Free Rad. Res. Comms., Vol. 6, No. 1, pp. 29–38
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PROTECTION BY DESFERRIOXAMINE AGAINST HISTOPATHOLOGICAL CHANGES OF THE LIVER IN THE POST-OLIGAEMIC PHASE OF CLINICAL HAEMORRHAGIC SHOCK IN DOGS: CORRELATION WITH IMPROVED SURVIVAL RATE AND RECOVERY

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(Received July 15th, 1988; in revised form October 20th 1988)

Haemorrhagic shock was produced in anaesthetized dogs, by rapid arterial bleeding to mean arterial blood pressure 35 mmHg, and maintained oligaemic for 4 h followed by return of withdrawn blood(ROWB). Dogs were observed for 72 h after ROWB for survival and recovery, and, for histopathological (HP) studies on liver, dogs were sacrificed 2 h after ROWB in non-survival experiments. Desferrioxamine mesylate (25 mg/kg) was administered intra-muscularly at 2,3 and 4 h after blood loss in survival experiments and for HP studies the drug was given at 4 h in one group and at 2 h plus 4 h after blood loss in the second group. With the drug given at 3 or 4 h, survival was 70% and 100% while in the 2 h and the untreated groups it was 50%. Recovery was rapid in all the drug treated survivors, few became conscious within 30 min, showed slight activity by 4–6 h, all were almost normally active by 24 and fully so by 72 h after ROWB. All the 5 control survivors remained unconscious/drowsy upto 24 h; 3 were sluggish at 72 h. By group analysis, serum iron elevation during the oligaemic and at the end of the post-oligaemic phase was less in the drug-treated animals. HP changes of shock in the liver studied by light microscopy, were markedly reduced in severity and were less prevalent in the drug-treated dogs. The salutory effects of desferrioxamine may be due to inhibition of iron catalyzed free-radical production and tissue damage, through its strong iron chelating action. It may have a therapeutic advantage in this emergency condition without the disadvantages of toxicity inherent in prolonged use.

KEY WORDS: Haemorrhagic shock, iron catalyzed free-radical production, desferrioxamine, recovery, liver, histopthology.

INTRODUCTION

Irreversibility of shock states continues to be a therapeutic challenge in the management of the critically-ill. The role of oxygen free-radicals in tissue damage is a new dimension to the irreversibility factors. Elevation of serum iron due to its release from ferritin in the hypoxic liver of haemorrhagic shock(HS) animals[1] is suggestive of the possible role of iron in this shock state. Rapid recovery and increased survival rate of standard and clinical haemorrhagic shock dogs given desferrioxamine(DF), an iron chelator by i.v.infusion, has been reported earlier.[2,3]

Increased generation of activated oxygen species,[4] excessive decompartmentalization of iron,[5] and, possible interaction between iron metabolism and free-radical

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generation[6] and formation of hydroxyl radical(·OH),[7–9] is the likely 'triad' operative in the low flow state of the tissues in HS.

Early appearance of histopathological(HP) changes in the organ of shock animals,[10] and their persistance (upto 8 days in the liver[11]) reflects a relationship between extensive tissue damage and irreversibility in the oligaemic and post-oligaemic phase of HS. Generation of oxygen free-radicals is enhanced in the ischaemia and reperfusion injury of the tissues.[12–16] Improved survival rate and recovery with DF administered i.v.in the oligaemic phase of HS[2,3] makes it a promising therapeutic possibility in its management. In the present study the effect of desferrioxamine on post-oligaemic shock has been investigated with the drug administered intra-muscularly. In chronic iron overload diseases, s.c. infusion and i.m.routes are reported to be equally or more effective than the intravenous route.[17,18]

MATERIALS AND METHODS

Haemorrhagic Shock(HS) Model

Healthy mongrel dogs of either sex (b.wt.9–17 kg), observed for 10 days for pre-experimental activity, feeding and bowel habits, were fasted for 12 h before the experiment. HS was produced by the method reported earlier,[3] by which dogs remained oligaemic for 4 h after rapid arterial bleeding to 35 mmHg mean arterial blood pressure(MABP), followed by return of withdrawn blood(ROWB) stored in a suitable anticoagulant mixture in 4:1 ratio. Initial MABP and the bleeding volume(BV) were noted for each dog.

