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Metal induction of haem oxygenase without concurrent degradation of cytochrome *P*-450

Protective effects of compound SKF 525A on the haem protein

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The induction of hepatic haem oxygenase (EC 1.14.99.3) by a series of metals, organometals and metalloporphyrins was examined *in vivo* in the presence of compound SKF 525A, which is known to complex with the prosthetic group of cytochrome *P*-450. Concurrent administration of SKF 525A and an inducing metal did not affect the extent and time course of haem oxygenase induction. The decrease in cytochrome *P*-450 content normally associated with metal administration was, however, prevented, indicating that haem oxygenase induction by metals can proceed without the significant labilization of the haem moiety of cytochrome *P*-450. In addition, the integrity of this haem protein can be maintained by chemical means in the presence of sustained high activities of haem oxygenase.

A number of metal ions, either in the form of the inorganic elements (Maines & Kappas, 1974, 1975, 1977*a*; De Matteis & Unseld, 1976) or bound to organic moieties, e.g., organotins (Rosenberg *et al.*, 1980) and metalloporphyrins (Drummond & Kappas, 1981*a*), are potent inducers *in vivo* of haem oxygenase, the rate-limiting enzyme in the catabolism of haem to bile pigment (Tenhunen *et al.*, 1968, 1969). Concomitant with the induced activities of this enzyme there occur substantial decreases in cellular cytochrome *P*-450 content; and, with certain metals, characteristic perturbations of ALA synthase activity (Maines & Kappas, 1975, 1977*a*). With single doses of inorganic metal ions these alterations of haem metabolism generally revert to normal within approx. 72h (Maines & Kappas, 1977*a*), although with single doses of certain organified metals the alterations in haem metabolism may last as long as 10 days or more (Rosenberg *et al.*, 1980; Drummond & Kappas, 1980*a*).

The proximate site and mechanism by which metals induce haem oxygenase are not known (Maines & Kappas, 1977*a*; De Matteis, 1978). We have postulated that these agents may act directly to induce this enzyme and that the associated decreases observed in cellular cytochrome *P*-450 content largely reflect the increase in haem oxygenase activity (Maines & Kappas, 1977*a*). Alternative mechanisms of induction could include metal labilization of the haem moiety of cytochrome *P*-450, with the released haem then initiating the induction process (Bissell & Hammaker, 1976). To explore this possibility we have examined in this study the effects of compound SKF 525A on the induction of haem oxygenase by a variety of inorganic and organometals. SKF 525A is a compound that is known to complex with the prosthetic group of cytochrome *P*-450, thus preventing the labilization of cytochrome *P*-450 haem and impairing its ability to bind CO and also its catalytic activity (Schenkman *et al.*, 1972). The SKF 525A–cytochrome *P*-450 complex can be split by ferricyanide, regenerating the intact haem protein and its catalytic and CO-binding capacity (Buening & Franklin, 1976).

The results of this study demonstrate that metals can potently induce hepatic haem oxygenase without altering the cellular content of cytochrome *P*-450 haem when SKF 525A is administered concurrently with the inducing agent. Thus it is highly unlikely that the proximate mode of action of metals in the induction of haem oxygenase involves, as an essential event, the labilization of cytochrome *P*-450 haem; and conversely cytochrome *P*-450 haem can, by suitable chemical means, clearly be protected against degradation by even markedly elevated activities of haem oxygenase.

Abbreviations used: ALA, δ-aminolaevulinate; TCHH, tricyclohexyltin hydroxide; DEDC, diethyltin.

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Materials and methods

Materials

Male Sprague–Dawley rats (175–225 g) purchased from Taconic Farms, Germantown, NY, U.S.A., were used throughout this study. TCHH and DEDC were gifts from M.T. Chemical Co., Rahway, NJ, U.S.A. SKF 525A was a gift from Smith, Kline and French Laboratories, Philadelphia, PA, U.S.A. Co–protoporphyrin was purchased from Porphyrin Products, Logan, UT, U.S.A. All other chemicals were of the highest grade available and were purchased from either Fisher, Pittsburg, PA, U.S.A., or Sigma, St. Louis, MO, U.S.A.

