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METAL IONS CATALYTIC FOR FREE RADICAL REACTIONS IN THE PLASMA OF PATIENTS WITH FULMINANT HEPATIC FAILURE

PATRICIA J. EVANS[1], ROBERT W. EVANS[2], ADRIAN BOMFORD[3], ROGER WILLIAMS[3] and BARRY HALLIWELL[1]

[1]Pharmacology Group, University of London King's College, Manresa Road, London SW3 6LX UK [2]Division of Biochemistry and Molecular Biology, United Medical and Dental Schools of Guy's and St. Thomas's Hospitals, Guy's Hospital, London SE1 9RT UK and [3]Institute of Liver Studies, Kings College Hospital, London SE5 9PJ UK

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We propose that the frequency and severity of multi-organ failure (MOF) in fulminant hepatic failure (FHF) involves free radical damage caused by the presence of circulating iron and copper ions, catalytic for free radical reactions. The presence of such metal ions is demonstrated by using the sensitive bleomycin and phenanthroline assays. Antioxidant therapy, e.g., using chelating agents that prevent metal ions from stimulating free radical reactions, may have benefit in the treatment of FHF and its consequences.

KEY WORDS: Fulminant hepatic failure, iron, copper, bleomycin, liver, free radicals.

INTRODUCTION

Fulminant hepatic failure (FHF) is the most severe form of acute liver disease, with a high mortality rate and frequent progression to multi-organ failure (MOF)[1,2]. Causes of FHF include overdoses of paracetamol, viral hepatitis, halothane hepatitis, and idiosyncratic reactions to drugs[1,3]. The development of MOF is independent of the aetiology and has been suggested to be associated with increased production of cytokines and eicosanoids, such as the leukotrienes[4,5]. Treatment of FHF requires high-intensity clinical management, but the prognosis is poor[1-3]. Liver transplantation is an effective therapeutic option, but only if a suitable organ is available[1,3].

The liver is the largest store of iron in the human body and plays a central role in the metabolism of iron and copper[6,7]. Like most other organs, liver takes up transferrin-bound iron from the plasma and unloads iron from it for use in the synthesis of iron-containing proteins[7]. It has been shown in animals that liver also possesses an efficient system for the uptake of low molecular mass non-transferrin-bound iron ions, such as iron bound to citrate or to glutamate[8-10]. For example, cell injury can result in release of intracellular iron[11,12], which may eventually be removed from the circulation by the liver[8-10]. Removal of such low-molecular-mass iron complexes is essential because they catalyze damaging free radical reactions[12], such as lipid peroxidation and formation of highly-reactive hydroxyl radicals from superoxide radicals ($O_2\cdot^-$) and H_2O_2. For example, $O_2\cdot^-$ and H_2O_2 are generated by activated phagocytes[12]. These catalytic iron complexes can be measured in plasma by the bleomycin assay[11,13,14,15].

The liver also plays a key role in copper metabolism.[6] It synthesizes the protein caeruloplasmin, which contains at least 95% of the copper in human plasma and is an important plasma antioxidant[16,17]. Dietary copper ions are thought to enter the circulation bound to albumin, to amino acids or to small peptides. The liver rapidly absorbs this copper so that the content of non-caeruloplasmin copper in plasma from healthy subjects (as measured by the phenanthroline assay[18]) is at or close to zero[18,19]. This sequestration of copper is important because non-caeruloplasmin copper might also catalyze potentially-damaging free radical reactions[17]. For example, it could cause peroxidation of low-density lipoproteins, which can accelerate atherosclerosis[20].

We have applied the bleomycin[15] and phenanthroline[18] assays to look for "catalytic" iron and copper in plasma from patients with FHF, and to study the effects of liver transplantation.

MATERIALS AND METHODS

Blood samples were obtained during routine procedures upon patients whose diagnosis of FHF was confirmed by an experienced consultant physician (see Tables 1 and 2). Blood was drawn into heparinized tubes and centrifuged immediately to produce plasma: this was stored at −20°C and analyzed within eight days to avoid artefacts from release of iron or copper ions from plasma proteins during prolonged storage or handling[18]. Samples were analyzed for phenanthroline-detectable copper as in[18,19], for bleomycin-detectable iron as in[15] and for caeruloplasmin (as its enzymic ferroxidase activity) as in[19]. Transferrin saturation was measured as in[21]. Peroxidation of liposomes was measured as in[22], using 100 μl aliquots of plasma in each assay. Peroxidation was measured by the thiobarbituric acid (TBA) test and results are expressed as absorbance at 532 nm.

