Virchows Arch. A Path. Anat. and Histol. 372, 89–95 (1976)

Virchows Archiv A

Myocardial Degeneration in Mice Treated with Dibutyryl Cyclic AMP and/or Theophylline*

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Summary. Fibrinoid degeneration of myocardial fibrils was induced in BALB/c mice treated from 2 months of age with weekly injection of dibutyryl cyclic AMP and/or theophylline. No evidence of cellular reaction or vascular occlusion was found.

Key words: Myocardial necrosis – Cyclic AMP.

Introduction

Cyclic AMP (cAMP) is the second membrane messenger in many hormonal (Boston and Perlman, 1971) and immune (Watson, 1975) reactions. In vitro experiments have furthermore shown that exogenous cAMP may alter the phenotype of some malignant cells to that of normal cells (Gazdar et al., 1972). Trying, in vain, to use cAMP in vivo as an anti-leukemic agent we found myocardial lesions in mice treated with a synthetic cAMP which enters cells (Falbriand et al., 1967), and/or with theophylline, which inhibits the phosphodiesterase-degrading endogenous cAMP (Boston and Perlman, 1971). This observation may be relevant to human pathology since it is believed that a state of chronically enhanced level of cAMP may occur in human disease (Exton and Park, 1968).

Material and Methods

Inbred female BALB/c mice and AKR mice (Staats, 1972) were fed mouse pellets and water ad lib. At 2 months of age they started receiving a weekly i.p. injection of 25 or 500 μg N^6-2,0-dibutyryl-A 3.5 MP, Cat. No. 51205 NAAT, Boehringer Mannheim, and/or 25 μg theophylline, Pharmacopea Danica. At the start of pharmacon treatment one group of BALB/c mice were infected with 10^4 XC units of a Rauscher leukemia virus (Rauscher, 1962). This batch had been tested by Microbiological Associates, Bethesda, Md., and by blind passage in newborn mice to be free of contamination with the cardiotropic (Woodruff and Woodruff, 1974) coxsackie virus.

* This investigation was supported by the Danish Heart Association, Rigsforeningen til Gigtens Bekæmpelse, and Daell Fonden

The author is indebted to Dr. Fischer Hansen for critical reading of the manuscript

Table 1. Myocardial degenerations in AKR and BALB/c mice treated with cAMP and/or theophylline

Strain	Group	Treatment (once a week)	No. of mice	Mean survival time ± s (months)	No. of cor investigated	No. with cardiac degenerations	χ^2 P with Yates' correction
BALB/c	A	Diluent	20	Killed	20	0	
		Theophylline 25 µg	10	10 months	8	1	
		cAMP 25 µg	10	old	9	3	<0.01
		cAMP 500 µg	10	(not ill)	10	7	<0.01
		The +AMP 25+ 25 µg	10		9	4	<0.01
		The +AMP 25+500 µg	10		8	5	<0.01
	B	Diluent	10 (8)[a]	Survivors (2)[b]	10	0	
		Theophylline 50 µg	10 (9)	Killed (2)	10	3	
		cAMP 1000 µg	10 (7)	18 months (2)	10	2	
		The +cAMP 50+1000 µg	10 (5)	old (6)	10	5	<0.01
	C	Rauscher leukemia virus once before pharmacon		Died ill with virus-induced leukemia			
		Diluent	20	7.9±3.6	13	1	
		Theophylline 25 µg	15	8.2±4.2	10	4	
		cAMP 25 µg	15	10.2±4.7	11	8	<0.01
		cAMP 500 µg	15	8.5±3.0	12	8	<0.01
		The +AMP 25+ 25 µg	15	7.7±2.7	12	8	<0.01
		The +AMP 25+500 µg	15	9.9±3.1	10	8	<0.01
AKR	D			Killed ill with spontaneous leukemia			
		Diluent	20	6.6±1.4	18	0	
		Theophylline 25 µg	10	6.6±1.1	6	1	
		cAMP 500 µg	10	5.3±0.7	10	3	<0.01
		The +AMP 25+500 µg	10	6.3±0.3	7	1	

