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EFFECT OF 1,10-PHENANTHROLINE AND DESFERRIOXAMINE *IN VIVO* ON PROLYL HYDROXYLASE AND HYDROXYLATION OF COLLAGEN IN VARIOUS TISSUES OF RATS*

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Abstract—We studied prolyl hydroxylase (PH) activity in various tissues of rats after systemic and local administrations of 1,10-phenanthroline and Desferrioxamine. We assayed PH in tissue extracts without adding iron to the incubation medium; we ascertained hydroxylation of collagen by measuring ^3H release from biosynthetically 3,4-^3H-proline-labeled collagen substrate. We studied the effect of both Fe^{2+} and Fe^{3+} chelating agents in tissues with a relatively high rate of collagen synthesis such as fetal skin and skin of newborn rats, 17-β-estradiol-stimulated uterus of immature rats, and carrageenan granuloma tissue. In addition, in some organs we studied collagen hydroxylation by measuring the ratio of proline to hydroxyproline either in highly purified samples or in the digest from samples treated with protease-free collagenase. Finally, the extent of underhydroxylation of collagen from some tissues of rats treated with either one or both chelating agents was determined using a 3,4-^3H-proline-labeled collagen as a substrate to partially purified PH. Neither systemic nor local injections of 1,10-phenanthroline or Desferrioxamine alone inhibited PH in any of the models. But simultaneous administration of both agents inhibited PH and hydroxylation of collagen in some models. Although local injections of 1,10-phenanthroline into granuloma tissue did not inhibit PH activity, hydroxylation of collagen synthesized in the granuloma from 4 to 16 hr after injection was reduced significantly. This seeming discrepancy is explained by the finding that PH is active even without the addition of Fe^{2+} in the assay medium; a strong reducing environment (α-ketoglutarate, ascorbic acid) reduces Fe^{3+}, thus providing ferrous ions essential for PH activity. Both forms of iron, therefore, must be chelated simultaneously to affect PH activity in the assay system. In some experiments, within 8 hr after systemic administration of 1,10-phenanthroline, the activity of PH in skin almost doubled and remained significantly elevated for 40 hr. The mechanism responsible for this phenomenon is not known. The results of this study indicate that previously reported inhibitory effects of some Fe^{2+} chelating agents on collagen synthesis in various models of fibrosis may not be related to inhibition of PH or to collagen hydroxylation.

THE MECHANISM of action of iron chelating agents such as 2,2'-dipyridyl, 1,10-phenanthroline or Desferrioxamine on synthesis of collagenous hydroxyproline and hydroxylysine in isolated systems of tissue slices or fibrogenic cells in tissue cultures has been described.[1–4] By chelating ferrous or ferric ions, prolyl and lysyl hydroxylase are inhibited and hydroxylation of collagen is prevented. Underhydroxylated collagen is not passed across cell membranes normally, and intracellular accumulation of such collagen inhibits further synthesis, possibly by a negative feedback mechanism.[3,4]

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Moreover, underhydroxylated collagen is abnormally susceptible to digestion by collagenase and tissue proteases and has reduced thermal stability.[5,6] Chelating agents, therefore, have been tested in various models of fibroproliferative inflammatory lesions for specific inhibition of collagen formation. Presently, data are available on inhibition of collagen formation by 1,10-phenanthroline and 2,2′-dipyridyl in hepatic cirrhosis,[7–9] wound granulation tissue,[8,10,11] carrageenan granuloma[12] and peritendinous adhesions.[13] Although the mechanism of action *in vivo* has not been reported, some investigators have assumed that interference with hydroxylating enzymes (as in systems *in vitro*) is a key factor. Tyrosine hydroxylase, an enzyme similar to prolyl hydroxylase as far as cofactors and mechanism of action are concerned, is inhibited *in vivo* by administration of 2,2′-dipyridyl.[14]

The primary goal of this study was to ascertain if chelating agents relatively specific for Fe^{2+}, such as 1,10-phenanthroline or 2,2′-dipyridyl, and chelating agents specific for Fe^{3+}, such as Desferrioxamine, would inhibit prolyl hydroxylase and prevent hydroxylation of collagen in intact animals.