Drug Investigated

Desferrioxamine mesylate(Desferal) from (CIBA-Geigy Pharmaceuticals was administered intramuscularly as a freshly prepared 10% solution in normal saline.

Experimental Groups and Parameters of Observation

Survival experiments There were 4 groups of 10 dogs each, i) the control group, and the three DF-groups, with the drug given at: (ii) 2 h, (iii) 3 h and (iv) 4 h after bleeding. Survival rate for each group was the number of dogs surviving beyond 72 h after ROWB. Survival time of each dog during 24 h after ROWB (Post-oligaemic phase) was noted and the total 24 h survival time and the Mean $\pm$ SD of each group calculated. Post-oligaemic recovery pattern was determined at different time intervals after ROWB in terms of, return of consciousness, level of activity, feeding and bowel movements. Serum iron elevation during the oligaemic and at the end of 2 h post-oligaemic phase was calculated in relation to the pre-bleeding(initial) value for each animal, and from this the Mean $\pm$ SD % change for each group was obtained. Serum iron was estimated by the method of Ramsay[19] which calculates total iron without need for correction for DF.[20] Influence of initial MABP, BV (ml/kg) and serum iron rise on 24 h survival time, the 72 h survival rate and recovery, was assessed by group analysis and the statistical significance was determined by modified student's t-test for unequal numbers.

Histopathological(HP) changes of shock in liver

HP changes of shock were studied in survival experiments in three groups of 6 dogs each: (i) control group, (ii) drug (25 mg/kg i.m.) given at the end of 4 h oligaemic phase, and, (iii) drug given in two doses (25 mg/kg each) at the end of 2 h and 4 h of the oligaemic phase. Liver was removed at the end of the 2 h post-oligaemic phase and immediately immersed in 10% formalin for subsequent sectioning (sections of 5–6 u thickness) and staining with haematoxylin and eosin for HP study by light microscopy.

RESULTS

Survival beyond 72 h and recovery during 72 h after ROWB (Table 1)

All the dogs which received DF at the end of 4 h oligaemic phase survived beyond 72 h while only 50% survived with the drug at 2 h and in the control group. All the five control survivors remained unconscious/drowsy upto 24, and, at 72 h two were drowsy and three were sluggish. Amongst the twenty two DF-treated survivors, three were conscious within 30 min, all (except two) by 4–6 h, all were almost normal by 24 h and fully active by 72 h. The recovery was rapid in the 3 and the 4 h DF groups and dogs took milk feed soon after return of consciousness, while most of the controls had poor feeding upto 72 h. All the controls had bloody diarrhoea or blackish stools in the first 24–48 h, persisting in some upto 72 h. In most of the DF-treated dogs, diarrhoea was less severe and bowel movements were nearly normal by 48 h. There is no correlation of the survival time and rate with the initial MABP and BV.

Group Survival Time over 24 h after ROWB (Table 1)

During 24 h after ROWB, more dogs survived in the 3 and the 4 h DF-groups as compared to the control group and the individual DF dogs survived longer. The 4 h DF-group has the maximum total (240 h) and the mean (24.0 h) survival time, and, that of the control group is minimum (122 h 03 min) as the total, and (12.05 h) as the mean. The differences between the groups are obvious, however, statistically significant only between the 4 h and 2 h DF-groups, and the 4 h and the control group.

Changes in Serum Iron(SI) Levels (Tables 1 and 2)

During the 4 h oligaemic phase of HS, both in the survival (Table 1) and non-survival experiments (Table 2), the control and the groups given the drug at the end of 4 h oligaemia, showed a higher mean serum iron elevation than the respective (Table 1 and 2) drug-treated groups given DF at 2 or 3 h after bleeding, and, the difference from the 2 h group is statistically significant. At the end of 2 h post-oligaemic phase (Table 2) the two dose (at 2 h + 4 h) DF-group had the minimum mean value and % age rise of SI, and the difference from the control and the single dose (at 4 h) DF-group is statistically significant.

