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Reduction of traumatic brain injury-induced cerebral oedema by a free radical scavenger

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Abstract

Oxygen derived free radicals have been proposed to be in part responsible for the cerebral oedema resulting from head injury. In the present study the effects of free radical suppression with MDL 74,180 (2,3-dihydro-2,2,4,6,7-pentamethyl-3-(4-methylpiperazino)-methyl-1-benzofuran-5-ol dihydrochloride), an α-tocopherol analogue free radical scavenger, on the development of cerebral oedema resulting from head injury has been assessed. Fluid percussion head injury in rats caused a regional oedema 48 h after injury. Infusion of MDL 74,180 for 2 h after the injury significantly attenuated oedema development in a dose-related manner. Using magnetic resonance imaging, cerebral oedema development was monitored in head injured mice. Oedema was apparent 4 h after head injury and was greatest in the vicinity of the olfactory bulb and surrounding the ventricles. Treatment with MDL 74,180 (1–10 μg/kg intravenously, administered 3–5 min after the injury) significantly reduced the oedema development. MDL 74,180 is a potential treatment for the oedema caused as a result of head injury

Keywords: Head injury; Edema; Antioxidant free radical scavenger; Magnetic resonance imaging

1. Introduction

The consequences of traumatic injuries to brain or spinal cord have long been recognized, but until recently it was thought that they result from the initial mechanical event, are instantaneous and irreversible. Studies performed in the last 10 years, however, have provided evidence which suggests that the mechanical insult gives rise to the primary event which results in secondary autodestructive tissue damage. It now appears that some elements of diffuse axonal injury are processes rather than events, that excitotoxicity, prostanoid production, free radical formation and hence lipid peroxidation all play a role in traumatic brain injury and that much of the brain damage that follows trauma may be caused by secondary insults that occur minutes, hours or days after the original injury (Miller, 1993).

In the first few minutes after experimental fluid percussion injury in the cat, brain prostaglandin levels increase secondary to the release of arachidonic acid and accelerated arachidonate metabolism, a pathway which leads to superoxide anion production (Ellis et al., 1981). About the same time activation of brain phospholipase C occurs (Wei et al., 1982) and a consequent rise in tissue levels of cyclogenase products of arachidonic acid including free radicals. More direct evidence for oxygen radical formation after experimental brain injury was provided by Kontos and Wei (1986), who demonstrated that the radicals are produced not only in cerebral blood vessel walls but also probably by leukocytes and macrophages that accumulate in the brain starting after 3–4 h and peaking 24 h after the injury.

Blood brain barrier damage and the resulting oedema is inevitable following central nervous tissue injury caused by physical trauma and ischaemia. Following head injury there is a secondary cerebral vasodilatation together with a loss of vascular reactivity. Evidence suggests that the cerebral microvasculature is a major target of this secondary tissue damage which is initiated largely by free radicals (Kontos and Povlishock, 1986). These authors suggested that the principle mediators of the microvascular damage are superoxide anions. Schettini et al. (1989) described a canine model which demonstrated considerable

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improvement in cerebral blood flow, brain oedema and histological damage after treatment with intravenous (i.v.) superoxide dismutase, and Levasseur et al. (1989) reported improved survival and outcome in rats subjected to fluid percussion injury after treatment with superoxide dismutase. Ikeda et al. (1989) reported that free and polyethylene glycol-superoxide dismutase did not prevent the development of brain oedema in cats after a cortical freezing lesion, but superoxide radicals were detected in the cold lesion. Similarly, Chan et al. (1986) reported, in cold-induced injury in rats, an early elevation of superoxide radicals followed by permeability changes in the blood brain barrier and development of oedema in injured brain. Administration of liposome entrapped copper-zinc superoxide dismutase (i.v.) both 5 min before or 5 min after the injury reduced the levels of superoxide anions and ameliorated the blood brain barrier permeability changes and brain oedema. Muizelaar (1993) and Muizelaar et al. (1993) have reported that in severely head injured humans, death and vegetative state occurred twice as often in the 26 patients receiving placebo compared with the group receiving polyethyleneglycol-superoxide dismutase (10000 U/kg bolus; $P < 0.03$).

Recent data indicate hydroxyl radicals are important in the initiation of vascular endothelial damage and the subsequent break down in the blood brain barrier by triggering the peroxidation of membrane polyunsaturated fatty acids (Hall et al., 1993; Smith et al., 1994).

