

# Mitoxantrone prevents disease relapse in a rat model of multiple sclerosis

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## Introduction

Mitoxantrone (Novantrone) is an immunosuppressive drug that impacts both T and B cell proliferation. In 2000, mitoxantrone was approved by the FDA for the treatment of multiple sclerosis (MS). Indeed, clinical data suggests that the drug markedly reduces relapse rate in patients with very active relapsing remitting MS (relapses reduced by up to 70-80%) and significantly reduces the accrual of disability over 2 years in patients with relapsing remitting MS.

However, there is no animal data showing the beneficial effect on the disease relapse when mitoxantrone treatment is initiated only after the occurrence of the first attack.

## Objectives

The objectives of the present work were to evaluate the effect of mitoxantrone in an animal model that mimics the relapsing remitting form of MS, especially the impact of the treatment on the disease relapse. In addition, the direct neuroprotective potential of mitoxantrone, independent to its action on T and B cell function, was assessed in LPS-stimulated cocultures of glia-neurons.

## Material & Methods

### Animal model of EAE:

Experimental autoimmune encephalomyelitis (EAE) serves as animal model of multiple sclerosis. EAE can be induced in rats or mice by immunization with myelin antigens combined with adjuvant. The relapsing remitting form of EAE (RR-EAE) is obtained in DA rats following inoculation with syngeneic spinal cord homogenate. The development of clinical disability is assessed by a scoring system as follow:  
**0** = no abnormality; **0.5** = distal weakness of the tail; **1** = complete weakness of the tail; **2** = mild weakness in one or two hindlimbs; **3** = moderate paraparesis of one or two hindlimbs; **4** = severe paraparesis with incontinence; **5** = total paraplegia. A score of 0.5 is added (or subtracted) when the clinical sign is between two scores.

### Mitoxantrone treatment

0.5 mg/kg mitoxantrone was administrated intraperitoneally (i.p.) on daily basis to EAE rats as preventive and curative therapy. In the **preventive regimen**, mitoxantrone treatment was initiated at day 8 post-immunization when there is still no obvious sign of disease symptoms. In the **curative therapy**, mitoxantrone treatment was initiated at day 17 during the remission period when there is still no obvious sign of disease relapse. The control EAE rats received an i.p. injection of 0.9 % NaCl starting from day 8 post-immunization.

### Cellular model of brain inflammation and neuronal death

Coculture of glia-neurons from rat embryos exposed to lipopolysaccharide (LPS) induces a rapid and marked release of various inflammatory mediators into the medium including nitric oxide, TNF- $\alpha$ , IL-1 $\beta$  and ultimately leads to the death of neurons. In the present study, brains of rat embryos at 15 days of gestation were dissected to harvest the ventral mesencephalic flexure used for the cell preparations. The cell preparations comprising neurons and glia cells were cultured in 96 well plates containing culture medium and maintained at 37°C in 5 % CO<sub>2</sub>-95 % air atmosphere.

