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Semotiadil improves survival of rats with monocrotaline-induced pulmonary hypertension: comparison with diltiazem

Takashi Takahashi [a,*], Tsugiyasu Kanda [b], Susumu Imai [a], Tadashi Suzuki [c], Isao Kobayashi [b], Kazuhiko Murata [a]

[a] *Second Department of Internal Medicine, Gunma University School of Medicine, Maebashi, Gunma 371, Japan*
[b] *Department of Laboratory Medicine, Gunma University School of Medicine, Maebashi, Gunma 371, Japan*
[c] *College of Medical Care and Technology, Maebashi, Gunma 371, Japan*

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Abstract

We compared the effects of semotiadil, a novel Ca^{2+} channel blocker, with those of diltiazem on survival and regression of right ventricular hypertrophy and media thickening of pulmonary arteries in a rat model of pulmonary hypertension. Pulmonary hypertension was induced by a single injection of monocrotaline (80 mg/kg). Four weeks later, after pulmonary hypertension was confirmed, oral administration of semotiadil (10, 30, or 100 mg/kg/day) or diltiazem (100 or 300 mg/kg/day) was initiated. The rats were observed for 3 weeks. Survival was significantly longer in the group that received semotiadil 100 mg/kg/day than in the groups treated with diltiazem 100 or 300 mg/kg/day. Media thickness and smooth muscle area in pulmonary arteries were significantly less in rats treated with semotiadil 100 mg/kg/day than in animals treated with diltiazem 100 mg/kg/day. The right ventricle to left ventricle mass ratio, right ventricular wall thickness, and right ventricular myocardial fiber diameter were equal in these two groups. Semotiadil 100 mg/kg/day improved the survival of rats, which responded with a significant regression of right ventricular hypertrophy and media thickening of pulmonary arteries in comparison with rats treated with diltiazem 100 or 300 mg/kg/day.

Keywords: Semotiadil; Ventricular hypertrophy, right; Media thickening; Pulmonary hypertension

1. Introduction

Primary pulmonary hypertension is a progressive disease that is incurable (Dresdale et al., 1951). Right heart failure is the cause of death in 64% of patients with primary pulmonary hypertension (Grossman and Braunwald, 1992). Ca^{2+} channel blockers have been investigated as therapy for primary pulmonary hypertension in humans (Kambara et al., 1981) and experimental models (Young et al., 1983) and have been shown to decrease both pulmonary artery pressure and right ventricular hypertrophy. A recent study has indicated that high doses of Ca^{2+} channel blockers, such as nifedipine and diltiazem, may improve survival over a 5-year period in patients with primary pulmonary hypertension, who respond with reductions in pulmonary artery pressure and pulmonary vascular resistance (Rich et al., 1992).

Semotiadil fumarate ((+)-(*R*)-3,4-dihydro-2-[5-methoxy-2-[3-[*N*-methyl-*N*-[2-[(3,4-methylene-dioxy) phenoxyl] ethyl] amino] propoxy] phenyl]-4-methyl-3-oxo-2*H*-1,4-benzothiazine hydrogen fumarate) is a novel Ca^{2+} channel blocker. It is structurally different from other such blockers, including the 1,4-dihydropyridine derivatives and diltiazem (Miyawaki et al., 1990), and has been shown to be more vasoselective than diltiazem (Miyawaki et al., 1991; Nishimura et al., 1990). We have found that semotiadil 100 mg/kg/day inhibits the development of right ventricular hypertrophy and media thickening of pulmonary arteries significantly more effectively than diltiazem 300 mg/kg/day in monocrotaline-induced pulmonary hypertension in

* Corresponding author. Second Department of Internal Medicine, Gunma University School of Medicine, 3-39-15, Showa-machi, Maebashi, Gunma 371, Japan. Tel.: +81-272-20-7111; fax: +81-272-20-7720.

