mg/kg
(dpi)
b
Angle of inclined plane (degrees)
(week)
c
Normal
Vehicle
MP
Minocycline
d
Grid error (%)
Vehicle
Minocycline
MP

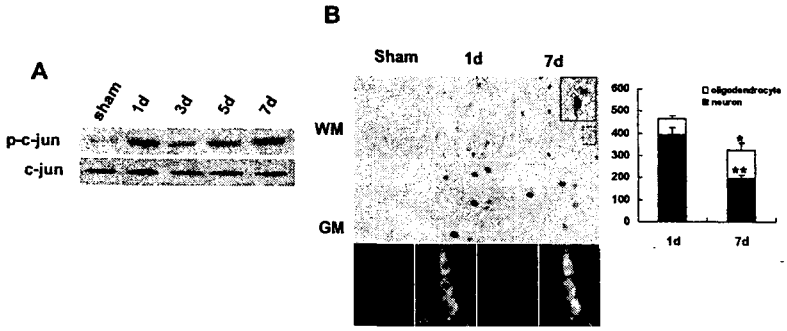
Estrogen effect on functional recovery after SCI



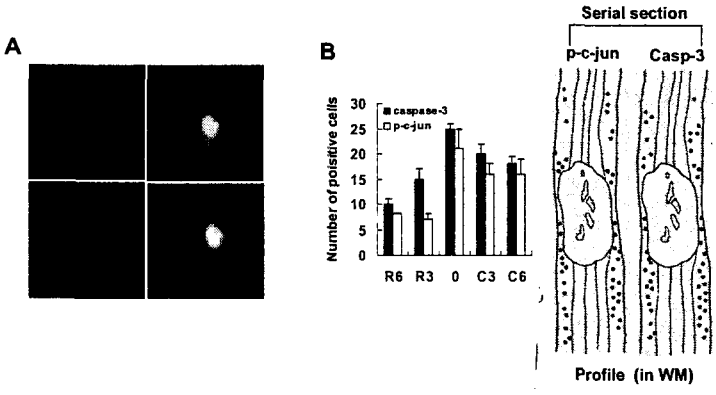
Estrogen reduces oligodendrocyte apoptosis at 7d after SCI