MATERIALS AND METHODS

Animals and experimental models

Group I. After day 10 of gestation, eight pregnant Sprague–Dawley rats (300 g body weight) were injected intraperitoneally every other day for 10 days with 2 mg/100 g body weight of 1,10-phenanthroline (Smith Chemical Co., Ohio). We killed all rats on day 20 of gestation. After we determined the weight of the fetus, we removed and pooled the skin in two samples from each litter.

Group II. We injected 36 Sprague–Dawley rats (female, 60 g body weight) intraperitoneally with a single dose of 2 mg/100 g body weight of 1,10-phenanthroline. We killed these rats at various time intervals after injection (see Table 2). We minced a sample of skin from the dorsal neck region and a segment of small intestine and washed them in cold saline. We perfused the liver *in situ* with 20 ml of cold saline and analyzed it for the iron content; we measured calcium in the serum.

Group III. We injected forty 19-day-old Sprague–Dawley rats (female, 30 g body weight) with 0·2 ml of a 5% ethanol–saline solution containing 5 μg 17-β-estradiol for 3 consecutive days according to the method of Salvador and Tsai.[15,16] We injected control rats with 5% ethanol in saline. (Table 3 shows various time intervals of 1,10-phenanthroline administrations.) The uterine horns and cervix of each rat were excised, washed in cold saline, blotted, and weighed. We pooled three uteri to provide sufficient tissue for PH assay and digestion with purified collagenase.

Group IV. We injected 2-day-old rats from the same litter (8 g body weight) intraperitoneally every 6 hr with 0·2 mg 1,10-phenanthroline or 2 mg Desferrioxamine (Desferal, Ciba), or a combination of both drugs. The dose was selected according to relative toxicity of the drugs. The LD_{50} for 1,10-phenanthroline is 29·3 mg/kg (i.v. mouse); the LD_{50} for Desferrioxamine under similar conditions is 287 mg/kg.[8] Multiple chelating agents administered simultaneously are more toxic than single agents. Because newborn rats receiving two agents did not survive longer than 24 hr, PH activity was assayed 5 hr after injection of both drugs. We assayed tissue from rats receiving only one drug for PH activity 24 hr after injection.

Group V. We formed dorsal air pouches in 11 Sprague–Dawley rats (male, 180 g

body weight) by injecting them with air; into these pouches, we injected 5 ml of 1% λ carrageenan (potassium salt, Renj 5055, Marine Colloids Inc.) in sterile saline as described by Robertson and Schwartz[17] and by Nakagawa *et al.*[12] Eight days after injection of carrageenan, we injected 1,10-phenanthroline (2 mg/100 g body weight) into the granuloma at intervals shown in Table 6. We assayed the PH in one aliquot of tissue; in another aliquot, we measured the extent of collagen hydroxylation.

Prolyl hydroxylase assay

We assayed PH directly in three tissues—skin, intestine and uterus. Tissues were washed in iced saline, blotted and weighed. We homogenized tissues in a Brinkman Polytron for 30 sec in a medium containing 0·23 M sucrose, 0·014 M Tris-HCl buffer (pH 7·5), 50 μg/ml phenylmethylsulfonylfluoride and 50 μM dithiothreitol. After we centrifuged the homogenate at 4° for 20 min at 15,000 *g*, we assayed the supernatant for PH activity. Using the method of Lowry *et al.*,[18] we determined protein content. The procedure for assaying the activity of PH was essentially that of Hutton *et al.*[19] with modifications by Chvapil and Ehrlich.[20] In this assay, we used 3,4-^{3}H-proline-labeled collagen synthesized by 10-day-old chick embryo tissue in the presence of 2,2′-dipyridyl (1 mM) as a substrate. Two ml of incubation medium contained 0·5 ml supernatant, 500,000 dis/min of ^{3}H substrate, 0·05 M Tris-HCl buffer (pH 7·5), 4 mg catalase, 15 mg bovine serum albumin, 1 mM ascorbic acid and 0·5 mM α-ketoglutarate. We added no ferrous iron to the medium, since we wanted to test the availability of endogenous tissue iron for PH activity. We ran blanks similarly without α-ketoglutarate. Incubation lasted 60 min at 37° with shaking in unstoppered distillation tubes. Trichloroacetic acid (TCA) added to a final concentration of 0·3 M stopped the reaction. We vacuum-distilled and counted released ^{3}H in a Triton X-1000 toluene in a Nuclear Chicago Mark II scintillation counter.