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rats. Some of the results were presented at the Joint XIIth World Congress of Cardiology and XVIth Congress of the European Society of Cardiology (Takahashi et al., 1994).

We compared the effects of semotiadil and diltiazem at given various doses on survival and right ventricular hypertrophy and media thickening of pulmonary arteries in a rat model of pulmonary hypertension.

2. Materials and methods

2.1. Preparation of animal model

Monocrotaline (lot 73H0759; Sigma, St. Louis, MO, USA) 80 mg/kg body mass was injected subcutaneously into 150 male Wistar rats weighing 100 g. Preliminary experiments indicated that 80 mg/kg was the appropriate dose with regard to survival and induction of cardiopulmonary injury, as compared with 20, 40, 60, and 100 mg/kg. Monocrotaline was dissolved in 1 M HCl at a concentration of 100 mg/ml, neutralized with 1 M NaOH, diluted with distilled water, and then injected at a concentration of 10 mg/ml.

2.2. Assessment of pulmonary hypertension

To confirm pulmonary hypertension, the following examinations were performed 4 weeks after the subcutaneous injection of monocrotaline in 10 rats.

2.2.1. Right ventricular systolic pressure

The rats were anesthetized with α-chloralose and urethane, which are known to have minimal effects on the cardiovascular system. Then right ventricular systolic pressure was measured by puncturing the apex of the heart from the peritoneal space.

2.2.2. Organ mass

The heart was removed immediately from the killed animals, weighed, and then divided into the ventricles and the atria. The free walls of right ventricle and left ventricle were separated and weighed, and the ratio of the mass of the free wall of the right ventricle to that of the left ventricle was calculated. Body mass and lung mass also were determined. The mass ratios of the heart, lungs, right ventricle, and left ventricle to the body were calculated.

2.2.3. Right ventricular wall thickness

A cut was made in the center of the longitudinal dimension of the right ventricular free wall, and the right ventricular wall thickness was measured.

2.2.4. Right ventricular myocardial fiber diameter

The cut sections of the right ventricular wall were fixed in ethanol and then stained with hematoxylin-eosin for light microscopy. We randomly selected 10 myocardial fibers from one section per rat and measured the fiber diameter at the level of the fiber nuclei in the longitudinal sections.

2.2.5. Percentage media thickness of pulmonary arteries

The left lung of each animal was fixed in ethanol, and sections were stained with hematoxylin-eosin for light microscopy. We randomly selected six peripheral small pulmonary arteries (< 50 μm in diameter) from the upper and lower lung fields of each rat and measured the thickness of the media arterial layer and the external diameter. The percentage media thickness was calculated according to Cassis et al. (1992) as $[(2 \times \text{media thickness})/\text{external diameter}] \times 100$.

2.2.6. Percentage smooth muscle cell area in pulmonary arteries

Each section of the lung was stained for α-smooth muscle actin with immunohistochemical techniques (Dako-Smooth Muscle Actin 1A4, lot 062; Dakopatts, Denmark). In the same peripheral small pulmonary arteries used for the measurement of media thickness, we measured the percentage of smooth muscle cell area in the vessels by computerized analysis. The calculation was (smooth muscle area/vascular area) $\times$ 100.

2.3. Treatment protocol

Of the original 150 rats, 19 (12.7%) died within 4 weeks after the injection of monocrotaline. The pretreatment group consisted of the 10 rats studied before the administration of Ca^{2+} channel blockers, to confirm the presence of pulmonary hypertension. After the assessment of monocrotaline-induced pulmonary hypertension, we randomly divided the living rats into Ca^{2+} channel blocker-treated and untreated groups. Semotiadil (SD-3211, lot 302; Daiichi Pharmaceutical, Tokyo, Japan) and diltiazem (lot 80H0426; Sigma, St. Louis, MO, USA) were the Ca^{2+} channel blockers. The remaining 121 rats were divided as follows: 17, 18, and 25 rats into the 10, 30, and 100 mg/kg/day semotiadil groups, respectively; 25 and 18 rats into the 100 and 300 mg/kg/day diltiazem groups; and 18 into the untreated control group. These agents were each dissolved in methanol, and the pH of the solution was adjusted to 4.2 with 1 M HCl. The drugs were administered orally in drinking water on a daily basis. Semotiadil was given in calculated doses of 10, 30, or 100 mg/kg/day and diltiazem in calculated doses of 100 or 300 mg/kg/day. The doses were based on the antihypertensive effect of semotiadil in experimental hyper-

