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DRUG THERAPY OF ACUTE PULMONARY INSUFFICIENCY

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The oral or parenteral administration of eriodictyol prevented the appearance of signs of acute pulmonary insufficiency provoked by the following procedures: inhalation of 25% carbon dioxide in oxygen by mice, intraperitoneal injection of paraquat in rats, intravenous injection of endotoxin in rats and intravenous infusion of iodoacetamide in monkeys. In the last-mentioned procedure, there was a reduction in pulmonary compliance,

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respiratory minute volume and arterial oxygen tension which was prevented by prior administration of eriodictyol. In rats, the decrease in pulmonary compliance and respiratory minute volume produced by either paraquat or endotoxin was prevented by the prior administration of eriodictyol. The postmorten examination of the three animal species indicated an elevated moisture and hemoglobin content and a decrease in phospholipid concentration in the lung. The intensity of these changes was reduced in the animals that were pretreated with eriodictyol.

Benzoylcarbinolmorpholineacetate hydrochloride (Mobecarbe) is effective in preventing pulmonary lesions induced by inhalation of carbon dioxide in mice. This compound prevented as well as reversed the pulmonary lesions provoked by endotoxin in rats, and by hemorrhagic shock and dextran infusion in monkeys. The functional and chemical changes in the lung are similar to those occurring in patients with acute pulmonary insufficiency. Benzoylcarbinolmorpholineacetate hydrochloride is the first compound that is capable of reversing or correcting signs of acute respiratory insufficiency in 3 animal species.

DRUG THERAPY OF ACUTE PULMONARY INSUFFICIENCY

a. Summary of work actually performed

This three-year contract was awarded with the primary purpose of developing drugs for the treatment of acute pulmonary insufficiency. The experiments consisted of testing chemical compounds on various animal models. Two compounds, eriodictyol and mobecarbe, have been demonstrated to prevent or reverse experimentally induced acute pulmonary insufficiency in animals.

b. Summary of data with examples and methods used to obtain data

The data essentially consist of measurements of pulmonary compliance, pulmonary hemorrhage and pulmonary edema in mice, rats, dogs and monkeys. These parameters are summarized in Tables in the appended manuscripts.

c. Results and conclusions relating to mobecarbe

Benzoylcarbinolmorpholineacetate hydrochloride (mobecarbe) is effective in preventing pulmonary lesions induced by inhalation of carbon dioxide in mice. This compound prevented as well as reversed the pulmonary lesions provoked by endotoxin in rats, and by hemorrhagic shock and dextran infusion in monkeys. The functional and chemical changes in the lung are similar to those occurring in patients with acute pulmonary insufficiency. Benzoylcarbinolmorpholineacetate hydrochloride is the first compound that is capable of reversing or correcting signs of acute respiratory insufficiency in three animal species.

d. Results and conclusions relating to eriodictyol

The oral or parenteral administration of eriodictyol prevented the appearance of signs of acute pulmonary insufficiency provoked by the following procedures: inhalation of 25% carbon dioxide in oxygen by mice, intraperitoneal injection of paraquat in rats, intravenous injection of endotoxin in rats and intravenous infusion of iodoacetamide in monkeys. In the last-mentioned procedure, there was a reduction in pulmonary compliance, respiratory minute volume and arterial oxygen tension which was prevented by prior administration of eriodictyol. In rats, the decrease in pulmonary compliance and respiratory minute volume produced by either paraquat or endotoxin was prevented by the prior administration of eriodictyol. The postmortem examination of the three animal species indicated an elevated moisture and hemoglobin content and a decrease in phospholipid concentration in the lung. The intensity of these changes was reduced in animals that were pretreated with eriodictyol.

e. Final conclusion

Two drugs have been developed to treat experimental acute pulmonary insufficency in animals. A request to fund clinical studies in human subjects has been made. Investigational New Drug (IND) applications have been filed with and approved by the Food and Drug Administration.

f. Manuscript relating to mobecarbe

(See following)

TREATMENT OF EXPERIMENTAL ACUTE RESPIRATORY INSUFFICIENCY BY BENZOYLCARBINOLMORPHOLINEACETATE HYDROCHLORIDE (MOBECARBE)[1]

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[1] This investigation was supported in part by the Shock and Trauma Unit, Medical Research and Development Command of the Department of the Army, under Contract No. DADA-17-71-C-1060.

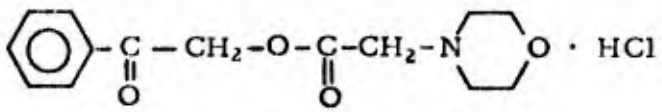
SUMMARY

Benzoylcarbinolmorpholineacetate hydrochloride (Mobecarbe) is effective in preventing pulmonary lesions induced by inhalation of carbon dioxide in mice. This compound prevented as well as reversed the pulmonary lesions provoked by endotoxin in rats, and by hemorrhagic shock and dextran infusion in monkeys. The functional and chemical changes in the lung are similar to those occurring in patients with acute pulmonary insufficiency. Benzoylcarbinolmorpholineacetate hydrochloride is the first compound that is capable of reversing or correcting signs of acute respiratory insufficiency in 3 animal species.

INTRODUCTION

In the course of testing compounds for treatment of chloroquine-resistant Plasmodium berghei infection in mice, it became apparent that some of them were effective in preventing injury to the lung induced by carbon dioxide (Aviado, 1969). The naphthoquinones were the first group identified as possessing both antimalarial and anti-edema effects in rodents (Aviado and Cambar, 1969). The search was then extended to include other forms of pulmonary lesions such as those induced by paraquat in the rat and alloxan or alphanaphthylurea in the dog. The most active compound was eriodictyol, which prevented the appearance of pulmonary lesions (Aviado and Bacalzo, in press). Eriodictyol, however, was only a prophylactic agent and was ineffective when administered subsequent to the appearance of abnormalities in lung function.

This report describes the discovery that benzoylcarbinolmorpholineacetate hydrochloride (BCMA), a drug sold by Carlo Erba in Italy for treatment of systemic capillary disease, is also effective in the treatment of pulmonary lesions induced in animals. Unlike eriodictyol, BCMA is of value not only prophylacticly but as a therapeutic agent as well. Its chemical structure is as follows:



Benxoylcarbinolmorpholineacetate hydrochloride

The experimental lesions in the lung of animals influenced by BCMA are similar to those in the human lung in acute pulmonary insufficiency. This syndrome is difficult to treat. The problems in managing separately shock, pulmonary embolism, pulmonary edema, pulmonary congestion, atelectasis and hypoxia are combined in this syndrome. Therapy of acute respiratory insufficiency consists of

administering one or more of the following: vasopressors, bronchodilators, corticosteroids, diuretics and oxygen (Bacalzo and Aviado, in press). Each type of drug intended to correct one type of pulmonary lesion would exaggerate the others so that the outcome of the use of drugs has been unsatisfactory. That a single drug, such as BCMA, would reverse all pulmonary lesions would be an outstanding contribution in therapy of this syndrome.

METHODS

Acute pulmonary insufficiency in mice. A total of 91 male mice (Swiss strain) were used in this investigation. The method of Aviado and Cambar (1969) -- exposing mice in a glass chamber filled with 25% carbon dioxide in oxygen -- was used. After 5 minutes' exposure, the mice were removed and killed immediately by an intraperitoneal injection of 0.1 ml of 25% sodium cyanide solution. The body was weighed, and the lungs were removed and weighed, dried in an oven (180° C) for 24 hr, and then reweighed. The moisture content of the lung was calculated as follows: wet weight minus dry weight ÷ wet weight x 100. The following groups of 3 or 5 mice each were examined: control (not exposed to carbon dioxide); exposed to 25% carbon dioxide; and pretreated 30 min prior to carbon dioxide exposure by intraperitoneal injection of one of the following. BCMA, prednisolone, hydrocortisone, sodium salicylate or indomethacin.

Acute pulmonary insufficiency in rats. A total of 21 male rats (Wistar strain) were used in this investigation. Two procedures were utilized to induce pulmonary insufficiency. In the first, 5 rats, used as controls, were anesthetized with an intraperitoneal injection of a mixture of urethane and allobarbital, 200 and 50 mg/kg respectively, and then sacrificed for lung examination. In the 3 remaining groups of 5 rats each, the animals were similarly anesthetized and measurements of pulmonary function were recorded. An intravenous injection of endotoxin (Lipopolysaccharide B of Escherichia coli) in a dose of 1 mg/kg was administered. After measurement of pulmonary function and aortic blood pressure, the rats were sacrificed and the lungs analyzed for moisture and phospholipid content. One group of rats received an intravenous injection of 25 mg/kg of BCMA 30 min prior

to the injection of endotoxin whereas the other group of rats received BCMA after endotoxin injection.

Acute pulmonary insufficiency in monkeys. A total of 33 male monkeys (Macaca mulatta) were used. They were anesthetized by intravenous injection of 30 mg/kg of sodium pentobarbital. In addition to measurement of pulmonary function, the aortic blood pressure was measured via a femoral catheter, and blood was collected from a second femoral arterial catheter for analysis of pH, oxygen tension and carbon dioxide tension with a Radiometer apparatus.

One form of pulmonary insufficiency was elicited by an infusion of iodoacetamide at a rate of 10 mg/kg/min until the monkey died. The lungs were removed, and a 5 g sample was used for determination of moisture content, a 1 g sample for analysis of phospholipid content, and the rest for measurement of static compliance. This consisted of suspending the lung in a glass chamber and exerting negative pressure for inflating the lung. The changes in pressure and volume of the lung were used to estimate compliance. Some of the monkeys were pretreated with 10 mg/kg BCMA administered intraperitoneally, daily for 3 days prior to infusion of iodoacetamide.

A second form of pulmonary insufficiency was provoked by bleeding the animal, maintaining the mean aortic blood pressure at 30 mm Hg for 30 min, and injecting low molecular weight dextran (40, 000). The infusion rate was 40 ml/kg/min for 30 or 60 min. BCMA was injected after 30 min of infusion, and the monkey was sacrificed 30 min later.

Measurement of pulmonary function in rats and monkeys. The trachea of the anesthetized rat was exposed and cannulated with a polyethylene catheter. The nitrogen-dilution technique, consisting of allowing the rat to rebreathe from a chamber filled with pure oxygen and 10% potassium hydroxide to absorb carbon dioxide, was used to measure functional residual capacity. After 7 min of rebreathing, the equilibrated gas mixture was analyzed in a Scholander gas analyzer and the functional residual capacity calculated from a calibration curve derived by equilibrating known volumes of air with the apparatus. The same apparatus was also used to measure the total oxygen consumed by the rat. An intrapleural catheter was next inserted into the 6th intercostal space in such a way that its outside end was attached to a differential pressure transducer, the other side of which connected to the trachea. Air flow was measured by a pneumotachometer attached to a plastic plethysmograph which contained the rat. The signals of the transpulmonary pressure and air flow, displayed on an oscilloscope screen, recorded pulmonary resistance, while those of transpulmonary pressure and tidal volume were used, following the method of Aviado and Palacek (1967), to estimate pulmonary compliance.

The same general technique was used in the anesthetized monkey. Transpulmonary pressure was measured by an intrapleural catheter inserted via the 5th intercostal space and another catheter attached to the trachea. A pneumotachometer was attached to the tracheal cannula, and the velocity of air flow was integrated to derive the tidal volume.

Chemical analysis of the lung. An aliquot portion of the lung weighing approximately 1 g was minced and homogenized with chloroform-methanol (2:1) mixture, with the total volume adjusted to 20 ml. The lipids were extracted by filtering the homogenate, adding 4 ml of 0.05% calcium chloride solution, separating the lower phase, and then diluting that lower phase to a volume of 20 ml by the addition of chloroform-methanol mixture. An amount of 25 µl of the extract was digested by heating at 180° C for 30 min, following the addition of 0.6 ml of 70% perchloric acid. Color was developed by adding 3 ml of distilled water, 0.5 ml of 2.5% ammonium molybdate, and 1 ml of 10% ascorbic solution, and the resultant mixture was heated for 5 min in boiling water. Optical density was read at 797 mµ by spectrophotometer, and total phospholipids were calculated by multiplying the phosphorus value by 25.