Determination of hydroxyproline/proline (Hyp/Pro) ratio as an index of hydroxylation of collagen

To determine the degree of hydroxylation of synthesized collagen under the influence of chelating agents by directly measuring the content of hydroxyproline and proline, isolation of pure collagen is essential. We purified collagen by digesting the tissue with protease-free bacterial collagenase. Chromatographically purified collagenase was purchased from Worthington Biochemical Corp. and purified further on Sephadex G-200 to eliminate nonspecific protease activity, as described by Peterkofsky and Diegelman.[21] Digestion of homogenized tissue by purified collagenase took 60 min at 37°. We added 25 μg collagenase to each mg of tissue protein. Peptides derived from digestion of collagen remained in the supernatant after the precipitation of noncollagenous protein and undigested collagen with 5% TCA and 0·25% tannic acid[21] (see Table 3). Under these conditions, approximately 60 per cent of collagenous hydroxyproline was released within 1 hr of digestion. We found that the ratio of Hyp/Pro in collagenase digest was negligibly affected by differing times of digestion.

Measurement of hydroxylation of collagen

We used another method to evaluate the extent of hydroxylation of collagen. The method is based on the finding that, *in vivo*, 3,4-^{3}H-proline-labeled collagen, if under-

hydroxylated (due in this study to the effect of chelating agents), could be used as a substrate for PH. During additional hydroxylation, some tritium is released and measured as tritiated water by the procedure described above. The substrate used in these experiments was derived from carrageenan granuloma injected with 1,10-phenanthroline. We incubated approximately 2 g granuloma tissue with 5 ml culture medium containing 200 μCi 3,4-^{3}H-proline (New England Nuclear) for 180 min at 37° with constant shaking.[21] We incubated another aliquot with 2 mM 2,2'-dipyridyl. We extracted collagen from homogenized tissue with 0·5 M acetic acid for 16 hr at 4°. Extracts were dialyzed against three changes of 0·01 M acetic acid for 48 hr. We used aliquots containing approximately 200,000 dis/min as a substrate for PH,[22] which was isolated and partially purified from 8-day-old chicken embryos as described elsewhere.[19]

Other analyses

Using the method of Jackson and Cleary,[23] we isolated and purified acid-soluble collagen from embryonal skin. We measured serum calcium directly by flame photometry. We measured hepatic iron after digestion of dry tissue in nitric acid on a Perkin–Elmer atomic absorption spectrophotometer, model 305.

RESULTS

Effect of 1,10-*phenanthroline on pregnant rats and fetuses*

Neither PH nor collagen hydroxylation in fetal tissue was affected significantly by treatment of pregnant female rats with 1,10-phenanthroline (Table 1). Fetuses stayed alive and we did not observe any resorption. We measured almost identical high levels of PH activity in skin from control fetuses and fetuses from rats treated with 1,10-phenanthroline. Acid-soluble collagen, isolated and purified from skin, contained 0·15 per cent tyrosine, indicating little or no contamination with noncollagenous proteins. The ratio of hydroxyproline to proline in hydrolyzed collagen was similar in samples from controls and from rats treated with 1,10-phenanthroline. Because all pregnant rats were injected only 24 hr before fetal skin was assayed for PH, data are incomplete as far as the effect of time is concerned.

TABLE 1 EFFECT OF 1,10-PHENANTHROLINE ADMINISTRATION TO PREGNANT RATS ON PROLYL HYDROXYLASE IN FETAL RAT SKIN*

	Control (3)†	Phenanthroline (5)†
Body wt of mothers (g)	360 ± 14·4	334 ± 7·3
Body wt of fetuses (g)	5·02 ± 0 12	4·29 ± 0·42
Prolyl hydroxylase		
(dis/min/mg dry tissue)	45,950 ± 5300	53,230 ± 5000
(dis/min/mg protein)	231,000 ± 17,930	256,000 ± 5000
Acid-extractable collagen		
Hyp/Pro	0·88 ± 0·09	0·79 ± 0·11

* Female Sprague–Dawley rats were injected i.p. with 1,10-phenanthroline (2 mg/100 g) every other day from the tenth day of gestation and were killed on the estimated day 20 of gestation.

† Three litters of controls contained 36 fetuses plus five litters of 1,10-phenanthroline-treated rats contained 50 fetuses. Data on the activity of PH are based on six (controls) or ten (1,10-phenanthroline) independent assays of probed skin.

