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# POLYAMINE SYNTHESIS BLOCKADE IN MONOCROTALINE-INDUCED PNEUMOTOXICITY*

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**Abstract**—Based on the documented regulatory role of polyamines in cell growth and differentiation, we have proposed that these organic cations are involved with the development of monocrotaline (MCT)-induced hypertensive pulmonary vascular disease. Two lines of evidence support this hypothesis: (1) MCT causes progressive increases in lung polyamine contents which are temporarily related to the development of cardiopulmonary abnormalities, and (2) blockade of polyamine synthesis with the site-selective enzyme-activated inhibitor, α-difluoromethylornithine (DFMO), attenuates development of medial arterial thickening, increased pulmonary arterial pressure, and right ventricular hypertrophy. To evaluate the mechanism of DFMO protection, the present study assessed when, during the course of MCT-induced pneumotoxicity, DFMO exerts its salutary effects, and determined if the protection afforded by DFMO could be reversed through supplementation with exogenous polyamines. To address the first issue, rats were treated with 30 mg/kg MCT and, 10 days after administration when lung polyamine contents were augmented and when pulmonary edema was evident, DFMO treatment was initiated as a 2% solution in the drinking water. In animals receiving MCT only, lung polyamine contents were elevated and right ventricular hypertrophy was evident at both 20 and 35 days after treatment. DFMO treatment initiated at day 10 attenuated the increases in putrescine and spermidine but not spermine and reduced the degree of right ventricular hypertrophy at both the 20- and 35-day time points. To determine if the blockade by DFMO could be reversed by supplementation with exogenous polyamines, animals were treated simultaneously with MCT and DFMO as described above and the immediate precursor to the polyamines, ornithine, was added to the drinking water as a 2% solution. Relative to animals receiving MCT and DFMO, ornithine supplementation increased lung polyamine contents to levels normally associated with MCT treatment only. Ornithine also reversed the protection against right ventricular hypertrophy normally afforded by DFMO. These observations indicate that the salutary effects of DFMO in MCT-induced pulmonary hypertension cannot be ascribed solely to interference in the early events after MCT treatment and that restoration of lung polyamine contents to high levels by supplementation with exogenous ornithine reverses DFMO protection against sustained pulmonary hypertension. It is concluded, therefore, that polyamines play a central role in delayed responses of lung cells underlying the development of MCT-induced sustained pulmonary hypertension.

A single subcutaneous injection of monocrotaline (MCT) in rats is associated with an early phase of endothelial injury as detected histopathologically and by altered endothelial functions [1–3]. At times coincident with development of endothelial injury, lungs isolated from MCT-treated rats also exhibit vascular hyperreactivity to selected pressor stimuli [4, 5]. These abnormalities are followed by progressive structural remodelling of the pulmonary vascular bed, sustained pulmonary hypertension, and right ventricular hypertrophy [6, 7]. Biochemical mechanisms which couple the initial insult(s) provoked by MCT to this spectrum of cellular responses are not delineated completely.

Prompted by findings that MCT-induced hypertensive pulmonary vascular remodeling is related to hyperplasia and hypertrophy of certain resident lung cells [8], we hypothesized that the polyamines, a family of intracellular organic cations which are essential for cell proliferation and differentiation [9], would play a central pathogenic role. The evidence supporting involvement of polyamines in MCT-induced pulmonary hypertension is reasonably convincing. Monocrotaline treatment is associated with increases in the lung activities of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase, the two rate-limiting enzymes in polyamine biosynthesis, and with accumulation of polyamines in lung tissue [10, 11]. These changes are temporally related to development of sustained pulmonary hypertension and right ventricular hypertrophy. Also in accord with our hypothesis, blockade of ornithine decarboxylase activity with the site-selective, enzyme-activated inhibitor, α-difluoromethylornithine (DFMO), attenuates the MCT-induced increase in putrescine and spermidine and abolishes or attenuates medial arterial thickening, increased pulmonary arterial pressure, and right ventricular hypertrophy [11, 12]. In addition to these events, in which cell proliferation and differentiation can be readily implicated, DFMO also blocks the early manifestations of MCT treatment, including development of pulmonary edema and vascular hyperreactivity [12, 13].

