tracting muscle [12] and unpublished observation). Our results also suggest that the potent anti-aggregatory agent PGD_1 (derived from 20 : 3ω5) warrants additional study of its biological activity. These studies indicate that specifically tailored PG analogues may be synthesized which would exert specific anti-thrombotic effects without a concurrent indication of other biological activity.

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Elevated serum copper concentration in monocrotaline pyrrole treated rats with pulmonary hypertension

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Monocrotaline pyrrole (MCTP) is an active metabolite of the plant toxin, monocrotaline (MCT) [1]. When administered to rats, MCTP produces pulmonary vascular injury, pulmonary hypertension, and right ventricular enlargement by unknown mechanisms [2–5]. Because much of the pathophysiology of MCTP-induced pulmonary hypertension is similar to that observed in humans suffering from primary pulmonary hypertension, the MCTP-treated rat provides a useful animal model for studying this human disease.

It has been reported recently that the concentration of copper in the serum of patients with primary pulmonary hypertension is greater than in the normal population [6]. It was of interest, therefore, to determine whether serum copper concentration also increases in this animal model of pulmonary hypertension. Accordingly, changes in the serum concentration of copper were examined in MCTP-treated rats. The serum concentrations of a number of other elements were measured to determine whether any of these also changed with the development of pulmonary hypertension.

Materials and methods

Male, Sprague–Dawley rats (CF:CD(SD)BR) (Charles River Laboratories, Portage, MI) weighing 230–280 g were used in these studies. They were housed on corn cob bedding in plastic cages kept in an animal isolator (Contamination Control, Inc., Lansdale, PA) so that the rats breathed only HEPA*-filtered air. A 12-hr light/dark cycle and conditions of controlled temperature and humidity were maintained.

MCTP was synthesized from monocrotaline (MCT) (TransWorld Chemicals, Washington, DC) via an *N*-oxide intermediate, as described by Mattocks [7], and it was dissolved in *N*,*N*-dimethylformamide (DMF). Rats received either MCTP (3.5 mg/kg) or DMF vehicle via the tail vein on day 0, and then they were killed on day 3, 5, 8, or 14. Rats were anesthetized with sodium pentobarbital, and pulmonary arterial pressure (PAP) was measured as described previously [4]. A 3.5 French umbilical vessel catheter was introduced through the right jugular vein, carefully advanced into the right ventricle, and then gently manipulated into the pulmonary artery [8]. Pressure was measured with a Statham P23ID pressure transducer and was recorded on a Grass model 7 polygraph.

After determination of PAP, blood was collected from the abdominal aorta into glass syringes. The blood was allowed to clot at room temperature for approximately 2 hr, and then it was spun in a centrifuge (600 *g*, 10 min). The serum was collected and stored frozen (−4°) in plastic tubes until analysis for copper as described below.

Right ventricular enlargement (RVE) was assessed as an increase in the ratio of the weight of the right ventricle to the weight of the left ventricle plus septum [9].

The serum was prepared for determination of copper by mixing it with twice the volume of concentrated nitric acid (Baker instra-analyzed grade), and then the mixture was ashed overnight at 90–100°. The concentrations of copper and several other elements were determined in the samples by inductively-coupled argon plasma emission spectroscopy

* HEPA = high efficiency particulate air.

(ICAP) according to the method of Stowe *et al.* [10], using yttrium (10 ppm; atomic absorption standard solution, Aldrich Chemicals, Milwaukee, WI) as an internal standard. The results are expressed as ppm (i.e. μg/l). The detection limit for copper was 0.05 ppm.

Data are expressed as mean ± SEM. Data were analyzed using a Student's *t*-test or using a completely random one-way analysis of variance (ANOVA), as indicated. Individual comparisons were made using the least significant difference test (lsd). Homogeneity of variance was tested using the F_{max} procedure [11]. In all instances, the criterion for significance was $P < 0.05$.