Methods

Treatment of animals and preparation of microsomes. Rats were injected subcutaneously with the chlorides of cobalt (Co^{2+}) and nickel (Ni^{2+}) at a dose of 250 μmol/kg body wt., cadmium chloride (Cd^{2+}; 25 μmol/kg body wt.) antimony potassium tartrate (Sb^{3+}, 82 μmol/kg body wt.), sodium arsenate (As^{3+}, 50 μmol/kg body wt.) or potassium chloroplatinate (Pt^{4+}, 125 μmol/kg body wt.) dissolved in saline. Solutions of metalloporphyrins (125 μmol/kg body wt.) were prepared by dissolving the compound in a small volume of 0.1 M-NaOH, adjusting the pH to 7.4 with 1 M-HCl and making up the final volume with saline. Metalloporphyrin solutions prepared in this manner were administered subcutaneously within 10 min of preparation. Control rats in experiments involving inorganic metal salts and metalloporphyrins received an equal volume of saline. TCHH and DEDC were dissolved in 95% ethanol and injected subcutaneously in a single dose (15 mg/kg body wt.). Control animals received an equal volume of the solvent ethanol (1 mg/kg body wt.) and were identical with saline-treated animals in all parameters studied. SKF 525A was dissolved in saline at a dose of 50 mg/kg body wt. and administered intraperitoneally simultaneously with the metal.

Animals were allowed free access to water but were starved for 16 h before killing by decapitation. Livers were perfused *in situ* with ice-cold 0.9% NaCl, removed and homogenized in 3 vol. of potassium phosphate buffer (0.1 M, pH 7.4) containing sucrose (0.25 M). The homogenate was centrifuged at 9000 **g** for 20 min. The precipitate was washed twice with potassium phosphate buffer (0.1 M, pH 7.4) before assaying for ALA synthase activity. The 9000 **g** supernatant was centrifuged at 100 000 **g** for 60 min; the cytosol served as a source of biliverdin reductase, whereas the microsomal pellet was washed once and resuspended in potassium phosphate buffer (0.1 M, pH 7.4) at a protein concentration of 15–20 mg/ml.

Enzyme assays. The activities of ALA synthase and haem oxygenase were assayed as previously described (Sassa *et al.*, 1979; Maines & Kappas, 1978). Bilirubin produced in the latter assay was calculated by using an absorption coefficient of $40\,mM^{-1}\cdot cm^{-1}$ between 464 and 530 nm. Potassium ferricyanide (to a final concentration of 50 μM) was added, before bilirubin determination, to the haem oxygenase reaction mixture derived from SKF 525A-treated animals to dissociate the SKF 525A–cytochrome *P*-450 complex, which interferes with the spectral determination of small amounts of bilirubin. Potassium ferricyanide itself did not interfere with the spectral determination of bilirubin.

Spectral studies. Cytochrome *P*-450 was measured from the reduced-minus-CO difference spectrum with sodium dithionite as the reducing agent and an extinction coefficient of $91\,mM^{-1}\cdot cm^{-1}$ between 450 and 490 nm (Omura & Sato, 1964). Cytochrome *P*-450 determinations on microsomes derived from animals treated wtih SKF 525A were carried out in a similar manner after first treating the microsomal suspension with potassium ferricyanide (50 μM) as previously described (Buening & Franklin, 1976). All spectral studies and enzyme assays were performed on an Aminco–Chance DW2A spectrophotometer in the split-beam mode.

Protein concentration was determined by the method of Lowry *et al.* (1951), with crystalline bovine serum albumin as standard. All experiments were conducted a minimum of three times and the results are expressed as means ± S.D. The standard *t* test was used in the analysis of the data and a value of $P < 0.05$ was regarded as denoting significance.

Results

Effect of SKF 525A on cytochrome P-450 content and haem oxygenase induction in liver after metal administration

The ability of SKF 525A to bind to cytochrome *P*-450 and the requirement for ferricyanide to dissociate the SKF 525A–haem protein complex thus permitting the complete spectral detection of cytochrome *P*-450 as previously described (Buening & Franklin, 1976) is illustrated in the difference shown between the cytochrome *P*-450 value in animals treated with the metal alone and the metal plus SKF 525A (Table 1). In every instance the cytochrome *P*-450 concentration of tissue samples from animals treated with metals alone did not change with ferricyanide treatment (results not shown), whereas in all SKF 525A-treated animals spectrally detectable amounts of cytochrome *P*-450 increased approx. 40–50% after ferricyanide treatment, as reported previously (Buening & Franklin, 1976). Ferricyanide treatment also restored to normal values the catalytic activity of cytochrome *P*-450 in SKF 525A-treated animals, confirming a previous report (Buening & Franklin, 1976).