Effect of 1,10-*phenanthroline on PH activity as a function of time*

We studied the effect of 1,10-phenanthroline on PH activity in 3-week-old rats in group II. Over a period of 8 hr, the activity of PH in skin almost doubled and remained elevated for an additional 40 hr (Table 2). No change in hepatic iron (total iron) concentration took place. Within 30 min after injection of 1,10-phenanthroline, all rats developed generalized tremors lasting approximately 90 min; serum calcium was normal. Such neuromuscular activity suggests that tyrosine hydroxylase was inhibited, lowering norepinephrine sufficiently to cause extrapyramidal choreiform movements, as others have recorded.[14,24]

After administration of 1,10-phenanthroline for a period of 3 days (six injections) or after two injections of 1,10-phenanthroline in a single day, we observed an increase in PH activity in the uterus of immature rats treated with 17-β-estradiol. Injection of 1,10-phenanthroline 4 hr before killing did not affect PH activity (Table 3).

TABLE 2. ACTIVITY OF PROLYL HYDROXYLASE IN THE SKIN OF YOUNG RATS AT DIFFERENT TIMES AFTER 1,10-PHENANTHROLINE ADMINISTRATION*

Time	Skin Prolyl hydroxylase (10^3 × dis/min/mg prote)	Serum Ca (mg/100 mμg/g dry substance)	Liver Fe
0	13·1 ± 1·4	8 58 ± 0·04	485 ± 41
1	13·5 ± 2·2		
2	14·2 ± 0·1	8·54 ± 0·14	443 ± 97
4	16·1 ± 3·1	8·93 ± 0·19	488 ± 11
8	24·6 ± 2·2		
16	21·1 ± 3·5	8·95 ± 0·16	504 ± 30
24	18·2 ± 2·4	8·45 ± 0·14	451 ± 70
48	24·3 ± 2·4	8·95 ± 0·27	517 ± 60

* Sprague–Dawley rats (60 g body wt) were injected i.p. with a high dose of 1,10-phenanthroline (2 mg/100 g). There were three rats for every time period, six rats at zero time. After exsanguination, the liver was perfused through the portal vein with 20 ml of ice-cold saline

Simultaneous administration of 1,10-*phenanthroline and Desferrioxamine*

Desferrioxamine (2 mg/rat) also increased PH activity in newborn rats (Table 4). Desferrioxamine and 1,10-phenanthroline injected simultaneously in half of the original doses resulted in the death of all rats in less than 16 hr. For this reason, we performed PH assays 5 hr after injection of both agents. In these experiments, PH activity was significantly inhibited ($P < 0{\cdot}001$) in skin and gut (Table 4). Either 2,2′-dipyridyl or Desferrioxamine added to the culture medium can further inhibit PH activity in skin (Table 5). In the presence of 0·5 mM 2,2′-dipyridyl, we observed almost 100 per cent inhibition of collagenous proline hydroxylation. Desferrioxamine was less effective at a 2 mM concentration and caused approximately 80 per cent inhibition.

Effect of local injections of 1,10-*phenanthroline into carrageenan granuloma at different time intervals*

Direct assay of PH in granulation tissue produced by carrageenan was the same in 1,10-phenanthroline-treated rats as in controls, regardless of the time interval after

TABLE 3. EFFECT OF 1,10-PHENANTHROLINE ON THE ACTIVITY OF PROLYL HYDROXYLASE AND SOME OTHER INDICATORS IN IMMATURE UTERUS OF RATS INJECTED WITH 17-β-ESTRADIOL

Group*	Body wt (g)	Uterus wet wt (mg)	Total protein (mg/uterus)	Total Hyp (μg/uterus)	PH (dis/min/mg protein)	Hyp/Pro†
I. Control, 0·1 ml of 5% ethanol–saline	36·2 ± 1·54	33·2 ± 2·66	2·86 ± 0·22	240 ± 0·5	1400 ± 70	1·11 ± 0·09
II. 17-β-Estradiol, 5 μg‡	39·1 ± 1·46	92·5 ± 2·81	7·21 ± 0·25	535 ± 22	28,600 ± 470	1·07 ± 0·18
III. 17-β-Estradiol + 1,10-phenanthroline, 2/day for 3 days‡§	42·5 ± 1·18	74·5 ± 2·26	5·46 ± 0·02	565 ± 19	33,300 ± 560	1·02 ± 0·14
IV. 17-β-Estradiol + 1,10-phenanthroline, 2/day for 1 day‡§	38·0 ± 1·39	79·9 ± 2·58	5·27 ± 0·56	600 ± 24	34,300 ± 3000	1·02 ± 0·14
V. 17-β-Estradiol + 1,10-phenanthroline, 1 injection 4 hr before killing‡§	36·7 ± 1·28	93·7 ± 3·00	6·43 ± 0·03	595 ± 22	27,100 ± 1100	0·96 ± 0·07