The present study addressed two important ques-

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tions regarding the involvement of polyamines in MCT-induced pulmonary hypertension. First, it is not known whether blockade by DFMO of the early effects of MCT is necessary for protection against sustained pulmonary hypertension. In this context, it is conceivable that one of the early events in MCT-induced pneumotoxicity, such as pulmonary edema or vascular hyperreactivity, could be important in the promotion of lung cell hyperplasia and hypertrophy and, ultimately, sustained pulmonary hypertension [4, 7]. By inhibiting such key early events, DFMO could forestall evolution of hypertensive pulmonary vascular disease in a manner unrelated to direct involvement of polyamines in the remodeling process *per se*. If this is true, then polyamine synthesis blockade applied after the onset of MCT-induced pneumotoxicity but before the development of sustained pulmonary hypertension should be without beneficial effects. Conversely, if DFMO administered well after the onset of MCT-induced pneumotoxicity retains its protective effects, this would imply that at least some of the delayed cellular events underlying hypertensive pulmonary vascular remodeling were polyamine dependent. A second unresolved issue pertains to the reversibility of the DFMO blockade through supplementation with exogenous polyamines. If polyamines are involved with the hypertensive effects of MCT, it should be possible to reverse the blockade afforded by DFMO by elevating lung polyamine contents through supplementation with exogenous polyamines.

## METHODS

*Experimental plan.* Initial experiments determined if DFMO would forestall development of pulmonary hypertension even if treatment were initiated after the onset of MCT-induced pneumotoxicity. As markers of the onset of MCT-induced pneumotoxicity, we assessed, at 10 days post MCT administration (in the absence of DFMO treatment), the wet left lung to total body weight ratio as an index of pulmonary edema formation, examined histopathologic characteristics of the lung, and evaluated lung polyamine contents as a biochemical index of the onset of lung injury. The extent of right ventricular hypertrophy also was determined at this time to confirm that sustained pulmonary hypertension had not yet developed. Based on the results of these studies, DFMO treatment was initiated at 10 days post MCT administration. Separate groups of animals were killed at 20 or 35 days post MCT and evaluated for the extent of right ventricular hypertrophy as evidence of sustained pulmonary hypertension and for lung polyamine contents to confirm the efficacy of MCT to augment, and DFMO to suppress, polyamine synthesis. The later time point was included specifically to determine if the salutary effects of DFMO were maintained.

As part of the 35-day study noted above, additional groups of animals were introduced to determine if supplementation with exogenous ornithine would reverse the inhibitory effects of DFMO on development of right ventricular hypertrophy through restoration of elevated lung polyamine contents. One group of MCT plus DFMO-treated animals was given ornithine continuously via the drinking water. A second group of MCT-treated animals was given ornithine in the absence of DFMO administration. Ornithine administration was begun at 10 days post MCT, i.e. at the same time as DFMO treatment was initiated, and animals were killed 25 days thereafter. Lung polyamine contents were assessed and the extent of right ventricular hypertrophy was determined to confirm the efficacy of the ornithine treatment protocol to restore the elevated lung polyamine contents and engender development of pulmonary hypertension respectively.

*Animal model and experimental measurements.* Adult male Sprague–Dawley rats weighing 272 ± 10 g (mean ± SE) and segregated into groups of four to six animals were used in these experiments. Groups receiving MCT were given a single s.c. injection of 30 mg/kg of the alkaloid. Control animals received an equivalent volume of vehicle. MCT and its vehicle were prepared as described previously [14]. DFMO treatment, which was initiated 10 days after administration of MCT or its vehicle, was given continuously as a 2% solution in the drinking water [11–13] for the duration of the experiment. In addition, on day 10 only, animals received a loading dose of DFMO given as four s.c. injections of 400 mg/kg each over a 10-hr period [11–13]. Animals not receiving DFMO received its vehicle in the same dosing regimen. Some animals received ornithine either in combination with MCT only or in combination with MCT plus DFMO. In either case, ornithine treatment was initiated at 10 days after MCT and given continuously as a 2% solution in the drinking water for the duration of the study. During the course of these experiments, all animals were provided with food and water *ad lib.* and maintained in a environmentally-controlled room with a 12-hr photoperiod.

