Transforming growth factor-beta (TGF- β) in acute injuries of the spinal cord

J. F. Quinlan,
R. W. G. Watson,
G. Kelly,
P. M. Kelly,
J. M. O'Byrne,
J. M. Fitzpatrick

*From the Mater
Misericordiae
Hospital, Dublin,
Republic of Ireland*

Injuries to the spinal cord may be associated with increased healing of fractures. This can be of benefit, but excessive bone growth can also cause considerable adverse effects.

We evaluated two groups of patients with fractures of the spinal column, those with neurological compromise (n = 10) and those without (n = 15), and also a control group with an isolated fracture of a long bone (n = 12). The level of transforming growth factor-beta (TGF- β), was measured at five time points after injury (days 1, 5, 10, 42 and 84).

The peak level of 142.79 ng/ml was found at day 84 in the neurology group (p < 0.001 vs other time points). The other groups peaked at day 42 and had a decrease at day 84 after injury (p $\leq$ 0.001).

Our findings suggest that TGF- β may have a role in the increased bone turnover and attendant complications seen in patients with acute injuries to the spinal cord.

Studies on patients with head injuries have shown that the healing response of fractures is enhanced in direct proportion to the severity of the neurological injury.¹ While the cause of this is unclear, neural mechanisms have been proposed. The response may be increased to the extent that heterotopic ossification occurs.^{1,2}

Heterotopic ossification was first described as a complication of neural disorders in 1918.³ It has been associated with a wide variety of spinal disorders and occurs in 15% to 40% of patients with injury to the spinal cord.^{4,6} Wittenberg, Peschke and Botel⁷ reported an incidence of 20%. Experimental work to date has concentrated on animal models with induced lesions of central and peripheral nerves.⁸⁻¹⁰

While the benefits of increased fracture healing in patients with such injuries is obvious, there are also side-effects such as joint stiffness and fusion¹¹⁻¹⁶ and calculi of the renal tract.¹⁷⁻¹⁹

The transforming growth factor-beta (TGF- β) family is a group of multifunctional polypeptide growth factors²⁰ which have a broad range of activity in bone tissue, connective tissue and the immunological system. Bone and platelets contain almost 100 times more TGF- β than any other tissue and osteoblasts have the greater number of TGF- β receptors.^{21,22} In rat calvarial and human osteoblast cell lines, TGF- β has been found to increase cell proliferation.^{23,24}

Bostrom and Asnis²⁵ proposed that the TGF- β family has a role in the development, induction

and repair of bone. The first *in vivo* studies demonstrating the stimulatory effects of TGF- β on the formation of bone used injections of TGF- β into fetal rat and mice calvaria and found a marked increase in bone formation.^{26,27} Further animal studies have shown increased osteoblastic stimulation using local and systemic administration of TGF- β ²⁸⁻³¹ as well as specific delivery mechanisms such as a methylcellulose gel carrier.³² To date, there have been no studies which have examined the level of this known osteogenic cytokine in patients with injuries to the spinal cord.

Our aim, therefore, was to investigate whether patients with acute spinal-cord injuries had increasing levels of TGF- β during the immediate period after injury and whether these levels were higher than those seen in similar groups without neurological compromise.

Patients and Methods

Consecutive patients admitted to our institution were recruited. They were divided into two groups, namely those with fractures of the spinal column and neurological compromise, referred to as the neurology group (n = 10), and those with fractures and no neurological compromise, referred to as the no neurology group (n = 15). A further group with an isolated fracture of a long bone, referred to as the long-bone group (n = 12), was also included as a control. The groups had similar mean ages and gender distribution. There were ten males

■ J. F. Quinlan, MCh, AFRCS
I, Orthopaedic Registrar
■ P. M. Kelly, FRCS(Orth),
Orthopaedic Registrar
■ J. M. O'Byrne, MCh,
FRCS(Orth), Consultant
Orthopaedic Surgeon
■ J. M. Fitzpatrick, MCh,
FRCS I, FRCS G, FRCS,
Professor of Surgery
School of Medicine and
Medical Science
Mater Misericordiae
University Hospital, Eccles
Street, Dublin 7, Republic
of Ireland.