tensive rats (Takada et al., 1991). We gave semotiadil in actual doses of 9.3 ± 1.4, 30.4 ± 2.6, or 99.5 ± 7.8 mg/kg/day, and diltiazem in doses of 100.9 ± 8.9 or 297.6 ± 11.3 mg/kg/day. Control rats were administered vehicle alone.

2.4. Assessment of drug effectiveness

We observed the survival of rats in the various groups for 3 weeks after the beginning of treatment. We then examined the same parameters that were measured pretreatment: right ventricular systolic pressure, organ mass, right ventricular wall thickness, right ventricular myocardial fiber diameter, percentage media thickness of pulmonary arteries, and percentage smooth muscle cell area in pulmonary arteries.

2.5. Statistical analysis

Data are expressed as means ± S.D. Kaplan-Meier survival curves were made for each of the groups. The probability of survival at 1, 2, and 3 weeks was estimated, and differences in survival among the groups were determined by log-rank test. The average values of each parameter of pulmonary hypertension were calculated in each group. Differences among the pretreatment and treatment groups in the various parameters were assessed by an unpaired two-tailed t-test. A P value < 0.05 was considered statistically significant.

3. Results

The Kaplan-Meier survival curves are shown in Fig. 1. All 18 untreated rats were dead within 8 days. One of 17, 1 of 18, and 11 of 25 rats in the 10, 30, and 100 mg/kg/day semotiadil groups survived, as did 5 of 25 and 1 of 18 in the 100 and 300 mg/kg/day diltiazem groups, respectively. All treatment groups had significantly better survival rates than the untreated group. The group that received semotiadil 100 mg/kg/day had significantly better survival rates than the groups treated with diltiazem 100 mg/kg/day ($P < 0.05$) or 300 mg/kg/day ($P < 0.01$). The survival rates in the semotiadil 100 mg/kg/day group were 58, 51, and 44% at 1, 2, and 3 weeks, respectively.

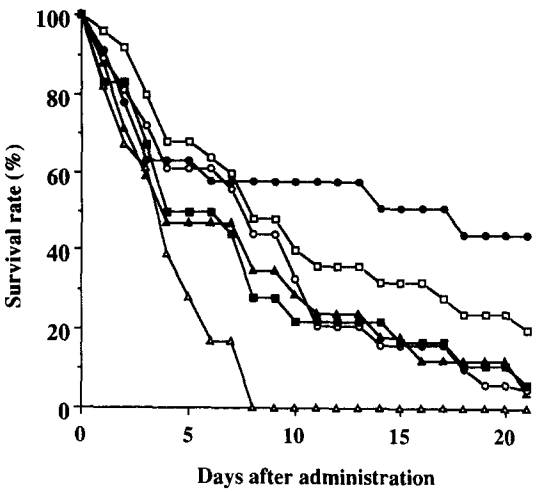

Fig. 1. Survival curves of rats that were untreated (open triangles) or treated with semotiadil at 10 (solid triangles), 30 (open circles), or 100 (solid circles) mg/kg/day or diltiazem at 100 (open squares) or 300 (solid squares) mg/kg/day for 3 weeks.