After the animals were killed with an overdose of i.p. sodium pentobarbital, the heart and lungs were excised rapidly. The extent of right ventricular hypertrophy, an index of sustained pulmonary hypertension, was determined as the ratio of the weight of the right ventricular free wall normalized to the weight of the left ventricle plus septum. The wet weight of the left lung was normalized to the total body weight and taken as an index of lung water accumulation [15]. In some instances, the right lung was perfused *in situ* at a pressure of 20 cm $H_2O$ with neutral buffered formalin and, after staining with hematoxylin and eosin, processed for routine light microscopy. Light microscopy was used to confirm the presence of perivascular cuffing indicative of edema and lung inflammation, both of which are typical of MCT-treated rat lungs.

Polyamine contents in the left lung of treated and untreated animals were evaluated according to slight modifications of procedures described by Stefanelli and co-workers [16] and Kabra *et al.* [17]. In brief, lungs were homogenized with an Ultra-Turrax homogenizer (full speed, 3 bursts for 10 sec each) in 5 vol. of 0.2 N $HClO_4$. The homogenates were then centrifuged at 40,000 *g* for 20 min. The resulting supernatant fraction was dansylated, transferred to a Bond-Elut $C_{18}$ column, and dansylated polyamines were eluted with 1500 μl methanol. Samples (25 μl)

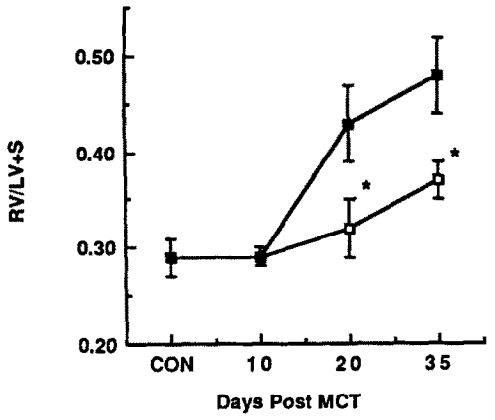


Fig. 1. Effect of DFMO treatment initiated at day 10 on development of right ventricular hypertrophy (RV/LV + S) in monocrotaline (MCT)-treated rats. Vehicle-treated rats (N = 6) were studied at the same times as MCT-treated rats, but because values did not differ significantly as a function of time, they were pooled for representation and designated as controls (CON). MCT-treated animals (N = 12) were treated identically until day 10 when DFMO administration was initiated in six animals (□–□) while the other six animals did not receive DFMO (■–■). Values are means ± SE. Key: (*) Significantly ($P < 0.05$) reduced compared to rats receiving MCT in the absence of concomitant DFMO treatment.

were then injected onto a 5 μm Beckman ODS HPLC column (4.6 mm × 25 cm), and polyamines were quantitated using a Beckman model 157 fluorescence spectrophotometer. The limit of detection for putrescine, spermidine, and spermine was 1 pmol. Polyamine contents were normalized to the total weight of the left lung [11, 12].

*Statistics.* Data are expressed as the mean ± the standard error of the mean. Differences between experimental groups were assessed using one- or two-way analysis of variance combined with the Newman–Keuls' test for multiple comparisons. A P value equal to or less than 0.05 was considered to denote statistical significance.

## RESULTS

At 10 days post MCT administration, prior to initiation of DFMO treatment, the wet left lung-to-body weight ratio had increased significantly ($P < 0.05$) from $1.86 \pm 0.08 \times 10^{-3}$ in control rats (N = 6) to $2.31 \pm 0.01 \times 10^{-3}$ in animals given MCT (N = 8). Histopathologic evaluation revealed findings typical of MCT-induced pneumotoxicity (see, for example, Ref. 1). Both perivascular cuffing, indicative of pulmonary edema, and a prominent inflammatory cell infiltration were evident in lungs from MCT-treated rats but not control animals (data not shown). As shown in Fig. 1, there was no evidence for development of right ventricular hypertrophy by day 10 post MCT insofar as the ratio, RV/LV + S, did not differ significantly between control and MCT-treated animals. Lung polyamine contents were augmented within 10 days of MCT administration, however. As shown in Fig. 2, the contents of putrescine and spermidine, but not spermine, were augmented significantly in animals receiving MCT 10 days previously.