■ R. W. G. Watson, PhD,
Senior Lecturer
School of Medicine and
Medical Science
■ G. Kelly, PhD, Senior
Lecturer
School of Mathematical
Sciences
University College Dublin,
Belfield, Dublin 4, Republic
of Ireland.

Correspondence should be
sent to Dr R. W. G. Watson;
e-mail:
william.watson@ucd.ie

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Fig. 1a

Fig. 1b

Fig. 1c

The level of TGF- β per patient per time point in a) the neurology, b) the no neurology and c) the control groups.

and no females with a mean age of 32.9 years in the neurology group, 12 males and three females with a mean age of 31.9 years in the no neurology group and nine males and three females with a mean age of 40 years in the long-bone group. A total of 33 (89.2%) patients had sustained their injuries in either road-traffic accidents (17) or falls (16). The patients in the neurology and no neurology groups had fractures at similar vertebral levels.

The study had been approved by the local ethics committee and written consent had been obtained from the patients.

The level of TGF- β assessed from venous blood samples was taken at five time points after the injury: day 1 (0 to 2),

day 5 (4 to 6), day 10 (9 to 11), day 42 (35 to 49) and day 84 (77 to 91). A total of 146 individual serum samples was obtained, representing 78.9% of the total available samples. The main reasons for missing samples were delay in transfer to our tertiary referral unit at the first time point and failure of follow-up at the later time points (Table I).

The samples were transported on ice and centrifuged at 1100 rpm for ten minutes and then separated and frozen at -80°C for batch analysis.

Each serum sample was analysed in duplicate for TGF- β using a commercially-available enzyme-linked immunosorbent assay (ELISA) kit (R+D Systems Europe Ltd, Abingdon, UK) in accordance with the manufacturer's

instructions. All samples were taken from the -80°C freezer and thawed at room temperature. The results were expressed as ng/ml.

Statistical analysis. The TGF- β levels were examined giving a profile for each subject. A quadratic function of time was fitted to the data of each subject separately, resulting in estimates for the subject-specific intercept and the two subject-specific slopes. This was done using a mixed-effects model through the mixed procedure in the statistical software SAS (SAS Institute Inc., Cary, North Carolina). The fixed effects were the regression parameters (intercept and two slopes) which were assumed to be the same for all subjects in a particular group (yielding mean profiles), while the random effects were the subject-specific regression parameters. A mean profile which was quadratic over time was fitted to each group and subjects within a group varied randomly about this mean. The repeated observations over time within each subject were specified to have an unstructured covariance matrix. Simpler forms for the covariance matrix were also fitted. Checking of the model was carried out by examination of the studentised residuals, influence statistics and normal probability plots. Restricted maximum likelihood was used to estimate the model.³³ A *p* value of less than 0.05 was considered significant.

Results

The results for the neurology group at the first three time periods i.e. day 1 (9 samples analysed), day 5 (9) and day 10 (10) after injury, were similar with means of 73.91 ng/ml, 73.15 ng/ml and 71.54 ng/ml, respectively. At day 42 (9) after injury, the value rose to a mean of 95.66 ng/ml.

The mean level in this group rose again and peaked at day 84 (4) after injury at 142.79 ng/ml. The level then increased linearly over time ($p < 0.001$). The profiles over time for each patient are shown in Figure 1.

A different pattern was seen in the no neurology group. The lowest recorded mean level of 64.03 ng/ml was seen at day 1 (6) after injury. The level peaked at day 42 (10) after injury at a mean of 112.52 ng/ml. At the final time point, day 84 (14) after injury, it fell slightly to 103.51 ng/ml. The linear progression noted in the neurology group thus was not seen in the no neurology group and this was significant, i.e. in the no neurology group, the progression was quadratic ($p = 0.001$; Fig. 2).

In the long-bone group there was an early rise at day 1 (10) after injury with a mean value of 96.31 ng/ml. At day 5 (11) and day 10 (10) after injury the level was 54.57 ng/ml and 99.32 ng/ml, respectively.