Table 1. *Effect of SKF 525A on haem oxygenase activity and cytochrome P-450 content in liver microsomes*
A minimum of four animals were used for each point. The animals were treated and the assays performed as described in the Materials and methods section. Animals were killed 16h after metal ion administration. Cytochrome *P*-450 assays on microsomes from SKF 525A-treated animals were done after treating the suspension with 50 μM-potassium ferricyanide.

Metal	Haem oxygenase (nmol of bilirubin/h per mg of protein)	Cytochrome *P*-450 (nmol/mg of protein)
Control	2.62 ± 0.18	0.79 ± 0.03
SKF 525A	3.90 ± 0.68	0.88 ± 0.03
Co^{2+}	19.65 ± 1.82	0.47 ± 0.05*
Co^{2+} + SKF 525A	24.09 ± 1.28	0.71 ± 0.04
Cd^{2+}	18.79 ± 2.10	0.39 ± 0.01*
Cd^{2+} + SKF 525A	14.96 ± 1.30	0.83 ± 0.01
Sb^{3+}	20.54 ± 2.56	0.47 ± 0.01*
Sb^{3+} + SKF 525A	22.09 ± 2.53	0.81 ± 0.08
As^{3+}	14.39 ± 3.24	0.53 ± 0.03*
As^{3+} + SKF 525A	16.95 ± 2.51	0.79 ± 0.02
Pt^{4+}	10.89 ± 0.73	0.58 ± 0.01*
Pt^{4+} + SKF 525A	9.80 ± 0.90	0.80 ± 0.05
Ni^{2+}	14.98 ± 1.11	0.61 ± 0.04*
Ni^{2+} + SKF 525A	13.80 ± 1.89	0.82 ± 0.02
Fe–protoporphyrin	14.57 ± 1.00	0.69 ± 0.01*
Fe–protoporphyrin + SKF 525A	14.43 ± 1.01	0.86 ± 0.04
Co–protoporphyrin	12.71 ± 0.84	0.39 ± 0.04*
Co–protoporphyrin + SKF 525A	12.58 ± 0.94	0.68 ± 0.05
DEDC	7.55 ± 0.88	0.57 ± 0.06*
DEDC + SKF 525A	8.58 ± 0.57	0.70 ± 0.01

* $P < 0.05$ (difference between treated and control).

The ability of SKF 525A to complex with cytochrome *P*-450 haem was employed to examine the relation between the haem oxygenase-inducing action of a series of inorganic metals and their ability to decrease cytochrome *P*-450 content *in vivo*. Adminstration of single doses of Co^{2+}, Cd^{2+}, Ni^{2+}, As^{3+}, Sb^{3+} and Pt^{4+} led in every instance to a substantial decrease (25–50%) in the content of hepatic cytochrome *P*-450 measured at both 8 (results not shown) and 16h (Table 1) after metal administration. Simultaneous administration of SKF 525A with each of the metal inducers of haem oxygenase, however, prevented the occurrence of these decreases in haem protein content. In addition the decreases in cytochrome *P*-450 content associated with organometal (DEDC) or metalloporphyrin (Fe–protoporphyrin or Co–protoporphyrin) administration were almost entirely prevented by simultaneous treatment with SKF 525A without impairing the haem oxygenase-inducing activity of these organometal compounds. Administration of a single dose of SKF 525A alone produced a slight but insignificant increase at 16h in cytochrome *P*-450 content in comparison with control values (Table 1), confirming previous observations (Buening & Franklin, 1976).