[a] Survivors [b] Leukemic

The animals were observed 6 days a week and killed when ill. Lung, liver, spleen, kidney, lymph nodes, thymus, thyroid gland, diaphragm and four pieces of cor were taken for microscopy. Fixation was done with Lillie's acetic acid-alcohol-formalin solution, and paraffin embedding was used. All organs were stained with H & E and periodic acid-Schiff (PAS). Hearts showing abnormalities were furthermore stained with PAS following pretreatment with α- plus β-amylase (Sigma AG, Cat. No. A-7655) and/or saliva, and also stained with toluidine blue 0.1% at pH 3 (Reintoft and Christensen, 1972), Mowry's alcian blue technique at pH 2.5 and the colloidal iron staining a.m. Hale in Mowry's modification, and Frazer-Lendrum staining with Picro-Mallory and Mallory's phosphotungstic acid haemotoxylin (PTAH), alcaline Congo red, Ziehl-Neelsen, thionin pH 3 and Sudan black B.

Results

Treatment with cAMP and/or theophylline much increased the incidence of usually peripherally located areas of myofibril degenerations in the cor (Fig. 1). The involved myofibrils contained intracytoplasmatic granular diastase and pepsin-resistant PAS-positive material under an intact sarcolemma. The fibrils also stained with Frazer and sometimes with PTAH, but not with the other stains used. Inflammatory cells and thrombi were not observed. Degenerating myofibers were not detected in the idaphragm.

Myocardial lesions occurred independent of leukemia in uninfected parmacon-treated BALB/c mice although treatment for a very long period with both theophylline and cAMP seemed to provoke leukemia as well as myocardial lesions in these mice (group B). The 55 leukemia virus–infected, pharmacon treated

Fig. 1. Myocardium of 10-month-old BALB/c female mouse treated for 8 months with cAMP plus theophylline. Darker staining myofibrils occur in a focal area and dispersed among normal-appearing myofibrils of peripheral part of left ventricular wall. PAS technique $\times$ 140

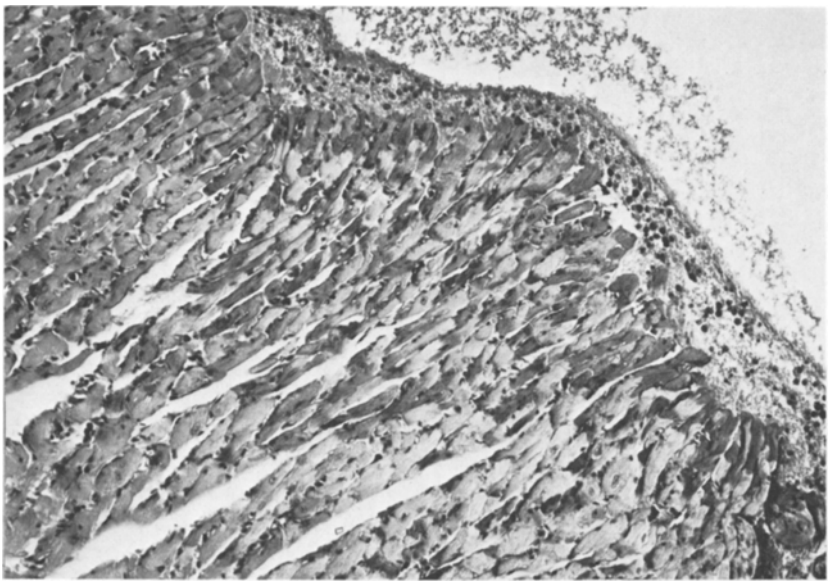

Fig. 2. Myocardium of 12-month-old BALB/c mouse treated with cAMP for 10 months. In this case the darker-staining fibrils lie mixed with normal fibrils near the central lumen. PAS technique ×140