Levels of endogenous antioxidants, such as α-tocopherol, retinoic acid, ubiquinones, and ascorbic acid are reduced following CNS trauma (Lemke et al., 1990). The reduction in the endogenous antioxidant defence may allow uncontrolled lipid peroxidation of cell membranes and cell death. α-Tocopherol has been shown to exert neuroprotective effects in experimental models of traumatic spinal cord injury (Anderson et al., 1985; Saunders et al., 1987) and traumatic brain injury (Clifton et al., 1989; Hall and Yonkers, 1989; Wei et al., 1981). Yoshida et al. (1983) have demonstrated that diet supplementation with vitamin E reduced compression-induced oedema in rats, whereas depletion of dietary vitamin E lead to a 50% greater swelling ratio of the right cerebellum to the left. (water content of the right compressed cerebellum compared to the left side which was not compressed). However, one limitation of α-tocopherol in the acute situation is its lipophylicity, although Stein et al. (1991) have reported that intracerebral administration of α-tocopherol containing liposomes facilitates recovery in rats with bilateral lesions of the frontal cortex. Nevertheless, other free radical scavengers such as methyl prednisolone, tirilazad and *N*-acetylcysteine as well as superoxide dismutase, as mentioned above, have been shown to improve neurological outcome after experimental head injury (Hall, 1985; Hall et al., 1988; Ellis et al., 1991) and to attenuate hippocampal oedema contralateral to the site of head trauma (McIntosh et al., 1992).

MDL 74,180, 2,3-dihydro-2,2,4,6,7-pentamethyl-3-(4-methylpiperazino)-methyl-1-benzofuran-5-ol dihydrochloride, was selected from a series of α-tocopherol analogues on the basis of its radical scavenging properties. Its IC_{50} as an inhibitor of spontaneous lipid peroxidation in rat brain homogenate was determined to be 0.45 ± 0.06 μM and its relative rate constant for reaction with superoxyl radicals was equal to $10.7 \pm 2.9 \times 10^4$ M^{-1} s^{-1} (Grisar et al., 1995). MDL 74,180 has been shown to inhibit ex vivo lipid peroxidation in mouse brain, demonstrating brain penetration and to reduce the effects of head injury in mice (Grisar et al., 1995). In the present study the effects of MDL 74,180 on the development of regional cerebral oedema following lateral fluid-percussion injury in the rat, and blunt head injury in the mouse have been examined. Regional brain oedema in the rat was determined by wet weight/dry weight methodology and magnetic resonance imaging (MR imaging) to visualise and measure oedema development in the mouse.

2. Materials and methods

Male OFA rats (300–400 g) and male NMRI mice (19–23 g; Iffa Credo, Lyon, France) were used. Water and food were provided ad libitum before and after each part of the experiment. Animals were kept under controlled conditions with respect to light, temperature and humidity.

2.1. Fluid percussion brain injury in the rat

The technique used is similar to that described by Dixon et al. (1987).

2.1.1. Surgery

Rats were anaesthetised with chloral hydrate (350 mg/kg). A femoral vein was cannulated for compound infusion. After the animal was placed in a stereotaxic frame, the scalp and temporal muscles were separated and a 2 mm craniotomy over the left parietal cortex, equidistant (5 mm) from bregma and lambda and 4 mm from the sagittal suture was carried out. The dura was left intact at this opening. Into the opening was placed a 2.1×38 mm Luer-Lok fitting, a needle hub from which the stainless steel part had previously been removed to within 1–2 mm of the plastic Luer-Lok fitting, which was fixed rigidly to the skull with dental cement.

2.1.2. Fluid percussion injury

The fluid percussion device was composed of a Plexiglas cylindrical reservoir, 60 cm long, which was attached at one end to a cork-covered Plexiglas piston mounted on 'o' rings. The opposite end of the reservoir was fitted with a transducer and a 5 mm tube that terminated with a male Luer-Lok fitting. At the time of injury the device was connected to the female Luer-Lok fitting on the rat's head,

which had been chronically implanted 24 h previously. With the entire system filled with saline at 37°C a metal pendulum was released from a predetermined height to strike the piston producing a pressure pulse, measured in atmospheres.

2.1.3. Experimental protocol

24 h later rats were lightly anaesthetised with ether and subjected to lateral fluid percussion injury through the cannula using a pressure of 2.8 atm. MDL 74,180, or saline (vehicle control) were infused i.v. (0.01–3.0 mg/kg per h) into the femoral vein beginning 3–5 min after the injury for a 2 h period. These doses were selected on the basis that they were shown to produce the largest decrease in infarct size after middle cerebral artery occlusion and reperfusion.

