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Original Articles

Cardiomyopathy in Leukemia, with Reference to Rubidomycin Cardiotoxicity

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Summary. *Sixteen patients who had died with leukemia were studied at autopsy between September 1975 and February 1977. Special attention was given to degenerative changes in the heart. Five of the patients died of cardiac failure, all with no or only slight leukemic infiltration in various organs at autopsy. Five patients showed basophilic necroses in the myocardium, and two of these also showed necroses in the bone marrow. The myocardial lipofuscin was significantly ($P < 0.01$) higher in the autopsies of leukemic patients (mean age 45 years) than in autopsies performed at the Department of Forensic Medicine in 18 cases of accidental death (mean age 36 years). No dose-response relationship could be found between the amount of myocardial lipofuscin and the total dose of rubidomycin. Eight of twelve patients with malignant lymphoma (mean age 45 years) also had increased amount of myocardial lipofuscin.*

Introduction

Infiltration of malignant cells in the myocardium has long been considered the most important cause of disturbed cardiac function in leukemia and lymphoma [26, 29, 30]. The ECG findings have been attributed to infiltration and hemorrhages in the heart [21]. Viral myocarditis complicating leukemia has also been recognized [34]. Since the introduction of potentially cardiotoxic drugs in leukemia [1] the cardiac function has again been highlighted [33]. Indirectly, hypokalemia, which is common in leukemia [18, 23], might also influence the cardiac function. The purpose of this study was to estimate the effects of leukemic infiltration, rubidomycin, and hypokalemia on the myocardium in leukemia through a careful search for histological evidence of damage to the myocardial cells.

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Materials and Methods

Leukemia

Twelve patients with acute myeloid leukemia, two with acute lymphatic leukemia, and two with chronic myeloid leukemia, one of whom had a blastic transformation, were investigated at autopsy (Table 1). The study was consecutive and lasted from September 1975 to February 1977. Some of these patients have been described elsewhere [19]. The mean age of the whole group was 57 years, and that of the seven patients born in 1920 or later was 45 years.

Controls

Lymphoma. From an earlier study [19], 12 patients under 60 years of age and with lymphoma were studied. Their mean age was 46 years (Table 1). This group is included only for the purpose of determining the role of cytostatics on the myocardial lipofuscin content.

Forensic Cases. Twenty randomly chosen forensic cases served as controls. Two of them were later excluded since they had cardiac disease. The cause of death in the remaining 18 cases was suicide or accident. Their mean age was 36 years.

Heart

At autopsy the hearts were weighed and routine macroscopic dissection was performed. Several specimens were taken for histology from the walls of ventricles and auricles. Histologically, the specimens were scrutinized for leukemic infiltration, evidence of infection, vacuolization of myocardial cells, necrosis, hypertrophy or hypotrophy of the cells, and amount of lipofuscin deposition.

For determination of the relative amount of lipofuscin, sections were taken from the left ventricular myocardium and embedded in paraffin as routine specimens: 5-μm sections were made and stained with hematoxylin-eosine. The lipofuscin pigment in the heart muscle was identified by staining with periodic acid-Schiff, Sudan Black B, and hematoxylin-eosin. Routine iron staining was negative.

The hearts from the controls were prepared in the same manner as described above. Forensic control sections were mixed with the sections from the leukemic patients and all sections were counted blind by the same pathologist.

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Table 1. Description of the patients

Diagnosis	Number of patients	Sex		Mean age (range) in years	Mean duration of disease (range) in months
		M	F		
Leukemia		11	5	57 (29–74)	13[a] (1–48)
Acute non-lymphatic	12				
Acute lymphatic	2				
Chronic myeloid	2				
Lymphoma		8	4	46 (28–56)	51 (10–204)
Hodgkin's	6				
Non-Hodgkin's	6				

[a] Preleukemic state excluded in two patients

An integrating disc with a grid containing 100 intersections (Leitz integrating disc II) was used, and counting of the lipofuscin pigments was performed under 800 × magnification with a regular light microscope. The intersections not overlying myocardial fibers, interstitial tissue, or lipofuscin pigments were left out.