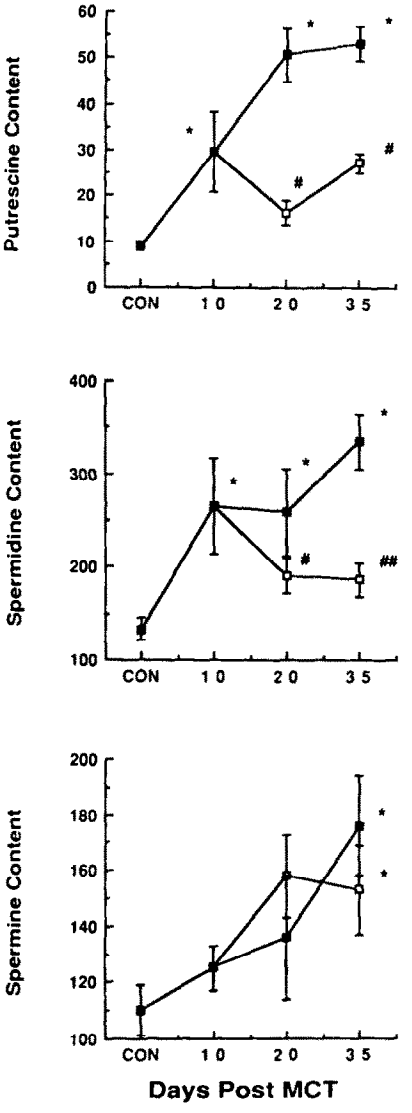


Fig. 2. Effect of DFMO treatment initiated at day 10 on monocrotaline (MCT)-induced increases in lung putrescine (top panel), spermidine (middle panel), and spermine (bottom panel) content. Polyamine contents are reported in nanomoles per left lung (mean ± SE). Vehicle-treated rats (N = 6) were studied at the same times as MCT-treated rats, but because values did not differ significantly as a function of time, they were pooled for representation and designated as controls (CON). MCT-treated animals (N = 12) were treated identically until day 10 when DFMO administration was initiated in six animals (□–□), while the other six animals did not receive DFMO (■–■). Key: (*) Significantly ($P < 0.05$) increased compared to controls; (#) significantly ($P < 0.05$) greater than controls but reduced in comparison to rats receiving MCT in the absence of concomitant DFMO treatment; and (##) not significantly different from controls but reduced in comparison to rats receiving MCT in the absence of DFMO treatment.

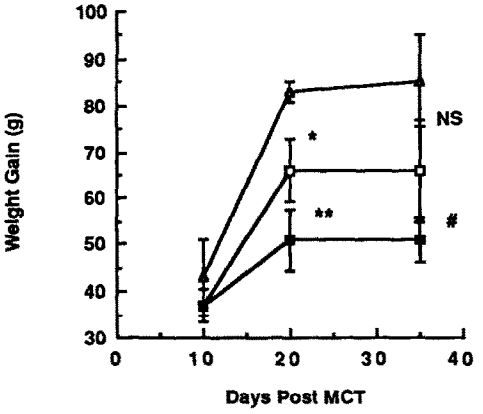


Fig. 3. Comparison of body weight in control rats (△–△) with that in rats treated with monocrotaline (□–□) and in rats treated with monocrotaline plus DFMO (■–■). DFMO treatment was initiated at day 10 post monocrotaline. Values are means ± SE; N = 6 for each point. Key: (*) significantly ($P < 0.05$) reduced compared to control animals; (**) significantly ($p < 0.05$) reduced compared to control. (Different from MCT at $P = 0.19$); and (#) significantly ($P < 0.05$) reduced compared to controls but not in comparison to MCT-treated rats.

