THE EFFECT OF β-AMINOPROPIONITRILE ON SILICOTIC PULMONARY FIBROSIS IN THE RAT

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THIS study was designed to test the feasibility of the control of fibrous tissue formation by drugs. Attempts at preventing deposition of collagen must be directed either at inhibiting its synthesis, which includes cross-linking of chain sub-units, or at controlling the enzymatic breakdown of collagen fibres. Should either neutral carbohydrates or acid mucopolysaccharides eventually be shown to prove essential for fibrogenesis, they will present a further potential target. This study is an attempt to inhibit the cross-linking of the component chains of the tropocollagen molecule with the lathyrus factor, β-amino-propionitrile (BAPN) as inhibiting agent. Pulmonary silicosis in the rat was used as a model system of a severe and progressive pathological fibrotic condition. The results favour the view that control of fibrosis may be an attainable goal. A brief summary of these results has been previously published (Levene and Bye, 1964).

MATERIALS AND METHODS

First experiment.—Fifty adult male Wistar rats weighing 350–400 g. were injected intratracheally under ether anaesthesia with 45 mg. alkali-etched quartz of 0·5–5 μ Stokes' diameter (the generous gift of Drs. G. Nagelschmidt and I. Bergman of the Safety in Mines Research Establishment, Sheffield) suspended in 0·6 ml. normal saline (Zaidi, King,Harrison and Nagelschmidt, 1956). Twelve similar rats were included as normal, non-silicotic controls. All animals were fed on Purina chow. Six days after the quartz injection they were equally divided into 2 groups, 1 group being treated 3 times weekly with 150 mg. BAPN (the generous gift of Abbott Laboratories, North Chicago, Illinois, U.S.A.) dissolved in 1 ml. sterile distilled water, given intraperitoneally, and the other group—the untreated silicotic controls—being given 1 ml. sterile distilled water intraperitoneally 3 times weekly.

All animals were weighed 3 times weekly and, 4 weeks after the administration of silica, half of the BAPN-treated and untreated silicotics were killed; the remainder, including the normal controls, were killed after a further 4 weeks. Thus, the 4-week BAPN-treated animals had received a total of 1·65 g. BAPN each and the 8-week group had received 3·50 g.

After death the lungs were weighed separately, half of their number being kept for collagen analysis and the remainder for histological examination. Specimens for collagen analysis were hydrolysed for 3 hr. at 140° in 1 ml. 6 N HCl in a sealed tube and the hydroxyproline content measured by the method of Neuman and Logan (1950). For histology the lungs were fixed in neutral formol saline and wax sections were stained with haematoxylin and eosin, Van Gieson and Verhoeff's elastic stain, periodic acid-Schiff, mucicarmine and toluidine blue.

Second experiment.—Three subsidiary tests were performed in order to determine the optimal length of time for BAPN treatment of silicotic rats. In this experiment 210 female

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Wistar rats weighing 200 g. were divided into 3 equal groups and rendered silicotic as previously described, but were maintained for a period of 150 days. In the first group BAPN treatment was given for the first 40 days and discontinued for the last 110 days; in the second group treatment was given for the last 40 days only and in the third group treatment was maintained throughout the 150 days. Half of the number of animals in each group were kept as untreated silicotic controls (Table I). All animals were weighed weekly and after death the lungs were weighed and a sample taken for histology.

TABLE I.—*Experiments to Determine the Optimum Length of BAPN Treatment of Silicotic Rats*

Experiment 2	Treatment		
Group 1	(a) Normal controls	(12)	BAPN treatment given for first 40 days (2·0 g./animal)
	(b) BAPN-treated control	(14)	
	(c) Silicotic controls	(21)	
	(d) BAPN-treated silicotics	(24)	
Group 2	(a) Silicotic controls	(12)	BAPN treatment given for last 40 days (2·2 g./animal)
	(b) BAPN-treated silicotics	(15)	
Group 3	(a) Silicotic controls	(17)	BAPN treatment given throughout 150 days (5·3 g./animal)
	(b) BAPN-treated silicotics	(23)	

Number of animals surviving the 150 days, indicated in parentheses

RESULTS

Effects of silicosis

Compared to normal controls, silicotic rats failed to gain weight, the difference being significant (Table II). There was a significant rise in the fresh weight and total collagen content of both right and left lungs of the silicotic rats (Tables III and IV).