Post-Oligaemic Histopathological changes in the Liver (Table 2, Figure 1,2 and 3)

In the control group the HP changes of shock in the liver, removed after the post-

TABLE 1

Effect of Desferrioxamine mesylate (25 mg/kg i.m.) administered at different hours(h)* after rapid arterial bleeding of anaesthetized dogs to MABP 35 mmHg and maintained oligaemic for 4 h, on serum iron rise above the pre-bleeding (initial) values, group survival time during 24 h, and survival and recovery pattern over 72 h, after return of withdrawn blood (ROWB). Correlation of survival and recovery with serum iron elevation, MABP† and bleeding volume (BV)‡

Drug administration (h)* after bleeding		Serum Iron (ug/100 ml)		Survival time upto 24 h after ROWB			Survival beyond 72 h after ROWB	
		Before Oligaemia	At the end of 4 h Oligaemia					
		Initial Mean ± SD	Rise Mean ± SD (%)	Mean ± SD (h)	Group (h)	total (min)	Survivors (no.)	Return of consciousness and recovery of survivors over 72 h at (h) after ROWB
Control	(10)	107.1 ± 6.78	[a]51.25 ± 4.81 (6)	12.05[x] ± 12.59	120	30	5	24 h: 3 UN 2 DR 72 h: 2 DR 3 SL, feed poor
2 h*	(10)	114.0 ± 5.01	[b]16.93 ± 10.48 (8)	13.22[x′] ± 11.64	132	15	5	4–6 h: 2 SL 24 h: all fairly mobile, feed + 72 h: all fully active, feed normal
3 h*	(10)	111.2 ± 6.5	[c]39.83 ± 13.44	17.04[y] ± 11.25	169	45	7	One CN within 30 min 4–6 h: all CN 4 SL 24 h: all almost normally mobile 72 h: all fully active
4 h*	10)	111.0 ± 5.98	[d]57.15 ± 8.47	24.00[y′] ± 0.00	240	–	10	Two CN within 30 min 4–6 h: 2 UN 8 SL 24 h: all fully active and later.

No. of dogs in parentheses: P Values: a–b & d–b = < 0.001; c–b = < 0.01; x–y′ & x′–y′ = < 0.01. UN: Unconscious; CN: Conscious; DR: Drowsy; SL: Sluggish

The differences between Mean ± SD of:

1. Initial MABP† (mmHg) of controls (110.9 ± 6.13) and DF-groups (105.5 ± 28.59; 122.5 ± 33.18 and 108.5 ± 17.38),
2. BV‡ (ml/kg) of controls (21.06 ± 7.9) and DF-groups (18.93 ± 4.55; 21.89 ± 2.52 and 19.45 ± 7.48). are statistically insignificant.

TABLE 2

Effect of Desferrioxamine mesylate (25 mg/kg i.m.) administered at different hours(h)* after rapid arterial bleeding of anaesthetized dogs to 35 mmHg and maintained oligaemic for 4 h, on serum iron (SI) rise above the pre-bleeding (initial) values, during oligaemic phase and at the end of 2 h after return of withdrawn blood, and, on the histopathological changes in the liver removed at the end of 2 h post-oligaemic phase.

Drug administration after bleeding (h)*	†Serum Iron (μg/100 ml) Before Oligaemia (Initial) Mean ± SD	At the end of 4 h Oligaemia Over 2% age rise over initial level) Mean ± SD %	At the end of 2 h post-oligaemic phase Mean ± SD %	Degree and prevalence of histopathological changes in the liver
Control (6)	88.82 ± 20.47	[a]63.83 ± 11.44	[a']117.7 ± 7.23	Marked dilatation of centrilobular vein (CLV) with peri-centrilobular vein pressure changes (P-CLVPC), sinusoidal compression, cellular break up, translucent and vacuolated hepatocytes – a predominant picture in four dogs, in few areas in one and changes mild in one. Moderate neutrophilic infiltration present in all and marked in one.
4 h* (6)	104.7 ± 75.90	[b]74.68 ± 14.05	[b']99.69 ± 21.23	CLV, P-CLVPC and other changes of moderate prevalence and severity in four and mild in two, interspersed with near normal lobular pattern.
2 h* (6) + 4h*	78.4 ± 14.18	[c]49.95 ± 11.48	[c']73.07 ± 13.57	CLV, P-CLVPC and other changes were mild in four and moderate in two, predominantly so in most areas, interspersed with near normal lobular pattern.