Development of right ventricular hypertrophy in MCT-treated animals receiving normal drinking water or drinking water supplemented with 2% DFMO starting at day 10 after MCT is shown in Fig. 1. Whereas rats receiving MCT in the absence of DFMO developed pronounced right ventricular hypertrophy by days 20 and 35 post MCT, animals treated with the polyamine synthesis inhibitor failed to exhibit right ventricular hypertrophy at day 20 and, at day 35, the RV/LV + S ratio still remained significantly below that associated with MCT-treated animals.

Lung polyamine contents in control animals, in MCT-treated animals, and in MCT-treated rats receiving DFMO beginning at day 10 are shown in Fig. 2. As noted above, DFMO administration was initiated at a time when the lung contents of putrescine and spermidine in MCT-treated rats were elevated. DFMO initiated at this time attenuated the increase in both putrescine and spermidine to levels below that associated with MCT treatment alone but slightly above those found in the lungs of control rats. Lung contents of spermine were not elevated above control until day 35 post MCT and were unaffected by concomitant DFMO administration.

Increases in body weight in control animals and in animals treated with MCT in the absence or presence of DFMO administration are shown in Fig. 3. Prior to day 10, all three groups of animals gained weight at approximately the same rate. After day 10, however, control animals gained a significantly greater amount of weight than did either group of MCT-treated animals. In addition, though not attaining statistical significance ($P = 0.19$), MCT-treated animals receiving DFMO tended to gain less weight than animals receiving MCT in the absence of DFMO. At 35 days post MCT treatment, control animals had gained significantly more weight than did animals receiving MCT plus DFMO. The weight gain in animals receiving MCT only did not differ from controls at this time. The weight gain in MCT-treated animals at the 35-day time point did not differ from animals receiving both MCT and DFMO.

To determine if the protective actions of DFMO against MCT-induced sustained pulmonary hypertension could be ascribed to inhibition of lung polyamine synthesis, additional experiments sought to overwhelm the blockade by DFMO and engender lung polyamine accumulation by supplementation with exogenous ornithine. As described previously, ornithine alone or ornithine plus DFMO was added to the drinking water at 10 days after MCT administration, and the animals were studied 25 days thereafter. Figure 4 shows the extent of right ventricular hypertrophy in control animals, MCT-treated animals, animals receiving MCT plus DFMO at day 10, and the two ornithine treatment groups noted above. As expected, MCT was associated with a considerable degree of right ventricular hypertrophy which was attenuated by DFMO treatment initiated at day 10. Importantly, concomitant administration of ornithine reversed the protection afforded by DFMO to the extent that the degree of right ventricular hypertrophy did not differ significantly from rats given MCT in the absence of DFMO. Rats treated with MCT and ornithine developed right ventricular hypertrophy to the same degree as animals given MCT only. Ornithine alone failed to promote right ventricular hypertrophy (data not shown).

Lung polyamine contents in animals given the above combinations of MCT, DFMO and ornithine are shown in Table 1. Monocrotaline elevated the contents of all three polyamines whereas concomitant administration of DFMO attenuated the increase in putrescine and spermidine but not spermine. Addition of ornithine to the MCT and DFMO treatment regimen partially restored the increases in both putrescine and spermidine, though not to the same levels observed in rats receiving MCT only.

Increases in body weights associated with the MCT, DFMO, and ornithine treatment regimens are shown in Fig. 5. At 35 days post MCT administration, treated animals tended to gain less weight than controls, although this difference did not attain statistical significance ($P = 0.24$). The weight gain in animals receiving DFMO plus MCT did not differ from MCT alone but was significantly less than the weight gain exhibited by control rats. Importantly, animals receiving the combination of MCT, DFMO and ornithine gained the same amount of weight as did animals receiving MCT plus DFMO. Animals receiving MCT plus ornithine alone gained the same amount of weight as control animals.