Although SKF 525A prevented the substantial decreases in cytochrome *P*-450 associated with treatment with all of the inorganic metal compounds shown in Table 1, the extent of haem oxygenase induction produced by these metals, determined 16h after administration of the inducers, was unaffected by the presence of SKF 525A (Table 1). The extent of enzyme induction (3–5-fold) by the organometals DEDC, Fe–protoporphyrin and Co–protoporphyrin was also unaffected by the administration of SKF 525A (Table 1). SKF 525A administration alone produced a slight but insignificant increase of haem oxygenase activity (Table 1).

The ability of this drug to complex with, and thus prevent degradation of, cytochrome *P*-450 haem, despite the concurrent presence of high haem oxygenase activity, was used to study the enzyme-induction process during the initial 8h period after metal administration, a time when enzyme activity is known to increase rapidly and during which cytochrome *P*-450 content declines significantly (Maines

& Kappas, 1975). During this early time period the following metals were examined: Co^{2+}, a metal ion that is a substrate for ferrochelatase (Labbe & Hubbard, 1961) and that can thus form endogenously a Co–protoporphyrin IX chelate (Sinclair *et al.*, 1979); Sb^{3+}, a metal ion that is not enzymically incorporated into protoporphyrin IX; Fe–protoporphyrin, which has been suggested to induce haem oxygenase by labilizing the haem of cytochrome *P*-450 (Bissell & Hammaker, 1976) and DEDC, an organotin that might labilize cytochrome *P*-450 haem through a direct effect on the cytochrome or on the membranes of the endoplasmic reticulum (Rosenberg *et al.*, 1981).

Effect of simultaneous Co^{2+} and SKF 525A administration on haem oxygenase and ALA synthase activities and cytochrome P-450 content in liver

With combined Co^{2+} and SKF 525A treatment haem oxygenase activity increased rapidly (Fig. 1), reaching values more than 2- and 5-fold higher than initial values at 2 and 8 h respectively, as expected (Maines & Kappas, 1975). ALA synthase exhibited the characteristic initial inhibition followed by a rebound increase in activity (Maines & Kappas, 1975; De Matteis & Gibbs, 1976). Throughout the initial 5 h after metal ion administration, when the rate-controlling enzymes of haem degradation and synthesis were undergoing the characteristic perturbations produced by the metal ion, cytochrome *P*-450 content was not significantly changed. At 8 h there was an insignificant decline in haem protein content (approx. 10%), which was markedly less than the decrease usually observed [25–30% at this time (results not shown)] in animals treated with Co^{2+} alone. The induction of haem oxygenase by Co^{2+} occurred without significant change in cytochrome *P*-450 content when cytochrome *P*-450 haem was complexed with SKF 525A; in addition ALA synthase activity, in the presence of the drug, exhibited the typical sequence of changes normally associated with Co^{2+} administration.

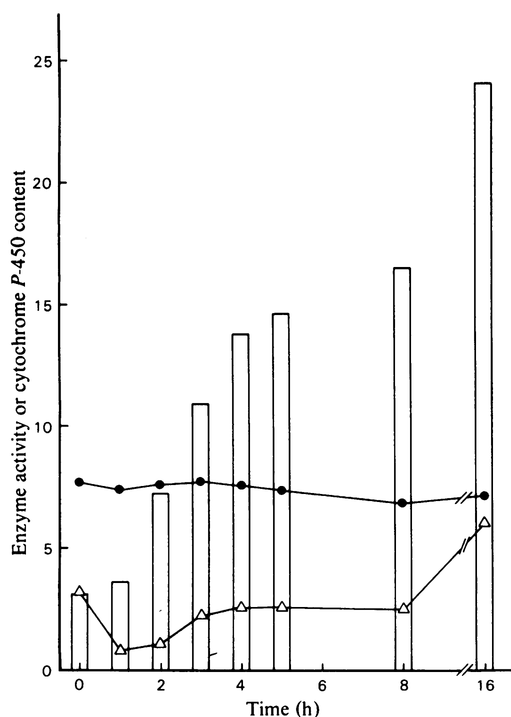

Fig. 1. *Time course of effects of simultaneous administration of Co^{2+} and SKF 525A on hepatic haem oxygenase and ALA synthase activities and cytochrome P-450 content*

Co^{2+} was administered subcutaneously as $CoCl_2$ (250 μmol/kg body wt.) and SKF 525A intraperitoneally (50 mg/kg body wt.). Three animals were used for each time point. The assays were performed as described in the Materials and methods section. Open columns, haem oxygenase activity (nmol of bilirubin formed/h per mg of protein); △, 10 × ALA synthase activity (nmol of ALA/h per mg of protein); ●, 10 × cytochrome *P*-450 content (nmol/mg of protein).