Similar to the results in the no neurology group, the mean level in the long-bone group peaked at day 42 (9) after injury at 112.71 ng/ml. At day 84 (6), it decreased to 102.28 ng/ml. These results did not represent a significant linear increase ($p = 0.001$) as was seen in the neurology group.

When the results of the various groups were analysed at day 1 after injury, it was seen that the mean level in the neu-

Fig. 2

The level of TGF- β in each group throughout the period of study.

Table I. Patients lost to follow-up at relevant time intervals

	Time (days)				
	1	5	10	42	84
Neurology	1	1	0	1	6
No neurology	9	0	1	5	1
Long bone	2	1	2	3	6

rology group (9 samples analysed) (73.91 ng/ml) was higher than that in the no neurology group (6) (64.03 ng/ml), but lower than that of the long-bone group (10) (96.31 ng/ml) (Fig. 2).

A similar pattern was seen at day 5 after injury. The mean recorded level of TGF- β for the neurology (9), no neurology (15) and long-bone groups (11) was 73.15 ng/ml, 68.79 ng/ml and 54.57 ng/ml, respectively.

There was a different trend at day 10 after injury in the neurology group (10) which had a lower mean level (68.27 ng/ml) than the no neurology group (14) (101.75 ng/ml) and the long-bone group (10) (99.32 ng/ml).

The mean level of TGF- β recorded at day 42 after injury was not significantly different. The level recorded for the neurology group (9), no neurology group (10) and long-bone group (9) was 95.66 ng/ml, 112.52 ng/ml and 112.71 ng/ml, respectively. However, in the case of the last two groups, the results at day 42 after injury represented the peak values seen.

At day 84 after injury, the mean value for the neurology group (4) was 142.79 ng/ml which represented a significant linear increase over the five time periods ($p < 0.001$). The mean level of 103.51 ng/ml in the no neurology group and of 102.28 ng/ml in the long-bone group (6) represented a

decrease from their respective values at 42 days after injury, thus not showing a linear increase ($p \leq 0.001$) as was seen in the neurology group.

The diagnostic checks revealed no departures from the fitted model.

Discussion

In our study the patients in the neurology group all received intravenous methylprednisolone according to the protocol of the American Spinal Injuries Association^{34,35} (30 mg/kg in 500 ml of dextrose over 15 minutes followed by an infusion of 5.4 mg/kg/per hour in dextrose continued for 23 hours).

The uniformity of the levels of TGF- β at days 1, 5 and 10 after injury suggests that the steroids are influencing the levels of TGF- β . This makes comparison between the three groups difficult and liable to bias.

A further confounding factor was the effect on inflammatory markers of intramedullary reaming in the long-bone group. This was performed in 11 of the 12 patients.³⁶

At day 42 there was no difference between the mean level in the three groups.

At day 84 the mean level for the neurology group was at its peak. This represented a statistically-significant linear increase in TGF- β throughout the time points in this group ($p < 0.001$) and contrasted with the level in the other two groups which peaked at day 42 after injury and thus showed a significant non-conformity with the observed linear increase ($p \leq 0.001$).

We believe that this increase at day 84 after injury validated the aims for our experiment. The unwanted side-effects of increased bone turnover seen in the patients in the neurology group are long-term problems and our results suggest that TGF- β may have a role as a clinical marker. In addition, the fact that heterotopic ossification can be present as early as four weeks after spinal injury³⁷ suggests that the process is well underway by 84 days after injury. A further long-term study is necessary to determine if these effects persist.

The clinical significance of these results is further suggested by virtue of the relative uniformity of the results within each group at each time point as shown by the respective SDs. When the concentration of TGF- β in relation to osteoblasts^{21,22} and the effect that it has on increased bone turnover and fracture healing are considered,^{23,24,29,30} we believe that these results support our theory that TGF- β has an active role in the mediation of the side-effects of acute injuries to the spinal cord. Furthermore, we feel that the possible role of TGF- β as a marker for increased bone turnover in patients with such injuries warrants further investigation.

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