## DISCUSSION

There are two principal observations of this study: (1) DFMO treatment initiated 10 days after MCT reversed the increase in lung putrescine and spermidine and attenuated development of right ventricular hypertrophy, and (2) supplementation with exogenous ornithine partially abrogated the blockade afforded by DFMO as evidenced by restoration

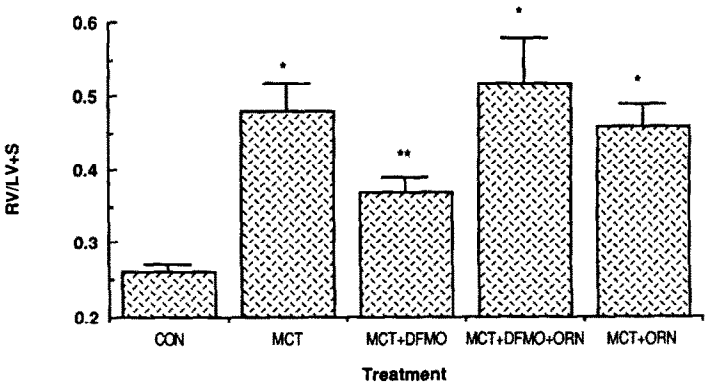


Fig. 4. Effect of ornithine (ORN) on attenuation by DFMO of right ventricular hypertrophy (RV/LV + S) in monocrotaline (MCT)-treated rats. Values are means ± SE; N = 6 for each experimental group. Key: (*) significantly ($P < 0.05$) increased compared to control (CON) animals; (**) significantly ($P < 0.05$) increased compared to control but significantly ($P < 0.05$) reduced in comparison to animals receiving MCT only. MCT + DFMO + ORN, or MCT + ORN.

Table 1. Effects of DFMO and ornithine on lung polyamine contents in rats treated 35 days previously with monocrotaline

| Treatment | Polyamine content (nmol/left lung) | | |
|---|---|---|---|
| | Putrescine | Spermidine | Spermine |
| Control | 8.85 ± 0.97 | 132.7 ± 12.8 | 75.5 ± 5.0 |
| MCT* | 53.1 ± 3.8† | 335.6 ± 29.0† | 176.4 ± 18.7† |
| MCT + DFMO‡ | 27.2 ± 2.2†,§ | 187.5 ± 18.4§ | 153.2 ± 16.2 |
| MCT + DFMO + ORN‖ | 32.6 ± 2.9† | 257.2 ± 38.9† | 170.8 ± 25.7† |
| MCT + ORN‖ | 45.9 ± 7.8† | 218.1 ± 21.9§ | 118.9 ± 11.1§ |

Values are means ± SE, N = 6.

* Animals received a single s.c. injection of 30 mg monocrotaline (MCT) per kg body weight and were studied 35 days thereafter.

† Significantly different from controls at $P < 0.05$.

‡ DFMO was administered as a 2% solution in the drinking water starting at day 10 post MCT and continuing until 35 days post treatment. In addition, on day 10 post MCT only, animals received four s.c. injections of DFMO administered as described in the text.

§ Significantly different from MCT at $P < 0.05$.

‖ Ornithine (ORN) was given as a 2% solution in the drinking water starting at day 10 post MCT treatment.

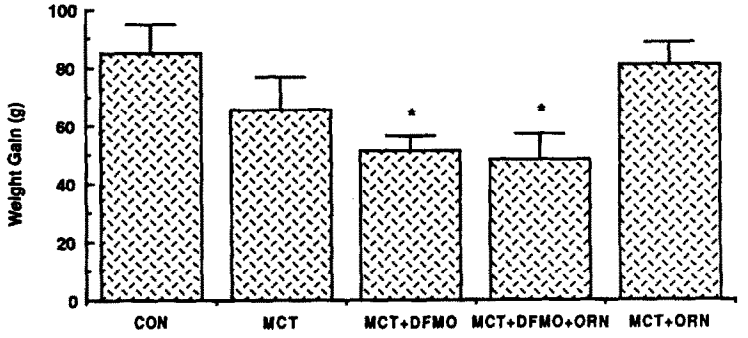


Fig. 5. Body weight gain in control rats (CON), animals receiving monocrotaline (MCT), MCT + DFMO, MCT + DFMO + ornithine (ORN), or MCT + ORN. Animals were treated with MCT or its vehicle 35 days previously. Values are means ± SE; N = 6 for each experimental group. Key: (*) significantly ($P < 0.05$) reduced compared to control animals.