TABLE II.—*Effect of BAPN Treatment on the Growth of Rats after Silicosis of 4 and 8 Weeks Duration*

	Treatment	Average weight gain ± s.e.m. (g.)	*P* values	
4 weeks	Normal controls	+ 80·8 ±6·1 (12)	< 0·05	< 0·05
	Silicotic controls	+ 15·7 ±29·6 (6)		
	Silicotics treated with BAPN	+ 31·7 ±8·9 (6)	> 0·5	
8 weeks	Normal controls	+ 129·2 ± 7·9 (12)	< 0·05	< 0·05
	Silicotic controls	+ 21·8 ± 26·2 (8)		
	Silicotics treated with BAPN	+ 60·9 ± 12·5 (7)	0·4–0·2	

Numbers of animals used indicated in parentheses

Histologically the silicotic lungs generally showed fibrosis at 4 weeks as well as an intense cellular reaction—necrotic macrophages, marked mast cell and plasma cell reaction with many immature elements (plasmablasts). At 8 weeks there was a typical advanced silicotic reaction, with some fibrohyaline nodules; there was still extensive macrophage necrosis and an intense plasma and mast cell reaction. A moderate amount of PAS-positive material was present interstitially. Most of the lungs also showed a marked suppurative bronchitis and some had large encapsulated abscesses.

TABLE III.—*Effect of BAPN Treatment on the Fresh Weight of the Lungs of Rats after Silicosis of 4 and 8 Weeks Duration*

Treatment		Right lungs: Average fresh lung weight ± s.e.m. (g.)	Right lungs: P values	Left lungs: Average fresh lung weight ± s.e.m. (g.)	Left lungs: P values
Normal controls		1·52 ± 0·10 (11)		0·91 ± 0·10 (12)	
4 weeks	Silicotic controls	3·21 ± 0·75 (6)	0·20–0·10	1·68 ± 0·42 (6)	> 0·50
	Silicotics treated with BAPN	2·04 ± 0·20 (6)		1·29 ± 0·14 (6)	
8 weeks	Silicotic controls	4·16 ± 0·67 (8)	0·10–0·05	2·11 ± 0·31 (8)	= 0·05
	Silicotics treated with PABN	2·48 ± 0·46 (7)		1·30 ± 0·20 (7)	

Number of lungs used indicated in parentheses.

TABLE IV.—*Effect of BAPN Treatment on the Total Collagen Content of the Lungs of Rats after Silicosis of 4 and 8 Weeks Duration*

Treatment	Right lungs: Total collagen content ± s.e.m. (mg.)	Right lungs: P values		Left lungs: Total collagen content ± s.e.m. (mg.)	Left lungs: P values	
Normal controls	21·28 ± 1·40 (5)	< 0·05		11·95 ± 1·13 (6)	< 0·05	
Silicotic controls —including 4- and 8-weeks specimens	76·10 ± 18·10 (6)		< 0·05	21·86 ± 3·15 (8)		< 0·05
Silicotics treated with BAPN— including 4- and 8-weeks specimens	34·73 ± 1·83 (6)	< 0·05		16·75 ± 1·60 (7)	0·2–0·1	

Number of lungs used indicated in parentheses.

Effect of BAPN

Compared with normal controls, BAPN-treated rats, like silicotic rats, also failed to gain weight; their fresh lung weights however, unlike the silicotics, remained normal (Table V).

TABLE V.—*Effect of BAPN on Growth and Fresh Lung Weight of Normal and Silicotic Rats*

Treatment	Average weight gain ± s.e.m. (g.)	P values		Fresh weight of both lungs ± s.e.m. (g.)	P values	
Normal controls	+ 50·0 ± 5·33 (6)	< 0·05		1·35 ± 0·08 (6)	0·4	
BAPN-treated controls	+ 20·8 ± 5·88 (9)		< 0·05	1·44 ± 0·06 (9)		< 0·05
Silicotic controls	+ 24·8 ± 7·12 (10)	> 0·50		4·67 ± 0·58 (10)	< 0·05	

Number of samples indicated in parentheses.

Effect of BAPN treatment on silicosis

BAPN treatment of silicotic rats increased their average weight gain, though not significantly (Table II). It also lowered the average fresh weights of the right and left lungs the differences being statistically recognisable by 8 weeks though not yet at 4 weeks (Table III); however, the lung weights never reached normal levels. Similarly, BAPN treatment lowered the total collagen content of silicotic lungs, the difference being significant in the case of right lung though not quite significant at the 5 per cent level in the case of the left lung (Table IV); treatment never succeeded in lowering the collagen content to normal levels.

Histologically, BAPN treatment appeared to have no effect on the nodular reaction or on the macrophage necrosis; the mast cell reaction and possibly the plasma cell reaction was somewhat diminished and there appeared to be a decreased amount of interstitial PAS-positive material. The suppurative reaction commonly found in the untreated silicotics was considerably less frequent and large abscesses were rarely observed.

Lung weight/collagen content correlation

A correlation coefficient of 0·95 was found to exist between the fresh weight of lungs, whether normal, untreated or treated, right or left, and their total collagen contents, indicating a linear relationship (Fig.).