2.1.4. Determination of cerebral oedema

48 h later the rats were killed, the brain removed and the olfactory bulbs, area of pre and frontal cortex, parietal cortex, striatum, septum (including the lateral ventricle) and ventral hippocampus were isolated. Preliminary experiments demonstrated that oedema, following lateral fluid percussion injury in the rat, was already fully developed after 48 h. All brain areas were weighed, and dried during a 48 h period at 200 torr and 80°C. The percentage water was calculated as:

$$\%\ \text{water} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100$$

2.2. Blunt head injury in the mouse

2.2.1. Head injury method

The technique used is similar to that described by Hall et al. (1988). Each mouse was held by the dorsal skin of the neck and its head carefully positioned under the injury apparatus, with the chin resting firmly on the base of the apparatus. The injury weight was then released, falling freely to strike a Teflon impounder resting on the top of the head. In all of the pharmacological studies, a 50 g weight was dropped 18 cm resulting in the same impact injury. Groups were composed of six mice which were treated after injury as described below.

2.2.2. Drug evaluation

MDL 74,180 (0.001 and 0.01 mg/kg) was investigated in male NMRI mice, administered i.v. as a bolus injection into a tail vein 3–5 min after head injury. These particular doses were selected on the basis of previous findings in which it was shown that MDL 74,180 produced a 'bell'-shaped dose response relationship with respect to 'grip time'. The maximum prolongation of the grip time occurred with a dose of 0.01 mg/kg which increased the grip time by 158% compared to the saline-treated injured group.

MDL 74,180 was dissolved in saline, which was used to treat injured-control animals. Sham injured animals received an i.v. injection of saline but were not subjected to the head injury.

2.2.3. MR imaging

At various times, from 1 to 36 h, after head injury, mice were anaesthetised with an intramuscular injection of ketamine (100 mg/kg; Uva) and xylazine (2 mg/kg; Bayer). Animals were fixed on a support which permitted good reproducibility of their position in the imaging coil. Air was blown into the apparatus during acquisition to avoid hypoxia.

Magnetic resonance images were performed on a Bruker MSL 200 spectrometer equipped with a vertical wide bore magnet and with micro-imaging accessories: gradient coils and unit and image processor. A 40 mm diameter resonator, proton tuned, was used for acquisition of images.

All images realised were 256 × 256, 1 mm thick and 4 × 4 cm wide. Different acquisition sequences providing different contrasts were used. A guide sagittal slice was followed by the acquisition of a set of five coronal slices at 2 mm intervals. The first section was taken at the level of the olfactory bulb.

A gradient echo, T_1- and T_2^*-weighted, fast imaging, sequence was first used, with an echo time (T_E) of 10 ms and a repetition time (T_R) of 379 ms. Two spin echo sequences were then acquired, with $T_E = 30$ ms. The first sequence was either a T_2-weighted, fast imaging, RARE sequence, with a rare factor equal to 4 and T_R equal to 3190 ms to visualize oedema through T_2 increase, or a multiecho CPMG sequence, eight echos, $T_R = 1900$ ms. This last sequence allowed the computation of T_2 images. The second spin echo image, one echo, used a short-recovery time $T_R = 690$ ms, to obtain T_1-weighted images. All images were transferred and translated to TIFF format for measurement of the oedematous area. A programme was developed to convert the original image files (native format from Bruker) into a standard image file format (TIFF). The intensity values (24 bits) were mapped to gray values (8 bits). Oedematous areas were measured by means of an image analysis processing system (Imaging Research Inc. Brock University, St. Catherines, Ontario, Canada L2S 3A1) on a PC computer.

3. Results

3.1. Fluid percussion brain injury in the rat

Fluid percussion injury resulted in a significant regional oedema 48 h after injury, in the right olfactory bulb, contralateral to the injury site (Fig. 1), parietal cortex, both ipsilateral and contralateral to the injury site and ventral

Table 1
Regional brain oedema measured by the wet weight/dry weight method 48 h after lateral fluid percussion head injury in rats

Brain region	Saline-treated injured rats		Sham-injured rats	
	Left side	Right side	Left side	Right side
Olfactory bulb	81 ± 3 [b]	85 ± 5	80 ± 5	79 ± 4 [b]
Frontal cortex	79 ± 2	81 ± 2	78 ± 2	80 ± 6
Parietal cortex	81 ± 3	80 ± 3	77 ± 2	77 ± 3 [a]
Striatum	76 ± 2	76 ± 2	73 ± 2	72 ± 3 [b]
Ventral hippocampus	78 ± 4	81 ± 4	75 ± 5	77 ± 6

The results are expressed as the mean ± S.D. of 7–10 rats in each group. [a] $P < 0.05$. [b] $P < 0.01$ when compared to the oedema occurring on the right side of saline-treated injured animals.