Effect of simultaneous Sb^{3+} and SKF 525A administration on haem oxygenase and ALA synthase activities and cytochrome P-450 content in liver

The combined administration of Sb^{3+} and SKF 525A produced a slow steady increase in haem oxygenase activity during the first 3 h after treatment, followed by a rapid increase, culminating at 8 h in levels of enzyme activity 7–8-fold higher than control values (Fig. 2). The time course and extent of hepatic haem oxygenase induction were similar to those we have previously reported for antimony (Drummond & Kappas, 1981*b*). Throughout the period when haem oxygenase activity was increasing slowly (0–3 h) and then rapidly (3–8 h) the content of cytochrome *P*-450 was not altered significantly. At 8 h the content of this haem protein, in the presence of SKF 525A, was unchanged when compared with initial levels, whereas in the absence of SKF 525A the content of cytochrome *P*-450 is known to be decreased substantially by Sb^{3+} treatment alone (Drummond & Kappas, 1981*b*). The overall extent of haem oxygenase induction was unaffected by the presence of SKF 525A (Table 1). ALA synthase activity exhibited the initial inhibition followed by the rebound increase, which we have previously described after antimony administration (Drummond & Kappas, 1981*b*).

Simultaneous administration of Ni^{2+} and SKF 525A resulted in a characteristic marked increase in

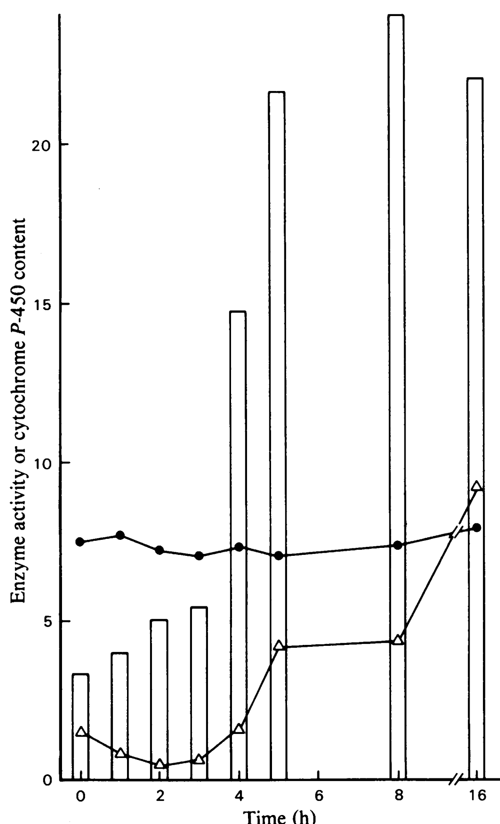

Fig. 2. *Time course of effects of simultaneous administration of Sb^{3+} and SKF 525A on hepatic haem oxygenase and ALA synthase activities and cytochrome P-450 content*

Sb^{3+} was administered subcutaneously as antimony potassium tartrate (82 µmol/kg body wt.) and SKF 525A intraperitoneally (50 mg/kg body wt.). Three animals were used for each time point. The assays were performed as described in the Materials and methods section. Open columns, haem oxygenase activity (nmol of bilirubin formed/h per mg of protein); △, 10 × ALA synthase activity (nmol of ALA/h per mg of protein); ●, 10 × cytochrome *P*-450 content (nmol/mg of protein).

hepatic haem oxygenase activity (4-fold at 8 h) and alterations in ALA synthase activity similar to those previously reported (Maines & Kappas, 1977*b*); these enzymic changes occurred in the absence of significant changes in cytochrome *P*-450 content over the time period studied (0–8 h). In the absence of SKF 525A, Ni^{2+} produced an equivalent induction response of haem oxygenase and a decrease of approx. 25% in cytochrome *P*-450 at 16 h (results not shown) as observed previously (Maines & Kappas, 1977*b*).

