We investigated the therapeutic effect of vitamin D3 in a rat diffuse axonal injury model. A total of 60 male Sprague–Dawley rats weighing 175 – 200 g were anaesthetized and subjected to head trauma using Marmarou’s impact-acceleration model. The rats were then separated into two groups; one group was treated with vitamin D3 and the other with saline for up to 4 days after the head trauma. Rats from both groups were killed 1, 3 or 8 days post-injury. The brains were examined histopathologically and scored according to the level of neuronal, vascular and axonal damage. There were no significant differences between the groups after 1 or 3 days, but evaluation after 8 days revealed a significant improvement in the group treated with vitamin D3. Our data indicate that vitamin D3 has a beneficial effect in diffuse axonal injury and may be useful in the management of this condition.

KEY WORDS: VITAMIN D3; DIFFUSE AXONAL INJURY; RAT

Introduction

Diffuse axonal injury (DAI) is thought to be the most common cause of a persistent vegetative state and severe disability after closed head injury; patients are rendered comatose immediately on impact but no focal lesions are detected. Traumatic forces can induce two types of axonal injury: primary and secondary axotomy. At the moment of impact some axons suffer immediate axonotmesis (primary axotomy). Other axons suffer some degree of functional impairment that can evolve over a variable time period after the injury (secondary axotomy). The processes leading to secondary axotomy include structural weakness of the axoplasmic membrane and ionic disturbances.

One aim when managing DAI is to rescue the axon from secondary damage. Vitamin D3 could have neuroprotective effects, acting via neurotrophin synthesis, modulation of neuronal Ca2+, inhibition of inducible nitric oxide synthase synthesis and upregulation of γ-glutamyl transpeptidase activity. In this study, we examined the therapeutic effect of vitamin D3 in a rat DAI model and showed that systemic administration of vitamin D3 ameliorated DAI histopathologically.

Materials and methods

ANIMALS

Animals were handled in accordance with the international guidelines for animal research of the World Health Organization as adopted by the Laboratory Animal Centre, Ataturk University, Turkey. A total of 60 male Sprague–Dawley rats weighing 175 – 200 g were used for the study. The rats were
anaesthetized with 30 mg/kg sodium thio-
pental intraperitoneally (Abbott, Istanbul, 
Turkey) and were allowed to breathe 
spontaneously throughout the procedure. 
Head trauma was induced using Marmarou’s 
impact-acceleration model.

TREATMENT PROTOCOLS
Rats were randomly divided into two groups: 
the vitamin D₃ treatment group and the 
saline control group. The vitamin D₃ 
treatment group and saline control group 
were then further divided into three 
subgroups. These subgroups received daily 
injections of either 1 µg/kg vitamin D₃ 
intraperitoneally (calcitriol, Sigma, St Louis, 
MO, USA) or 1 ml/kg saline intraperitoneally 
for 1, 3 or 4 days, respectively, post-injury. 
The subgroups receiving injections for 1, 3 or 
4 days were killed after 1, 3 or 8 days, 
respectively, and their brains were removed 
and sectioned.

HISTOPATHOLOGICAL ASSESSMENT
Sections were prepared with haematoxylin 
and eosin and silver stains for light 
microscopic studies. They were examined by 
a pathologist who was blinded to the groups.

Neuronal, vascular and axonal damage 
were scored using a five-point ordinal scale 
(0, none; 1, minimal; 2, mild; 3, moderate; 
4, marked; 5, severe). The semiquantitative 
scores reflect the approximate percentage of 
neuronal, vascular and axonal changes 
observed in the section (1, ≤ 5%; 2, 6 – 20%; 
3, 21 – 50%; 4, 51 – 75%; 5, 76 – 100%).

DATA ANALYSIS
Data analysis was performed using SPSS 
version 10.0 for Windows (SPSS Inc., 
Chicago, IL, USA). The ordinal rankings of 
histological damage were analysed using the 
Mann–Whitney U-test. A P-value < 0.05 was 
considered to be significant.

Results
On gross pathological observation, the 
brains looked normal, with no contusion or 
focal lesions apart from mild subarachnoid 
haemorrhage in the basal cisterns.

Histopathologically, severe oedema in the 
cerebral cortex, vascular congestion, 
subarachnoid haemorrhage and brainstem 
oedema were observed in the saline controls 
1 day post-injury (Fig. 1A). Similar but less

**FIGURE 1:** Photomicrographs of rat cerebral cortex under the site of impact, 1 day 
after head trauma: (A) saline control; (B) treated with vitamin D₃. Neuronal injury, 
cerebral oedema and vascular congestion are less severe in the specimen from the rat 
treated with vitamin D₃. Haematoxylin and eosin, magnification × 100
severe findings were observed in the corresponding vitamin D₃ treatment group (Fig. 1B). There was no statistically significant difference between the histopathological scores of these two groups. By 3 days post-injury, the severity of the cerebral cortical neuronal degeneration, oedema, vascular congestion and subarachnoid haemorrhage had lessened in the saline control group (Fig. 2A) and the vitamin D₃ treatment group (Fig. 2B). Again, there was no statistically significant difference between the histopathological scores of the two groups.