* There were three 19-day-old Sprague–Dawley rats in each group.
† Refers to hydroxylation index determined from the tissue digested by pure collagenase.
‡ 17-β-Estradiol was administered subcutaneously for 3 consecutive days.
§ 1,10-Phenanthroline was injected i.p. twice daily as an aqueous solution at the dose of 2 μg/100 g/day according to the scheme given above.

TABLE 4. EFFECT OF ADMINISTRATION OF Fe^{2+} OR Fe^{3+} CHELATING AGENTS *in vivo* ON THE ACTIVITY OF PROLYL HYDROXYLASE IN THE SKIN AND GUT OF NEWBORN RATS

Group*	Body wt	Prolyl hydroxylase (dis/min/mg protein) Skin	Gut
I. Control	9·2	9700	1760
II. 1,10-Phenanthroline†	6·9	18,000‡	3060‡
III. Desferrioxamine§	8·6	15,200‡	3200‡
IV. 1,10-Phenanthroline + desferrioxamine¶	9·1	6700‖	

* Two-day-old rats, four/group.
† 0·2 mg/rat every 12 hr, killed 24 hr after the first injection.
‡ $P < 0·001$ as compared to control
§ 2 mg/rat every 12 hr, killed 24 hr after the first injection.
‖ $P < 0·01$ as compared to control.
¶ 0·1 mg 1,10-phenanthroline and 1 mg desferrioxamine injected simultaneously. Rats were killed 5 hr later.

TABLE 5. EFFECT OF Fe^{2+}, 2,2'-DIPYRIDYL OR DESFERRIOXAMINE ADDITION TO ASSAY MEDIUM FOR PROLYL HYDROXYLASE IN THE SKIN OF NEWBORN RATS TREATED *in vivo* WITH SOME CHELATING AGENTS.

Addition to the medium*	Prolyl hydroxylase (dis/min/mg protein) Control	1,10-Phenanthroline treated	Desferrioxamine treated
None	9720	17,970	15,225
Fe^{2+} (1 mM)	8730	15,810	10,675
Desferrioxamine (2·0 mM)	1310	2600	1060
2,2'-Dipyridyl (0·5 mM)	190	630	0

* Total volume of 2 ml; for composition see Methods.

TABLE 6. EFFECT OF LOCAL INJECTIONS OF 1,10-PHENANTHROLINE INTO CARRAGEENAN GRANULOMA ON THE ACTIVITY OF PROLYL HYDROXYLASE AND HYDROXYLATION OF COLLAGEN

Time of 1,10-phenanthroline injection before killing (hr)	PH Direct assay (dis/min/mg protein)	Hydroxylation of collagen (dis/min/mg dry substance) Control	+2,2'-Dipyridyl (2 mM)	+Fe^{2+} (0·1 mM)
Control*	6500 ± 1000	2·85 ± 0·55	36·0	5·1
26		3·00 ± 0·71	40·7	4·1
18	6400 ± 820	16·9 ± 5·7†	129‡	2·7
4	6000 ± 1100	33·9 ± 9·7‡	111‡	27·0‡

* There were three samples of granuloma tissue analyzed in each group. 1,10-Phenanthroline (2 mg/100 g body wt) was injected into granuloma tissue at times given in the table.
† $P < 0·01$ as compared with control.
‡ $P < 0·001$ as compared with control.

injection (Table 6). A discrepancy occurred between the direct assay for PH and the indirect determination of hydroxylation of collagenous polypeptide (Table 6). The most pronounced inhibition of hydroxylation was in tissues injected with 1,10-phenanthroline 4 or 16 hr before killing. When we added 2,2'-dipyridyl to the culture medium, we observed an additive inhibitory effect upon proline hydroxylation. Iron added to the incubation medium abolished this effect. These data indicate that 4 hr

after local injection of 1,10-phenanthroline into a carrageenan granuloma, the tissue still contains enough chelating agent to bind iron subsequently added to the medium.