Total No. of dogs in Parentheses
†SI estimation in 5 dogs each group.
P values:

$\left.\begin{matrix} a\text{–}a' \\ a'\text{–}c' \end{matrix}\right\} = < 0.001$ $\left.\begin{matrix} c\text{–}c' \\ b'\text{–}c' \end{matrix}\right\} = < 0.05$

$b\text{–}c = < 0.02$

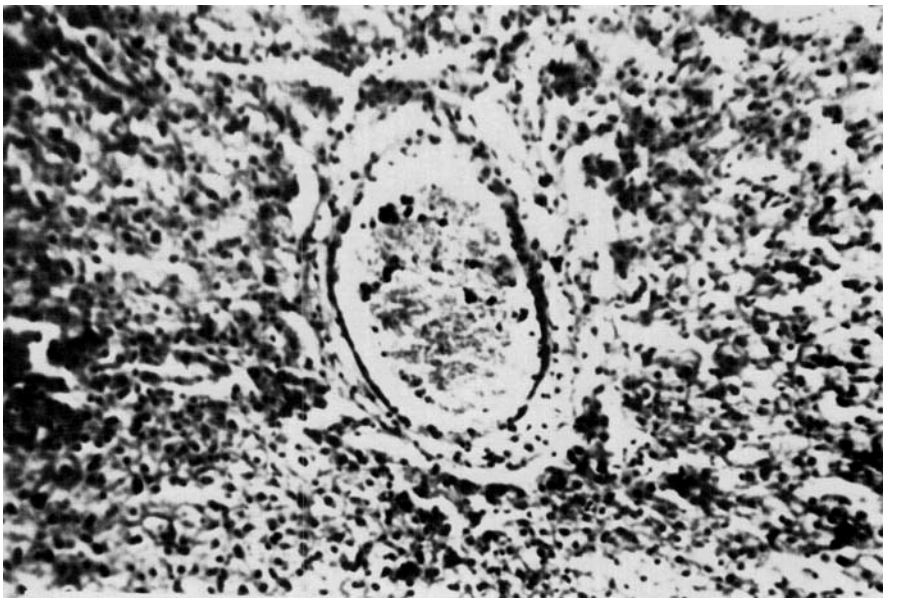

FIGURE 1 Photomicrograph of the liver of a control dog, removed at the end of 4 h oligaemic followed by 2 h post-oligaemic phase of haemorrhagic shock (HS): showing very marked centrilobular vein (CLV) dilatation with peri-centrilobular vein pressure changes (P-CLVPC). Loss of normal lobular pattern, obliteration of sinusoids and vacuolation of hepatocytes is predominant (H & E × 100).

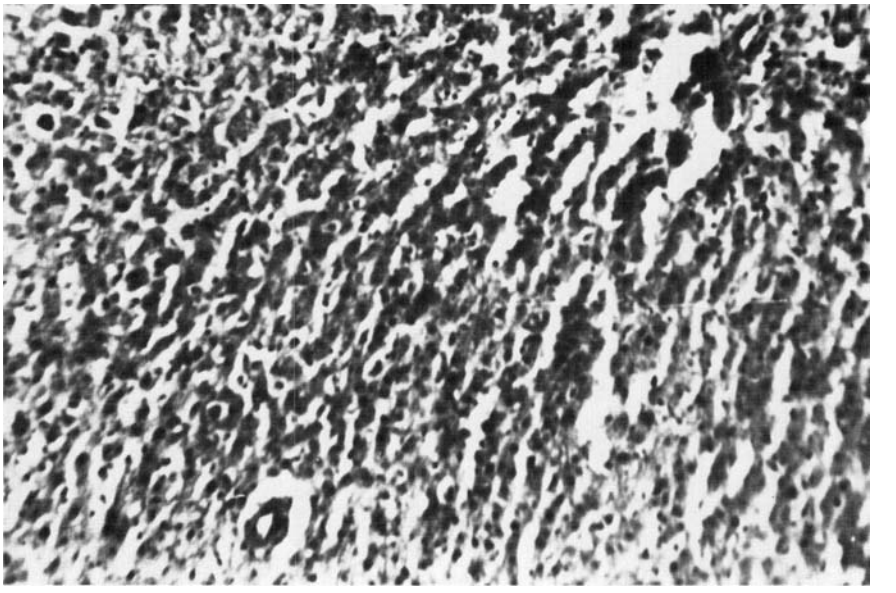

FIGURE 2 Photomicrograph of the liver of a single dose DF-treated HS dog, removed 2 h after desferrioxamine injection and return of withdrawn blood (interval subsequent to bleeding same as in the control dog). There is moderate dilatation of the centrilobular vein with partial peri-centrilobular vein pressure changes. Sinusoidal spaces and normal lobular pattern are evident and few vaculotated cells are seen (H & E × 100).