In each section, 10–12 randomly selected visual fields were counted, making the total number of 'effective' intersections per section about 1000. As a check on the reliability of the counting process, 40 visual fields were counted in two different sections and the result proved to be normally distributed.

In all patients a subjective estimation of the lipofuscin content was done by grading + (normal), ++ (normal to increased), and +++ (increased).

Pulmonary Edema

The presence of pulmonary edema was determined by macroscopic investigation, weight, and the microscopic picture. Pulmonary edema was graded according to the findings: 0 (none), ++, and +++.

Clinical data were taken from the charts and from the clinical laboratory. In two cases only incomplete clinical charts were available.

Results

Lipofuscin

The individual lipofuscin values are shown in Table 2. The mean relative number of lipofuscin pigment grains in the leukemia group was 15.7 ± 6.0 (SD), compared with a mean of 7.7 ± 3.9 (SD) in the forensic control group. The difference is highly significant ($P < 0.001$). Since the amount of lipofuscin pigment is age-dependent a separate comparison was made including only patients born 1920 and later. Their mean lipofuscin value was 15.6 ± 6.4 (SD), and this difference is also significant ($P < 0.01$).

There is fairly good agreement between the semiquantitative values and the subjective estimation (Table 2). This fact is therefore used in the lymphoma controls (Table 3).

Table 2. Myocardial lipofuscin count in leukemia

Patient	Lipofuscin counting			Subjective estimation
	Number of points (N)	Effective intersections[a] (E)	N/E × 100	
1	41	966	4.2	+
2	247	1010	24.5	++
3	202	953	21.2	+
4	122	925	13.2	++
5	151	986	15.3	++
6	139	1000	13.9	+++
7	160	958	16.7	++
8	152	904	16.8	++
9	178	940	18.9	++
10	126	871	14.5	++
11	145	948	15.3	++
12	70	995	7.0	+
13	210	994	21.1	+++
14	126	890	14.2	++
15	269	1026	26.2	++
16	78	1000	7.8	+

[a] Produced by Leitz integrating disk II
Patients 1–7 were born in 1920 or later

Table 3. Relationship between myocardial lipofuscin in lymphoma (< 60 years), and duration of disease, hypokalemia and relevant cytostatics

Patients		Lipofuscin	Duration mean	Hypokalemia	Cytostatics Mean value in mg	
n	Age (mean)				Anthracyclines[a]	Cyclophosphamide
4	48	+	90 months	0%	192	7,700
8	45	++	57 months	38%	370	9,500

[a] Adriamycin and rubidomycin

Myocardial Damage

Two cases had signs, seen clinically and at autopsy, of coronary heart disease; one had an old infarction and the other a diffuse myocardial fibrosis. One patient had a recent infarction, near which there was a massive leukemic infiltration. A massive leukemic infiltration was seen in one other patient, while six further patients had slight leukemic infiltration in the myocardium.

Five patients had small basophilic necroses of the myocardial cells. These necroses were not associated with infarctions. One patient with candida septicemia had disseminated septic embolism including the heart. One case had fibrinous pericarditis with leukemic infiltration. Two other patients had pericardial leukemic infiltration.

Hypotrophy – subjectively estimated – of the myocardial cells was seen in seven cases. Vacuolization of the myocardial cells was seen in two cases.

Cause of Death

Nine patients had no or slight general leukemic cell infiltration, which was judged to be insufficient to cause death. Five patients had signs of cardiac and circulatory failure as the main cause of death, and in one case this was a contributory cause. In nine patients organ congestion was found. Three of these did not have signs of left ventricular failure on X-ray before death. Septicemia was found in five patients and fatal hemorrhage in five cases.

Clinicopathologic Correlations

In Fig. 1 the lipofuscin values are plotted against the total dosages of rubidomycin. There is no statistical dose-response correlation.

Patients with pulmonary edema graded 0 had a mean lipofuscin value of 11.8 and those with pulmonary edema graded +++ a value of 17.4. However, there was no statistically significant difference.