RESULTS

As no drug has been previously reported to be effective in treating acute pulmonary insufficiency, there was no standard drug to which the action of BCMA could be compared. Our approach has been to experimentally provoke pulmonary insufficiency in four ways and determine the influence of BCMA in each modality.

A. Acute Respiratory Insufficiency in Mice.

The inhalation of 25% carbon dioxide elicited signs of pulmonary insufficiency. Both moisture content and lung weight expressed as a percent of body weight were elevated. This increase in both parameters was confirmed by the gross appearance of congestion and edema of the lung similar to that reported previously in mice that had either been infected with Plasmodium berghei or received an injection of lethal amounts of epinephrine (Aviado and Cambar, 1969).

The influence of pretreatment with BCMA is summarized in Table 1. While the intraperitoneal injection of 0.1 mg/kg did not prevent carbon dioxide-induced pulmonary insufficiency, injection of 0.5, 1.0, 5, 10, 25 or 50 mg/kg did significantly lower the moisture content of the lung compared with that of nontreated mice which inhaled carbon dioxide. Yet, though BCMA prevented carbon dioxide-induced pulmonary congestion and edema, the continued elevation in lung weight of mice treated with BCMA indicates that this compound did not prevent all signs of pulmonary insufficiency resulting from inhalation of lethal concentrations of carbon dioxide.

Pretreatment with anti-inflammatory agents. Table 2 summarizes the influence of selected anti-inflammatory agents on carbon dioxide-induced pulmonary

insufficiency. Prednisolone and hydrocortisone prevented the increase in moisture content of the lung; sodium salicylate and indomethacin did not. The significance of these observations is discussed below.

B. Acute Pulmonary Insufficiency in Rats.

The rat was used because of the availability of techniques for measuring the mechanical properties of the lung and of a procedure -- intravenous injection of endotoxin -- known to cause acute pulmonary insufficiency experimentally. Four groups of rats were tested: (1) control; (2) intravenous injection of 1 mg/kg endotoxin; (3) intravenous injection of 25 mg/kg BCMA 30 min prior to injection of endotoxin; and (4) endotoxin followed 30 min later with an injection of BCMA.

Endotoxin only. The results summarized in Tables 3 and 4 indicate that the intravenous injection of endotoxin caused an increase in pulmonary resistance and decreases in pulmonary compliance, respiratory minute volume, blood pressure, and heart rate. These measurements, taken 15 min after injection, were statistically significant when compared with the preinjection data. Thirty minutes later, the rats were sacrificed, and the lungs were found to contain more moisture but less phospholipids than were characteristic of the nontreated controls. The postmortem and antemortem examinations indicated that endotoxin caused the typical signs of acute pulmonary insufficiency.

Pretreatment with BCMA. The group of rats that received BCMA prior to injection with endotoxin did not develop all the signs of acute pulmonary insufficiency. There was no increase in pulmonary resistance, and no decreases in pulmonary compliance, respiratory minute volume, blood pressure and/or heart rate. The

last-mentioned parameter instead showed tachycardia. Postmortem examination of the lung showed no elevation in moisture content (wet lung weight to dry lung weight ratio, and wet lung to body weight ratio). Phospholipid content was reduced, and its level (18.5 mg) was intermediate between the levels for endotoxin only treatment (16.9 mg) and controls (20.3 mg).

Endotoxin followed by BCMA. In the last group of rats, acute respiratory insufficiency was induced by endotoxin and confirmed by the post-endotoxin measurements. The subsequent injection of BCMA caused a reversal of 3 signs: pulmonary resistance, pulmonary compliance and respiratory minute volume. While hypotension and bradycardia persisted after the injection of BCMA, both were less intense than they were prior to BCMA administration (Tables 3 and 4). After sacrificing the animals, the lungs were examined. No signs of fluid accumulation were noted, but the level of phospholipid content was still low (15.1 mg).

C. Acute Pulmonary Insufficiency in Monkeys using Iodoacetamide.

The substances known to produce pulmonary insufficiency in the dog were tested in the monkey. Alloxan and alphanaphthylthiourea failed to produce lesions. Iodoacetamide provoked signs of acute pulmonary insufficiency. The monkeys were divided into 5 groups: (1) control; (2) intravenous injection of 25 mg/kg BCMA; (3) infusion of 10 mg/kg/min of iodoacetamide; (4) pretreatment with 10 mg/kg BCMA intraperitoneally for 3 days followed by iodoacetamide infusion; and (5) reversal of the sequence, i. e., iodoacetamide followed 15 min later with an intravenous injection of 25 mg/kg BCMA. The results for each group are summarized in Tables 5 and 6.

Iodoacetamide infusion. The first group of 3 monkeys were used as controls; ante- and postmortem data were collected. The second group of 3 monkeys received a continuous infusion of 10 mg/kg/min of iodoacetamide. The lethal doses were as follows: 410, 420, and 440 mg/kg. Prior to death, iodoacetamide caused a significant reduction in pulmonary compliance, a fall in mean aortic blood pressure, bradycardia, reduction in blood pH, and a decrease in oxygen tension of the arterial blood. An increase in pulmonary resistance and a decrease in respiratory minute volume also occurred, but neither was statistically significant. Postmortem examination of the lung revealed a higher moisture content and lower phospholipid content as compared with the lungs from control monkeys. The static compliance of the lung was also different in the monkeys that succumbed after the infusion of iodoacetamide. The critical inflating pressure of the lungs for iodoacetamide-infused monkeys was higher than in the normal lung.

Pretreatment with BCMA. The group of monkeys that was pretreated with BCMA for 3 days responded to iodoacetamide differently from the animals that had not been pretreated. The lethal dose of iodoacetamide was 567.0±61.7 mg/kg, which was significantly greater than the lethal dose of 410.0±17.3 mg/kg for animals without pretreatment. The following signs of pulmonary insufficiency did not appear in the monkeys given BCMA prior to indoacetamide infusion: decrease in pulmonary compliance, slowing of the heart rate, fall in arterial blood pH, increase in moisture content of the lung, and elevation of critical inflating pressure. Thus, the administration of BCMA prevented the development of acute respiratory insufficiency. The cause of death remained terminal hypotension, however.

Iodoacetamide followed by BCMA. Before considering the influence of BCMA on iodoacetamide-treated monkeys, it is pertinent to note that the intravenous injection of BCMA did not influence any of the antemortem or postmortem measurements. In the last group of monkeys, BCMA (25 mg/kg i. v.) was administered 15 min after iodoacetamide infusion was begun. The mean lethal dose of iodoacetamide was 430. 0±65. 1 mg/kg, which was not significantly different from the lethal dose for nontreated monkeys.

Nonetheless, the rise in critical inflating pressure and increase in moisture content of the lung, together with no decrease in pulmonary compliance and respiratory minute volume, suggest that although the animals still died of hypotension and bradycardia, prior injection of BCMA confers greater protection against the deleterious effects of iodoacetamide infusion than does administration of BCMA after infusion has begun.

D. Hemorrhagic Shock in Monkeys.

The fourth and last model consisted of bleeding the monkeys and causing them to sustain a hypotension of 30 mm Hg for either 30 or 60 min. The monkeys in shock for 30 min received an infusion of low molecular weight dextran for 60 min. In addition, some monkeys, after hemmorhagic shock and infusion, received an injection of 25 mg/kg BCMA, singly or in combination with hesperidin and vitamin C, which is the commercial preparation for BCMA. The results of these experiments are summarized in Tables 7 to 9.

Hemorrhagic shock. The first group of monkeys was used as controls. Repeated measurements of lung function taken 30 or 60 min apart remained unchanged. The second group of monkeys was bled and sustained shock for 60 min. At the end of this period, the animals were sacrificed and their lungs analyzed. No changes in moisture content or in pulmonary function were noted. There was a reduction in blood pH, pCO_2 and pO_2, but the acidosis was the only change that was statistically significant.

Hemorrhagic shock and dextran. The third group of monkeys sustained hypotension for 30 min and was then infused with dextran for 60 min. The end result was pulmonary insufficiency characterized by increased pulmonary resistance, decreased pulmonary compliance, increased moisture content of the lung, decreased phospholipid content, and elevated critical inflating pressure. The blood gas content and pH were unchanged.

Hemorrhagic shock, dextran and BCMA. The fourth group of monkeys were bled and infused with dextran as the third group had been, but differed from that group in that they received additionally a subsequent injection of BCMA. Post-infusion measurements showed a reversal of most of the signs of pulmonary insufficiency. There was no decrease in pulmonary compliance, no increase in moisture content, no decrease in phospholipid content and no increase in critical inflating pressure. These observations indicate that BCMA can correct signs of pulmonary insufficiency.

The fifth and last group of monkeys was used to evaluate the effects of BCMA as it is marketed commercially. The results indicate that the mixture also effectively reverses the signs of acute pulmonary insufficiency, i.e., the presence of hesperidin and ascorbic acid did not interfere with the efficacy of BCMA.

DISCUSSION

The claim that BCMA is effective in preventing or reversing experimental pulmonary insufficiency is based on the following findings: (1) prevention of carbon dioxide-induced increase in moisture content in mice; (2) prevention and/or reversal of endotoxin-induced increase in moisture content and decrease in pulmonary compliance in rats; (3) increase in lethal dose of iodoacetamide in monkeys; and (4) prevention and/or reversal of decrease in compliance, increase in moisture content, and decrease in phospholipid content in monkeys that have been subjected to hemorrhagic shock and infusion of dextran solution. In the last group of observations, noting no increase in critical inflating pressure suggests no reduction in compliance occurred.

The pulmonary action of BCMA has not been previously reported. While this unique action is also exerted by corticosteroids, BCMA has no systemic action similar to hydrocortisone. In comparisons of BCMA with other anti-inflammatory agents, both protect systemic capillaries (Tommasini et al., 1963), but, in the lung, salicylates and indomethacin exert no protective action against carbon dioxide. It appears that the action of BCMA is independent of its reported effects on systemic circulation.

There is sufficient information to justify clinical testing of BCMA in patients suffering from acute pulmonary insufficiency. The literature on BCMA and on acute pulmonary insufficiency is enclosed as Appendix I and II, respectively.

