Short report

Acetyl salicylic acid augments functional recovery following sciatic nerve crush in mice
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Abstract

Cyclin-dependent kinase 5 (CDK-5) appears to play a significant role in peripheral nerve regeneration as CDK-5 inhibition retards nerve regeneration following nerve crush. Anti-inflammatory drug acetyl salicylic acid elevates CDK-5 and reduces ischemia – reperfusion injury in cultured neurons. In this study we have evaluated the effect of acetyl salicylic acid on functional recovery following sciatic nerve crush in mice. Eighteen Swiss albino mice underwent unilateral sciatic nerve crush. Test animals received acetyl salicylic acid (100 mg/kg/day, n = 6 or 50 mg/kg/day, n = 6) and control animals (n = 6) received normal saline for 14 days following surgery. Functional recovery was assessed with improvement in Sciatic Function Index, nociception and gait. In comparison with normal saline treatment, acetyl salicylic acid (100 mg/kg/day) significantly improved functional recovery following sciatic nerve crush. Anti-inflammatory drug acetyl salicylic acid appears to be a promising agent for treating peripheral nerve injuries and hence elucidation of its neuroprotective pathways is necessary.

Background

The injured adult mammalian peripheral nerves, in contrast with axons injured inside central nerve tracts, show vigorous regeneration [1]. The exact physiological and molecular signals involved in inducing the regenerative process are largely unknown. In addition to the induction of transcription factors, adhesion molecules, growth associated proteins and structural components required for axonal elongation, intracellular signalling molecules that control cell cycle and differentiation appears to play a major role in nerve regeneration process [1].

Cyclins and the cyclin dependent kinases (CDKs) play a central role in regulating the cell cycle progression in all eukaryotic organisms [2]. Cyclin-dependent kinase – 5 (CDK-5) is a member of these cyclin-dependent kinase family of serine/threonine kinases. CDK-5 along with its activators, p35 and p39, is predominantly expressed in post-mitotic neurons [3]. CDK-5 appears to be involved in active reorganization of the actin cytoskeleton during neurite outgrowth [4]. Enhanced CDK-5 activity and expression of p35 are associated with differentiation of cultured neuronal cells as well as accelerated neurite outgrowth [4]. Namgung et al [5] reported a high expression of CDK-5 and p35 in regenerating nerves. In their experiments inhi-
bition of CDK-5 activity, through CDK-5 inhibitors rocos-
vitine and olomoucine, led to reduction in CDK-5 activity
and retardation of nerve regeneration [5].

Non-steroidal anti-inflammatory agent acetyl salicylic
acid (ASA), in addition to its well known inhibitory action
on cyclooxygenases, affects several cellular signalling
pathways involved in regulation of cellular proliferation
and differentiation [6]. One of the newly identified
actions of ASA is being the induction of p 35 synthesis and
activation of CDK-5 [6]. ASA has shown a neuroprotective
effect in an in-vitro model of neuronal ischemia reper-
fusion injury [6]. However its effect on peripheral nerve
injuries is unknown.

In this study we have evaluated the effect of ASA at two
doses (100 mg/kg/day and 50 mg/kg/day) on functional
recovery following peripheral nerve injury using mouse
sciatic nerve crush model.

The following drugs were used for this study: Urethane
(Sigma, USA), Normal Saline (Baxter, India) and ASA
(Alta Labs, India).

Swiss albino mice (25 – 30 gms) of both sexes were ran-
domly allocated into three different treatment and control
groups. Animals received food and water ad libitum and
were kept on a 12-hour light/dark cycle. Animals were
kept under the accordance of protocols approved by the
institutional animal care and use committee.

Mice were subjected to sciatic nerve crush as described ear-
lier [7]. In brief adult mice were anesthetized with 150
mg/100 g intraperitoneal urethane. The area above the
right lower thigh was shaved and sterilized with betadine
and 70% surgical spirit. A 1 cm incision was made in the
skin above the lower thigh between the gluteus maximus
muscle and the biceps femoris muscle. The muscles were
removed and crushed for 20 sec. The holder was rotated 90°
and the sciatic nerve exposed. Sciatic nerve was placed in a
1 mm wide needle holder and teased apart with scissors and the sciatic nerve exposed.

Evaluation of sciatic function index (SFI) [8] and gait [7]
was done on day 0 i.e. before surgery and on days 1 and15
following surgery. Mice were held by the chest and their
hind feet were pressed down onto a stamp pad soaked
with water soluble black ink. Mice were immediately
allowed to walk along a confined walkway 6 cm wide by
30 cm long with a dark shelter at the end of the corridor
leaving its foot prints on the paper that is cut to the appro-
priate dimensions and placed on the floor of the corridor.
The tracks were evaluated for three different parameters:
(1) distance from the heel to the third toe, the print length
(PL); (2) distance from the first to the fifth toe, the toe
spread (TS); and (3) distance from the second to the fourth
toe, the intermediary toe spread (ITS). All three
measurements were taken from the experimental (E, under-
going sciatic nerve crush) and normal (N) limbs. Using the following formula derived by Bain et al [9] SFI
was calculated as,

\[ SFI = -38.3 \left( \frac{EPL - NPL}{NPL} \right) + 109.5 \left( \frac{ETS - NTS}{NTS} \right) + 13.3 \left( \frac{EIT - NIT}{NIT} \right) - 8.8 \]

The SFI was analysed as: An SFI equal to 100 indicates sig-
nificant impairment, whereas an SFI oscillating around 0
is considered to reflect normal function.