The five patients with basophilic necroses all had high lipofuscin values (mean 19.6). Three of these patients also had signs of cytostatic damage in a triple bone marrow sample [19]. One patient had not been given rubidomycin but a total of 21.1 g cyclophosphamide. The other four patients with basophilic necroses had been given a mean of 550 mg rubidomycin in total. The five patients who had signs of cardiac failure as a major cause of death displayed high lipofuscin values (mean 18.7). Two of them had basophilic necroses in the myocardium. A mean total dose of 574 mg rubidomycin had been given to the patients with cardiac failure, and they all had only slight general leukemic infiltration or none at all.

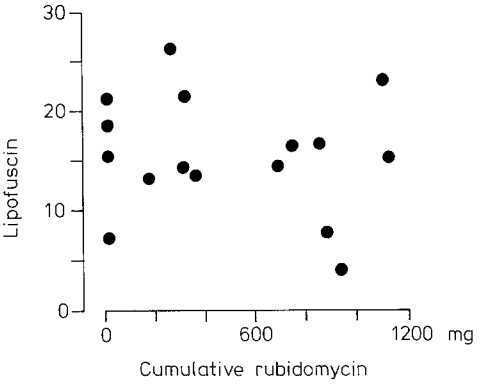

Fig. 1. Absence of correlation between accumulated rubidomycin dose and myocardial lipofuscin. $r = -0.12$

Hypokalemia occurred intermittently in 8 of 14 patients. In eight patients the total body potassium was studied [18]. Sixteen measurements were made, five patients being measured more than once. Five of the eight patients had a decreased value for potassium in the lean body mass in one or more measurements, and one had an increased value. Of these five patients, four had a high lipofuscin and one a normal lipofuscin value.

Among the patients with lymphomas, hypokalemia occurred only in those judged to have an increased amount of lipofuscin (Table 3).

Discussion

The results show significantly higher myocardial lipofuscin levels in leukemic patients born in 1920 and later than in the forensic cases. The mean age is 9 years higher in the leukemic group, but the difference in lipofuscin content is too large to be explained by this difference.

The method used in this study to determine the amount of lipofuscin is not as specific as the techniques based on fluorescence and electron microscopy. However, this method has been used before [7, 32], and we feel that the comparison with the forensic cases permits at least a semiquantitative conclusion about myocardial lipofuscin in leukemia.

The increased lipofuscin content in the myocardial cells seems to be a sign of cardiomyopathy, since the patients with basophilic necroses, which are a reported sign of cardiotoxicity [3, 4, 20], all had high lipofuscin values. Other subgroups, which partly overlapped with each other, namely cardiac death and massive pulmonary edema, also had high lipofuscin values.

Our hypothesis is that an increased myocardial lipofuscin content reflects a cardiomyopathy in leukemia. If this hypothesis is valid, cardiomyopathy (clinical or subclinical) is much more common in leukemia than has been reported [33].

The results indicate that rubidomycin (Fig. 1) and other cardiotoxic drugs are not the only causes of increased lipofuscin accumulation in the hearts of leukemia and lymphoma patients. Potassium depletion might be an additional cause. In lymphoma the possibility of radiation-induced heart disease must be considered [12].

It is not surprising that a dose-response relationship is lacking between the myocardial lipofuscin content and the total rubidomycin dosage, since lipofuscin accumulation is the result of various factors, as indicated below. Since lipofuscin accumulation is age-dependent and an absence of lipofuscin is regularly found in the hearts of subjects below the age of 10 years [32], it would be of interest to study patients in childhood leukemia from this angle.

The individual susceptibility to cardiotoxic drugs shown in these results and elsewhere [2] remains to be explained. Possibly hypokalemia [11], which is common in leukemia [18, 23], might contribute to the cardiac damage in leukemia and also help to explain the obvious individual sensitivity to rubidomycin. The hypothesis that the cardiotoxicity of anthracyclines depends on the digitalis-like properties [6, 15] supports the suggestion that electrolyte disturbances must be considered.

Myocardial damage might also be caused by the leukemic infiltration per se in the myocardium. Eight out of 16 patients had infiltration in the heart; in one case this was associated with infarctions. In the other cases the degree of leukemic infiltration was rather limited, however. This traditional explanation of cardiac dysfunction seems to play a minor role in leukemia compared with the cardiotoxicity due to cytostatics and electrolyte disturbances.