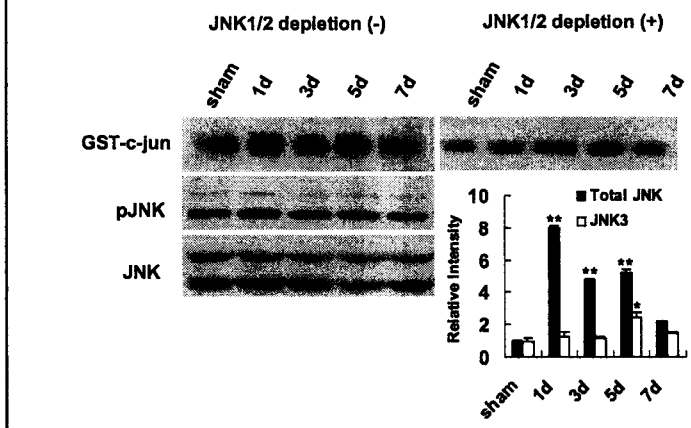


Delayed increase of c-jun phosphorylation in oligodendrocyte after SCI

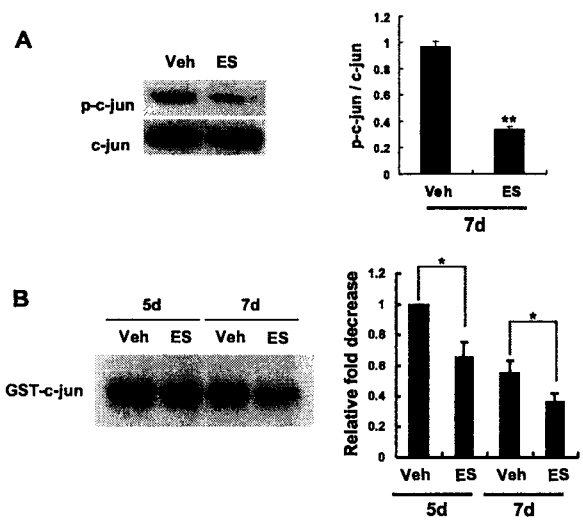


Phosphorylated c-jun may involved in oligodendrocyte apoptosis

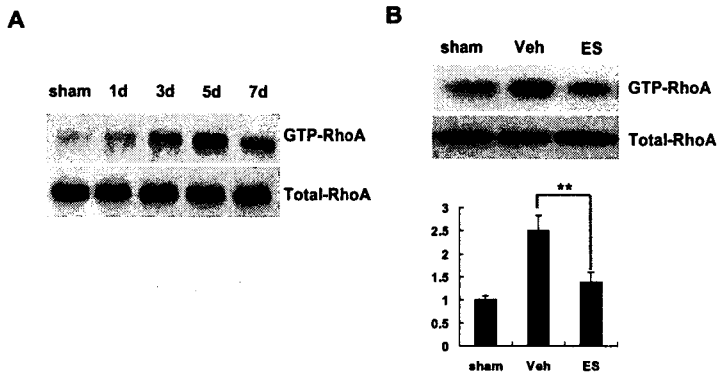




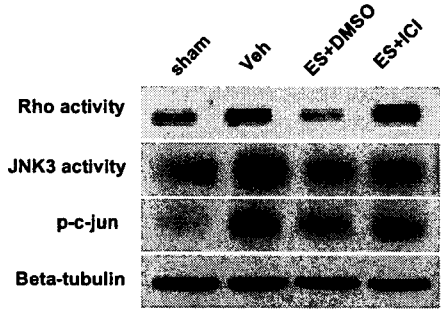
Estrogen inhibit JNK3 activation followed c-jun phosphorylation