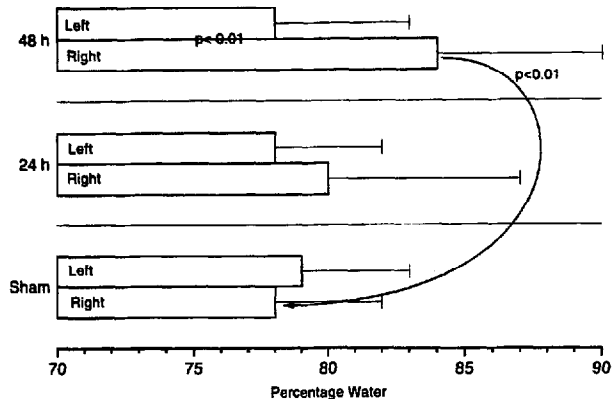

Fig. 1. Development of oedema in the two sides of the olfactory bulb following lateral fluid percussion head injury and sham injury in rats. The oedema was measured by the wet weight /dry weight method and is expressed as percentage water. Oedema development was measured in different groups of rats 24 and 48 h after the head injury. The results are expressed as the mean ± S.D. of ten animals.

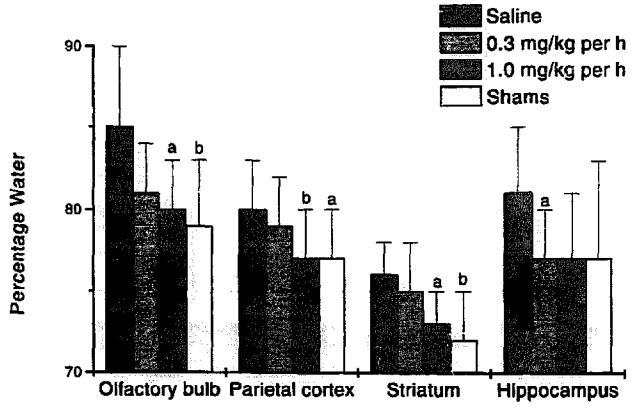

Fig. 2. The effect of MDL 74,180 infusion (2 h) on regional brain oedema development 48 h after lateral fluid percussion head injury in the rat The infusion commenced 3–5 min after the head trauma. Only the effects in areas on the right side of the brain are shown. The oedema, measured by the wet weight/dry weight method, is expressed as percentage water and the results are expressed as the mean ± S.D. of 7–10 rats in each group. [a] $P < 0.05$, [b] $P < 0.01$ when compared to saline-treated injured animals by ANOVA with application of Dunnet's multiple comparisons test.