Results and discussion

The serum copper concentration of control rats did not change with time after treatment; therefore, the data were pooled and are represented as a single mean (Fig. 1). The same is true of PAP and RVE of control rats. The concentration of copper in the serum of MCTP-treated rats was first increased at day 8, and it remained elevated through day 14 (Fig. 1A). Pulmonary hypertension was first observed in MCTP-treated rats at day 14 (Fig. 1B). RVE, which is thought to be a response to a sustained elevation in pulmonary vascular pressure in this model, was also evident at day 14 (Fig. 1C). The tendency toward increased serum concentration with increasing PAP was not observed with other elements (Table 1).

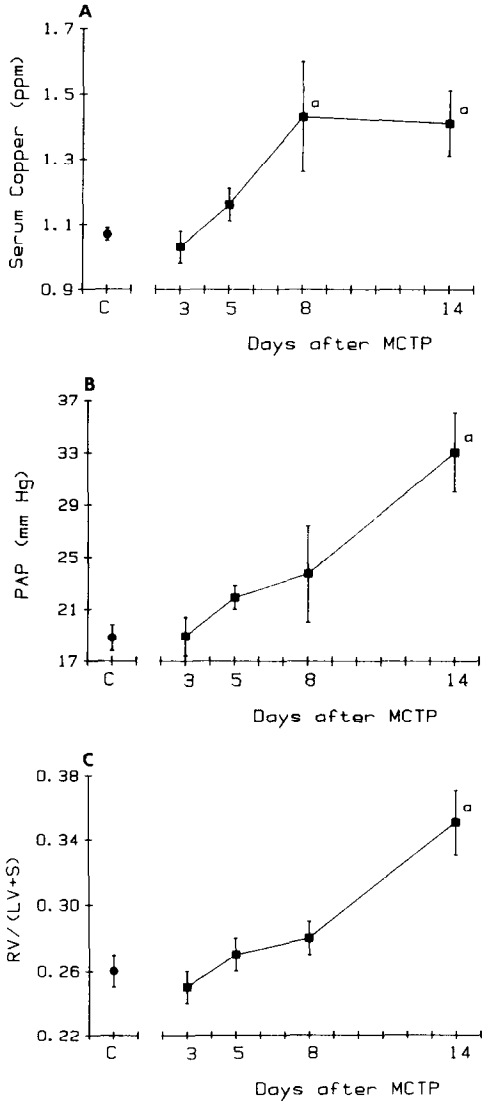

Fig. 1. Serum copper (A), pulmonary arterial pressure (PAP) (B), and right ventricular enlargement (C) in MCTP-treated rats. On day 0, rats were treated with MCTP (3.5 mg/kg) or DMF and were killed 3, 5, 8 or 14 days later (N = 3–10). DMF controls (C; filled circle) were pooled for comparison (N = 14–21). a = significantly different from DMF control (ANOVA, $P < 0.05$).

In DMF control rats, the serum concentrations of several elements (iron, magnesium, phosphorus, zinc and potassium) tended to be higher on day 3 than on subsequent days. Although the cause of this difference was not determined, it is possible that the increase observed at day 3 was an effect of administration of the vehicle. Serum copper concentration in control rats at day 3 was not different from that of subsequent days. In MCTP-treated rats, the difference on day 3 (vs day 5 or 8) was statistically significant ($P < 0.05$) only for phosphorous.

Elevated serum concentrations of copper have been associated with primary pulmonary hypertension in humans [6], and in this study a similar observation was made in rats using a model of chronic pulmonary hypertension. Following treatment with MCTP, increases in serum copper concentration preceded the development of pulmonary hypertension and right ventricular enlargement in rats (Fig. 1) and correlated significantly with increases in pulmonary arterial pressure ($r = 0.65$, $P < 0.05$). Copper exists in several forms in plasma, including forms bound to transcuprein for short-term transport, and to ceruloplasmin for long-term transport and storage [12]. The different forms of copper in the serum were not determined in this study; therefore, it is not possible to discern the relationship between these and pulmonary hypertension.

One explanation for the increase in serum copper concentration may be the inflammation observed in lungs of rats treated with MCTP [4], since copper incorporated into ceruloplasmin accumulates in the plasma during an inflammatory response [12, 13]. Copper also affects some functions of the platelet such as uptake of certain amino acids [14] and 5-hydroxytryptamine [15]. Although it is unclear how these properties of copper may relate to the development of pulmonary hypertension, this observation may be important because blood platelets have been implicated in MCTP-induced pulmonary hypertension [16].