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APPENDIX I

BENZOYLCARBINOLS

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APPENDIX F

ENDOTOXEMIA, HEMORRHAGE & RESUSCITATION

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TABLE 1

Influence of benzoylcarbinolmorpholineacetate HCl (BCMA) on carbon dioxide-induced pulmonary insufficiency in mice (Mean ±SE)

	Dose (mg/kg)	No. of Mice	Body Wt. (g)	Lung Wt. % BW	P versus Control	P versus CO_2	Lung Moisture (%)	P versus Control	P versus CO_2
Control		3	19.7 ±1.45	0.67 ±0.03			77.7 ±0.33		
Carbon Dioxide 25%		3	18.7 ±1.20	1.1 ±0.12	<0.001		82.3 ±1.20	<0.001	
BCMA	0.1	3	19.0 ±1.15	0.93 ±0.03		NS	81.0 ±0.58		NS
	0.5	3	18.7 ±0.88	1.0 ±0.03		NS	76.0 ±0.58		<0.001
	1.0	3	16.0 ±0.58	0.90 ±0.10		NS	79.7 ±0.33		<0.05
Control		5	30.8 ±1.16	0.62 ±0.02			79.4 ±0.68		
Carbon Dioxide 25%		5	21.4 ±2.16	0.94 ±0.12	<0.001		83.8 ±1.77	<0.05	
BCMA	2.5	5	21.6 ±0.81	0.94 ±0.02		NS	81.2 ±1.16		NS
	5.0	5	21.6 ±0.81	0.86 ±0.04		NS	79.0 ±0.71		<0.05
	10.0	5	29.2 ±0.37	0.64 ±0.07		<0.01	79.6 ±0.68		<0.05
Control		3	22.3 ±1.86	0.73 ±0.03			73.3 ±2.93		
Carbon Dioxide 25%		3	21.7 ±2.03	1.0 ±0.06	<0.001		81.7 ±1.67	<0.05	
BCMA	25.0	3	24.0 ±2.65	0.8 ±0.00		<0.001	78.0 ±0.00		<0.05
	50.0	3	19.3 ±1.76	1.0 ±0.00		NS	78.0 ±0.58		<0.05

TABLE 2

Influence of anti-inflammatory agents on carbon dioxide-induced pulmonary insufficiency in mice (Mean ±SE)

	Dose (mg/kg)	No. of Mice	Body Wt. (g)	Lung Wt. % BW	P versus Control	P versus CO_2	Lung Moisture (%)	P versus Control	P versus CO_2
Control		3	19.7 ±1.45	0.67 ±0.03			77.7 ±0.33		
Carbon Dioxide 25%		3	18.7 ±1.20	1.1 ±0.12	<0.001		82.3 ±1.20	<0.001	
Prednisolone	1.0	3	16.7 ±1.33	1.0 ±0.19		NS	77.7 ±0.67		<0.01
	10.0	3	16.7 ±1.45	1.0 ±0.15		NS	78.7 ±0.33		<0.01
Hydrocortisone	1.0	3	15.0 ±1.29	1.1 ±0.09		NS	78.0 ±0.41		<0.001
	5.0	3	21.3 ±3.84	0.97 ±0.12		NS	78.7 ±0.67		<0.001
	10.0	3	16.3 ±0.88	1.1 ±0.03		NS	80.7 ±0.33		NS
Sodium Salicylate	250.0	3	18.7 ±1.20	1.0 ±0.03		NS	79.7 ±0.88		NS
Control		3	22.3 ±1.86	0.73 ±0.03			73.3 ±2.91		
Carbon Dioxide 25%		3	21.7 ±2.03	1.0 ±0.06	<0.001		81.7 ±1.67	<0.05	
Indomethacin	1.0	3	24.7 ±1.76	0.93 ±0.09		NS	78.3 ±0.33		NS
	5.0	3	26.3 ±1.86	0.87 ±0.07		NS	77.3 ±1.76		NS
	10.0	3	20.7 ±2.33	1.1 ±0.06		NS	79.3 ±2.33		NS

TABLE 3

Influence of benzoylcarbinolmorpholineacetate HCl (BCMA) on endotoxin-induced pulmonary insufficiency in rats

Procedure Dose	Rat No.		Body Wt. (g)	P versus Control	Wet Lung Wt. % BW	P versus Control	WLW (g) / DLW (g)	P versus Control	Phospho-lipids	P versus Control
Controls	1		176		0.64		4.95		21.3	
	2		188		0.66		4.78			
	3		176		0.60		4.95		19.3	
	4		174		0.70		4.70		20.3	
	5		180		0.69		4.70			
		Mean	178.8		0.66		4.82		20.3	
		S. E. M.	±2.50		±0.02		±0.06		±0.58	
Endotoxin	6		174		0.74		5.12		16.2	
1 mg/kg i.v.	7		174		0.72		5.16		17.6	
	8		202		0.85		5.52			
	9		198		0.77		5.49			
	10		176		0.96		5.26		16.8	
		Mean	184.8		0.81		5.31		16.9	
		S. E. M.	±6.25	NS	±0.04	< 0.001	±0.08	< 0.001	±0.41	< 0.001
BCMA	11		184		0.66		4.83		19.4	
25 mg/kg i.v.30 min	12		200		0.89		5.22		17.2	
before endotoxin	13		196		0.65		4.87		18.8	
1 mg/kg i.v.	14		186		0.65		4.82			
	15		201		0.92		5.06			
		Mean	193.4		0.75		4.96		18.5	
		S. E. M.	±3.54	< 0.001	±0.06	NS	±0.08	NS	±0.66	< 0.05
Endotoxin	16		180		0.65		4.87		14.3	
1 mg/kg i.v.followed by	17		190		0.78		5.11		16.8	
BCMA 25 mg/kg i.v.	18		188		0.74		4.92		14.2	
	19		176		0.60		4.75			
	20		180		0.66		4.82			
		Mean	182.8		0.69		4.89		15.1	
		S. E. M.	±2.65	NS	±0.03	NS	±0.06	NS	±0.85	< 0.001

TABLE 4

Pulmonary function in rats following administration of endotoxin and benzoylcarbinolmorpholineacetate HCl (BCMA)

Procedure / Dose	Exp. No.		Pulmonary Resistance (cm H_2O/ml/sec) C	E	B	Pulmonary Compliance (ml/cm H_2O) C	E	B	Respiratory Minute Volume (ml/min) C	E	B	Blood Pressure (mm Hg) C	E	B	Heart Rate (beats/min) C	E	B
Endotoxin	6		0.27	0.31		0.30	0.17		281	195		140	20		440	320	
1 mg/kg i. v.	7		0.37	0.44		0.35	0.26		236	273		115	45		370	260	
	8		0.24	0.29		0.35	0.27		290	212		110	25		350	300	
	9		0.27	0.49		0.39	0.22		253	224		120	30		400	310	
	10		0.25	0.37		0.43	0.28		298	196		110	15		330	290	
		Mean	0.28	0.38		0.36	0.24		271.6	220.0		119.0	27.0		378.0	296.0	
		S. E. M.	±0.02	±0.04		±0.02	±0.02		±11.7	±14.3		±5.6	±5.1		±19.3	±10.3	
		% Change	+36%			-33%			-19%			-77%			-22%		
		P vs C	< 0.05			< 0.001			< 0.01			< 0.001			< 0.001		
BCMA	11		0.29	0.31		0.36	0.35		292	316		125	110		360	375	
25 mg/kg i. v.	12		0.18	0.17		0.61	0.69		316	310		110	100		320	310	
30 min before	13		0.24	0.23		0.43	0.42		304	280		130	120		400	360	
endotoxin	14		0.21	0.23		0.48	0.42		310	264		125	115		360	330	
1 mg/kg i. v.	15		0.22	0.28		0.35	0.28		245	234		130	125		380	370	
		Mean	0.23	0.24		0.45	0.43		293.4	280.8		124.0	114.0		368.0	349.0	
		S. E. M.	±0.01	±0.02		±0.05	±0.07		±12.7	±15.1		±3.7	±4.3		±13.6	±12.5	
		P vs C	NS			NS			NS			NS			NS		
Endotoxin	16		0.27	0.29	0.28	0.48	0.35	0.43	326	229	288	110	15	96	420	310	330
1 mg/kg i. v.	17		0.33	0.59	0.42	0.35	0.14	0.28	296	312	234	115	20	100	360	260	300
followed by	18		0.28	0.31	0.27	0.35	0.24	0.32	286	239	312	110	25	100	300	220	290
BCMA	19		0.20	0.28	0.20	0.43	0.35	0.42	328	264	280	125	40	98	370	300	310
25 mg/kg i. v.	20		0.22	0.26	0.22	0.42	0.38	0.42	338	286	364	130	30	110	340	290	300
		Mean	0.26	0.35	0.28	0.41	0.29	0.37	314.8	266.0	295.6	118.0	26.0	100.8	358.0	276.0	306.0
		S. E. M.	±0.02	±0.06	±0.04	±0.03	±0.05	±0.03	±10.1	±15.2	±21.3	±4.1	±4.3	±2.4	±19.6	±16.3	±6.8
		P vs C		NS		< 0.05	NS		< 0.01	NS		< 0.001	< 0.001		< 0.01	< 0.05	
		P vs E											< 0.001			NS	

TABLE 5

Blood analysis and postmortem examination of the lung in monkeys following administration of iodoacetamide and benzoylcarbinolmorphineacetate (BCMA)

Preparation	No of Monkeys		Arterial Blood Analysis									Postmortem examination of the lung			Mean lethal dose (mg/kg
			pH			pCO_2 (mm Hg)			pO_2 (mm Hg)			WLW/BW	WLW/DLW	CIP (-cm H_2O)	
			C	I	%Δ	C	I	%Δ	C	I	%Δ				
C	3	Mean	7.33	7.33	0	45	43	- 4	86	95	+10	7.10	4.67	-11.3	
		SE	±0.03	±0.04		± 1.2	± 0.9		± 2.6	± 7.4		±0.36	±0.11	± 0.33	
		p verses control		NS			NS			NS					
B 25 mg/kg i.v.	3	Mean	7.36	7.34	- 0.3	48	47	- 2	83	85	+ 2	7.37	4.80	-11.7	
		SE	±0.04	±0.03		± 4.1	± 4.4		± 3.5	± 2.4		±0.60	±0.29	± 0.67	
		p verses control		NS			NS			NS					
Iodoacetamide 10 mg/kg/min i.v.	3	Mean	7.40	7.27	- 2	37	25	-33	99	87	-12	9.84	4.85	-15.2	410
		SE	±0.03	±0.06		± 3.2	± 6.4		± 7.6	± 6.6		±0.44	±0.18	± 1.34	± 17.
		p verses control		<.05			NS			NS					
BCMA 10 mg/kg i.p. for 3 days followed by iodoacetamide 10 mg/kg/min	3	Mean	7.38	7.30	- 1	48	49	+ 3	86	76	-11	8.25	4.68	-12.0	267
		SE	±0.03	±0.05		± 2.1	±11.3		± 2.3	±12.7		±0.24	±0.02	± 0.57	± 61.
		p verses control		NS			NS			NS					<.05
Iodoacetamide infusion followed by BCMA 25 mg/kg i.v.	3	Mean	7.37	7.24	- 2	38	44	+15	87	77	-11	8.70	4.74	-14.7	430
		SE	±0.05	±0.02		± 1.5	± 8.7		± 5.5	±12.1		±0.23	±0.15	± 1.76	± 65.
		p verses control		<.05			NS			NS					NS

TABLE 6

Influence of iodoacetamide and benzoylcarbinolmorphineacetate (BCMA) on pulmonary function in monkeys

Procedure	No of Monkeys		Pulm. Resistance (cm H_2O/l/sec) C	I	%Δ	Pulm. Compliance (ml/cm H_2O) C	I	%Δ	Resp. Minute Volume (ml/min) C	I	%Δ	Mean Arterial Pressure (mm Hg) C	I	%Δ	Heart Rate (beats/min) C	I	%
Control	3	Mean	37.6	37.6	0	4.4	4.4	0	370	450	+22	126	119	- 6	157	180	+
		SE	± 5.4	± 5.4		±0.9	±0.9		± 65.3	± 76.2		± 8.3	± 16.7		± 14.6	± 17.3	
		p verses control		NS			NS			NS			NS			NS	
BCMA 10 mg/kg i.v.	3	Mean	30.5	40.8	+34	4.8	4.4	- 8	378	357	- 6	120	109	- 9	197	190	-
		SE	± 7.0	± 5.8		±0.4	±1.0		± 94.3	± 64.5		± 13.2	± 15.9		± 3.3	± 5.8	
		p verses control		NS			NS			NS			NS			NS	
Iodoacetamide 10 mg/kg/min i.v.	3	Mean	22.7	27.2	+20	9.0	7.2	-20	364	270	-26	132	28	-79	183	75	-
		SE	± 3.2	± 2.6		±1.0	±0.4		± 55.6	± 78.2		± 6.0	± 1.7		± 8.8	± 2.9	
		p verses control		NS			<.001			NS			<.001			<.001	
BCMA 10 mg/kg i.p. for 3 days followed by Iodoacetamide 10 mg/kg/min	3	Mean	21.8	26.5	+22	4.4	4.3	- 2	170	290	+71	115	48	-58	163	163	
		SE	± 0.8	± 1.9		±0.2	±0.2		± 27.8	± 74.3		± 5.8	± 18.3		± 8.8	± 12.0	
		p verses control		<0.5			NS			NS			<.001			NS	
Iodoacetamide infusion followed by BCMA 25 mg/kg i.v.	3	Mean	27.7	46.7	+69	4.9	5.0	+ 2	267	192	-28	113	28.3	-75	187	93	
		SE	± 3.7	±14.2		±0.5	±0.4		± 74.5	± 31.3		± 10.9	± 1.7		± 18.6	± 23.3	
		p verses control		NS			NS			NS			<.001			<.01	