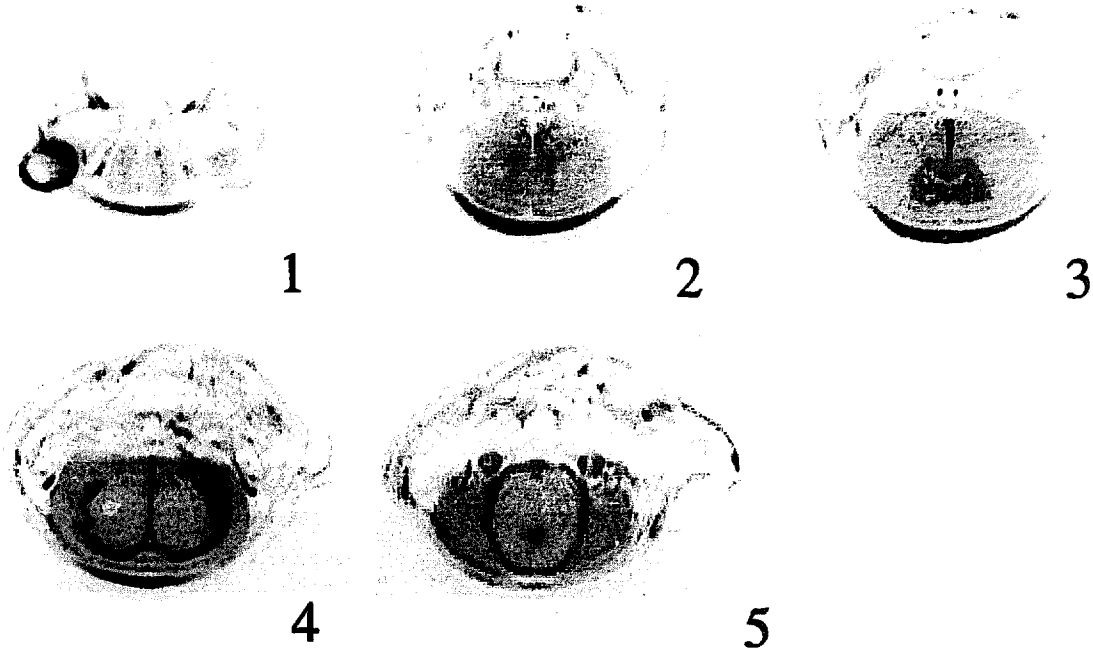

Fig. 3. T_2-weighted images of brain oedema after blunt head injury in mice. The surface oedema was measured on five consecutive coronal images of forebrain, where it is apparent that 4 h after the traumatic injury most of the oedema is present on image 1, the olfactory bulb, and a periventricular oedema particularly on image 3, the lateral ventricle.

hippocampus, ipsilateral and contralateral to the injury site (Table 1).

Infusion of MDL 74,180 for 2 h after head injury significantly attenuated oedema development in the right olfactory bulb, parietal cortex, striatum and ventral hippocampus (Fig. 2). The most effective dose was 1 mg/kg per h reducing the level of oedema in the above mentioned areas to that apparent in sham-injured animals. No significant decrease in brain oedema after infusion of MDL 74,180 was evident in areas on the left, injured side of the brain.

3.2. Blunt head injury in the mouse

Using MR imaging cerebral oedema development was monitored in the same mice 4, 20, 24 and 28 h after head injury. The surface oedema was measured on five consecutive coronal images of forebrain (Fig. 3), where it is apparent that 4 h after the traumatic injury the percentage oedema was greatest on image 1, the olfactory bulb, and a periventricular oedema particularly on image 3, which corresponds to the lateral ventricle (Fig. 3).

Treatment with MDL 74,180 (1 and 10 μg/kg) administered i.v. 3–5 min after head injury had no significant effect on the total brain water content, as assessed by MR imaging 4 and 20 h after the injury, although the higher dose of 10 μg/kg tended to reduce the oedema. When the oedema was expressed as a percentage, 4 h after the injury both doses induced a significant reduction in the oedema development at the level of the olfactory bulb ($P < 0.05$) and the 10 μg/kg dose significantly reduced the oedema at the level of the ventricular system ($P < 0.05$; Fig. 4).

20 h after injury and treatment with the radical scavenger there was no statistical difference between the oedema of treated animals and the saline-treated controls.

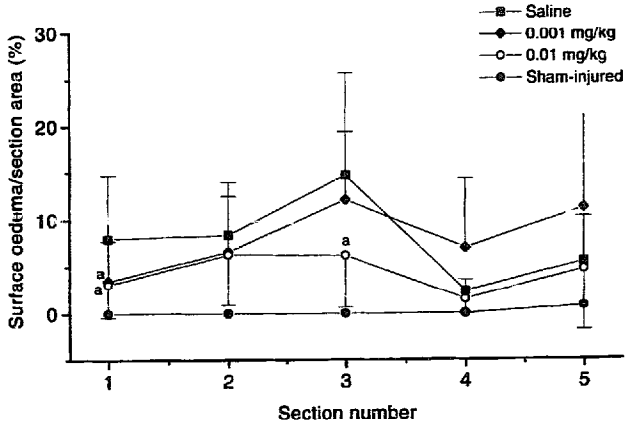

Fig. 4. The effects of MDL 74,180 injection i.v. 3–5 min after blunt head injury in mice on brain oedema development 4 h after the injury. The results are expressed as the mean of 7–10 mice in each group and were compared by ANOVA with application of Dunnet's multiple comparisons test. [a] $P < 0.05$ when MDL 74,180 treated animals are compared to saline treated injured animals.

4. Discussion

The results of the present study demonstrate that acute treatment with MDL 74,180 reduced brain oedema following traumatic brain injury, both in the rat after fluid percussion injury and the mouse after blunt head injury.