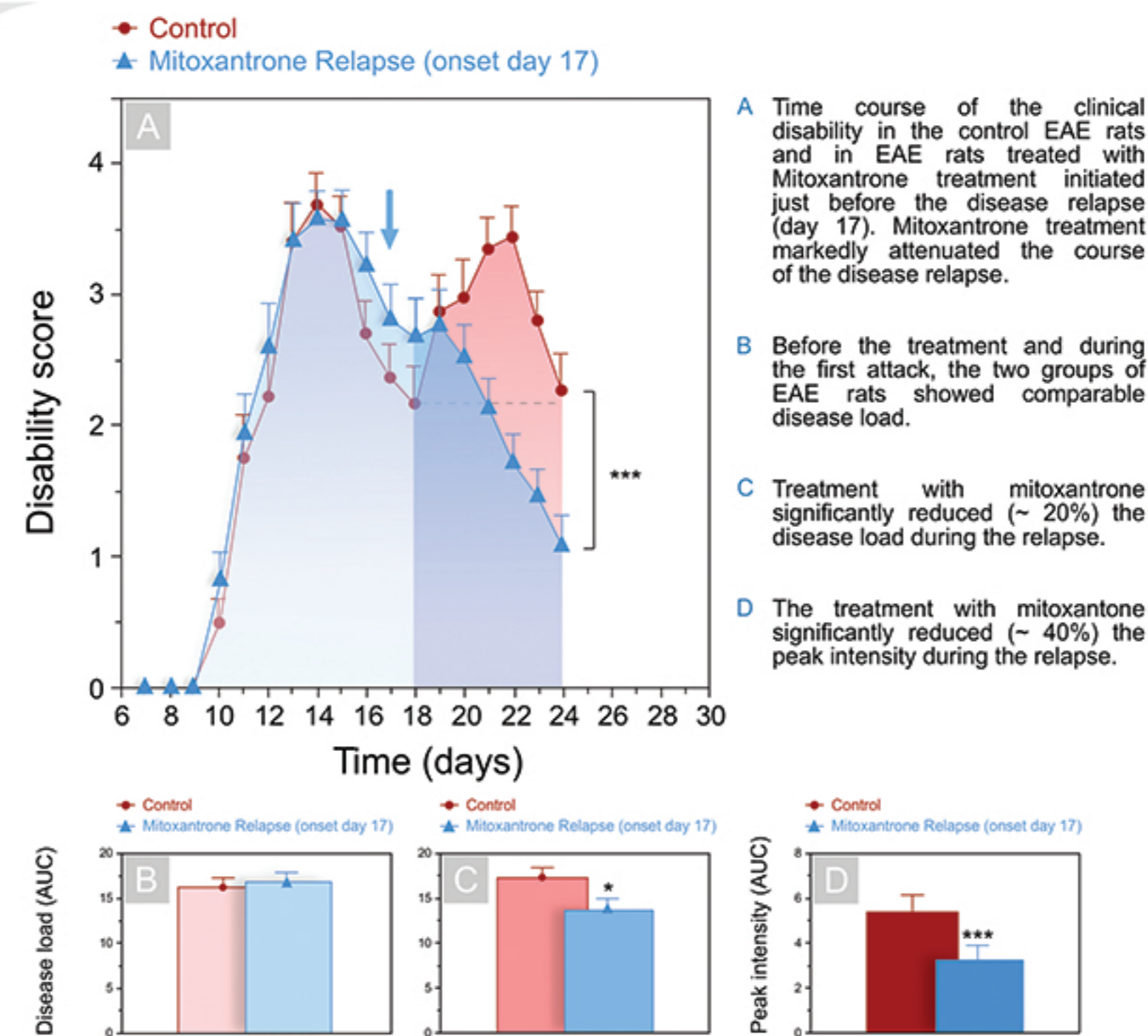
### Mitoxantrone treatment and LPS stimulation of cocultures

After 7 days, cocultures were exposed to different concentrations of mitoxantrone (0.3-100 nM). 30 minutes later, 10 ng/ml of LPS was added to stimulate the coculture. The release of nitric oxide, TNF- $\alpha$  and IL-1 $\beta$  are measured in the supernatant 24h later using their respective detection kits. After 5 days, the extent of neuronal injury was indirectly measured by the density of surviving dopaminergic neurons (tyrosine hydroxylase positive cells) in the coculture.