TABLE 7

Treatment with benzoylcarbinolmorphineacetate (BCMA) of hemorrhagic shock in monkeys: pulmonary function*

Procedure	No of Monkeys		Pulmonary Resistance (cm H_2O/l/sec) C	H	D	B	Pulmonary Compliance (ml/cm H_2O) C	H	D	B	Respiratory Minute Volume (ml/min) C	H	D	B
Control	3	Mean	37.6	37.6			4.4	4.4			370	459		
		SE	± 5.4	± 5.4			±0.9	±0.9			± 65.3	± 76.2		
		%Δ		0%				0%				± 22%		
		p vs C		NS				NS				NS		
Hemorrhagic Shock	3	Mean	24.4	24.5			4.3	6.7			401	412		
		SE	± 5.8	± 5.8			±0.3	±1.6			± 76.7	± 44.4		
		%Δ		+ 0.4%				+56%				+ 3%		
		p vs C		NS				NS				NS		
Hemorrhagic Shock and Dextran Infusion	3	Mean	38.4	28.9	56.7		4.0	5.4	3.0		308	283	326	
		SE	± 2.0	± 6.1	± 6.9		±0.4	±0.8	±0.6		± 17.2	± 46.7		
		%Δ		-25%	+48%			+35%	-25%			- 8%	+ 6%	
		p vs C		NS	<.05			<.05	<.05			NS	NS	
		p vs H			<.01				<.05				NS	
Hemorrhagic Shock and Dextran Infusion and BCMA	3	Mean	24.6	18.6	28.1	24.6	5.1	6.7	3.7	5.1	183	184	189	225
		SE	± 3.7	± 2.9	± 6.4	± 3.7	±0.6	±0.6	±0.4	±0.5	± 34.5	± 20.2	± 26.1	± 67.8
		%Δ		-23%	+11%	0%		+34%	-28%	0%		+ 0.7%	+ 5%	+ 18%
		p vs C		NS	NS	NS		<.05	<.05	NS		NS	NS	NS
		p vs D				NS				<.05				NS
Hemorrhagic Shock and Dextran Infusion and BCMA mixture	3	Mean	31.4	28.4	32.2	31.1	4.7	5.2	4.4	4.8	402	313	327	281
		SE	± 3.4	± 3.3	± 5.1	± 7.9	±0.9	±0.7	±0.8	±0.9	± 75.2	±100	± 77.9	± 72.8
		%Δ		-10%	+ 3%	- 1%		+11%	- 6%	+ 2%		-22%	-19%	-30%
		p vs C		NS	NS	NS		NS	NS	NS		NS	NS	NS
		p vs H			NS	NS			NS	NS			NS	NS
		p vs D				NS				NS				NS

*C = Control
H = Hemorrhagic Shock; 60 min of sustained hypotension 30 mm Hg
D = Dextran Infusion; 40 ml/kg for 60 min
B = BCMA 25 mg/kg i.v.; 30 min after beginning of dextran

TABLE 8

Treatment with benzoylcarbinolmorphineacetate (BCMA) of hemorrhagic shock in monkeys: blood analysis*

Procedure	No of Monkeys		pH				pCO_2 (mm Hg)				pO_2 (mm Hg)			
			C	H	D	B	C	H	D	B	C	H	D	B
Control	3	Mean	7.33	7.33			45	43			86	95		
		SE	±0.03	±0.04			± 1.2	± 0.9			± 2.6	± 7.4		
		%Δ		0%				- 4%				+10%		
		p vs C		NS				NS				NS		
Hemorrhagic Shock	3	Mean	7.41	7.26			44	29			79	82		
		SE	±0.02	±0.04			± 1.7	±10.5			± 6.4	± 2.2		
		%Δ		- 2%				-33%				+ 4%		
		p vs C		<.001				NS				NS		
Hemorrhagic Shock and Dextran Infusion	3	Mean	7.39	7.32	7.29		43	39	44		95	106	94	
		SE	±0.02	±0.05	±0.07		± 2.2	± 3.2	± 5.0		± 8.2	± 5.2	± 5.0	
		%Δ		- 0.9%	- 1%			- 9%	+ 2%			+ 12%	- 1%	
		p vs C		NS	NS			NS	NS			NS	NS	
		p vs H			NS				NS				NS	
Hemorrhagic Shock and Dextran Infusion and BCMA	3	Mean	7.39	7.42	7.35	7.37	45	39	50	47	91	105	102	99
		SE	±0.02	±0.02	±0.00	±0.03	± 3.1	± 7.3	± 6.6	± 5.2	± 6.2	± 5.5	± 7.7	± 7.1
		%Δ		+ 0.4%	- 0.6%	- 0.2%		-15%	+11%	+ 4%		+ 16%	+11%	+ 9%
		p vs C		NS	<.05	NS		NS	NS	NS		NS	NS	NS
		p vs D				NS				NS				NS
Hemorrhagic Shock and Dextran Infusion and BCMA mixture	3	Mean	7.41	7.32	7.36	7.41	41	29	46	42	88	99	95	100
		SE	±0.02	±0.08	±0.04	±0.02	± 0.50	± 2.6	± 3.8	± 1.2	± 8.3	± 8.5	± 9.7	± 8.5
		%Δ		- 1%	-0.7%	0%		-29%	+12%	+ 4%		+10%	+ 6%	+ 11%
		p vs C		NS	NS	NS		<.001	NS	NS		NS	NS	NS
		p vs H			NS	NS			<.001	<.001			NS	NS
		p vs D				NS				NS				NS

*C = Control
H = Hemorrhagic Shock; 60 min of sustained hypotension 30 mm Hg
D = Dextran Infusion ; 40 ml/kg for 60 min
B = BCMA 25 mg/kg i.v.; 30 min after beginning of dextran

TABLE 9

Treatment with benzoylcarbinolmorphineacetate (BCMA) of hemorrhagic shock in monkeys: cardiovascular and postmortem examination of the lung*

Procedure	No of Monkeys		Mean Arterial Pressure (mm Hg) C	H	D	B	Heart Rate (beats/min) C	H	D	B	Postmortem Examination of the Lung WLW/BW	WLW/DLW	Critical inflating pressure (-cm H_2O)	Phospholipids (mg/g)
Control	3	Mean	126	119			157	180			7.10	4.67	-11.3	20.8
		SE	± 8.3	± 16.7			± 14.5	± 17.3			±0.36	±0.18	± 0.33	± 0.76
		%Δ		- 6				+ 15						
		p vs C		NS				NS						
Hemorrhagic Shock	3	Mean	114	35			183	173			6.97	4.82	-11.0	17.3
		SE	± 5.6	± 5.0			± 3.3	± 6.7			±0.60	±0.16	± 0.58	± 0.59
		%Δ		-69				- 5						
		p vs C		<.001				NS			NS	NS	NS	<.001
Hemorrhagic Shock and Dextran Infusion	3	Mean	110	27	82		183	173	160		8.25	5.50	-13.3	14.4
		SE	± 10.4	± 3.3	±18.8		± 16.7	± 12.0	± 15.3		±0.46	±0.27	± 0.33	± 0.39
		%Δ		-75	-25			- 5	- 33					
		p vs C		<.001	NS			NS	NS		<.05	<.01	<.001	<.001
		p vs H			<.01				NS					
Hemorrhagic Shock and Dextran Infusion and BCMA	3	Mean	108	36	103	100	150	168	138	150	7.44	4.76	-11.7	19.3
		SE	± 1.2	± 3.5	± 4.4	± 2.9	± 5.8	± 19.2	± 14.8	± 11.5	±0.04	±0.12	± 0.33	± 0.27
		%Δ		-67%	- 4%	- 7%		+ 12%	- 8%	0%				
		p vs C		<.001	NS	<.05		NS	NS	NS	NS	NS	NS	NS
		p vs D				NS				NS				
Hemorrhagic Shock and Dextran Infusion and BCMA mixture		Mean	115	30	92	82	200	213	177	173	7.63	4.73	-11.3	20.3
		SE	± 8.7	± 5.0	±13.6	±11.7	± 15.3	± 14.5	± 8.8	± 6.7	±0.51	±0.48	± 0.67	± 1.07
		%Δ		-74	-20	-28		+ 6	-11	-13				
		p vs C		<.001	NS	<.001		NS	NS	NS	NS	NS	NS	NS
		p vs H			<.001	<.001			<.05	<.05				
		p vs D				NS				NS				

*C = Control
H = Hemorrhagic Shock; 60 min of sustained hypotension 30 mm Hg
D = Dextran Infusion; 40 ml/kg for 60 min
B = BCMA 25 mg/kg i.v.; 30 min after beginning of dextran

g. Publication relating to Eriodictyol

(See reprint entitled "Prevention of Acute Pulmonary Insufficiency by Eriodictyol")

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PREVENTION OF ACUTE PULMONARY INSUFFICIENCY BY ERIODICTYOL[1]

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ABSTRACT

AVIADO, DOMINGO M., LEONARDO V. BACALZO, JR. AND MIROSLAW A. BELEJ: Prevention of acute pulmonary insufficiency by eriodictyol. J. Pharmacol. Exp. Ther. **189:** 157–166, 1974.

The oral or parenteral administration of eriodictyol prevented the appearance of signs of acute pulmonary insufficiency provoked by the following procedures: inhalation of 25% carbon dioxide in oxygen by mice, intraperitoneal injection of paraquat in rats, intravenous injection of endotoxin in rats and intravenous infusion of iodoacetamide in monkeys. In the last-mentioned procedure, there was a reduction in pulmonary compliance, respiratory minute volume and arterial oxygen tension which was prevented by prior administration of eriodictyol. In rats, the decrease in pulmonary compliance and respiratory minute volume produced by either paraquat or endotoxin was prevented by the prior administration of eriodictyol. The postmortem examination of the three animal species indicated an elevated moisture and hemoglobin content and a decrease in phospholipid concentration in the lung. The intensity of these changes was reduced in the animals that were pretreated with eriodictyol.

Acute pulmonary insufficiency is a syndrome characterized by edema, congestion, thrombosis and atelectasis of the lung. It results from respiratory burns, thoracic and nonthoracic trauma and shock. At the present time, there is no specific form of therapy for this condition. Management of the patient with acute pulmonary insufficiency has been entirely symptomatic and unsatisfactory (see references cited by Moore *et al.*, 1969, and Aviado, 1972).

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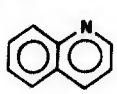
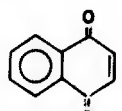
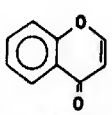
In the course of investigation of compounds for the treatment of chloroquine-resistant malaria, some antimalarial compounds were noted to protect mice from pulmonary edema induced by inoculation with *Plasmodium berghei*. The mechanism of antiedema action is not known. The protection includes other forms of pulmonary edema such as inhalation of 25% carbon dioxide in oxygen and injection of a lethal dose of epinephrine (see references cited by Aviado, 1969). The double-ring compounds with antimalarial activity have the following basic structures:



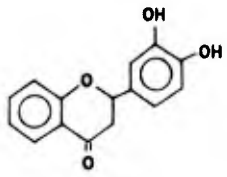
QUINOLINE



NAPHTHOQUINONE



CHROMONE



ERIODICTYOL

After examination of 25 compounds with the above basic structures, it became apparent that the quinolines had conspicuous antimalarial activity but did not prevent pulmonary edema, that the chromones had weak or no antimalarial activity but important antiedematogenic action and that the naphthoquinones exert both actions. The pharmacology of selected quinolines and naphthoquinones has been discussed elsewhere, specifically that of chloroquine (Aviado *et al.*, 1970), of lapinone (Aviado and Will, 1969) and of menoctone (Aviado and Cambar, 1969). Eriodictyol is a chromone which has a weak antimalarial activity but proved to exert a preventive action against pulmonary edema in mice. It has been isolated from leaves of yerba santa (*Eriodictyon californicum*) by Geissman (1940) and from lemon peel by Mager (1942). Its chemical name is 3′,4′,5,7-tetrahydroxyflavanone (see structure above).