After 8 days, evaluation of the saline control group yielded the same findings as seen after 3 days (Fig. 3A), whereas a significant improvement \((P = 0.04)\) was seen in the vitamin D₃ treatment group (Fig. 3B).

The mean histopathological scores for the two groups are shown in Fig. 4.

**Discussion**

Vitamin D₃ has been shown to have a number of effects that may be relevant for neuroprotection.

**FIGURE 2:** Photomicrographs of rat brainstem 3 days after head trauma: (A) saline control; (B) treated with vitamin D₃. Axonal injury is more severe in the saline control. Silver stain, magnification × 100

**FIGURE 3:** Photomicrographs of rat cerebral cortex 8 days after head trauma: (A) saline control; (B) treated with vitamin D₃. Significant improvement is seen in the cortex from the rat treated with vitamin D₃ compared with the saline control. Haematoxylin and eosin, magnification × 100
Neurotrophic factors are thought to play important roles in neuronal damage, and several reports indicate that vitamin \( \text{D}_3 \) may be involved in the regulation of these factors. It has been shown to reduce ischaemia-induced brain damage, possibly through the upregulation of glial cell line-derived neurotrophic factor (GDNF) mechanisms in the cortex. Vitamin \( \text{D}_3 \) is a potent inducer of trophic factors in vitro: it augments GDNF expression in \( C_6 \) glioma cells and increases expression of nerve growth factor (NGF) and transforming growth factor-\( \beta \) in neuroblastoma cells. Pre-treatment with vitamin \( \text{D}_3 \) has been shown to confer neuroprotection before head trauma, but its therapeutic effect after trauma is not known. In the present study, vitamin \( \text{D}_3 \) was not given before trauma for neuroprotection to reflect the fact that trauma is not usually foreseen.

Several drugs have been reported to upregulate NGF synthesis in brain tissue in different cell culture models. It appears that the various NGF-inducing agents act on different cell types via different pathways, showing the complexity of NGF regulation. The sphingomyelin pathway is thought to be activated by vitamin \( \text{D}_3 \). Elevated NGF levels have been reported after brain trauma. This is probably an endogenous protective mechanism to maintain neuronal survival in lesioned brain regions.

Treatment with GDNF has been shown to ameliorate brain oedema after transient focal ischaemia.

Treatment with vitamin \( \text{D}_3 \) significantly increases total and free calcium levels in serum. It has been reported that an increase in the \( \text{Ca}^{2+} \) level potentiates apoptosis, whereas application of \( \text{Ca}^{2+} \) before an insult induces protection by pre-conditioning the ischaemic organ.

Inducible nitric oxide synthase (iNOS) is increased in humans after traumatic brain injury. The dominant cell types that express iNOS after injury are vascular smooth muscle cells, neutrophils and microglia/macrophages. It has been shown that vitamin \( \text{D}_3 \) reduces iNOS in rat brain. The
central nervous system’s response to the ischaemic stress that accompanies trauma is an extremely complicated and controversial process. The inflammatory response is most pronounced during the period from 24 h to 7 days after the injury;\textsuperscript{1,2} we observed this phenomenon in our study.

The potential regulatory effects of vitamin D\textsubscript{3} on cerebral γ-glutamyl transpeptidase (γ-GT) activity have been demonstrated \textit{in vivo} and \textit{in vitro}, in whole brain homogenates, \textit{ex vivo} purified microvessels, an \textit{in vitro} reconstructed blood–brain barrier model, and cultured pericytes and astrocytes. The modulation of γ-GT activity in astrocytes by vitamin D\textsubscript{3} is of particular importance, since these cells are involved in the anti-oxidative process. This action of vitamin D\textsubscript{3} on γ-GT activity could, therefore, be associated with the elimination of toxic agents that cause degeneration and neuronal cell death, such as reactive oxygen or nitrogen intermediates.\textsuperscript{23 – 26}

In conclusion, we have demonstrated that systemic administration of vitamin D\textsubscript{3} protects the axons from secondary axotomy following brain trauma. This vitamin may, therefore, be useful in the management of DAI.

Conflicts of interest

No conflicts of interest were declared in relation to this article.

References


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