RESULTS

Plasma samples were obtained from twenty patients with an established diagnosis of FHF. Whereas plasma from healthy human subjects (or indeed from patients with diseases other than iron or copper overload) rarely contains "catalytic" iron or copper, the majority (18/20) of patients with FHF had plasma that was positive in the bleomycin assay (Tables 1 and 2). Transferrin was usually iron-saturated. Bleomycin-detectable iron was present in the majority of plasmas from patients whose FHF resulted from paracetamol overdosage or non-A, non-B hepatitis (Table 1). It was also present in the cases of Wilson's disease and alcohol abuse examined (Tables 1 and 2) but not in the single case of Budd-Chiari syndrome (Table 1). Phenanthroline-detectable copper was also present in the majority of patients, including the patient with Wilson's disease (as might be expected).

Several patients were sampled on more than one occasion and results were always comparable. Table 1 shows a representative result: five plasma samples taken on successive days from a patient with paracetamol-induced FHF.

Caeruloplasmin Activity

Caeruloplasmin was assayed by measuring its ferroxidase enzymic activity[19]. Levels in FHF patients were almost all sub-normal (Table 1), presumably reflecting impaired caeruloplasmin synthesis by the liver.

TABLE 1
"Catalytic" iron and copper in plasma of patients with FHF

No.	Patient	Aetiology	Outcome	Transplant	Plasma Parameters: Bleomycin-detectable iron μmoles/dm³	Transferrin saturation	Phenanthroline-detectable copper μmoles/dm³	Ferroxidase activity units/ml
1	41M	Budd-Chiari syndrome	Survived	Yes	0	U	0	0.26
2	39F	Paracetamol OD	Died	No	>5.0	-	0	0.10
3	26M	Paracetamol OD	Died	No	0.8	S	2.9	0.12
4	61F	Paracetamol OD	Died	No	0.2	S	4.4	0.17
5	31F	Paracetamol OD	Survived	Yes	3.2	S	0	0.34
6	27F	Paracetamol OD	Survived	No	0.95	S	4.0	0.16
7	20F	Paracetamol OD	Survived	No	1.2	S	2.1	0.19
8	19F	Paracetamol OD	Died	No	4.9	-	0	0.79
9	28M	Paracetamol OD	Died	No	0.85	-	0	1.06
10	35F	Non-A, Non-B hepatitis	Died 2 days post Tp	Yes	0	-	0	1.10
11	29F	Paracetamol OD	Survived	No	2.05	-	0	1.07
12	28F	Paracetamol OD	Died	No	6.0	-	0	0.83
					5.4	-	0	0.57
					3.8	-	0	0.38
					5.9	-	0	0.41
					5.5	-	0	0.27
13	67F	Paracetamol OD	Survived	No	6.2	-	0	0.81
14	F	Wilson's Disease	-	Yes	6.1	-	5.1	0.48
15	22F	Paracetamol OD	Survived	No	0.75	S	3.1	0.19
16	42M	Paracetamol OD	Survived	No	1.1	S	2.0	0.15
17	-	Non-A, Non-B hepatitis	Died	No	1.0	S	0	0.28
18	-	Non-A, Non-B hepatitis	Died	No	1.3	S	0.7	0.34
19	-	Non-A, Non-B hepatitis	Survived	No	1.0	S	2.3	0.07
20	36M	Alcohol abuse	Died	No	0.6	S	8.1	0.17
Control values in healthy subjects					0 (n > 250)	U (n > 250)	0 (n > 100)	1.20 ● 0.11 (n = 50)

U Transferrin not completely saturated.
S Transferrin saturated.
- Data not available.