Like other flavones or flavonoids, eriodictyol exerts an anti-inflammatory effect on the joints, skin and mucous membranes (see references cited by Gabor, 1972). There has been no previously reported attempt to examine the effect on acute pulmonary insufficiency of eriodictyol or of other anti-inflammatory agents. The experiments reported below indicate for the first time that it is possible to prevent acute pulmonary insufficiency in mice, rats and monkeys by prior administration of eriodictyol.

Methods

Acute pulmonary insufficiency in mice. A total of 50 male Swiss mice was used in this investigation. The method of Aviado and Cambar (1969) was used, which consists of exposing mice in a glass chamber filled with 25% carbon dioxide in oxygen. After 5 minutes' exposure, the mice were removed and killed immediately by the intraperitoneal injection of 0.1 ml of 25% sodium cyanide solution. This manner of sacrificing the animal was also used for the controls that were not exposed to carbon dioxide. The body was weighed and the lungs were removed and weighed, dried in an oven (180°C) for 24 hours and then reweighed. The moisture content of the lung was calculated as follows: [(wet weight minus dry weight ÷ wet weight) × 100]. The following groups of 5 mice each were examined: control group not exposed to carbon dioxide, group exposed to 25% carbon dioxide in oxygen and group exposed to 25% carbon dioxide in oxygen 30 minutes after pretreatment with an intraperitoneal injection of eriodictyol in doses of 1.5 or 10 mg/kg.

Acute pulmonary insufficiency in rats. A total of 57 male rats (Wistar strain) was used in this investigation. Two procedures were utilized to induce pulmonary insufficiency. The *first* was the method of Cambar and Aviado (1970), consisting of intraperitoneal injection of 10 mg/kg of paraquat dichloride. After 48 hours, the animal was anesthetized with an intraperitoneal injection of a mixture of urethan and allobarbital, 200 and 50 mg/kg, respectively. After measurement of pulmonary function (see below), the rat was killed by intraperitoneal injection of 0.1 ml of 25% sodium cyanide solution. The lungs were removed and the extent of pulmonary congestion was estimated by analysis for hemoglobin content by the cyanohemoglobin method. Some of the rats were pretreated with 100 mg/kg of eriodictyol administered orally, daily for 3 days, and others were treated with eriodictyol 24 hours after the administration of paraquat.

The *second* method was to infuse endotoxin intravenously in a dose of 1 mg/kg in anesthetized rats. After measurement of pulmonary function and aortic blood pressure, the rats were sacrificed and the lungs analyzed for moisture and phospholipid content. Some rats received an intraperitoneal injection of 100 mg/kg of eriodictyol 30 minutes prior to the injection of endotoxin.

Acute pulmonary insufficiency in monkeys. A total of 12 male monkeys (*Macaca mulatta*) were used. They were anesthetized by intravenous injection of 30 mg/kg of sodium pentobarbital. In addition to measurement of pulmonary function, the aortic blood pressure was measured *via* a femoral catheter, and blood was collected from a second femoral arterial catheter for analysis of pH, oxygen tension and carbon dioxide tension with a Radiometer apparatus. One of the following chemicals dissolved in saline was infused: alloxan, α-naphthylthiourea (ANTU) and iodoacetamide. The intravenous injection was started and continued until the monkey died. The lungs were removed and a 5-g sample was used for determination of moisture content and a 1-g sample for analysis of phospholipid content. The lung was sutured after the sample was obtained and the rest was used for measurement of static compliance. This consisted of suspending the lung in a glass chamber and exerting negative pressure

for inflating the lung. A syringe was used to remove air from the chamber and the pressure change was measured by a transducer. At the same time the increase in pulmonary volume was observed by the movement of a bubble in a burette attached to the tracheal cannula. The changes in pressure and volume of the lung were used to estimate compliance. Some of the monkeys were pretreated with 500 mg/kg of eriodictyol administered orally, daily for 3 days prior to the infusion of the agent for provoking acute respiratory insufficiency.

Measurement of pulmonary function in rats and monkeys. The trachea of the anesthetized *rat* was exposed and cannulated with a polyethylene catheter. For measurement of functional residual capacity, the nitrogen dilution technique was used, which consists of allowing the rat to rebreathe from a chamber filled with pure oxygen and 10% potassium hydroxide to absorb carbon dioxide. After 7 minutes of rebreathing, the equilibrated gas mixture was analyzed in a Scholander gas analyzer and the functional residual capacity was calculated from a calibration curve derived by equilibrating known volumes of air with the apparatus. The same apparatus was also used for measuring the total oxygen consumed by the rat. The next step was to insert an intrapleural catheter into the 6th intercostal space: its outside end was attached to a differential pressure transducer, while the other side of the transducer connected to the trachea. Air flow was not measured by a flow meter in the trachea which would readily be occluded by accumulation of moisture. Instead a pneumotachometer was attached to a plastic plethysmograph which contained the rat and the respiratory movements were measured by noting the volume of air moving in and out of the chamber. The signals of the transpulmonary pressure and air flow were displayed on an oscilloscope screen to measure pulmonary resistance, and those of transpulmonary pressure and tidal volume to estimate pulmonary compliance by a method described in an earlier paper (Ito and Aviado, 1968).

The same general technique was used in the anesthetized *monkey*. Transpulmonary pressure was measured by an intrapleural catheter inserted *via* the 5th intercostal space and another catheter attached to the trachea. A pneumotachometer was inserted directly into the tracheal cannula, and the velocity of air flow was integrated to derive the tidal volume.

Chemical analysis of the lung. An aliquot portion of the lung weighing approximately 1 g was minced and homogenized with chloroform–methanol (2:1) mixture, with the total volume adjusted to 20 ml. The lipids were extracted by a method described by Folch *et al.* (1957), which consists of filtering the homogenate, adding 4 ml of 0.05% calcium chloride solution and separating the lower phase which was diluted to 20 ml by the addition of chloroform–methanol mixture. The lipid phosphorus was measured according to the method of Rouser *et al.* (1970). Total phospholipids were calculated by multiplying the phosphorus value by 25 (Weinstein *et al.* 1969).

Sources of chemical compounds. Eriodictyol was obtained from Dr. Harry Salem (Director of Pharmacology and Toxicology, Cooper Research Laboratories, Cedar Knolls, N.J.). It was suspended in 0.5% Methocel in a concentration of 10%, which was used for oral administration and intraperitoneal injection.

The sources of the chemicals used for provoking acute respiratory insufficiency were as follows: endotoxin as Lipopolysaccharide B, *Escherichia coli* 0127:B8 from Difco Laboratories (Detroit, Mich.); alloxan, α-naphthylthiourea and 2-iodoacetamide from Eastman Kodak Company (Rochester, N.Y.). These compounds were dissolved in distilled water at a concentration of 0.1 to 1% for intravenous injection. Paraquat dichloride, a weed-killer, was obtained in its pure form from Dr. A. A. B. Swan (Imperial Chemical Industries Ltd. Alderley Park, Macclesfield, England).

Statistical analysis. The results of the experiments were analyzed by Student's t test (Goldstein, 1964). A P value $< .05$ was reported as significant.

Results

Acute Pulmonary Insufficiency in Mice

The inhalation of 25% carbon dioxide elicited signs of pulmonary insufficiency. There was an elevation of moisture content and of lung weight expressed as a percentage of body weight. This increase in both parameters was confirmed by the gross appearance of congestion and edema of the lung, similar to that reported previously in mice that had been infected with *P. berghei* or had received an injection of lethal amounts of epinephrine (Aviado and Cambar, 1969).

The influence of prior treatment with eriodictyol is summarized in table 1. The intraperitoneal injection of 1 mg/kg did not prevent pulmonary insufficiency induced by the inhalation of carbon dioxide. However, the injection of 5, 10, 25, 50 or 100 mg/kg resulted in a significant fall in moisture content of the lung compared with that of nontreated mice which in-

TABLE 1

Influence of eriodictyol on carbon dioxide-induced pulmonary insufficiency in mice

Values are mean ± S.E.

Procedure and Dose (i.p.)	Mouse No.	Body Weight	Lung Weight	Lung Weight	Lung Moisture Content
		g	*g*	*% body wt.*	*%*
Control	5	22.3 ±1.86	0.17	0.73 ±0.03	73.3 ±2.91
Inhalation 25% CO_2 in O_2	5	21.7 ±2.03	0.22[a] ±0.01	1.0[a] ±0.06	81.7[a] ±1.67
Eriodictyol, 1 mg/kg, plus inhalation 25% CO_2 in O_2	5	17.3 ±1.86	0.18[a] ±0.01	0.97[a] ±0.03	81.7 ±0.88
Eriodictyol, 5 mg/kg, plus inhalation 25% CO_2 in O_2	5	18.0 ±1.15	0.19[a] ±0.01	0.97[a] ±0.03	75.7[a] ±0.88
Eriodictyol, 10 mg/kg, plus inhalation 25% CO_2 in O_2	5	17.3 ±1.20	0.18 ±0.01	1.1[a] ±0.06	76.3[a] ±0.88
Control	5	20.8 ±1.16	0.13 ±0.01	0.62 ±0.02	79.4 ±0.68
Inhalation 25% CO_2 in O_2	5	20.8 ±1.28	0.19[a] ±0.01	0.92[a] ±0.05	83.2[a] ±0.49
Eriodictyol, 25 mg/kg, plus inhalation 25% CO_2 in O_2	5	20.2 ±1.28	0.18[a] ±0.01	0.92[a] ±0.07	79.4[a] ±0.40
Eriodictyol, 50 mg/kg, plus inhalation 25% CO_2 in O_2	5	21.2 ±2.06	0.19[a] ±0.01	0.92[a] ±0.07	79.4[a] ±0.81
Eriodictyol, 100 mg/kg, plus inhalation 25% CO_2 in O_2	5	22.2 ±0.51	0.20[a] ±0.00	0.84[a] ±0.02	78.6[a] ±0.93

[a] $P < .05$ compared to control group of mice.

haled carbon dioxide. This observation signifies that eriodictyol prevented an increase in moisture content which results from pulmonary congestion and edema. The weight of the lung relative to body weight remained elevated in the mice pretreated with eriodictyol, indicating that this compound did not prevent all signs of pulmonary insufficiency induced by the inhalation of lethal concentrations of carbon dioxide. The increase in moisture content is preventable whereas the increase in lung weight relative to body weight is not in this experimental model.

Acute Pulmonary Insufficiency in Rats

The rat was used because of the availability of techniques for measuring the mechanical properties of the lung and of procedures that cause acute pulmonary insufficiency.

Paraquat. In a previous report, the intraperitoneal injection of paraquat in the rat was reported to produce pulmonary congestion (Cambar and Aviado, 1970). This procedure has been repeated to determine the influence of prior oral administration of eriodictyol. The results summarized in table 2 indicate that, as compared with those obtained from the controls, the intraperitoneal injection of 10 mg/kg of paraquat elicited the following signs of acute pulmonary insufficiency: reduction in pulmonary compliance, increase in pulmonary resistance, decrease in tidal and minute volume and increase in the hemoglobin content of the lung. There was also a reduction in body weight and in total oxygen consumption but no change in functional residual capacity.