Copper is necessary for the synthesis or activity of a number of enzymes. For example, copper is required for the activity of dopamine-β-hydroxylase, a key enzyme in the synthesis of catecholamines. Acute administration of copper sulfate to sheep results in an increase in pulmonary arterial pressure and pulmonary vascular resistance which is prevented by alpha-adrenergic blockade or catecholamine depletion [17]. Another copper-dependent enzyme is lysyl oxidase, an enzyme required for the cross-linking of collagen and elastin [13, 18]. The appearance of collagen bundles in alveolar walls is associated with MCT treatment [19], and inhibition of collagen maturation by co-treatment with penicillamine, a copper chelator, reduces the ultrastructural changes due to MCT [20]. Thus, copper may contribute to several processes that could be involved in the response of rats to MCT or MCTP.

Although the nature of the link between elevations in serum copper and in pulmonary arterial pressure has not been established, it is of interest that the relation holds in humans with primary pulmonary hypertension as well as in rats in which pulmonary hypertension was induced by treatment with MCTP. This observation further supports use of MCTP-treated rats as a model for this human disease.

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Table 1. Concentrations of several elements in the serum of rats after treatment with MCTP

Days after treatment*	Rx	Elements (ppm)						
		Iron	Magnesium	Phosphorus	Zinc	Potassium	Sodium	Calcium
3	DMF	21 ± 7	31 ± 4	219 ± 35	2.3 ± 0.4	499 ± 112	3382 ± 612	116 ± 19
	MCTP	9 ± 1	24 ± 1	162 ± 4	1.7 ± 0.1	235 ± 14†	2631 ± 642	93 ± 4
5	DMF	7 ± 1	24 ± 1	153 ± 9	1.7 ± 0.1	202 ± 19	2587 ± 238	88 ± 4
	MCTP	8 ± 1	22 ± 1	144 ± 4	1.6 ± 0.1	188 ± 16	2661 ± 160	89 ± 2
8	DMF	7 ± 1	24 ± 1	152 ± 5	1.7 ± 0.1	184 ± 11	2474 ± 12	96 ± 1
	MCTP	10 ± 2	24 ± 2	142 ± 7	1.6 ± 0.2	231 ± 27	2395 ± 56	86 ± 2†
14	DMF	5 ± 1	20 ± 3	157 ± 4	1.6 ± 0.1	200‡	2861 ± 102	88 ± 2
	MCTP	7 ± 1	23 ± 1	150 ± 4	1.6 ± 0.1	277 ± 46	2806 ± 100	87 ± 2

* Rats received MCTP (3.5 mg/kg) or DMF vehicle i.v. on day 0 and were killed on day 3, 5, 8 or 14. Values represent mean ± SEM, N = 3–10.

† Significantly different from DMF on the same day (Student's *t*-test, $P < 0.05$).

‡ N = 2.

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Growth inhibition of melanoma cells by N-protected dopa derivatives

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Melanocytes possess a unique biochemical property, melanin synthesis [1]. The synthesis of melanin pigment from tyrosine is catalysed by tyrosinase (EC. 1.14.18.1) present in both normal and malignant melanocytes: tyrosine is hydroxylated to dopa and then oxidised to dopaquinone, and the latter is converted to melanin pigment in a complex series of spontaneous reactions [2].

Wick *et al.* [3] showed that dopa is selectively toxic to pigmented melanoma cells *in vitro*. Subsequently, Wick [4–6] showed that catecholic compounds related to dopa, e.g. dopa methyl ester and 3,4-dihydroxybenzylamine, possess significant antitumour effect against mouse and human melanomas *in vitro* and *in vivo*. Several attempts have been made to enhance the antimelanoma effect of these catechols [7–9], and new types of dopa derivatives have also been evaluated [10–12].

In this study, we examined the effects of *N*-protected dopa derivatives, *N*-acetyldopa and γ-glutamyldopa, on the growth of melanoma cells *in vitro* and *in vivo*.

Materials and methods

Catalase, superoxide dismutase (SOD), phenylthiourea (PTU), reduced glutathione (GSH) and L-dopa were purchased from Sigma Chemical Co. (St Louis, MO), and the