This study is the first, as far as the authors know to report an increased lipofuscin content in the myocardium of leukemia patients in general.

Lipofuscin is known to exist in most mammalian tissues. It has been shown to increase with age in neurons in humans [8] and in myocardial fibers in humans [32]. Certain reports are consistent with the fact that lipofuscin accumulates in nondividing cells such as neurons and myocardial cells [8, 13]. Lipofuscin granules are generally thought to be secondary lysosomes containing indigestible material with its origin in autophagolysosomes [9, 11, 13, 16].

This autophagocytosis occurs physiologically in normal cells as a mechanism of normal turnover of cell constituents [11]. Increased autophagy occurs in cells subjected to sublethal injury of various forms, including hypokalemia and vitamin E deficiency and also certain drugs and other agents [11, 35]. According to de Duve [9], increased autophagy is found in stressed cells. Müntzing and Nilsson [24] found numerous lipofuscin granules in malignant prostatic tissue after treatment with estrogens. Before therapy there had not been any visible lipofuscin in the tissue.

Increased amounts of lipofuscin are reported by Hibbs et al. [16] in the hearts of rats in which a myocardial injury was produced by the administration of epinephrine. They also found increased amounts of lipofuscin in human hearts with cardiomyopathy due to primary myocardial disease; this has also been reported by others [31].

Since lipofuscin is thought to accumulate as a result of sublethal injury to the cell, the increased amount of lipofuscin could also be interpreted as a marker of pathologic injury (acute or chronic) to the cell.

The concequences of the accumulation of lipofuscin in the myocardium are unknown. Many authors claim that the accumulation is linked to impaired function [31, 32], but there is still very little proof for this.

It is by now well known that anthracyclines are cardiotoxic in humans [3, 20, 22, 28]. Besides anthracyclines, cyclophosphamide in massive, intermittent doses has recently been reported cardiotoxic [5, 27].

Cardiovascular failure associated with anthracyclines is usually seen after a period of treatment, and can probably even develop after the treatment is ended [14], but is also encountered with a relatively acute onset [3, 20, 22].

Anthracycline-induced heart and circulatory failure is usually very resistant to conventional treatment [3, 22].

At autopsy, patients treated with anthracyclines and with cardiovascular failure display different kinds of degenerative changes. Histologically focal necrosis, usually basophilic, in the myocardium is the most commonly reported alteration in humans [3, 4, 14, 20]. This kind of necrosis has also been seen in animals treated with anthracyclines [17]. Activation of the interstitial tissue in the heart [20] and atrophy of the cardiac muscle cells [3, 4] are other findings. Ultrastructurally, myofibrillar degeneration, mitochondrial degeneration, and chromatin changes in the nuclei have been reported [3, 4]. Buja et al. [4] found higher myocardial lipofuscin in patients with leukemia treated with rubidomycin than in a control group. None of these changes, however, is specific for myocardial lesions produced by anthracyclines; all of them are also seen in other conditions [4, 5]. ECG changes linked to cardiotoxic drugs have been described [14, 20, 28, 33] but some of these changes, e.g., prolonged QT time have also been described in leukemia with toxoplasma myocarditis [34]. The knowledge of lipofuscin built up over the last century has been looked at with new interest following the lysosome research. Theoretically, there is a relationship between the lyso-

somes and lipofuscin. In recent pharmacological studies it has been demonstrated that the anthracyclines are lysosomotropic [10]. In fibroblasts these agents pass into the cell by endocytosis [25]. It is not known whether endocytosis also occurs in the myocardial cells or whether the receptor theory is applicable to them [25].

Conclusions

1. This study suggests that leukemic infiltration of the heart plays a minor role in cardiac fatalities in leukemia with modern treatment and that myocardial damage due to cytostatics and electrolyte disturbances is of more importance.

2. Leukemic patients have higher levels of lipofuscin in the myocardium than controls and this can be considered a sign of cardiomyopathy, which therefore seems to be more common in leukemia than previously thought.

3. No relationship could be seen between the total amount of rubidomycin given to the leukemic patients and the level of heart lipofuscin.

4. Determination of the amount of lipofuscin permits an estimation of the degree of nonspecific myocardial involvement.

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