The influence of oral administration of eriodictyol was investigated in three additional groups of rats. The animals that received 100 mg/kg of eriodictyol after the injection of paraquat still showed all the signs of pulmonary insufficiency except the increase in the hemoglobin content of the lung. It was not possible to prevent pulmonary insufficiency by administering eriodictyol 24 hours after the injection of paraquat.

The sequence of administration was reversed

TABLE 2

Influence of eriodictyol on paraquat-induced pulmonary insufficiency in rats

Values mean ± S.E.

Procedure and Dose	Rat No.	Body Weight	Functional Residual Consumption/ Weight	Pulmonary Compliance	Pulmonary Resistance	Tidal Volume	Respiratory Rate	Minute Volume	Oxygen Consumption	Pulmonary Hemoglobin
		g	*ml/kg*	*ml/cm H_2O*	*cm H_2O/ ml/sec*	*ml*	*/min*	*ml/min*	*ml/min*	*mg/100 g lung*
Control	17	246 ±2.3	9.3 ±0.35	0.16 ±0.006	0.71 ±0.024	3.0 ±0.09	91 ±2.2	280 ±14.0	4.2 ±0.11	2600 ±168.0
Paraquat, 10 mg/kg i.p.	10	226[a] ±4.6	9.6 ±0.58	0.07[a] ±0.003	1.28[a] ±0.043	2.3[a] ±0.11	78[a] ±1.7	189[a] ±9.1	3.3[a] ±0.11	2805[a] ±242.1
Paraquat, 10 mg/kg i.p. followed by eriodictyol 100 mg/kg p.o.	5	208[a] ±8.6	8.4 ±0.95	0.07[a] ±0.006	1.25[a] ±0.081	2.3[a] ±0.11	72[a] ±5.4	163[a] ±6.4	3.8[a] ±0.20	2675 ±90.0
Eriodictyol 100 mg/kg p.o. followed by paraquat 10 mg/kg i.p.	5	240 ±4.7	9.3 ±0.73	0.14 ±0.014	0.88[a] ±0.041	3.0 ±0.38	94 ±2.3	281 ±16.0	4.2 ±0.13	2624 ±50.0
Eriodictyol, 100 mg/kg p.o.	5	246 ±2.0	9.9 ±0.75	0.14 ±0.012	0.85[a] ±0.042	2.7 ±0.22	106 ±6.7	256 ±16.0	3.9 ±0.14	2583 ±219.7

[a] $P < .05$ compared to control group of rats.

TABLE 3

Influence of eriodictyol on endotoxin-induced pulmonary insufficiency in rats

Values are mean ± S.E.

Procedure and Dose	Rat No.	Body Weight	Lung Weight	Lung Weight	Lung Moisture Content	Lung Phospholipid Content
		g	*g*	*% body wt.*	*%*	*mg/g*
Control	5	178.8 ±2.50	1.18 0.04	0.66 ±0.02	79.4 ±0.24	20.3 ±0.58
Endotoxin, 1 mg/kg i.v.	5	184.8 ±6.25	1.49[a] 0.10	0.81[a] ±0.04	81.2[a] ±0.37	16.9[a] ±0.41
Eriodictyol, 100 mg/kg i.p. followed by endotoxin, 1 mg/kg i.v.	5	198.6 ±6.27	1.51[a] ±0.08	0.76[a] ±0.03	80.0 ±0.32	17.2[a] ±0.40

[a] $P < .01$ compared to control group of rats.

in the second group of rats. Eriodictyol (100 mg/kg) was administered orally, daily for 3 days, and then paraquat was injected intraperitoneally on the third day. There were no signs of pulmonary insufficiency detected in these rats. The only exception was an increase in pulmonary resistance; this also appeared in the last group of rats, which were treated with eriodictyol only and not with paraquat.

The increase in pulmonary resistance is an effect produced both by eriodictyol and by paraquat. The decrease in pulmonary compliance and in tidal and minute volume, and the increase in pulmonary hemoglobin content elicited by paraquat were prevented by prior oral administration of 100 mg/kg of eriodictyol.

Endotoxin. Three groups of rats were used to investigate the interaction between eriodictyol and endotoxin. Table 3 summarizes the results of postmortem examination. As compared with the nontreated controls, the rats that received 1 mg/kg of endotoxin intravenously showed a higher lung weight relative to body weight, an elevated moisture content and a lower phospholipid content. The group of rats that received an intraperitoneal injection of 100 mg/kg of eriodictyol prior to the intravenous injection of endotoxin showed a lung moisture content that was lower than that of the group that received endotoxin only. The lung weight and phospholipid content of rats treated with both eriodictyol and endotoxin were closer to those of the

TABLE 4

Responses to endotoxin and eriodictyol in anesthetized rats

Values are mean ± S.E.

Procedure and Dose	Pulmonary Compliance	Pulmonary Resistance	Respiratory Minute Volume	Mean Aortic Blood Pressure	Heart Rate
	ml/cm H_2O	*cm H_2O/ml/sec*	*ml/min*	*mm Hg*	*beats/min*
Endotoxin, 1 mg/kg i.v. (5 rats)					
Control	0.36 ±0.02	0.28 ±0.02	271.6 ±11.7	119.0 ±5.6	378.0 ±30.3
15 min after endotoxin	0.24* ±0.02	0.38* ±0.04	220.0* ±14.3	27.0* ±5.1	396.0* ±10.3
% Δ	−33	+36	−19	−77	−22
Eriodictyol 100 mg/kg i.p. followed by endotoxin, 1 mg/kg i.v. (5 rats)					
Control	0.47 ±0.02	0.27 ±0.02	279.6 ±10.6	122.0 ±2.5	357.0 ±8.0
15 min after endotoxin	0.45 ±0.02	0.28 ±0.01	274.4 ±22.1	128.0 ±2.5	381.0* ±8.1
% Δ	−4	+4	−2	+5	+7

* $P < .05$ compared to control.

control animals but the differences were still statistically significant, indicating that these measurements were also abnormal.

The antemortem observations relating to the intravenous injection of 1 mg/kg of endotoxin are summarized in table 4. Within 15 minutes after the injection, the following effects were observed: an increase in pulmonary resistance, a decrease in pulmonary compliance, a decrease in respiratory minute volume and a decrease in mean aortic blood pressure and slowing of the heart rate. The group of five rats that were pretreated with 100 mg/kg of eriodictyol 30 minutes prior to the intravenous injection of endotoxin behaved differently from the group of five rats that received only endotoxin. Pulmonary resistance, pulmonary compliance, respiratory minute volume and mean aortic blood pressure were within normal levels 15 minutes after the injection of endotoxin, indicating that eriodictyol prevented the appearance of functional signs of acute pulmonary insufficiency induced by endotoxin but did not prevent postmortem changes. The slowing of the heart rate observed after endotoxin injection was converted to an acceleration response in the rats that were pretreated with eriodictyol.

Acute Pulmonary Insufficiency in Monkeys

The experiments on the monkey were designed to obtain measurements of blood gases, in addition to the antemortem and postmortem observations of the lung similar to those described above for the rat. Since there was no animal model previously reported for the monkey, the initial experiments were directed to testing alloxan, ANTU and iodoacetamide, which have been reported previously to produce pulmonary edema and hypertension in the dog (Gruhzit *et al.*, 1951; Aviado and Schmidt, 1957).

Alloxan. Alloxan was injected by continuous infusion at the rate of 10 mg/kg/min in 3 monkeys. The effects on pulmonary resistance, pulmonary compliance and respiratory minute volume consisted of either an increase or a decrease. The monkeys died after the injection of the following total doses: 833, 900 and 1100 mg/kg, respectively. Terminal hypotension, respiratory depression and cardiac arrest occurred. Postmortem analysis for moisture content did not reveal any pulmonary edema or congestion.

α-Naphthylthiourea. The next group of three monkeys received a continuous intravenous infusion of 4 mg/kg/min of ANTU. There was consistently a fall in pulmonary compliance, an increase in pulmonary resistance and hypotension, but the accompanying changes in respiratory minute volume and heart rate were variable. The lethal doses were as follows: 160, 264 and 320 mg/kg, respectively. Pulmonary edema and congestion and pleural effusion were absent from the lungs when examined at autopsy.

Iodoacetamide. The third group of three monkeys received a continuous infusion of 10 mg/kg/min of iodoacetamide. The lethal doses were as follows: 410, 420 and 440 mg/kg. The range of doses was narrower than those for alloxan and ANTU described in the preceding paragraphs. Prior to death, iodoacetamide caused simultaneously the following effects that were statistically significant: reduction in pulmonary compliance, a fall in mean aortic blood pressure, bradycardia, reduction in blood pH and a decrease in oxygen tension of the arterial blood. An increase in pulmonary resistance and a decrease in respiratory minute volume also occurred which were not statistically significant. Postmortem examination of the lung revealed a higher moisture content and lower phospholipid

content as compared with the lungs from control monkeys. The static compliance of the lungs was also different in the monkeys that died from infusion of iodoacetamide. The critical inflating pressure of the lungs from iodoacetamide-treated monkeys was higher than in the normal lung (fig. 1).

Eriodictyol pretreatment and iodoacetamide. The fourth and last group of three monkeys received 500 mg/kg of eriodictyol orally for 3 days prior to the infusion of iodoacetamide (tables 5 and 6). The protective influence of eriodictyol was noted from the following factors: 1) increase in the lethal dose of iodoacetamide from 423.3 ± 8.8 to 536.7 ± 34.8 mg/kg; 2) absence of decrease in pulmonary compliance, increase in pulmonary resistance, depression of respiratory minute volume and bradycardia in response to iodoacetamide in eriodictyol-pretreated monkeys; 3) reduced intensity of hypoxemia, acidosis and hypotension in response to iodoacetamide; and 4) lower moisture content, lesser lung weight and higher content of phospholipid in monkeys pretreated with eriodictyol and injected with iodoacetamide, as compared with monkeys that received iodoacetamide only.

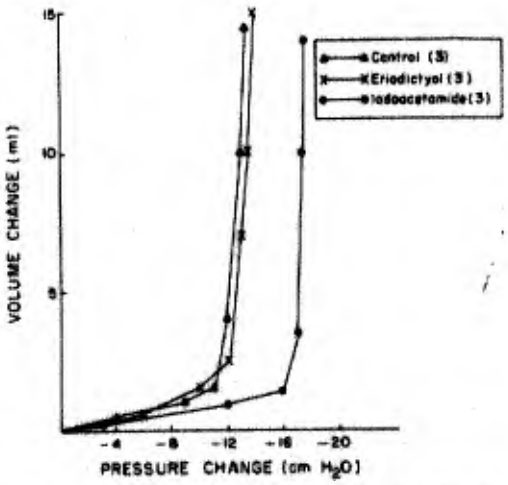


FIG. 1. Mean changes in volume and pressure of lungs removed from three groups of monkeys described in table 6. The critical inflating pressure of lungs from iodoacetamide-treated monkeys is higher than those from normal controls and eriodictyol-pretreated monkeys.

Discussion

Until the 1960's, the search for pulmonary drugs was limited to those that would be useful in the treatment of acute pulmonary edema. Three types of therapeutic agents were identified: the cardioactive drugs, for use when the edema followed left ventricular failure, central nervous system depressants for edema resulting from either the inhalation of carbon dioxide or experimental lesions of the midbrain and the sulfhydryl inhibitors for edema caused by the injection of ANTU (see references cited by Luisada, 1970). Although among the findings of investigators studying this last-mentioned category was the report of Meyer and Saunders (1949) that thiourea derivatives protected rats from ANTU poisoning by acting as competitive antagonists, none of these compounds was subsequently tested for possible use in other forms of experimentally induced pulmonary edema.

The increasing incidence during the past decade of acute pulmonary insufficiency involving edema, congestion, thrombosis and embolism necessitated modifications in the tests used in the search for therapeutic agents. In addition to the measurement of moisture content for edema, and hemoglobin content for congestion, measurements of pulmonary compliance in the living animal and static compliance in the excised lung as well as chemical analysis of the phospholipid content of the lung were developed. The last-mentioned parameter gauges pulmonary surfactant, which is deficient in the lung in acute pulmonary insufficiency (see references cited by Scarpelli, 1968).