The weights of the entire body, heart, both lungs, right ventricle, left ventricle, and the mass ratios of the organs to the body in the pretreatment and various treatment groups are shown in Table 1. The right ventricle was significantly ($P < 0.01$) smaller in the groups treated with semotiadil and diltiazem 100 mg/kg/day than in the pretreatment group. Body mass and the left ventricle were significantly larger in the

Table 1
Weight of the body, heart, lungs, right ventricle, and left ventricle in the pretreatment and various treatment groups

Groups		Body (g)	Heart (mg)	Lungs (mg)	Right ventricle (mg)	Left ventricle (mg)
Pretreatment	(n = 10)	208 ± 36	1123 ± 212	2428 ± 670	273 ± 52	403 ± 76
			(5.4 ± 0.9)	(12.3 ± 5.6)	(1.3 ± 0.2)	(1.9 ± 0.3)
Treatment	(mg/kg/day)					
Semotiadil	10 (n = 1)	226	1326	2391	231	438
			(5.9)	(10.6)	(1.0)	(1.9)
	30 (n = 1)	241	1289	2302	209	465
			(5.3)	(9.6)	(0.9)	(1.9)
	100 (n = 11)	324 ± 48 [b]	1236 ± 291	2180 ± 806	183 ± 54 [b]	556 ± 105 [b]
			(3.9 ± 1.0 [b])	(6.9 ± 3.1 [a])	(0.6 ± 0.2 [b])	(1.7 ± 0.3)
Diltiazem	100 (n = 5)	339 ± 36 [b]	1182 ± 149	2100 ± 529	160 ± 46 [b]	506 ± 55 [a]
			(3.5 ± 0.4 [b])	(6.2 ± 1.5 [a])	(0.5 ± 0.2 [b])	(1.5 ± 0.2)
	300 (n = 1)	368	980	2020	130	530
			(2.7)	(5.5)	(0.4)	(1.4)

Values are means ± S.D. The mass ratios of the heart, lungs, right ventricle, and left ventricle to the body are indicated in parentheses.
[a] $P < 0.05$, [b] $P < 0.01$ vs. pretreatment group.

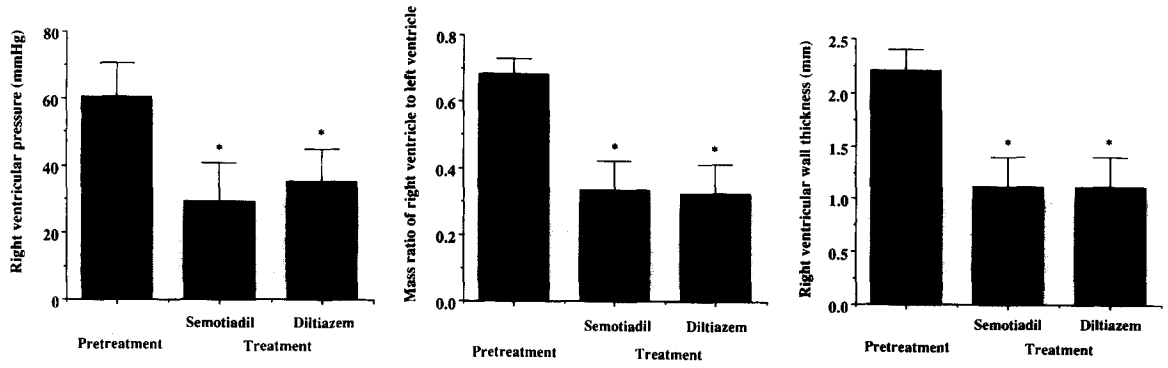

Fig. 2. Systolic pressure of the right ventricle, ratio of the mass of the free wall of the right ventricle to that of the left ventricle, and wall thickness of the right ventricle in rats treated with semotiadil at 100 mg/kg/day or diltiazem at 100 mg/kg/day. Values are means ± S.D. $^{*}P < 0.01$ vs. pretreatment group.

groups treated with semotiadil and diltiazem 100 mg/kg/day than in the pretreatment group. The mass ratios of the heart, lungs, and right ventricle to the body were significantly lower in the groups treated with semotiadil and diltiazem 100 mg/kg/day than in the pretreatment group.