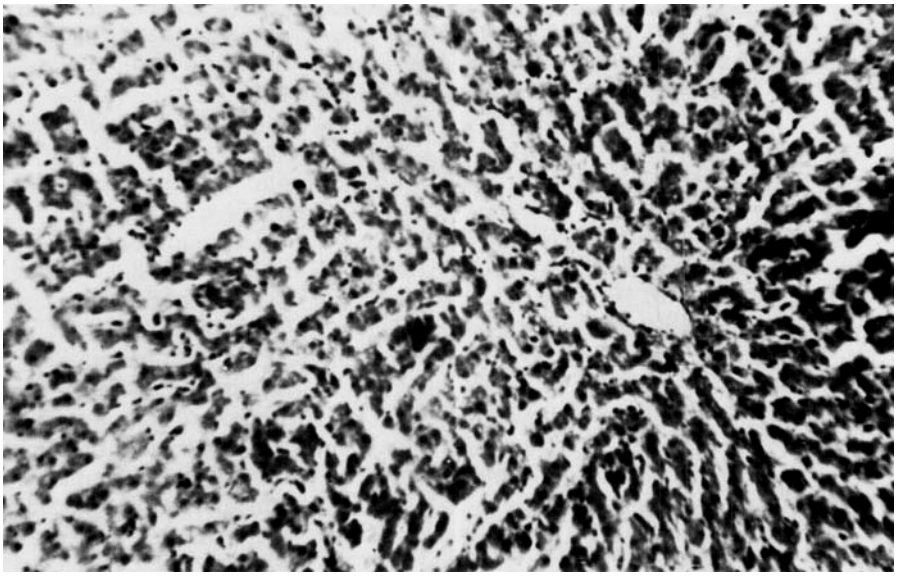

FIGURE 3 Photomicrograph of the liver of a two dose DF-treated HS dog, removed 2 h after the second desferrioxamine injection and return of withdrawn blood (as in the one dose group). First injection was given 2 h after bleeding. Centrilobular vein not dilated, lobular pattern and sinusoidal spaces appear near normal and vacuolation seen in very few cells (H & E × 100).

oligaemic phase of 2 h, were severe, predominant and occurred in all the dogs and the normal lobular pattern was scanty. In the DF-treated dogs, changes were predominantly moderate and mild respectively in the one dose and the two dose groups, and the normal lobular pattern was more prevalent.

DISCUSSION

State of shock following acute blood loss in the oligaemic phase may continue or even be perpetuated after replacement of lost blood,[21] and, this is termed as the post-oligaemic or post-infusion phase of HS. Irreversible post-infusion fall in blood pressure was attributed to a myocardial depressant factor (MDF) dectected in the plasma of experimental animals[22] released from pancreas[23] and intestine.[24] The role of superoxide radical ($\cdot O_2^-$) and its dismutation product H_2O_2, in ischaemia and reperfusion injury of tissues, and protection by superoxide dismutase,[12-14] adds a new dimension to the cause of irreversibility. The results of the present study on desferrioxamine in HS, have relevance to the possible role of iron catalyzed oxygen derived free-radicals in the organ damage in this shock state.

Widespread tissue ischaemia/hypoxia and acidosis are accompaniments of the oligaemic phase of HS, and, deterioration of the organ function initiated in this period extends into the post-oligaemic phase. Decreased ATP synthesis,[10,25] deranged ATP-dependant $Na^+ K^+$ pump of cell plasma membrane,[26] release of proteolytic enzymes from disrupted lysosomes possibly injuring more normal cells and sublethal mitochondrial damage seen in the liver within one hour of the bleeding,[10] and, increased

generation of superoxide radical ($\cdot O_2^-$) due to enhanced leakage of electrons from the damaged mitochondria[27,28] may well be the initial events, subsequently self perpetuating, in the progressive deterioration of HS. Any increase, in the generation of $\cdot O_2^-$ and consequently of hydroxyl radical ($\cdot OH$) from $\cdot O_2^-$ and its dismutation product H_2O_2 by the Haber–Weiss reaction, and of other powerful oxidants,[7–9] implicates iron,[29–31] and aggravates cell damage through lipid peroxidation[32] and its cytotoxic end products.[33] Acidosis of HS favours formation of $\cdot HO_2^-$ from $\cdot O_2^-$; the former crosses the biological membranes more easily, participates in lipid peroxidation,[34] keeps iron in Fe^{2+} state and is a most potent free-radical generator.[35] Recently, the ability of oxyhaemoglobin and methaemoglobin to generate $\cdot OH$ and another highly reactive species in the presence of H_2O_2, has been reported;[36,37] its relevance to shock state could be significant in the presence of haemorrhages in the hypoxic tissues.