The experiments reported above represent the first successful attempt to prevent the characteristic lesions of acute pulmonary insufficiency in four types of experiments in three animal species. The protection afforded by eriodictyol was inferred from the following observations: 1) prevention of an increase in moisture content in mice inhaling 25% carbon dioxide in oxygen; 2) prevention of the increases in moisture and hemoglobin content, and of the decreases in pulmonary compliance and tidal volume, in rats that have been treated with paraquat; 3) prevention of the decreases in pulmonary compliance and tidal volume and recovery from shock, in rats that have received an intravenous injection of endotoxin; and 4) prevention of decreases in pulmonary compliance and tidal vol-

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TABLE 5

Responses to intravenous infusion of iodoacetamide and oral pretreatment with eriodictyol

Values are mean ± S.E.

Procedure and Dose	Pulmonary Compliance	Pulmonary Resistance	Respiratory Minute Volume	Mean Aortic Blood Pressure	Heart Rate	Arterial Blood Gases		
						pH	P_{CO_2}	P_{O_2}
	ml/cm H_2O	*cm H_2O/l/sec*	*ml/min*	*mm Hg*	*beats/min*	*units*	*mm Hg*	*mm Hg*
Iodoacetamide, 10 mg/kg/min								
Control	9.7	25.4	310	122	165	7.36	33.3	88.7
	±0.3	±2.2	±26.5	±2.3	±7.6	±0.01	±1.3	±0.3
40 min after iodoacetamide	7.5[a]	29.9	200[a]	26.7[a]	70[a]	7.13[a]	34.0	65.0
	±0.3	±1.5	±48.7	±1.7	±5.8	±0.05	±13.5	±12.6
%Δ	−22	+20	−36	−78	−57	−3	+2	−27
Eriodictyol, 500 mg/kg p.o., for 3 days								
Control	6.0	23.9	370	140	170	7.41	35.7	99.0
	±0.58	±2.9	±38	±2.9	±10	±0.02	±3.3	±7.4
40 min after iodoacetamide	6.77	22.5	511	72[a]	185	7.27	25.3	81.3
	±0.50	±2.3	±141	±6.4	±2.9	±0.09	±6.6	±18.7
%Δ	+16	−5	+34	−48	+10	−2	−29	−18

[a] $P < .05$ compared with control value.

TABLE 6

Influence of eriodictyol on iodoacetamide-induced pulmonary insufficiency in monkeys

Values are mean ± S.E.

Procedure	Monkey No.	Lethal Dose of Iodoacetamide	Lung Weight	Lung Weight (% of Body Weight)	Lung Moisture Content	Lung Phospholipid Content (mg/g)	Critical Inflating Pressure
		mg/kg	*g*	*% body wt.*	*%*	*mg/g*	*−cm H_2O*
Control	3		13.5	0.65	78	16.7	11.08
			0.30	±0.06	±1.8	±0.90	±0.51
Iodoacetamide, 10 mg/kg/min i.v.	3	423.3	16.2[a]	1.07[a]	83[a]	14.5[a]	16.00[a]
		±8.8	±3.5	±0.02	±1.0	±0.55	±1.58
Eriodictyol, 500 mg/kg p.o. for 3 days followed by iodoacetamide, 10 mg/kg/min i.v.	3	536.7[b]	14.8[b]	0.83[b]	80	15.0	12.07[b]
		±34.8	0.6	±0.07	±1.3	±0.43	±0.29

[a] $P < .05$ compared to control group of monkeys.

[b] $P < .05$ compared to monkeys treated with iodoacetamide but not pretreated with eriodictyol.

ume, hypoxemia and acidosis and an increase in the lethal dose of iodoacetamide in monkeys. In rats and monkeys, postmortem examination of the lungs showed an elevation in moisture content and a decrease in phospholipid content following either endotoxin or iodoacetamide administration. The intensity of these effects was reduced in animals that had been given eriodictyol prior to the administration of the pulmonary insufficiency-provoking agent.

The reductions in pulmonary compliance seen in the iodoacetamide-treated monkeys, and in the endotoxin- and paraquat-treated rats, are the most significant parameters indicative of acute pulmonary insufficiency. This effect detected antemortem is related to increases in the

moisture and hemoglobin content of the excised lung. The reduction in phospholipid content represents a deficiency of surfactant which is characteristic of lungs that have been damaged by endotoxin, paraquat, carbon dioxide or iodoacetamide. Each of these procedures is known to produce pulmonary congestion and edema in some animal species (see references cited by Scarpelli, 1968).

The mode of action of the agents that provoke acute pulmonary insufficiency is partially known. A high concentration of carbon dioxide produces pulmonary edema by increasing capillary permeability (Poulsen, 1964). Although paraquat causes hemorrhagic and thrombotic lesions in the lung, the basic cause has not been identified (Cambar and Aviado, 1970). Endotoxin causes the release of serotonin, histamine and other biogenic amines which individually produce alveolar edema (Cahill *et al.*, 1965). Iodoacetamide, alloxan and ANTU cause pulmonary vasoconstriction and pulmonary edema by inhibiting sulfhydryl enzymes, a mechanism proposed by Gruhzit *et al.* (1951) on the basis of their observations in the canine lung. In the primate lung, as reported above, only iodoacetamide causes pulmonary insufficiency, whereas the two other sulfhydryl inhibitors do not. The injection of ANTU does not produce pulmonary edema in the monkey but does provoke this effect in the rat (Dieke and Richter, 1946; Richter, 1952).

The mode of action by which eriodictyol prevents acute pulmonary insufficiency is currently under investigation but has not yet been completely identified. Eriodictyol has been found to reduce the weight of granulomatous tissue resulting from the implantation of cotton pellets in the subcutaneous tissue of the rat, an effect indicative of an anti-inflammatory action similar to that of the corticosteroids and indomethacin. However, the latter agents do not prevent pulmonary lesions, and eriodictyol has no glucocorticoid activity so that its effect on the lung is independent of its systemic anti-inflammatory action. Eriodictyol blocks the effects of angiotensin and prostaglandins E_2 and F_2 on the smooth muscle of the colon and the coronary vascular bed. If this interaction occurs in the pulmonary vascular bed as well, it would explain eriodictyol's acting to prevent acute pulmonary insufficiency in animals.

Eriodictyol does not prevent pulmonary lesions in the rat previously given paraquat. Since eriodictyol is insoluble in water, it was not possible to administer it after signs of pulmonary insufficiency had developed in the monkey. The animal models described above are useful only in the detection of prophylactic agents. Until a compound suitable for intravenous injection is available, observations on the reversal of pulmonary lesions are not possible. The development of suitable laboratory tests described above will hopefully lead to improvement of the management of clinical forms of acute pulmonary insufficiency.

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h. Publication relating to Drug Therapy of Acute Pulmonary Insufficiency

(See reprint entitled "Pharmacologic Principles in the Treatment of Acute Respiratory Insufficiency")

Pharmacologic Principles in the Treatment of Acute Respiratory Insufficiency

By Leonardo V. Bacalzo, Jr., and Domingo M. Aviado

IN RECENT YEARS, acute respiratory insufficiency has become one of the most difficult syndromes to treat. The problems in managing separately shock, pulmonary embolism, atelectasis, and hypoxia are combined in this syndrome. The challenge to the physician is to ascertain that, in a particular patient, the benefit derived from a selected procedure to correct one lesion is greater than the risk of exaggerating another lesion. For example, the vasopressor drugs for treatment of systemic shock can be expected to elevate the pulmonary arterial blood pressure and further increase pulmonary edema. The corticosteroids which have been recommended to reverse pulmonary lesions are known to potentiate the harmful effects of the biogenic amines in the systemic circulation, thus bringing about a further progression of shock. Even the simplest procedure of oxygen therapy to increase the availability of oxygen is not totally harmless because high levels of oxygen have been demonstrated to produce lesions similar to those of acute respiratory insufficiency.[10]

This article attempts to discuss the principles of therapy in acute respiratory insufficiency by pointing out the desirable and undesirable consequences of the use of drugs. The specific details relating to dosages and the manner of administration have been omitted, since these can be derived from a recent publication.[2] It should be stated at the outset that, because of the extensive investigation of the pathophysiology and pharmacology of the lungs, new drugs will probably be obtainable in the near future. Several animal models are available for testing the activity of compounds, and these are briefly discussed here.

RECOGNITION OF THE ROLE OF BIOGENIC AMINES AND POLYPEPTIDES IN THE PATHOGENESIS OF PULMONARY LESIONS

It is pertinent to point out that what is now recognized as acute respiratory insufficiency in patients was described in laboratory animals in 1918 by the late Sir Henry Dale and the late Alfred Newton Richards, two pharmacologists who were collaborating in a project to study the role of histamine in the pathogenesis of shock.[7–9] They concluded that the injection of histamine alone was fatal to the cat because it produced pulmonary vasoconstriction, systemic shock, and respiratory arrest. The Dale–Richards phenomenon was subsequently elaborated upon by other investigators to include the following pathologic and functional changes: (1) increase in capillary permeability with exudation of plasma into the alveolar interstitial space, progressing to edema;[27,29] (2) capillary hemorrhage, thrombosis, and

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atelectasis, resulting in congestive atelectasis;[20] (3) vasoconstriction of pulmonary veins with increased shunting of blood through poorly ventilated areas;[6] and (4) bronchospasm and edema of the bronchial mucosa.[16] The ultimate effect is hypoxemia.

The functional changes in the Dale–Richards phenomenon are similar to those reported in patients suffering from acute respiratory insufficiency. There has been no recent attempt to determine whether histamine is the pathogenic agent in patients with acute respiratory insufficiency. Instead, the role of other biogenic amines and of polypeptides has been well documented.[1] Table 1 includes the catecholamines–norepinephrine, epinephrine, and dopamine–and two additional amines–serotonin and histamine. Only serotonin approaches histamine in potency and diversity of bronchopulmonary effects. Norepinephrine and epinephrine constrict the pulmonary vessels but, unlike histamine, they do not produce bronchospasm. Dopamine causes bronchospasm and is present in the lung of ruminants, but not in the human lung. All five biogenic amines cause aggregation of blood platelets, which promotes thrombosis.

The vasoactive polypeptides listed in Table 1 are bronchoconstrictors with variable effects on the pulmonary blood vessels. The slow-reacting substance,

Table 1. Biogenic Amines and Vasoactive Peptides

Biogenic Amines (1 to 5) Vasoactive Peptides (6 to 9)	Physiologic and Pharmacologic Significance	Bronchopulmonary Effects
1 Norepinephrine	Neurohumoral transmitter for sympathetic nerves (?) Released from other organs and metabolized in lungs Used in treatment of shock	Weak pulmonary arterial vasoconstrictor and potent bronchial arterial vasoconstrictor
2 Epinephrine	Neurohumoral transmitter for sympathetic nerves (?) Used in treatment of bronchial asthma	Bronchodilator, bronchial arterial vasoconstrictor and weak pulmonary arterial vasoconstrictor
3 Dopamine	High level in lung of ruminants Used in treatment of shock	Bronchoconstriction
4 Histamine	Released during anaphylactic response Important in defense mechanism	Bronchoconstriction and vasoconstriction, increased capillary permeability
5 Serotonin	Released from platelets and serotonin-containing tissues Stimulates pulmonary chemoreflexes Partakes in formation of erythropoietins Role in pulmonary embolism (?)	Bronchoconstriction and vasoconstriction
6 Slow-reacting substance	Released during anaphylactic and allergic response (?)	Bronchoconstriction
7 Bradykinin	Formed from plasma kallikrein of plasma kallikreinogen Role in inflammation and allergy (?)	Bronchoconstriction and vasodilatation
8 Kallidin	Formed from plasma kallidinogen Role in inflammation and allergy (?)	Bronchonstriction and vasodilatation
9 Angiotensin	Formed from plasma angiotensinogen Metabolized in the lung Used in treatment of shock	Weak pulmonary arterial vasoconstrictor
10 Prostaglandins	Released from the lung Prostaglandins E_1 and E_2 under clinical investigation for treatment of bronchial asthma	Bronchoconstriction or bronchodilatation

bradykinin, and kallidin have been implicated in the allergic and the inflammatory response of the lung, which have features similar to the pulmonary lesions of acute respiratory insufficiency. Angiotensin has a weak pulmonary arterial vasoconstrictor action as compared with its systemic effect. The prostaglandins are released from the lung and exert varied effects on the bronchial muscle and the pulmonary blood vessels.