Animals were allowed to walk on a platform as well as on
an inclined plane for 2 min each. Subjective scores were
assigned on the basis of hind limb movement and its pos-
ture while ambulating. Mice moving both the hind limbs
uniformly given 3, if the operated limb was moving with
deformity it received 2, scored as 1 if the operated limb
was moving seldom and 0 – when no movement was seen
in the operated hind limb.

Nociceptive function was evaluated by observing the
withdrawal reflex of the hind limb and vocalization in
response to noxious stimulation like mechanical stimula-
tion (pinch test) and pricking the plantar aspect of the lat-
eral part of the foot with a needle [7]. Animals were
evaluated daily, till the recovery of nociceptive function.

The entire regimen was repeated twice and then all the val-
ues from multiple experiments were averaged. Statistical
evaluation was conducted using multiple comparisons
and Mann Whitney U test. Data are depicted as mean ± sd.
‘P’ values < 0.05 were considered significant.

In all animals SFI score prior to surgery was 0, gait score
was three and nociceptive function was intact. Following
sciatic nerve crush in all animals, except sham controls,
SFI scores became 100 and gait scores were reduced to 0
and there was loss of nociceptive function. In sham con-
trols there was no change in SFI scores and gait scores,
while nociceptive function remained intact.
There was a spontaneous recovery of sensorimotor function in normal saline treated mice as shown by reduction in SFI scores, improvement in gait scores and recovery of nociceptive function (table 1). There was no significant difference, in the functional parameters, between animals treated with 50 mg/kg/day of ASA and animals receiving normal saline treatment. However animals treated with 100 mg/kg/day of ASA showed statistically significant reduction in SFI scores and improvement in gait and exhibited an early recovery in nociceptive function (table 1).

ASA is one of the most widely used analgesic, anti-pyretic and anti-inflammatory drug. ASA exerts these effects through inhibition of cyclooxygenases (COX) [6]. However novel COX-independent actions of ASA like, inhibition of excitatory amino acid release, NF-kappa beta (Nfkb) translocation to the nucleus and expression of inducible nitric acid synthase (iNOS) following cerebral ischemia are projecting ASA as a promising neuroprotective agent for treating stroke [10].

Our results show that ASA, at anti-inflammatory dose, significantly accelerates functional recovery following peripheral nerve crush. Even though ASA at 50 mg/kg/day dose showed marginally higher functional recovery it was not significant in comparison with normal saline treatment. Hence the neuroprotective action of ASA following peripheral nerve injuries appears to be dose-dependent with maximum benefit seen with 100 mg/kg/day.

Even though our preliminary study shows the neuroprotective action of ASA in peripheral nerve injuries, data regarding the molecular mechanisms leading to the neuroprotective action of ASA is still lacking. We have not given the histological and molecular evidence for neuroprotective action of ASA. This may be considered as a limitation to our study. Based on the previous reports describing the role of CDK-5 in nerve regeneration [5] and effect of ASA on CDK-5 [6] it may be assumed that ASA promotes nerve regeneration following peripheral nerve injury through activation of CDK-5. However ASA also affects prostaglandin synthesis, iNOS expression, Nfkb translocation, mitogen activated protein (MAP) kinase pathway etc which can modulate nerve regeneration following peripheral nerve injury. Hence understanding the molecular pathways leading to the neuroprotective action of ASA is necessary.

In conclusion our preliminary study shows that acetyl salicylic acid accelerates functional recovery following peripheral nerve injury and it appears to be a promising agent for treating peripheral nerve injuries. Further studies aimed at understanding the molecular mechanisms involved in the neuroprotective action of acetyl salicylic acid are required.

**Abbreviations**

1. CDK – Cyclin dependent kinases
2. ASA – Acetyl salicylic acid
3. SFI – Sciatic function index
4. PL – Print length
5. TS – Toe spread
6. IT – Intermediary toe spread
7. COX – Cyclooxygenase
8. NFKB – NF-kappa beta
9. iNOS – Inducible nitric oxide synthase
10. MAP kinase – Mitogen activated protein kinase

**Competing interests**

The author(s) declare that they have no competing interests.

**Authors’ contributions**

1. PKTS – Concept, Experiments, Data analysis, Manuscript preparation
2. PCG – Concept, Experiments, Data analysis
3. BKG – Concept, Experiments, Data analysis

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<table>
<thead>
<tr>
<th>Treatment groups (n = 6 animals in each group)</th>
<th>Improvement in SFI scores (in %, as on day 15)</th>
<th>Improvement in gait scores (in %, as on day 15)</th>
<th>Time taken for sensory recovery (number of days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>38.2 ± 1.8</td>
<td>25.12 +/- 4.8</td>
<td>17.80 +/- 3.7</td>
</tr>
<tr>
<td>Acetyl salicylic acid (50 mg/kg/day)</td>
<td>41.7 ± 2.4</td>
<td>31.3 ± 1.7</td>
<td>16.1 ± 3.1</td>
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<tr>
<td>Acetyl salicylic acid (100 mg/kg/day)</td>
<td>55.3 ± 1.7*</td>
<td>48.35 +/-1.7*</td>
<td>14.05 +/- 2.0*</td>
</tr>
</tbody>
</table>

Data are depicted as mean ± SD. ‘P’ values: < 0.05; * vs control (Mann-Whitney U test with multiple comparisons)
4. MGT – Concept, Data analysis, Manuscript preparation

References