TABLE 2
Serial estimations of plasma iron and copper in FHF following orthotopic liver transplantation

Patient	Aetiology	Days post transplant	Plasma Parameters: Bleomycin-detectable iron/μmol/dm^3	Transferrin saturation	Phenanthroline-detectable copper/μmol/dm^3	Ferroxidase activity
5	Paracetamol OD	Pre	3.2	S	0	0.34
		Post Hep*	2.2	S	0.7	0.29
		2	0.6	S	0	0.39
14	Wilson's disease	Pre	6.1	-	5.1	0.48
		7	0	-	0.7	0.56
		14	0	-	0	0.56
		35	0	-	0	1.15

*Patient underwent hepatectomy prior to organ availability.
- Data not available.

Effects of Transplantation

Two patients were followed before and after liver transplantation (Table 2). In both cases, levels of "catalytic" iron and/or copper decreased and eventually disappeared after transplantation.

Stimulation of Lipid Peroxidation by Plasma from FHF Patients

The iron and copper ions detected by the bleomycin and phenanthroline assays have been shown in other systems to be capable of stimulating free radical reactions[14,15,23,24]. In order to demonstrate this directly for plasma from FHF patients, their ability to stimulate lipid peroxidation was examined. In the presence of ascorbic acid, bovine brain phospholipid liposomes undergo peroxidation only in the presence of "catalytic" metal ions[12]. Plasma from healthy human subjects does not stimulate such peroxidation. In fact, it inhibits because of the chelation of traces of iron ions in the reagents by plasma constituents (Table 3). However, plasma samples from six different FHF patients failed to inhibit lipid peroxidation or actually stimulated it slightly. These results have been corrected for TBA reactivity in the plasma using "no liposome" controls (Table 3).

DISCUSSION

Severe liver dysfunction, as in FHF, causes derangement of the function of many other organs and often leads to MOF. Our data suggest one reason for this. In healthy humans, no "catalytic" iron or copper are present in blood plasma. This sequestration of metal ions is an important extracellular antioxidant defence system[12,16,17] in that it prevents $O_2^{\cdot -}$ and H_2O_2 (generated during several metabolic processes and by activated phagocytes) from forming highly-reactive $OH^{\cdot}$. MOF is known to be associated with increased complement and phagocyte activation, and possibly with increased free radical generation by other mechanisms, such as those involving the enzyme xanthine oxidase[25] and tumor necrosis factors. For example, TNFα stimulates $O_2^{\cdot -}$ generation by Kupffer cells[26]. Superoxide and H_2O_2 generated by these mechanisms could easily interact with "catalytic" iron and copper to cause

TABLE 3

Stimulation of liposomal lipid peroxidation by plasma from patients with fulminant hepatic failure.

Plasma (100 μl) was incubated for 20 min at 37°C with bovine brain liposomes in the presence of 100 μM ascorbate at pH 7.4 as described in[22].

Peroxidation is expressed as A_{532} arising from the TBA test. Results are corrected for any TBA-reactive material present in the plasma itself, or generated during its incubation, by using "no liposome" controls.

Patient number	Bleomycin-detectable iron μmol/dm^3	Amount of peroxidation A_{532}
Reaction mixture, no plasma	-	0.28
Normal plasma•	0	0.067
3	0.8	0.29
4	0.2	0.31
6	0.95	0.30
7	1.2	0.31
15	0.75	0.24
16	1.1	0.32
$FeCl_3$ (100 μM)	100	1.20

• Plasma from a healthy human subject.

severe tissue injury. At the same time, levels of plasma caeruloplasmin, an important antioxidant[16,17], are decreased (at least as measured by the protein's ferroxidase activity).

The origin of the bleomycin-detectable iron and phenanthroline-detectable copper is uncertain. It may be that they arise from injured extrahepatic tissues, and the liver is failing to take them up. Perhaps more likely, they could be released by the damaged liver, since hepatocytes are rich in these metals. In patients awaiting transplantation, improved short-term management is often achieved by removing the nonfunctional liver[27], suggesting that it is releasing toxic agents, of which "catalytic" iron and copper could be examples. The fact that both these metal forms disappear after transplantation suggests that functional human liver, like that of other mammals[8-10], can take up "catalytic" iron and copper. Perhaps the cautious use of chelating agents that bind these metal ions and inhibit free radical reactions[28], might be useful in the clinical management of FHF and MOF.

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