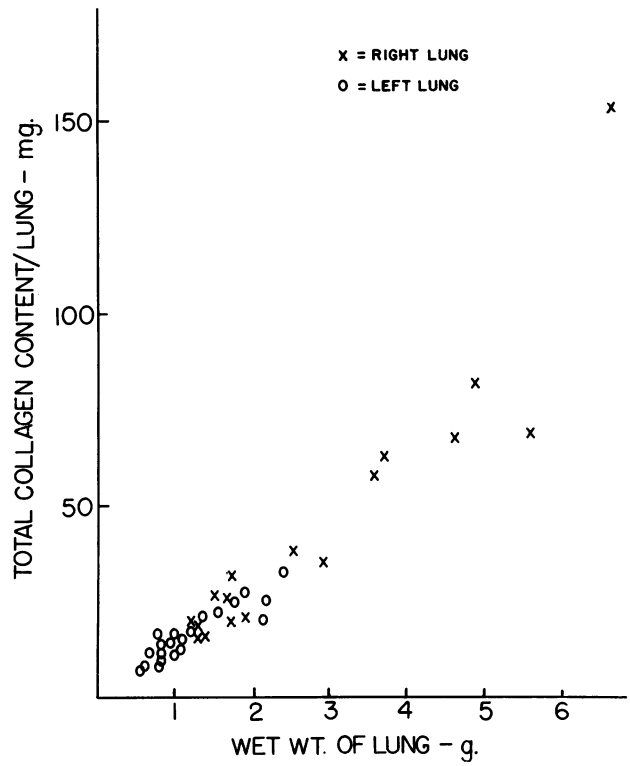

FIG.—Graph to show linear relationship between the wet weight of lungs from normal, silicotic, and BAPN-treated silicotic rats and their total collagen content; correlation coefficient—0·95.

Effect of duration of BAPN treatment

The previous results indicate that BAPN treatment of silicotic rats produces a fall in the weight and total collagen content of the lungs whilst the total body weight of the animals is increased. Since most of the injected quartz remained in the lungs (Nagelschmidt, Nelson, King, Attygalle and Yoganathan, 1957) providing a continuous stimulus to fibrosis, it was desirable to know whether the maintenance of the reduction in weight and collagen content required continuous BAPN treatment. In order to answer this, BAPN treatment was given to silicotic rats in the second experiment, at the beginning, the end and throughout the 150 days of the disease (see Materials and Methods). It was evident that in order for BAPN treatment of silicotic rats to lower their lung weights and consequently the amount of lung collagen significantly, it was necessary to maintain treatment over the whole period of the experiment (Table VI).

TABLE VI.—*Effect of Varying the Duration and Timing of BAPN Treatment on the Fresh Lung Weight of Rats after Silicosis Lasting* 150 *Days*

Experiment	Treatment	Fresh weight of both lungs ± s.e.m. (g.)	*P* values
1	Normal controls	1·35 ± 0·08 (6)	0·50–0·40
	BAPN-treated controls	1·44 ± 0·06 (9)	
2	Silicotic controls	4·67 ± 0·58 (10)	0·40–0·10
	Silicosis treated with BAPN for first 40 days	5·58 ± 0·57 (13)	
3	Silicotic controls	5·58 ± 0·50 (5)	0·20–0·10
	Silicotics treated with BAPN for last 40 days	4·68 ± 0·31 (13)	
4	Silicotic controls	4·88 ± 0·34 (9)	< 0·05
	Silicotics treated with BAPN continuously	3·76 ± 0·30 (19)	

Number of lungs used indicated in parentheses.

DISCUSSION

Various factors influence the degree of fibrosis produced in experimental pulmonary silicosis such as the type of silica used (Englebrecht, Yoganathan, King and Nagelschmidt, 1958; Saffiotti, Tommasini and Mayer, 1960), its particle size (Goldstein and Webster, 1966), the amount of silica injected (Chvapil and Holusa, 1966), the amount retained by the lungs (Nagelschmidt *et al.*, 1957), and the duration of the experiment. Given these variables, it is therefore difficult to compare the studies of different workers quantitatively. Nevertheless, the silicotic picture produced in the present study, using criteria of histology, fresh weight and collagen content of the lungs, accords well with that generally reported in previous studies.

The finding that one lung tends to suffer more than the other (Goldstein, Webster and Sichel, 1962; Goldstein and Webster, 1966) was confirmed; its occurrence probably reflects the asymmetry of bronchial anatomy in the rat. The close relationship between fresh lung weight and collagen was also confirmed (Goldstein, *et al.*, 1962; Chvapil and Holusa, 1966); their linear relationship was considered close enough to be of practical value.