The systolic pressure of the right ventricle in the pretreatment and treatment groups is shown in Fig. 2. The right ventricular pressure in the rats that received semotiadil 10 and 30 mg/kg/day, and diltiazem 300 mg/kg/day was 52, 45, and 30 mm Hg, respectively. The right ventricular pressure was significantly ($P <$ 0.01) lower in the groups treated with semotiadil and diltiazem 100 mg/kg/day than in the pretreatment group.

The ratio of the mass of the free wall of the right ventricular to that of the left ventricle in the pretreatment and treatment groups is shown in Fig. 2. The mass ratio of the right ventricle to the left ventricle in the rats that received semotiadil 10 and 30 mg/kg/day, and diltiazem 300 mg/kg/day was 0.53, 0.45, and 0.25, respectively. The mass ratio was significantly ($P < 0.01$) smaller in the groups treated with semotiadil and diltiazem 100 mg/kg/day than in the pretreatment group.

The wall thickness of the right ventricle in the pretreatment and treatment groups is shown in Fig. 2. The wall thickness of the right ventricle in the rats that received semotiadil 10 and 30 mg/kg/day, and diltiazem 300 mg/kg/day was 1.6, 1.4, and 1.0 mm, respectively. The right ventricular wall thickness was significantly ($P < 0.01$) smaller in the groups treated with semotiadil and diltiazem 100 mg/kg/day than in the pretreatment group.

The myocardial fiber diameter in the right ventricle in the pretreatment and treatment groups is shown in Fig. 3. The fiber diameter in the right ventricle in the rats that received semotiadil 10 and 30 mg/kg/day, and diltiazem 300 mg/kg/day was 18.3 ± 1.5, 16.9 ± 0.9, and 12.9 ± 0.8 μm, respectively. The right ventricular myocardial fiber diameter was significantly ($P <$ 0.01) smaller in the groups treated with semotiadil and diltiazem 100 mg/kg/day than in the pretreatment group.

The percentage media thickness of pulmonary arteries in the pretreatment and treatment groups is shown in Fig. 3. The percentage media thickness in the rats that received semotiadil 10 and 30 mg/kg/day, and diltiazem 300 mg/kg/day was 58.0 ± 4.2, 52.7 ± 5.9,

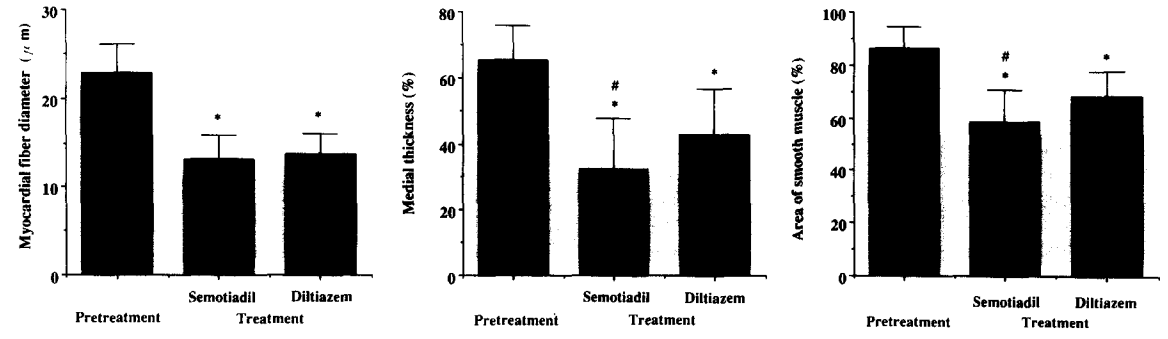

Fig. 3. Myocardial fiber diameter in the right ventricle, percentage media thickness of pulmonary arteries, and percentage smooth muscle cell area in pulmonary arteries in rats treated with semotiadil at 100 mg/kg/day or diltiazem at 100 mg/kg/day. Values are means ± S.D. $^{*}P < 0.01$ vs. pretreatment group. $^{\#}P < 0.05$ vs. diltiazem group.

and 42.0 ± 4.8%, respectively. The percentage media thickness was significantly ($P < 0.01$) less in the groups treated with semotiadil and diltiazem 100 mg/kg/day than in the pretreatment group. The percentage media thickness in the group that received semotiadil 100 mg/kg/day was significantly ($P < 0.05$) less than that in the group treated with diltiazem 100 mg/kg/day.