of lung polyamine contents to elevated levels and by development of right ventricular hypertrophy. Before discussing the significance and implications of these findings, it is appropriate to emphasize an important aspect of our experimental design. Inherent in our objective to determine if DFMO would exert salutary effects if administered after the onset of pneumotoxicity is the necessity of confirming that the early actions of MCT on the lung have indeed been expressed before development of pulmonary hypertension and application of the DFMO treatment. We selected 10 days post MCT as a reasonable point to initiate DFMO administration based upon previous reports that lung injury but not pulmonary hypertension would develop by this time (see, for example, Refs. 3, 6 and 7). In accord with these earlier studies, we found several changes indicative of lung injury in rats 10 days after MCT treatment; the wet lung-to-body weight ratio was increased, perivascular cuffing and lung inflammation were evident on light microscopic evaluation, and lung polyamine contents were elevated. Despite the presence of lung injury, there was no evidence that sustained pulmonary hypertension had developed by this time: the ratio RV/LV + S, an estimate of the degree of right ventricular hypertrophy, did not differ between control and MCT-treated rats. It could be argued that the RV/LV + S ratio is an indirect and, therefore, insensitive indicator of sustained pulmonary hypertension. However, while in some animal models of pulmonary hypertension the relation between right ventricular hypertrophy, hypertensive pulmonary vascular disease and pulmonary hypertension may be complex, these parameters seem to be closely linked in MCT-treated rats. For example, both Ghodsi and Will [6] and Meyrick and Reid [7] called attention to the fact that pulmonary arterial pressure in MCT-treated rats increases in parallel with hypertensive pulmonary vascular remodeling and with development of right ventricular hypertrophy. In view of these considerations, we concluded that lung injury was present in rats treated 10 days previously with MCT but that sustained pulmonary hypertension had not yet developed. Consistent with the objectives of this study, DFMO treatment was initiated at day 10 post MCT.

Monocrotaline is biotransformed by the liver to toxic metabolites, some of which are highly reactive, short-lived pyrroles [18], which produce pulmonary endothelial injury [1–3], a transient phase of pulmonary vascular hyperreactivity to selected pressor stimuli [4, 5] and, ultimately, hypertensive pulmonary vascular disease. The endothelial injury and vascular hyperreactivity are apparent within the first week after MCT administration. While the time–course for resolution of the endothelial injury has not been established [3], the vascular hyperreactivity appears to subside within about 2 weeks [4]. Hypertensive pulmonary vascular lesions and attendant sustained pulmonary hypertension develop progressively over a 2- to 3-week period [6, 7]. Although the specific contributions of the endothelial injury and vascular hyperreactivity to the eventual development of hypertensive pulmonary vascular disease are unknown, either or both may be essential for initiation and progression of the hypertensive vascular remodeling [4, 7]. We have reported previously that DFMO treatment initiated prior to MCT administration fails to influence the hepatic production of MCT-derived pyrroles [12] but prevents development of pulmonary edema [12], vascular hyperreactivity [13], and attenuated MCT-induced medial arterial thickening, pulmonary hypertension, and right ventricular hypertrophy assessed at day 21 post MCT [10, 11]. In the specific case of our finding that DFMO fails to interfere with hepatic production of MCT-derived pyrroles, it is relevant that more recently Lafranconi and Huxtable [19] reported the appearance of a long-lasting, ostensibly non-pyrrolic, hepatic metabolite of MCT capable of producing lung injury in *in vitro* systems. It is not known whether DFMO inhibits formation of this non-pyrrolic substance.