DISCUSSION

Contrary to expectations, these experiments revealed that systemic administration of 1,10-phenanthroline (Fe^{2+} chelator) and Desferrioxamine (Fe^{3+} chelator) *in vivo* increased rather than inhibited PH activity in three tissues. Eight hr after injection, we observed increased activity which lasted for 40 hr. Because of the relatively short time involved, increased synthesis of a new enzyme probably did not occur. Thus, we can speculate that 1,10-phenanthroline activates the inactive form of prolyl hydroxylase present in some tissues.[25–27] The mechanism is still unknown. Against such a concept, however, is the report of Mason *et al.*,[28] showing that the active form of cytochrome P-450 becomes inactive chromogen P-420 in the presence of bathophenanthroline.

Another explanation could be that concentrations of chelating agents in tissue after administration *in vivo* are too low to form a saturated 3:1 complex with loosely bound iron. Some unsaturated complexes of metal–chelating agents increase reactivity of the metal.[29] Even if loose ferrous ions are chelated completely in an intact animal, the assay for PH *in vitro*, which utilizes ascorbate and α-ketoglutarate in the medium, reduces Fe^{3+} to Fe^{2+}, which functions as an oxygen activator during hydroxylation, as Hobza *et al.* suggested.[30]

The reason tissue slices or cells, under tissue culture conditions, show inhibition of PH in the presence of divalent and trivalent iron chelators is probably explained by the actual concentration of chelating agents in the incubation medium. In a closed system of tissue culture, all of the labile iron can be chelated; thus, the hydroxylation is drastically reduced. In intact animals, however, Desferrioxamine is metabolized and excreted quickly.[31] Renal excretion data following metabolism of 1,10-phenanthroline are not complete, but we have observed rapid excretion in urine after a single injection (unpublished). Measurement of concentration of iron-chelating agents in extracellular fluids might clarify the data; effective concentrations may not have been reached in tissues. Data from experiments *in vivo* suggest that concentrations of 1,10-phenanthroline should be approximately 0·5 to 0·1 mM and concentrations of Desferrioxamine should range between 1 and 5 mM for optimal inhibition of PH.[3,12] But intraperitoneal administration of 2,2′-dipyridyl at a dose comparable to that used for 1,10-phenanthroline in this study inhibits activity of tyrosine hydroxylase in adrenal, brain and heart tissues of rats within 6 hr.[14] The tremors in rats treated with 1,10-phenanthroline and Desferrioxamine in experiments reported in this study suggest a similar inhibition. The differing susceptibility of PH and tyrosine hydroxylase to administration of some chelating agents *in vivo* may reflect the relative affinity of each enzyme to iron.

Inhibition of PH in the skin of newborn rats treated simultaneously with Fe^{2+} and Fe^{3+} chelators shows that it is possible to decrease PH activity in an intact animal. However, the dose required produces death of the animal within 24 hr. Despite local injections of relatively high doses of 1,10-phenanthroline, only partial inhibition of proline hydroxylation occurred. Further inhibition of proline hydroxylation could be achieved by adding 2,2′-dipyridyl to the incubation medium.

In summary, data revealed that neither PH activity nor hydroxylation of newly

formed collagen could be significantly inhibited after administration of chelating agents to intact animals and that only partial inhibition of collagen hydroxylation could be achieved after local injections of chelating agents in carrageenan granulomas. These data strongly suggest that such agents have little usefulness in the control of fibrosis in human beings. Jacobs *et al.* (unpublished) reached similar conclusions following studies in which iron chelators were also found to be ineffective in nontoxic doses in controlling fibrosis in the esophagi of dogs or in influencing mechanical properties in dermal scars of rats. The beneficial effect of 2,2'-dipyridyl or 1,10-phenanthroline administration on liver injury induced by silica[8] or ethionine[7] may not be related to chelating properties of these drugs at all, since both the nonchelating analogs (1,7-phenanthroline and 1,10-phenanthroline) were equally protective against the hepatotoxicity of ethionine in acute and chronic experiments.[32] Another mechanism of the protective effect of these agents may depend on their known interference with lipid peroxidation and related tissue damage.[33] Other mechanisms of the protective action of these agents against tissue injury are discussed elsewhere.[34]

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