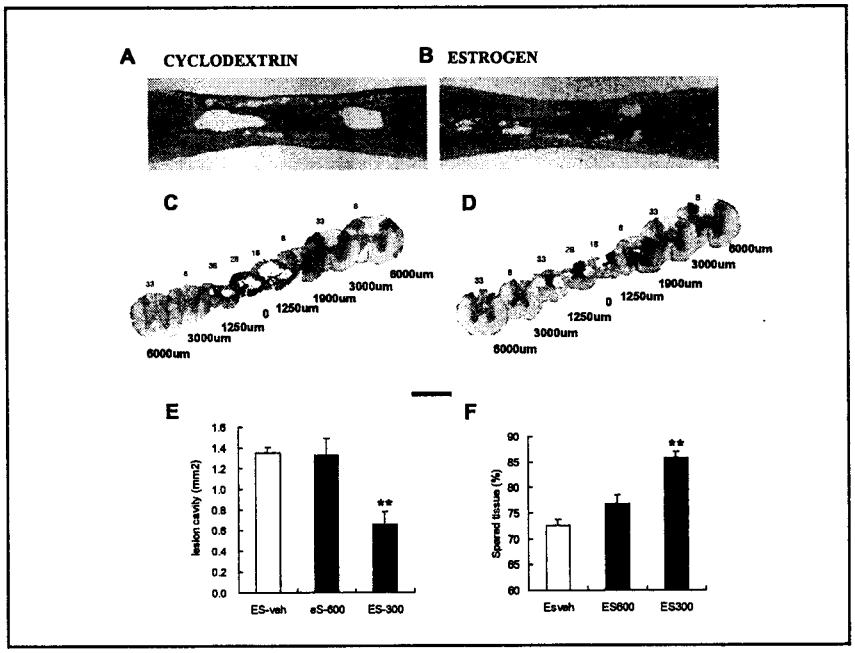


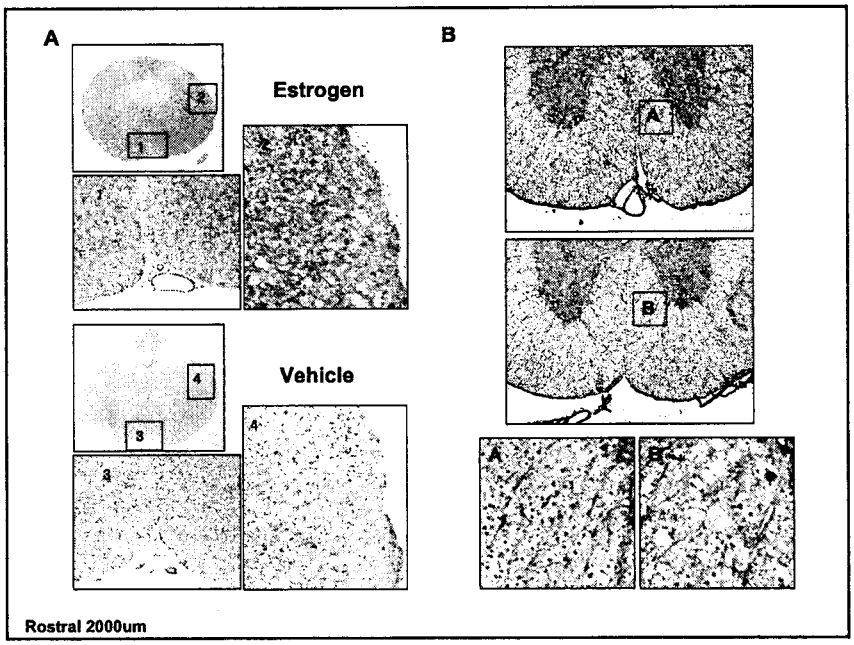
Estrogen inhibit rhoA activation

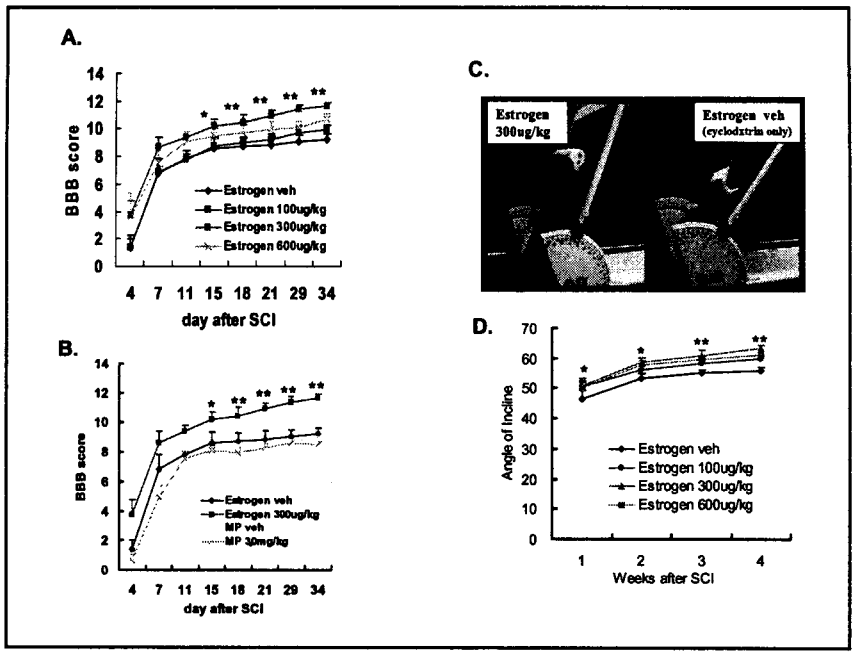


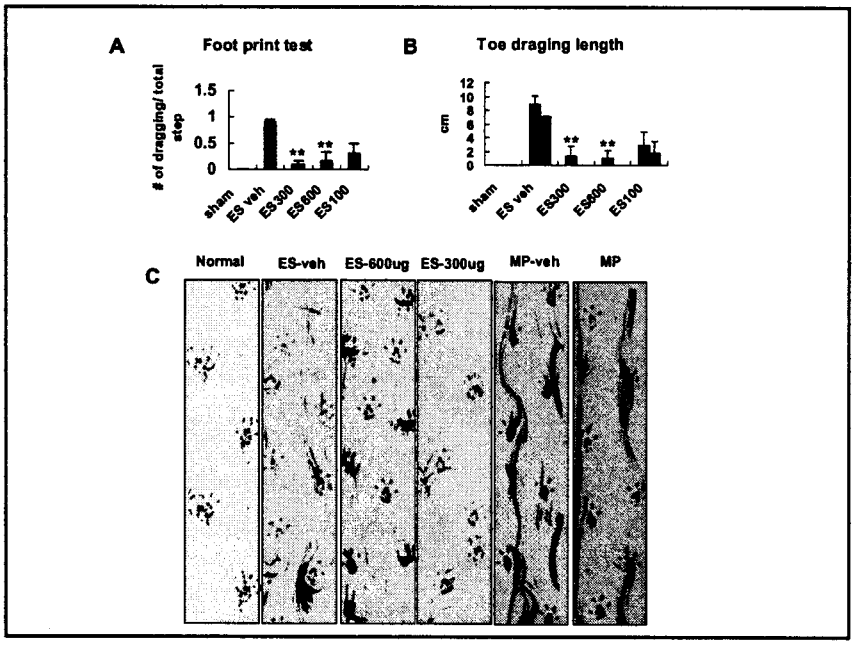
Estrogen receptor dependent protection

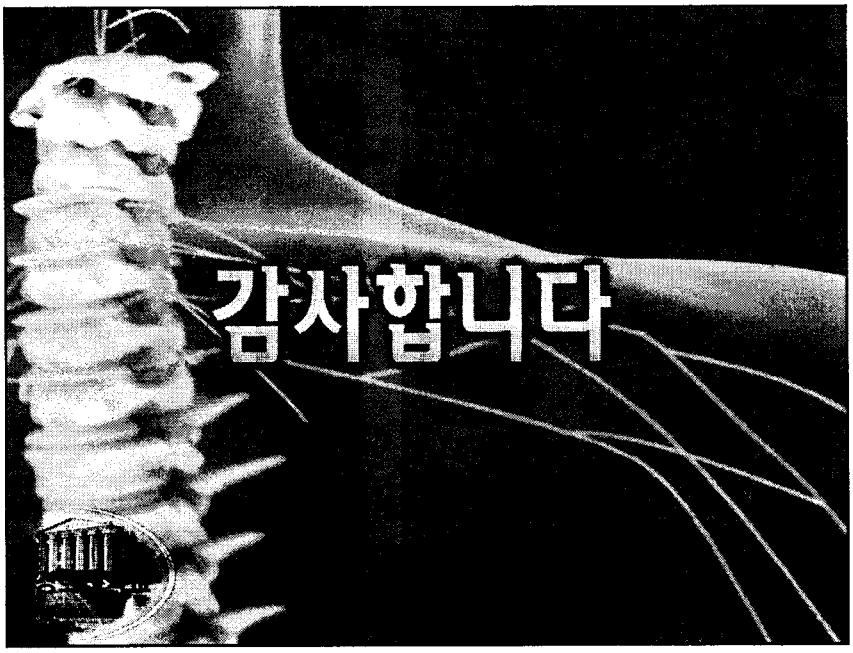



A
CYCLODEXTRIN
B
ESTROGEN
C
D
6000um
3000um
1900um
1250um
0
E
F
lesion cavity (mm2)
ES-veh
eS-600
ES-300
Spared tissue (%)
Esveh
ES600
ES300


A
B
Estrogen
Vehicle
Rostral 2000um


A.
BBB score
day after SCI
Estrogen veh
Estrogen 100ug/kg
Estrogen 300ug/kg
Estrogen 600ug/kg
B.
BBB score
day after SCI
Estrogen veh
Estrogen 300ug/kg
MP veh
MP 30mg/kg
C.
Estrogen 300ug/kg
Estrogen veh (cyclodxtrin only)
D.
Angle of Incline
Weeks after SCI
Estrogen veh
Estrogen 100ug/kg
Estrogen 300ug/kg
Estrogen 600ug/kg


A
Foot print test
# of dragging/ total step
sham
ES veh
ES300
ES600
ES100
B
Toe draging length
cm
sham
ES veh
ES300
ES600
ES100
C
Normal
ES-veh
ES-600ug
ES-300ug
MP-veh
MP


감사합니다