The percentage smooth muscle cell area in pulmonary arteries in the pretreatment and treatment groups is shown in Fig. 3. The percentage smooth muscle in the rats that received semotiadil 10 and 30 mg/kg/day, and diltiazem 300 mg/kg/day was 80.6 ± 3.5, 77.1 ± 5.8, and 65.3 ± 4.9%, respectively. The percentage smooth muscle was significantly ($P < 0.01$) less in the groups treated with semotiadil and diltiazem 100 mg/kg/day than in the pretreatment group. The percentage smooth muscle area in the group that received semotiadil 100 mg/kg/day was significantly ($P < 0.05$) less than that in the group treated with diltiazem 100 mg/kg/day.

4. Discussion

Monocrotaline induces pulmonary vascular changes, including endothelial cell damage, pulmonary vascular hyperreactivity, subsequent extension of smooth muscle into normally nonmuscularized pulmonary arterioles, and proliferation of smooth muscle in normally muscularized vessels (Meyrick and Reid, 1979). These events produce an increase in pulmonary vascular resistance and right ventricular hypertrophy (Meyrick and Reid, 1979). Monocrotaline-induced pulmonary hypertension therefore provides an appropriate model for studying the treatment of this disorder (Farhat et al., 1993). Our results with this model in rats indicated that semotiadil has potential efficacy for the management of pulmonary hypertension.

The survival of rats in the group that received semotiadil 100 mg/kg/day was significantly longer than that in the groups treated with diltiazem 100 or 300 mg/kg/day. The percentage media thickness and the percentage smooth muscle area in pulmonary arteries were significantly smaller in rats that received semotiadil 100 mg/kg/day than in animals treated with diltiazem 100 mg/kg/day. The mass ratio of right ventricle to left ventricle, right ventricular wall thickness, and right ventricular myocardial fiber diameter in the group treated with semotiadil 100 mg/kg/day were equivalent to those in the group that received diltiazem 100 mg/kg/day.

Nifedipine reduced right ventricular hypertrophy and media thickening of pulmonary arteries when used to reverse established hypoxic pulmonary hypertension in rats (Stanbrook et al., 1984). Thus, it could be predicted that semotiadil, another Ca^{2+} channel blocker, might also reverse right ventricular hypertrophy and media thickening of pulmonary arteries in rats with monocrotaline-induced pulmonary hypertension.

Ca^{2+} channel blockers have been classified into two pharmacologic groups with regard to tissue specificity (vascular smooth muscle versus myocardium). Dihydropyridines (nifedipine and nicardipine) are predominantly vasoselective, whereas nondihydropyridines (diltiazem and verapamil) are predominantly cardioselective (Singh et al., 1985; Taira, 1987). Semotiadil has been shown to be more vasoselective than diltiazem and more cardioselective than nifedipine (Miyawaki et al., 1991; Nishimura et al., 1990). The coronary vasodilator potency of semotiadil was 16 times that of diltiazem but less than that of nicardipine in Langendorff perfused rabbit hearts (Miyawaki et al., 1991). The antihypertensive effect of semotiadil was 5–7 times that of diltiazem but 2–3 times less than that of nicardipine in hypertensive and normotensive rats (Takada et al., 1991). Thus, semotiadil is intermediate between diltiazem and nicardipine in tissue selectivity.