Although there are agents which block the bronchopulmonary effects of the biogenic amines, they have not been used successfully in the treatment of acute respiratory insufficiency. The search has not yielded any agent which can block the effects of the vasoactive polypeptides. However, there is a continued effort to find blocking agents by testing them on animal models, in which the pulmonary lesions are induced by procedures other than the administration of biogenic amines and vasoactive polypeptides. The procedures include hemorrhagic shock,[1,17] oxygen deprivation,[20] and the administration of compounds that are toxic to the pulmonary tissues.[11] The details of these procedures as they relate to the development of a rational treatment of acute respiratory insufficiency are discussed in the following sections.

CORRECTION OF CIRCULATORY FAILURE OF PERIPHERAL ORIGIN

The initial consideration in the treatment of a patient with acute respiratory insufficiency is to correct the primary cause of circulatory failure. This is simple in hemorrhagic shock, but complicated if the shock is due to trauma, infection, or intoxication. It is more difficult to overcome the consequences of trauma, of bacterial endotoxin, or of poisoning.

Vasopressor Drugs

The hypotension accompanied by low cardiac output characteristic of patients with acute respiratory insufficiency can be corrected by the use of vasopressor drugs.[1] The primary aim is to increase cardiac output and elevate the blood pressure. Unfortunately, neither fluid replacement nor the vasopressor drugs ac-

Table 2. Vasopressor Drugs Arranged in the Order of Importance of Vasoconstriction Relative to Vasodilatation and Cardiac Stimulation

Generic Name	Type of Drug	Vasoconstriction	Vasodilatation	Cardiac Stimulation
Methoxamine	*alpha*	+ + + + +	0	0
Phenylephrine	*alpha*	+ + + + +	0	0
Angiotensin	(not sympathomimetic)	+ + + +	0	0
Norepinephrine	*alpha* and *beta*	+ + + +	0	+ +
Metaraminol	*alpha* and *beta*	+ + +	+ +	+
Cyclopentamine	*alpha* and *beta*	+ + +	+ +	+
Methamphetamine	*alpha* and *beta*	+ +	+	+ + +
Ephedrine	*alpha* and *beta*	+ +	+	+ + +
Isometheptene	*alpha* and *beta*	+ +	+ +	+ + +
Methylaminoheptane	*alpha* and *beta*	+ +	+ +	+ + +
Dopamine	*alpha* and *beta*	+ +	+ +	+ +
Mephentermine	*alpha* and *beta*	+	+ + +	+ +
Hydroxyamphetamine	*alpha* and *beta*	+	+ + +	+ +
Phenoxybenzamine	(block *alpha*)	0	+ + + +	+ + +
Isoproterenol	*beta*	0	+ + + +	+ + +

complish this effect without influencing the pulmonary circulation. There is an increase in pulmonary blood volume which serves to exaggerate further the pulmonary lesions. The drugs that are used in order to elevate the systemic arterial blood pressure are listed in Table 2. When the drug is administered in therapeutic doses to a patient in shock, the relative intensity of vasoconstriction responsible for the vasopressor effect can be rated from +++++ to 0. The highest rating is given to methoxamine (Vasoxyl), a sympathomimetic alpha-receptor stimulant which produces maximal vasoconstriction, but no vasodilatation or cardiac stimulation. At the other extreme is isoproterenol (Isuprel), a beta-receptor stimulant that has a rating of 0 for vasoconstriction and a maximal rating of ++++ for vasodilation and of +++ for cardiac stimulation. The other 11 drugs are sympathomimetics which are either alphamimetic, betamimetic, or a combination of both. Two nonsympathomimetic drugs are included in the table: angiotensin, a polypeptide which has a pure vasoconstrictive action like that of methoxamine; and phenoxybenzamine, a sympathetic alpha-blocking agent with vasodilator and cardiac-stimulating effects like those of isoproterenol.

Pulmonary Vascular Effects of Vasopressor Amines

All of the vasopressor drugs cause a rise in pulmonary arterial blood pressure either by vasoconstriction, in the case of alphamimetics and angiotensin, or by increasing pulmonary blood flow, in the case of betamimetics and phenoxybenzamine. The latter action may also be accompanied by pulmonary vasodilatation. However, this effect will cause an elevation of pulmonary capillary blood pressure and capillary congestion, and an increase in severity of congestive atelectasis. It has not been possible to compare alphamimetics and betamimetics in patients with acute respiratory insufficiency, because it has been difficult to have a reasonable number of matched patients available for study.

Fluid Replacement

Parenteral fluids are important in the management of a patient in shock, regardless of the cause. However, there is a potential danger of pulmonary edema developing from the overloading of the circulation. Furthermore, the pulmonary blood vessels are likely to store the fluid temporarily, which may lead to exudation of fluid into the pulmonary interstitial tissue. Recently, there has been increasing evidence from clinical experience in man[15] and experimental observations in animals[13,25] that acute respiratory insufficiency frequently follows successful resuscitation in low flow states.

REDUCTION OF PULMONARY FLUID

The primary aim in the therapy of congestive lesions in the lung is to reduce body fluids. This is the most obvious paradox in treating the acute respiratory insufficiency of a patient in shock. Fluid replacement for the treatment of shock is diametrically opposed to the fluid restriction in the treatment of pulmonary congestion and edema. The body fluid can further be reduced by the intravenous injection of diuretics, such as the sodium salt of ethacrynic acid (Edecrin),[14,21] and furosemide (Lasix).[11] The amount of blood in the lungs can also be reduced by the administration of a ganglion-blocking agent, such as trimethaphan camsylate (Ar-

fonad), which acts by shifting the blood to the periphery. Morphine sulfate, which is often administered in traumatized patients, has also a beneficial hemodynamic effect, consisting of a reduction in circulating blood volume.

A decision has to be made as to whether to restrict or force parenteral fluids. The answer depends on the relative severity of the pulmonary lesions. If oxygenation is adequate and there are minimal signs of edema, it is recommended to proceed with careful use of fluids and vasopressor agents to elevate the systemic blood pressure. In severe cases of respiratory insufficiency, reduction of pulmonary fluid should take precedence over the management of shock.

IMPROVEMENT OF PULMONARY FUNCTION

The most conspicuous sign of acute respiratory insufficiency is hypoxemia. Oxygen administered by inhalation is the most important single procedure. The basis for the administration of oxygen is to increase alveolar oxygen tension, thus compensating for reduced efficiency of the pulmonary gas exchange resulting from the pulmonary lesions.

There is no reason to suspect, however, that oxygen strikes at the primary cause of acute respiratory insufficiency. If there is no immediate improvement in blood oxygen tension, additional procedures can be performed, such as intermittent positive pressure breathing.[5] In the event that this procedure is not sufficient, bronchodilators such as isoproterenol[24] and aminophylline may be used. It should be noted that these drugs also increase pulmonary blood flow and dilate the blood vessels, which are not desirable in overcoming the congestive atelectasis. These bronchodilators also increase venous shunting in the lung, so that hypoxemia is augmented instead of being alleviated. If there is also embolization or thrombosis of the pulmonary artery, it becomes more difficult to correct the hypoxemia because there is a reduction of the gas exchange in the nonperfused portion of the lung, even though an adequate supply of oxygen is present in the alveolar air.

TREATMENT OF PULMONARY LESIONS

The lung of a patient with acute respiratory insufficiency is characterized by a deficiency in oxygen uptake. In most cases, there is a poor diffusion of oxygen, largely because of interstitial edema. Pulmonary capillaries are dilated and congested, and the alveolar walls are thickened with plasma exudate and fibrin. Pulmonary compliance is decreased, indicating a reduction in the distensibility of the lung. The fall in pulmonary compliance is associated with early elevation of the water content in the lung, defects in the surface activity of alveolar lining, and a decrease in phospholipid content.[12] Airway resistance is increased because of fluid exudation and swelling of the bronchioles. There is still no single drug known to reverse all these pathologic processes. It has been suggested that glucocorticoids, because of their antiinflammatory effect, may reduce the exudation. Specifically, the following steroids have been used: dexamethasone (Decadron),[22] hydrocortisone,[28] and methylprednisolone (Medrol).[23]

The basis for the use of glucocorticoids in the treatment of acute respiratory insufficiency is not clear. Clinical studies have been largely documentary, and there has been no comparable control group of matched patients not treated with glucocorticoids. In animal experiments, the mode of action of corticosteroids on the

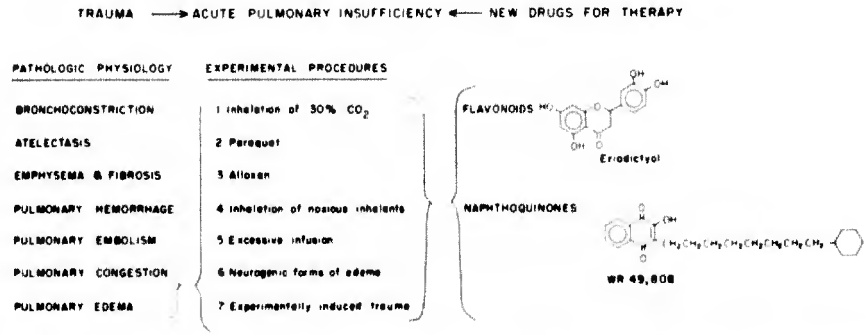

Fig. 1. Summary of experimental procedures which simulate in animals the clinical syndrome of acute respiratory insufficiency.

lung tissue has not been identified. The following mechanisms have been suggested: (1) prevention of sludging of leukocytes in the pulmonary capillaries;[18] (2) gluconeogenic effect of steroids;[19] (3) improvement of hemodynamic state of the systemic circulation; and (4) decrease in capillary permeability to fluids by antiinflammatory action. The first mechanism has been noted only in animals, the second mechanism has been demonstrated only in organs other than the lung, and the third mechanism cannot account for the appearance of pulmonary lesions in situations where abnormalities in the systemic circulation have already been corrected. The most likely mechanism, therefore, is the antiinflammatory action although it is not selective for the lung vessels. The glucocorticoids are effective in treating inflammation of most other areas. The glucocorticoids also exert systemic effects that are undesirable, among which is the accumulation of fluids and electrolytes in the tissues. The glucocorticoids also potentiate the effects of biogenic amines in the blood vessels, which may be harmful if these amines are the cause of the pulmonary lesions (see Section I).

Efforts continue to seek new drugs which may reduce capillary permeability selectively without the general systemic actions of glucocorticoids. In the course of searching for new drugs to treat chloroquine-resistant malaria, the naphthoquinones were observed to prevent or reduce pulmonary lesions produced in mice and rats by the parasite, by inhalation of carbon dioxide, or by injection of large doses of epinephrine.[3] The naphthoquinones have also been tested in other animal models listed in Fig. 1. So far, one naphthoquinone and eriodictyol have been found to be effective in reducing capillary permeability and preventing the various forms of experimental lesions in the lung. These studies are continuing and will hopefully lead to the introduction of drugs that have been developed specifically for the treatment of acute respiratory insufficiency. Meanwhile, the management of this syndrome will have to depend on the judicious use of fluid, steroid, cardiac, and ventilatory support.

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