Effect of Xymedon on Cell Survival in the System Sensory Neuron Schwann Cell

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Pyrimidine derivative xymedon inhibits neuronal death in L4-L5 spinal ganglia 30 days after ligation of rat sciatic nerve. After treatment with xymedon the number of neurons on the operated side decreased by 22.1% compared to that on the contralateral side, while in the control group this parameter decreased by 28.7%. At the same terms, the number of Schwann cells on the operated side after xymedon injection increased by 27.7% in comparison with that on the contralateral side, while in the control group this parameter decreased by 57.3%

Key Words: *neuron; Schwann cell; regeneration; xymedon*

Survival of neurons after trauma of the peripheral nerve is stimulated by neurotrophic factors (nerve growth factor, brain neurotrophic factor, neurotrophin-3) retrogradely transported from axons to perikaryons [7]. These factors are produced by nonneural and, especially, Schwann cells (SC) of the damaged nerve [3]. On the other hand, SC population and phenotype under conditions of regeneration are maintained by surviving neurons [7]. This effect is probably mediated via neuregulins [5, 9]. Our previous experiments on the model of sciatic nerve transection showed that pyrimidine derivative xymedon stimulates regeneration of myelinated fibers, increases the number of sensory neurons expressing apoptosis inhibitor Bcl-2, and maintains SC population in the area of potential axon growth [1,2,4]. The aim of the present study was to identify cells (neuron, SC, or both) responsible for the stimulating effect of xymedon. To this end we studied the effect of xymedon on neuronal survival and the number of SC after nerve ligation, *i. e.* disruption of direct communications between neurons and SC responsible for transport of neurotrophic factors along the regenerating axons.

MATERIALS AND METHODS

Experiments were carried out on 20 white male rats (180-200 g). The animals were intraperitoneally narcotized with ketamine (calipsol, Gedeon Richter, 150 mg/kg), and the left sciatic nerve was aseptically ligated in at the level of the lateral wing of the ilium. Experimental rats (n=10) received xymedon (1,2-dihydro-4,6-dimethyl -N-(β-oxyethyl)pyrimidone-2) in a dose of 30 mg/kg [1]. Thirty days after surgery, 6-mm fragments of both sciatic nerves were isolated at the level of the upper third of the thigh. The fragments were fixed in formalin and embedded in paraffin. SC were counted on 4-μ slices stained immunohistochemically with antibodies to protein S100 ($S100^+$-cells). Spinal L4-L5 ganglia on both sides were obtained from the same animals after laminectomy and neurons were counted as described previously [2]. The data were processed statistically using Student's t test.

RESULTS

On day 30 after ligation of the sciatic nerve the number of neurons in spinal ganglia (L4-L5) on the operated side in control rats decreased by 28.7% ($p<0.05$) in comparison with that on the contralateral side (Fig. 1), while in the experimental group this parameter decreased by 22.1% ($p<0.05$ compared to that on the ipsilateral side in the control group). These findings suggest that xymedon inhibits posttraumatic loss of sensory neurons after nerve ligation [2]. In animals treated with xymedon the number of neurons in L4-L5 spinal ganglia on the operated side surpassed

the number of neurons on the operated side and did not differ from that on the contralateral side of control animals [2]. Hence, after nerve ligation the effect of xymedon was less pronounced than after transection.

Previous experiments showed that posttraumatic neuronal death starts on day 7 after trauma and persisted for 6 months [6] mainly due to reduced retrograde transport of neurotrophic factors [7].

The intensity of immunohistochemical staining in $S100^+$-cells in the control group was lower than in experimental rats and much lower than in the contralateral nerve. Therefore, xymedon stimulates expression of S100 protein in SC.

On day 30 after ligation of the sciatic nerve, the number of $S100^+$ cells on the operated side in the control decreased by 57.3 % ($p<0.05$) compared to the contralateral side (Fig. 2), while in the experimental group this parameter increased by 27.7% ($p<0.05$) compared to the contralateral side.

On days 3-7 after trauma, SC in the distal nerve fragment started to proliferate [10], while some of them entered apoptosis [6]. When growing axons attained the distal portion of the nerve, the number of SC increases by more than 3 times compared to intact nerve. If axons did not come in contact with SC, the number of SC decreases. Our findings suggest that xymedon increased the number of SC in the distal portion of the nerve and this effect did not correlate with the number of neurons.

Our findings and published data suggest that xymedon inhibits posttraumatic loss of sensory neurons by acting both directly on neurons and through maintenance of SC population and stimulation of production of neurotrophic factors.

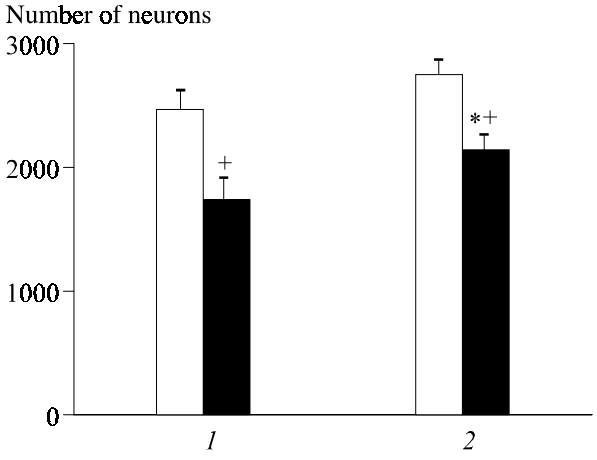

Fig. 1. Number of neurons in L4-L5 spinal ganglia on day 30 after ligation of the sciatic nerve. Here and on Fig.2.: open and dark bars correspond to the number of neurons on the contralateral and operated side, respectively; *1*) control, *2*) xymedon, $p<0.05$: *compared to the control, $^+$compared to the contralateral side.

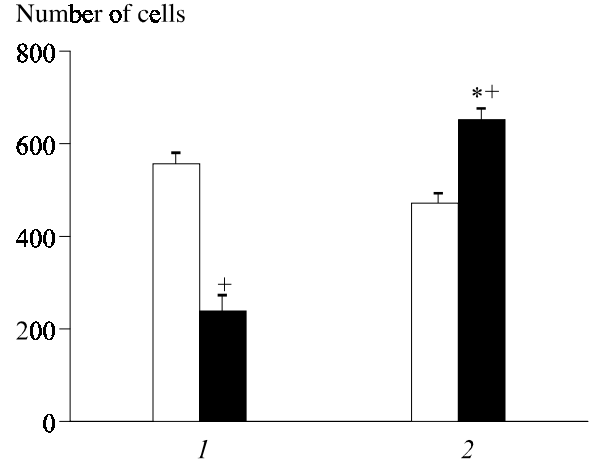

Fig. 2. Number of Schwann cells in the distal portion of the sciatic nerve on day 30 after ligation.

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