Based on the above considerations, a key question to emerge is whether blockade by DFMO of these early effects of MCT, i.e. hepatic biotransformation of MCT to toxic metabolites, endothelial injury and vascular hyperreactivity, is required for inhibition of the later occurring pulmonary hypertension. The present observations that initiation of DFMO treatment well after the onset of MCT-induced pneumotoxicity reversed the early increase in lung putrescine and spermidine and significantly blunted development of right ventricular hypertrophy suggest that prevention of these early events by DFMO probably is not essential for protection against MCT-induced pulmonary hypertension. In addition, the present observations indicate that at least some of the critical DFMO-sensitive events necessary for development of pulmonary hypertension occur relatively late after MCT administration. At this time, we can only speculate as to what these events might be. However, observations that proliferation and differentiation of several resident lung cells occur (e.g. fibroblasts, smooth muscle cells and endothelial cells) over this time frame in MCT-treated rats [8], coupled with the recognition that polyamines are essential regulators of these processes [9], suggest that some of the cellular mechanisms of hypertensive pulmonary vascular remodeling are polyamine dependent. In a similar context, insofar as the mitogenic actions of growth factors on certain target cells appear to be polyamine dependent (e.g. [20]) and because at least one growth factor (interleukin 1) has been detected in MCT-treated rat lungs [21], it is conceivable that some of the polyamine-dependent events are driven by growth factors. Clearly, the specific cellular events sensitive to the inhibitory effects of DFMO will require much additional study.

DFMO is a highly selective compound whose only known pharmacologic effect is inhibition of ornithine decarboxylase [22, 23]. Based largely on this selectivity, we have suggested that the protective actions of DFMO in MCT-induced pulmonary hypertension are most likely attributable to inhibition of polyamine synthesis. Nevertheless, in our previous reports as well as the present study we have noted that animals treated with DFMO plus MCT tended to gain less weight than did rats given MCT only. These differences in weight gain have not attained statistical significance, but their biological significance is considerably more difficult to assess.

This is particularly important in view of findings by Hayashi *et al.* [24] that dietary restriction, by an unknown mechanism, attenuates development of MCT-induced pulmonary hypertension. Two interrelated lines of evidence from the present study argue strongly against dietary restriction being the sole basis of the protective actions of DFMO. First, supplementation with exogenous ornithine reversed both the inhibition by DFMO of MCT-induced lung polyamine accumulation and the attenuation of MCT-induced right ventricular hypertrophy. This reversal was not related to a synergistic effect of ornithine and MCT since animals exposed to this treatment regimen did not develop more severe right ventricular hypertrophy than did animals receiving only MCT. These observations are consistent with a preliminary report by Hacker and Byus [25]. Second, the reversal of DFMO protection by ornithine was not associated with additional weight gain. Animals treated with MCT, DFMO, and ornithine did not gain more weight than animals treated with MCT plus DFMO. From these observations, the most reasonable conclusion is that DFMO protects against MCT-induced pulmonary hypertension through inhibition of polyamine synthesis in key lung cells.

The conventional strategy for confirming involvement of polyamines in the salutary effects of DFMO uses the product of ornithine decarboxylation, putrescine, or spermidine to bypass the effect of DFMO and augment polyamine contents. We conducted pilot studies using putrescine instead of ornithine, but, for unknown reasons, putrescine administration was associated with unacceptable mortality in rats with MCT-induced pneumotoxicity. Our present approach using ornithine was adapted from a preliminary communication by Hacker and Byus [25]. The exact mechanism by which ornithine augments polyamine contents in DFMO-treated rats is unknown. Because ornithine is taken up by cells via a specific transport system whereas DFMO gains entry through diffusion [23], it seems unlikely that the protective actions of ornithine are related to simple interference with DFMO uptake. A more plausible explanation is that ornithine effectively competes with DFMO for the common binding site on ornithine decarboxylase and thereby permits synthesis of putrescine and ultimately the other two polyamines.

In summary, results of the present study demonstrated that DFMO treatment initiated after the onset of MCT-induced pneumotoxicity reverses the increase in lung polyamines and, most importantly, forestalls development of right ventricular hypertrophy. Blockade of the early effects of MCT on the lung by DFMO is thus not essential for the salutary actions of the polyamine synthesis inhibitor on development of sustained pulmonary hypertension. In addition, because the protective actions of DFMO on MCT-induced changes in lung polyamine contents and development of right ventricular hypertrophy can be abrogated by concomitant administration of ornithine, we suggest that at least some of the late-occurring DFMO-sensitive events are polyamine dependent.

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