Activation of phagocytes due to tissue injury of haemorrhage and ischaemia/hypoxia,[4] enhanced oxidative metabolism and consequent tissue damage,[7,38–40] are factors conducive to early and sustained decompartmentalization of iron; this state of 'acute iron overload' in the present context, is enhanced by lowered threshold for transferrin saturation.[7] Serum iron rise (possibly a reflection of the decompartmentalized iron) during 4 h of oligaemia (Tables 1 and 2) confirms that reported by Janoff *et al.*[1] A superoxide radical ($\cdot O_2^-$) dependant release of iron from the liver ferritin[40] can be immediately used for the formation of $\cdot OH$ radical.[41] The reactions between superoxide and redox active iron assume toxicological significance in HS because of, (i) tissue hypoxia-initial cellular damage-release of lysosomal enzymes and further tissue damage, (ii) free-radical generation interacting with excessive decompartmentalisation of iron, and (iii) acidosis, an inevitable accompaniment of severe and prolonged low flow state.

A link between toxicity of activated oxygen and the normal and pathological metabolism of iron, and the value of iron chelation in preventing the chain reactions, has been suggested.[6,7] DF, a powerful iron chelator and a free radical scavenger,[42,43] firmly binds the Fe^{3+} salts, preventing their reduction, and hence production of $\cdot OH$ radical[44,45] and other oxidants.[46] Thus the detoxication of iron loaded cells[47] with DF would be continual, and the suppression of tissue damage reported in whole animals, hepatocytes and other cellular systems[8,48–50] and that observed in the present HP studies, expectedly sustained. Clinically, DF is used in chronic iron overload disease such as haemochromatosis[51] and in β-thalassaemia, a condition with transferrin saturation and excess iron[52,53] possibly mediating free-radical cell damage.[47] Desferrioxamine is a specific antidote for acute iron poisoning[54] and its use in a shock like episode in haemochromatosis has been speculated.[51]

A post-oligaemic salutory effect of desferrioxamine is evident from the more rapid recovery, longer survival time and higher survival rate with the drug at 3 or 4 h after blood loss (Table 1) and a smaller rise in serum iron levels during the post-oligaemic phase (Table 2) and reduction of the HP changes in the liver with the drug at 4 h and 2 plus 4 h (Figures 2 and 3). With DF given at the end of the oligaemic phase or a short time earlier, the iron catalyzed free-radical production and the tissue damage continuing in the post-oligaemic phase of HS are presumably more effectively arrested due to greater availability of the iron chelating agent in the immediate post-oligaemic phase of HS. Early return of consciousness and rapid recovery of the animals is suggestive of similar effect on the other vital organs and a salutory effect on CNS which is highly vulnerable to free-radical mediated tissue damage in the absence of iron binding antioxidants in CSF.[8]

The results of the present study with desferrixoamine, such as increase in the 72 h survival rate and 24 h survival time, rapid recovery, concomitant minimising of serum iron elevation and the histopathological changes (Tables 1 and 2; Figure 1, 2 and 3) are indirect evidence for the crucial role of iron in the severe tissue damage and irreversibility of HS, and support the speculation that this agent may emerge as a life saving drug for this critical clinical condition. Its efficacy by intramuscular route, in either phase of HS, is of distinct advantage in this emergency condition. Its strong chelating action,[55] of dubious vlaue due to cerebral and ocular toxicity[56–58] with prolonged use in chronic conditions,[59] could be of advantage for emergency use in the critically ill.

Acknowledgement

The technical assistance of Mr. Darshan Kumar and Mr. Sunder Lal for experimental work, Mr. Mohinderpal for serum iron estimation and of Mr. Santokh Singh and Mr. Raghbir Singh for histopathological studies is gratefully acknowledged.

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Accepted by Prof. B. Halliwell