In the rat a decrease in oedema was apparent 48 h after traumatic head injury, in particular in the right olfactory bulb, parietal cortex, striatum and hippocampus. The lack of a profound effect on the left side of the head may be due to greater irreversible tissue damage which occurs on the impact side, since in these areas greater haemorrhage and a more prolonged decrease in regional cerebral blood flow occurs as reported by McIntosh et al. (1992). Similarly, treatment with the 21-aminosteroid lipid peroxidation inhibitor, tirilazad (3 mg/kg i.v.) 15 min and 3 h after fluid percussion head injury induced a decrease in right hippocampal oedema, contralateral to the injury side, 48 h after injury (McIntosh et al., 1992).

Subsequent to fluid percussion head injury, there is a secondary cerebral arteriolar vasodilatation together with a loss of reactivity to vasoactive agents. Concomitantly a disruption of the blood brain barrier occurs resulting in sodium and protein accumulation and osmotic fluid expansion of the brain extracellular space. Suggestive evidence from experimental oedema models and the effects of exogenously applied antioxidants (see Section 1) support an involvement of oxygen-derived free radicals and lipid peroxidation in the development of post-traumatic brain oedema. In fact, Smith et al. (1994) have demonstrated a burst of hydroxyl radical formation immediately after cortical impact head injury, followed by an increase in both lipid peroxidation and blood brain barrier permeability. The blood brain barrier opened in a time-dependent fashion between 5 and 60 min after the injury. Treatment with tirilazad (10 mg/kg i.v. 5 min post injury) significantly attenuated the blood brain barrier disruption measured 30 min post injury (Smith et al., 1994).

Attenuation of the blood brain barrier disruption could also explain the mechanism by which MDL 74,180 reduced the cerebral oedema in the head injured rats. MDL 74,180, however, was more effective than tirilazad, in that it was able to reduce the oedema in more areas than just the hippocampus (McIntosh et al., 1992). This may be due to the fact that MDL 74,180 was infused for 2 h i.v., whereas the 21-aminosteroid was administered as two separate bolus injections i.v. Alternatively, as MDL 74,180 readily penetrates the blood brain barrier, it can be detected in brain tissue 30 min after s.c. administration (Bolkenius et al., 1996), the antioxidant could rapidly suppress the free radicals produced in injured brain.

Using MR imaging the development of brain oedema caused as a result of blunt head injury in mice has been followed, and the effects MDL 74,180 on the oedema development have been studied. In this particular mouse model the brain water content reaches a maximum at about

24 h after the injury, most of the oedema being present in the area of the olfactory bulb and surrounding the ventricles.

MDL 74,180 reduced the oedema development 4 h after head injury, but was without significant activity 20 h after the trauma. Such a transient reduction in oedema development can be explained by a relatively short duration of action of this particular antioxidant, or alternatively the dose used was inadequate to eliminate all the radicals. However, when infused over a 2 h period, as in the rat, a significant reduction in the percentage water was still evident after 48 h. Hall et al. (1993) have reported that in this mouse model hydroxyl radical formation occurs rapidly in the microvasculature and is very transient. Treatment with tirilazad (1 mg/kg i.v.) 5 min after the injury reduced the hydroxyl radical generation 10 min later.

MR imaging permits the visualisation and eventual measurement of oedema in small areas of brain, which under normal circumstances cannot be measured since the oedematous change becomes diluted by the bulk of normal tissue. Furthermore a positive, significant effect of the free radical scavenger MDL 74,180 on the oedema development has been demonstrated. Unfortunately only the oedema present at 2 mm intervals in a coronal plane was visualized, oedema development in sagittal sections was not considered. This is probably the explanation behind the large variance which surrounds the data.

Therefore, in the present study acute treatment with MDL 74,180 reduces brain oedema following traumatic brain injury, both after fluid percussion and blunt head injury, probably by suppressing the superoxyl radicals (Grisar et al., 1995) produced as a result of the initial injury (Kukreja et al., 1986) and hence, the ensuing hydroxyl radical formation and lipid peroxidation. The transient time course of free radical production as a result of head injury, means that the therapeutic window for treatment with a free radical scavenger is also very short. Therefore a blood brain barrier penetrating free radical scavenger that will interrupt the on-going lipid peroxidation and the cascade of events leading to a post-traumatic pathophysiology in head trauma would seem to be clinically relevant. However, further studies need to be carried out to ascertain how long after the injury the antioxidant can be administered and still produce beneficial effects.

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