Effect of simultaneous administration of Fe–protoporphyrin and SKF 525A on haem oxygenase and ALA synthase activities and cytochrome P-450 content in liver

Simultaneous administration of Fe–protoporphyrin and SKF 525A produced a steady sustained increase in haem oxygenase activity, which reached levels approx. 4-fold higher than control values at 8 h (Fig. 3). Throughout this period cytochrome *P*-450 content remained at its initial level. In comparison, the cytochrome *P*-450 content of animals treated with Fe–protoporphyrin alone decreased by approx. 20% at 8 h (results not shown). ALA synthase activity exhibited the characteristic inhibition associated wtih Fe–protoporphyrin administration.

Simultaneous administration of SKF 525A with CO–protoporphyrin resulted in a marked induction

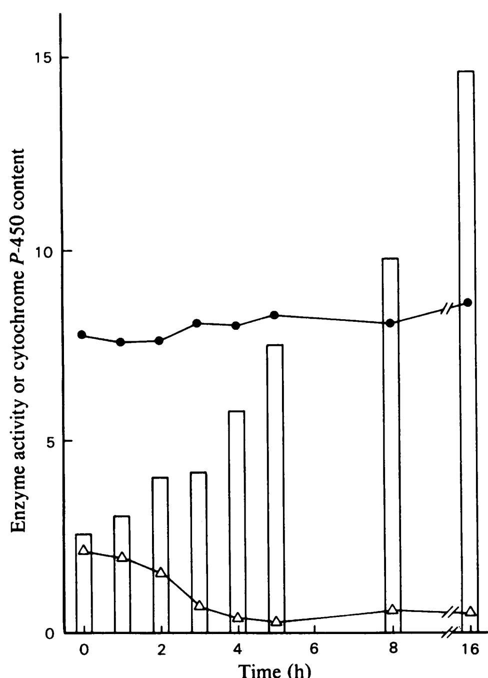

Fig. 3. *Time course of effects of simultaneous administration of Fe–protoporphyrin and SKF 525A on hepatic haem oxygenase and ALA synthase activities and cytochrome P-450 content*

Fe–protoporphyrin was administered subcutaneously at a dose of 125 µmol/kg body wt. and SKF 525A intraperitoneally at a dose of 50 µmol/kg body wt. Three animals were used for each time point. The assays were performed as described in the Materials and methods section. Open columns, haem oxygenase activity (nmol of bilirubin formed/h per mg of protein); △, 10 × ALA synthase activity (nmol of ALA/h per mg of protein); ●, 10 × cytochrome *P*-450 content (nmol/mg of protein).

of haem oxygenase at 8 h (seven times the normal value) and inhibition of ALA synthase activity without significant changes occurring in cytochrome *P*-450 content (approx. 10% decline). In the absence of SKF 525A, Co–protoporphyrin induced haem oxygenase to the same degree and decreased cytochrome *P*-450 content by nearly 50% (results not shown).

Effect of simultaneous administration of DEDC and SKF 525A on haem oxygenase and ALA synthase activities and cytochrome P-450 content in liver

Simultaneous treatment of animals with the organified tin compound DEDC and SKF 525A resulted in a steady increase in haem oxygenase activity with activities of the enzyme reaching a maximum of four times the normal value at 5–8 h. As previously noted (Rosenberg *et al.*, 1980) this organotin does not produce an increase in haem oxygenase as large as that produced by inorganic metals in liver, but the enzyme-inducing effect is sustained for a longer period of time. During the period when haem oxygenase activity was increasing, cytochrome *P*-450 increased slightly, but by 8 h cytochrome *P*-450 content was at its initial level; in animals treated wtih DEDC alone, cytochrome *P*-450 content had declined by approx. 20% at 8 h (results not shown). ALA synthase exhibited a slight decline in activity, which is characteristic of DEDC treatment.

SKF-525A administration was also able to maintain the integrity of spectrally detectable cytochrome *P*-450 up to 48 h on simultaneous treatment with the organotin TCHH (results not shown), without altering the extent of haem oxygenase induction produced by this organometal.