## Keypoints and Results

- Curative treatment markedly prevents the EAE disease relapse in the rat model of multiple sclerosis
- Preventive treatment with mitoxantrone markedly reduces the development of EAE disease in the rat model of multiple sclerosis
- Mitoxantrone does not attenuate the inflammation of activated CNS-resident cells and does not protect against the process of neuronal damage

Figure 1: Effect of Curative mitoxantrone treatment on the EAE disease of rats



Effect of Preventive mitoxantrone treatment on the EAE disease of rats

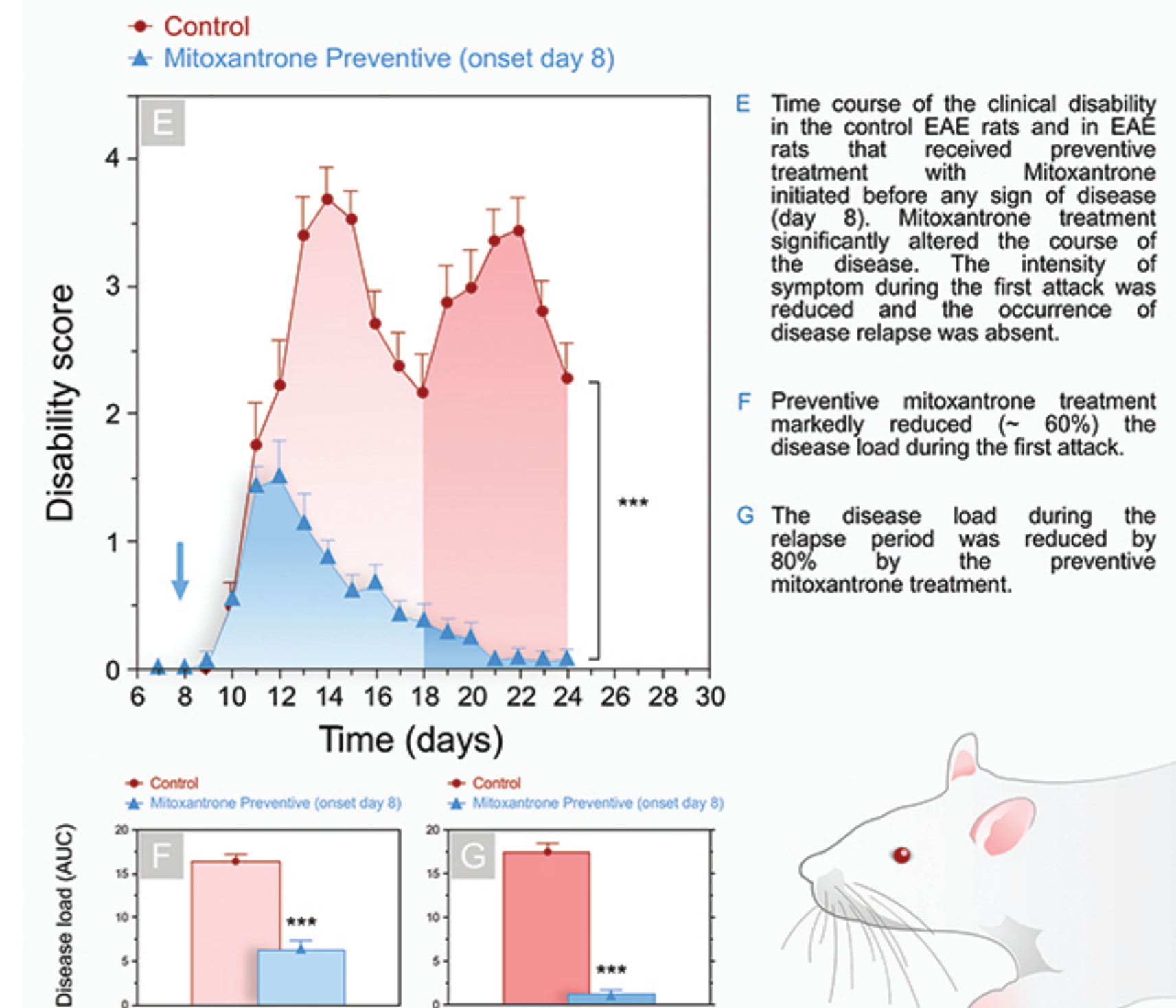
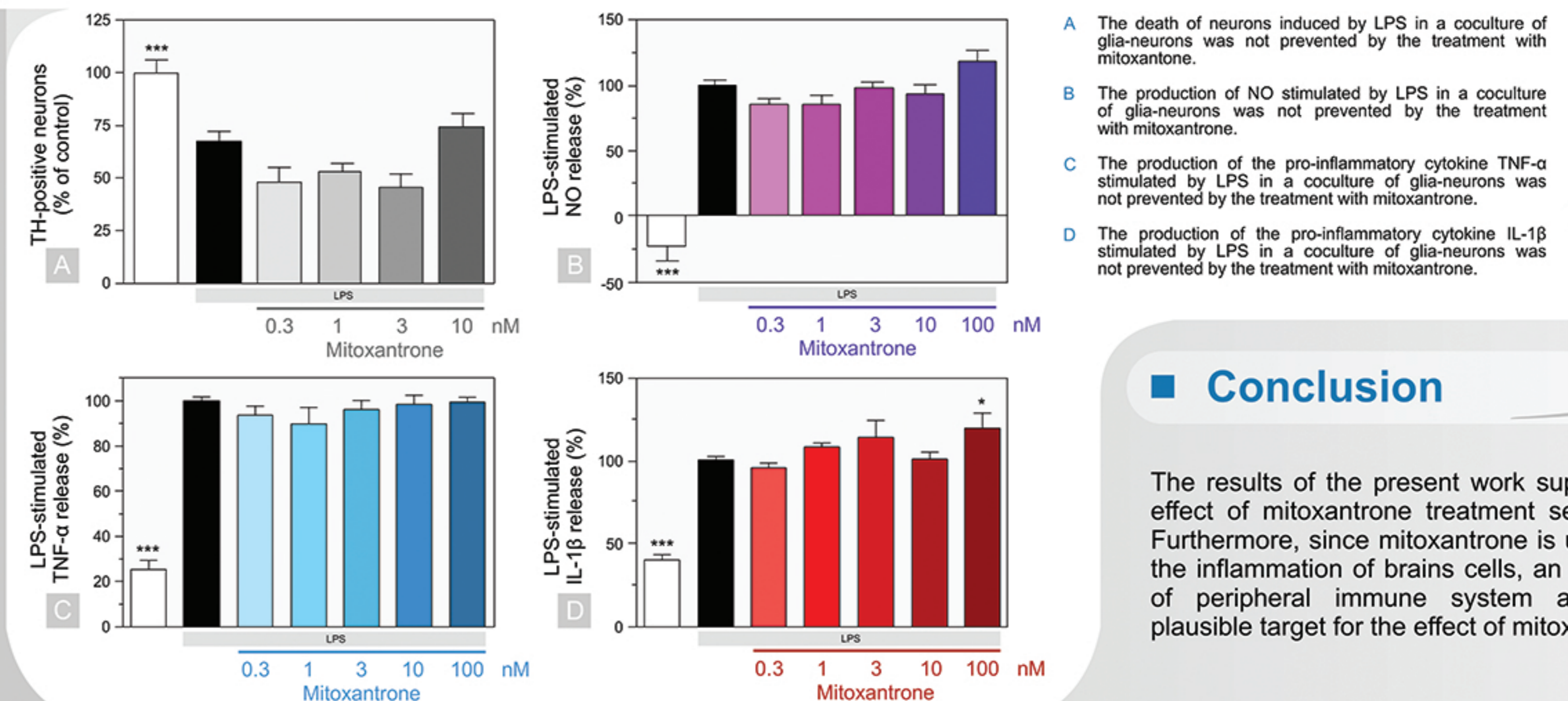


Figure 2: Effect of mitoxantrone exposure on rat primary cocultures of glia-neurons



## Conclusion

The results of the present work support the beneficial effect of mitoxantrone treatment seen in MS patient. Furthermore, since mitoxantrone is unable to attenuate the inflammation of brains cells, an immunomodulation of peripheral immune system appears the most plausible target for the effect of mitoxantrone in MS.