This study is not primarily concerned with either the pathogenesis or the

inhibition of silicosis, but it is rather an attempt to see whether the control of pathological fibrosis is feasible, particularly since it has been shown that it is possible to vary the rate of healing of divided tendons by the use of drugs (Morcos, 1962) and to prevent cirrhotic fibrosis of the liver in carbon tetrachloride-treated mice by the administration of a lathyrogen, amino-acetonitrile (Fiume and Favilli, 1961). In the case of silicosis, any attempt at inhibition must take account of the multiple pathogenetic factors of the disease, and the role of macrophages, fibroblasts, plasma cells and mast cells and the formation of fibrous tissue (Saffiotti, 1960, 1962; Saffiotti *et al.*, 1960; Vigliani and Pernis, 1963); based on this scheme varying degrees of success and failure have been recorded using chelating agents (Daniel-Moussard and Collet, 1960), aluminium compounds (James, Morris and Marks, 1960), the polycation oligo-N-methyl-morpholiniumpropyleneoxide (Frimmer, Hegner and Lukas, 1965), cortisone and its derivatives (Marenghi and Rota, 1954, Dinischiotu *et al.*, 1961, Rodkina, 1965; Talley and Burrows, 1963), polyvinylpyridine-N oxide (Schlipköter and Brockhaus, 1960; Beck, Santer, Bruch and Brockhaus, 1964; Chvapil, 1966; Stalder, Lawaczek and Liefländer, 1966) the lathyrogen amino-acetonitrile (Cavagna, Amante and Finulli, 1962; Stramignoni, Terracini, Battistini and Massobrio, 1964). In the case of amino-acetonitrile the reported results vary; Cavagna *et al.* (1962) found that it had no effect on either the histology or the collagen content of silicotic nodules of the liver and spleen of the mouse, but that it did reduce the collagen content of the normal liver and spleen; Stramignoni *et al.* (1964) found that treating rats which had been suffering from pulmonary silicosis for 120 days, with amino-acetonitrile, did not modify the histological picture. In the present study we have shown that BAPN will reduce the amount of collagen in a silicotic lung whilst increasing body weight, and that the most sensitive index of this BAPN effect is a drop in either fresh weight of the lungs or the collagen content, whereas the histology failed to show marked differences and appears to be of no assistance as an index at this level. We have also indicated that BAPN treatment must be continuously maintained for fibrosis to be affected; this confirms the results of Talley and Burrows (1963) who showed that whilst they could arrest the fibrotic process in mice suffering from silicosis of the liver by treating them with hydrocortisone, any cessation of treatment resulted in further fibrosis, since the original cause of the fibrosis, the quartz, still remained.

BAPN is known to inhibit the formation of the interchain cross-link essential to the formation of the tropocollagen molecule (Bornstein and Piez, 1966; Rojkind, Blumenfeld and Gallop, 1966; Bensusan, 1966; Fessler and Bailey, 1966) but it does not inhibit collagen synthesis (Levene and Gross, 1959; Levene, 1967); how then does it diminish the collagen content of the silicotic lung? One possible explanation is that a pathological, imperfectly cross-linked collagen may turn over more rapidly than normal collagen, whose turnover in the normal rat lung is particularly slow (Pierce, Resnick and Henry, 1967).

Rats with pulmonary silicosis are reported to accumulate considerable amounts of mucoproteins in their lungs (Shnaidman, 1966); we have confirmed this and also found a diminition in the amount of PAS-positive material in the lungs of our BAPN-treated rats, whereas in other systems BAPN usually increased the amount of PAS-positive material present (Hoof, 1963; Churchill, Gelfant, Lalich and Angevine, 1955; Krikos, 1964; Krikos and Orbison, 1960); we cannot explain this discrepancy on the basis of the present data.

In this study, the lungs of BAPN-treated animals seemed to suffer from fewer major lung abscesses than the control silicotics but the insufficient number of animals does not allow any general conclusion to be drawn on this issue. The whole problem of the significance of intercurrent inflammatory processes in the course of experimental silicosis needs to be further investigated. Many of the cellular reactions attributed to the effects of silica from studies in rats (*e.g.* Saffiotti *et al.*, 1960) probably represent a complex interaction of responses to silica and to various infectious agents. An assessment of the response to silica alone would be possible using germ-free animals.

SUMMARY

An attempt was made to inhibit pathological fibrosis by chemical means; as a model system of a severe, progressive fibrotic conditon, pulmonary silicosis was produced in rats by the intratracheal injection of quartz, resulting in the typical histological picture of silicosis, a drop in body weight, and a rise in the fresh weight and collagen content of both lungs.

Treatment of silicotic rats with β-aminopropionitrile, the lathyrus factor, significantly lowered the lung weight and its collagen content, whilst increasing the animals' body weight.

The partial arrest of the fibrotic process was maintained only so long as BAPN treatment was continued.

It is concluded that eventual control of the fibrotic process is a feasible goal.

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