Discussion

The present paper demonstrates that metals that induce haem oxygenase in liver can do so without necessarily diminishing the cellular content of cytochrome *P*-450. In these studies, the simultaneous administration of SKF 525A with an inducing metal served to protect the integrity of the haem protein against the substantial loss normally associated with metal administration without affecting the extent of the haem oxygenase induction response.

A single administration of SKF 525A results *in vivo* in the rapid complexing of approx. 40% of liver cytochrome *P*-450 (Buening & Franklin, 1976). A stable oxygenated complex of ferrous cytochrome *P*-450 is produced, which impairs CO binding and therefore the spectral determination of the haem protein (Schenkman *et al.*, 1972). Ferricyanide destroys this complex, thus permitting quantitative assay of the total amount of cytochrome *P*-450 available for CO binding (Buening & Franklin, 1976). This ability to measure cytochrome *P*-450 content after SKF 525A treatment permitted us to examine the relationship between metal induction of haem oxygenase and the associated changes in cytochrome *P*-450 that accompany this enzyme-induction process.

As noted previously, metal adminstration alone produces prompt and substantial increases in haem oxygenase activity in the liver, kidney and certain other tissues, and this induction of haem oxygenase is associated with major depletions of cellular cytochrome *P*-450 content (Maines & Kappas, 1974; 1975, 1977*a*; De Matteis & Unseld, 1976; Rosenberg *et al.*, 1980). In the present studies the effect of SKF 525A complexing of cytochrome *P*-450 on the alterations produced in this haem protein and in haem oxygenase activities by nine different metals was studied (Table 1). Six of the metals were inorganic elements and three were organometals, of which two were the protoporphyrin IX complexes of cobalt and iron. Each of these metal types has been previously shown to induce haem oxygenase in liver and to produce concomitant depletions of liver cell content of cytochrome *P*-450 (Maines & Kappas, 1974, 1975, 1977*a*; De Matteis & Unseld, 1976; Rosenberg *et al.*, 1980; Drummond & Kappas, 1981*b*); the data shown in Table 1 confirm these actions. However, as Table 1 also indicates, in every instance the simultaneous administration of SKF 525A with an inducer metal blocked the degradation of cytochrome *P*-450 without altering the induction response of haem oxygenase. The maximum decline in cytochrome *P*-450 produced by any of the inducers tested was approx. 10% and for most compounds no significant changes at all occurred in cytochrome *P*-450 content after combined metal and SKF 525A treatment. In the absence of SKF 525A decreases of cytochrome *P*-450 content in the range 25–50% were observed (Table 1). Thus, clearly SKF 525A protected the cytochrome *P*-450 haem moiety with which it was complexed from degradation by the highly induced activity of haem oxygenase; and conversely, the enzyme induction process elicited by the metals did not require the significant labilization of cytochrome *P*-450 to develop along its normal course and extent.

The data reported in Figs. 1–4 show the changes in haem oxygenase, cytochrome *P*-450 and ALA synthase produced by combined metal and SKF 525A treatment at frequent time-points within the initial 8 h period after the agents were administered as well as at the 16 h time point when peak enzyme induction effects are usually observed. The inducers tested, with SKF 525A, were Co^{2+}, Sb^{3+}, Fe–protoporphyrin, and the organotin compound, DEDC. As is evident from the data in these Figures SKF 525A completely protected cytochrome *P*-450

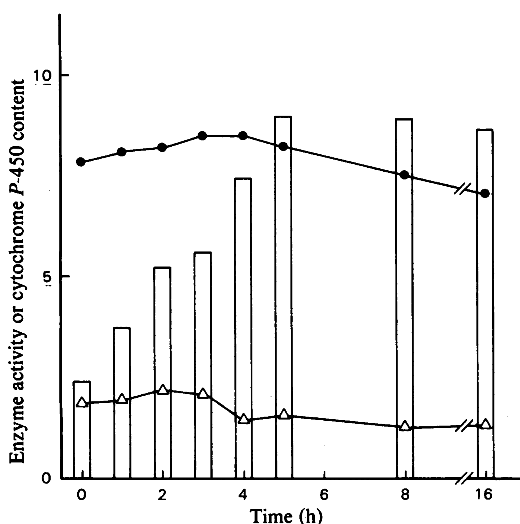

Fig. 4. *Time course of effects of simultaneous administration of DEDC and SKF 525A on hepatic haem oxygenase and ALA synthase activities and cytochrome P-450 content*