The antihypertensive potency of semotiadil lasts for at least 9 h, while that of the same dose of diltiazem persists for only 3 h in dogs with renal hypertension (Kageyama et al., 1991). The inhibition of Ca^{2+}-induced contractions in depolarized rabbit aortic strips by diltiazem, but not that by semotiadil, is reversed by washout of Ca^{2+} channel blocking agents (Nishimura et al., 1990). It has recently been reported as the mechanism of drug action that the long-lasting inhibition of Ca^{2+} current after removal of semotiadil in dispersed smooth muscle cells of the rabbit portal vein may be due to tight binding of semotiadil on the channel through a hydrophobic site (Teramoto, 1993). We believe that both the dominant vasoselectivity and the prolonged duration of semotiadil action may be responsible for its beneficial effects on survival and the regression of right ventricular hypertrophy and media thickening in comparison with those of diltiazem.

Regarding the regression of right ventricular hypertrophy and media thickening of pulmonary arteries, we did not evaluate drug effectiveness in the same rats. However, the rats in both the treated and untreated groups had pulmonary hypertension 4 weeks after the injection of monocrotaline. Another limitation of our study was that the observation period was short, only 3 weeks. However, we consider that the pulmonary hypertension induced by monocrotaline in this study was severe, because all rats in the untreated group died within 8 days after the start of the study. In addition, it was unclear whether 100 mg/kg/day was an optimal dose for semotiadil in terms of improvement in survival and the regression of right ventricular hypertrophy and media thickening. We should examine whether smaller doses of semotiadil are similarly effective. Adverse

effects of semotiadil at these doses need to be determined.

Ca^{2+} channel blockers appear to reverse left ventricular hypertrophy through an antihypertensive effect. The mechanism includes a decrease in total peripheral resistance and inhibition of Ca^{2+} metabolism, with absence of a stimulatory action on the sympathetic nervous system (Senda et al., 1990). These drugs may be applied therapeutically to reverse right ventricular hypertrophy induced by pulmonary hypertension. We should, however, examine other effects of semotiadil that are related to the mechanism of survival prolongation and regression of right ventricular hypertrophy and media thickening. Monocrotaline-induced pulmonary hypertension may be associated with perivascular inflammation and platelet activation (Wilson et al., 1992), so the effect of semotiadil on the inhibition of platelet aggregation needs to be studied.

We conclude that the oral administration of semotiadil at a dose of 100 mg/kg/day improved the survival of rats, which responded with a significant regression of right ventricular hypertrophy and media thickening of pulmonary arteries in comparison with rats treated with diltiazem at the dose of 100 or 300 mg/kg/day. Results suggest that a high oral dose of semotiadil may prove efficacious in treating patients with pulmonary hypertension and right ventricular hypertrophy.

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References

Cassis, L.A., P.E. Rippetoe, E.E. Soltis, D.J. Painter, R. Fitz and M.N. Gillespie, 1992, Angiotensin II and monocrotaline-induced pulmonary hypertension: effect of losartan (Dup 753), a nonpeptide angiotensin type 1 receptor antagonist, J. Pharmacol. Exp. Ther. 262, 1168.

Dresdale, D.T., M. Schultz and R.J. Michtom, 1951, Primary pulmonary hypertension. I. Clinical and hemodynamic study, Am. J. Med. 11, 686.

Farhat, M.Y., M.F. Chen, T. Bhatti, A. Iqbal, S. Cathapermal and P.W. Ramwell, 1993, Protection by oestradiol against the development of cardiovascular changes associated with monocrotaline pulmonary hypertension in rats, Br. J. Pharmacol. 110, 719.

Grossman, W. and E. Braunwald, 1992, Pulmonary hypertension, in: Heart Disease, ed. E. Braunwald (W.B. Saunders, Philadelphia, PA) p. 790.