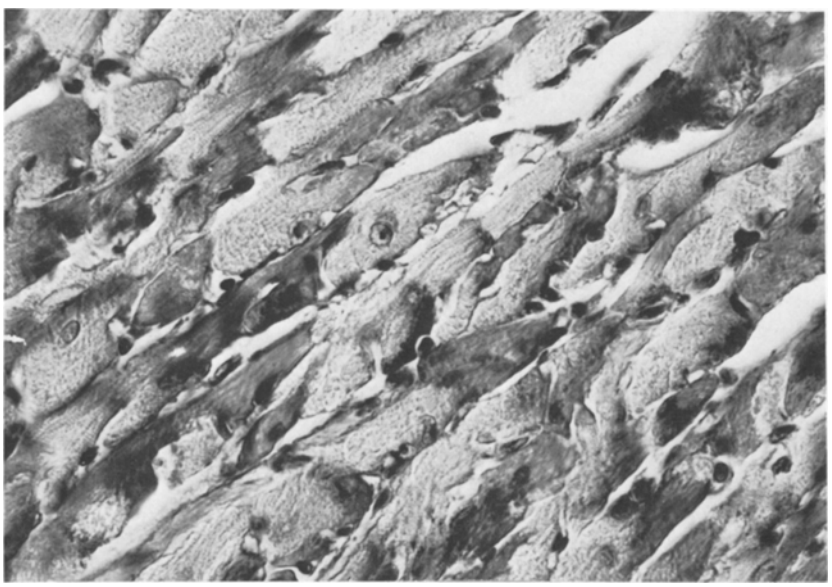

Fig. 3. Myocardium of same mouse as in Figure 2. Degenerated fibre with amorphous/granular appearance. Note normal morphology of adjacent fibres and no cellular infiltration. PAS technique ×2100

BALB/c mice that succumbed to leukemia at 8–12 months of age (group C) possibly had a higher ($P<0.05$) incidence of myocardial lesions than the 44 uninfected, pharmacon-treated BALB/c not ill mice which were killed at the age of 10 months (group A). All AKR mice developed leukemia, but only 5 showed degeneration of myocardial fibrils (group D).

cAMP and theophylline had no effect on survival time or leukemia development whether in Rauscher virus–infected BALB/c mice or in AKR mice developing spontaneous leukemia (groups C and D).

Discussion

We hold the myocardial lesions not to be post-mortem artefacts, firstly, because the mice were killed and the autopsy completed within a few minutes, secondly, because nearly all lesions occurred in pharmacon-treated animals, and thirdly, because the lesion is PAS-positive, and one of the alterations occurring during the first post-mortem hour is a loss of PAS stainability (Yokoyama et al., 1955).

The staining reactions indicate fibrinoid degeneration, with no evidence of glycogen, intracellular plasma glycoprotein (Kent, 1967), acid mucopolysaccharides, calcium, lipofuchsin (brown degeneration) or amyloid.

With the occurrence of cardiac lesions virtually restricted to the pharmacon-treated animals we must hold the pharmacons responsible. Since both exogenous cAMP and theophylline, which elevates the concentration of endogenous cAMP, are effective, we believe cAMP to be involved in the pathogenesis. cAMP has a positive inotropic effect like that of β-adrenergic catacholamines, is believed to mediate the effect of catacholamines (Sutherland and Robinson, 1966), and is involved in normal myocardial functions (Kukovetz and Pöch, 1970; La Raia and Morkin, 1974; Fabiato and Fabiato, 1975; Nawrath, 1976). Abnormal concentrations of cAMP may occur in pathologic conditions of the heart (Limas et al., 1974; Amer et al., 1974). A direct toxic effect on the myocardial fibers due to our chronic treatment with cAMP appears likely, since chronic treatment with catecholamines may produce multiple focal myocardial necroses (Selye, 1958; Rona et al., 1959). Occlusion of minute vessels leading to a late secondary PAS-positive hyalinization (Yokoyama et al., 1955) and/or an effect via an endocrine organ (Poisner, 1971), however, cannot be ruled out.

Leukemic cells can contribute to murine myocardial degeneration (Ebbesen et al., 1970), and leukemia virus may cause non-leukemic lesions (Durin and Arleigh, 1966; Ebbesen and Rask-Nielsen, 1967; Gardner et al., 1973). Leukemic cells or leukemia virus may therefore have contributed to our finding of the highest incidence of myocardial lesions in pharmacon-treated, virus-infected leukemic BALB/c mice.

Development of the degenerative lesions of secondary amyloidosis is also enhanced by treatment with cAMP (Ebbesen, 1974).

The lacking effect of cAMP on in vivo leukemia development is in accordance with some (Curtis et al., 1974) but at variance with other (Gericke and Chandra, 1969) reports on tumor growth in animals receiving exogenous cAMP.

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Received August 3, 1976