DEDC was administered subcutaneously at a dose of 30 μmol/kg body wt. and SKF 525A intraperitoneally at a dose of 50 mg/kg body wt. Three animals were used for each time point. The assays were performed as described in the Materials and methods section. Open columns, haem oxygenase activity (nmol of bilirubin formed/h per mg of protein); △, 10 × ALA synthase activity (nmol of ALA/h per mg of protein); ●, 10 × cytochrome *P*-450 content (nmol/mg of protein).

haem from degradation during the induction of haem oxygenase without altering the pattern or extent of the enzyme response to the four different inducers. In addition the perturbations of ALA synthase produced by Co^{2+} and Sb^{3+} occurred in their typical pattern (Maines & Kappas, 1975; De Matteis & Gibbs, 1976; Drummond & Kappas, 1981*b*) despite the absence of changes in cytochrome *P*-450 as did the expected inhibition of ALA synthase activity by Fe–protoporphyrin. These observations provide clear evidence that in the very early time period after metal administration, when the cellular metabolic events leading to haem oxygenase induction have already been triggered, as we have shown previously (Drummond & Kappas, 1980*b*), the labilization of cytochrome *P*-450 haem is not essential for the induction process.

It is known that a single administration of SKF 525A results *in vivo* in the rapid complexing of approx. 40% of total liver cytochrome *P*-450 (Buening & Franklin, 1976). The fact that SKF 525A–cytochrome *P*-450 complex formation to the extent of only 40% proves sufficient to protect total cellular haem protein content from degradative loss by the highly induced activities of haem oxygenase produced by metal treatment is thus of interest. The SKF 525A-complexed fraction of cytochrome *P*-450 appears, from these results (Table 1, Figs. 1–4), to be also that fraction of the total cellular haem protein content that is most vulnerable to degradation by haem oxygenase activity. Thus there seems to be a portion (approx. 40%) of cytochrome *P*-450 within the liver that is readily available both for binding by SKF 525A and also for degradation by haem oxygenase action. The remaining portion (approx. 60%) of cytochrome *P*-450 appears resistant to further binding with the drug and also to degradation, at early time periods at least, by haem oxygenase. Degradation of the latter fraction of cytochrome *P*-450 can occur of course, but apparently only after 16 h or more after metal administration; that is, at a time when haem oxygenase activities have increased substantially (Table 1, Figs. 1–4) and have remained high for a number of hours. That this fraction of cytochrome *P*-450 can in fact be subject to degradation by long sustained high levels of haem oxygenase activity has been shown in recent studies in this laboratory in which levels of hepatic cytochrome *P*-450 can be depleted by 85–90% for many weeks after appropriate Co–protoporphyrin treatment (Drummond & Kappas, 1981*c*).

The possibility has been suggested that agents that induce haem oxygenase including haem itself might act by first labilizing the haem moiety of cytochrome *P*-450, with the released haem then acting as the proximate inducing agent for the enzyme (Bissell & Hammaker, 1976). However, the almost complete loss of blocking effects of Mn^{2+} and Zn^{2+} on haem oxygenase induction (Drummond & Kappas, 1980*b*) when these elements are administered as little as 10 min after an inducing metal (such as Ni^{2+}, Cd^{2+} or Sn^{2+}) indicates that metal ion inducers of haem oxygenase must very quickly (within minutes) reach the regulatory site(s) of enzyme induction. This finding alone strongly suggests that labilization of cytochrome *P*-450 haem, with the released haem acting as the proximate inducing agent, is a highly unlikely mechanism for metal induction of haem oxygenase, since no significant perturbations of cytochrome *P*-450 within this early time period have so far been described. The present report, in which SKF 525A protects the integrity of cytochrome *P*-450 throughout the immediate period (0–16 h) after metal administration without affecting the extent of haem oxygenase induction, provides additional strong evidence that labilization of cytochrome *P*-450 haem is not an essential event in the initiation by metals of the induction process for

haem oxygenase in liver and probably in other tissues as well.

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