Kageyama, M., K. Nishimura, T. Takada, N. Miyawaki and H. Yamauchi, 1991, SD-3211, a novel benzothiazine calcium antagonist, alone and in combination with a beta-adrenoceptor antagonist, produces antihypertensive effects without affecting heart rate and atrioventricular conduction in conscious renal hypertensive dogs, J. Cardiovasc. Pharmacol. 17, 102.

Kambara, H., K. Fujimoto, A. Wakabayashi and C. Kawai, 1981, Primary pulmonary hypertension: beneficial therapy with diltiazem, Am. Heart J. 101, 230.

Meyrick, B. and L. Reid, 1979, Development of pulmonary arterial changes in rats fed *Crotalaria spectabilis*, Am. J. Pathol. 94, 37.

Miyawaki, N., T. Furuta, T. Shigei, H. Yamauchi and T. Iso, 1990, Electrophysiological properties of SD-3211, a novel putative Ca^{2+} antagonist, in isolated guinea pig and rabbit hearts, J. Cardiovasc. Pharmacol. 16, 769.

Miyawaki, N., T. Furuta, T. Shigei, H. Yamauchi and T. Iso, 1991, Cardiovascular characterization of SD-3211, a novel benzothiazine calcium channel blocker, in isolated rabbit hearts, Life Sci. 48, 1903.

Nishimura, K., N. Miyawaki, H. Yamauchi and T. Iso, 1990, Tissue selectivity of the novel calcium antagonist sesamodil fumarate in isolated smooth muscles and cardiac muscles, Arzneim.-Forsch. 40, 244.

Rich, S., E. Kaufmann and P.S. Levy, 1992, The effect of high doses of calcium-channel blockers on survival in primary pulmonary hypertension, New Engl. J. Med. 327, 76.

Senda, Y., H. Tohkai, M. Kimura, Y. Shida, S. Tsutsumi, H. Koshiyama, T. Tanaka, Y. Shimaji and T. Nogita, 1990, ECG-gated cardiac scan and echocardiographic assessments of left ventricular hypertrophy: reversal by 6-month treatment with diltiazem, J. Cardiovasc. Pharmacol. 16, 298.

Singh, B.N., S. Baky and K. Nademanee, 1985, Second-generation calcium antagonists: search for greater selectivity and versatility, Am. J. Cardiol. 55, 214B.

Stanbrook, H.S., K.G. Morris and I.F. McMurtry, 1984, Prevention and reversal of hypoxic pulmonary hypertension by calcium antagonists, Am. Rev. Respir. Dis. 130, 81.

Taira, N., 1987, Differences in cardiovascular profile among calcium antagonists, Am. J. Cardiol. 59, 24B.

Takada, T., N. Miyawaki, M. Kageyama, K. Matsuno, N. Ishida, H. Yamauchi and T. Iso, 1991, Antihypertensive effect of a novel calcium antagonist, SD-3211, in experimental hypertensive rats, J. Cardiovasc. Pharmacol. 18, 855.

Takahashi, T., T. Kanda, S. Imai, T. Suzuki and K. Murata, 1994, The beneficial effect of semotiadil on the development of right ventricular hypertrophy and medial proliferation in pulmonary arteries induced by monocrotaline in rats: comparison with diltiazem, Eur. Heart J. 15 (Abstr. Suppl.), 523 (abstract 2870).

Teramoto, N., 1993, Mechanisms of the inhibitory action of semotiadil fumarate, a novel Ca antagonist, on the voltage-dependent Ca current in smooth muscle cells of the rabbit portal vein, Jpn. J. Pharmacol. 61, 183.

Wilson, D.W., H.J. Segall, L.C. Pan, M.W. Lame, J.E. Estep and D. Morin, 1992, Mechanisms and pathology of monocrotaline pulmonary toxicity, Crit. Rev. Toxicol. 22, 307.

Young, T.E., B.S. Lundquist, E. Chesler and E.K. Weir, 1983, Comparative effects of nifedipine, verapamil, and diltiazem on experimental pulmonary hypertension, Am. J. Cardiol. 51, 195.