Alveolar type II cells in lungs of guinea pigs exposed to vinyl chloride and varying levels of vitamin C

Marian L. Miller, in memory of Martha J. Radike.

Abstract

Vinyl chloride exposure in guinea pigs, concomitant with the administration of either 2 of 50 mg vitamin C, increased the variability in size of lamellar bodies in alveolar type II cells. Very large lamellar bodies appearing amongst other more typically sized lamellar bodies. Tumor production was minimal (several papillary type lesions only in the vinyl chloride group), no tumors were found in liver.

Introduction

Vinyl chloride (VC) a known liver carcinogen, was investigated in guinea pigs receiving high and low amounts of vitamin C. It has been postulated that vitamin C will lower the incidence of tumors. Unlike the rat, however, liver tumors were not induced in guinea pigs by VC inhalation, and only small tumors in the lung were noted at autopsy. Tumors, and a random sample of the normal lung tissue were obtained for electron microscopy. The morphology and morphometry of the lung, the alveolar type II cell and in particular was examined.

One hundred and sixty male Hartley guinea pigs were exposed by inhalation to 600 ppm VC for 4 hours per day five days per week for one year. The specifics of this exposure are minimal as the experiment took place 25 years before this small report was put together. An equal number of age-matched animals breathed filtered air. Groups were subdivided as follows: eighty of the one hundred sixty animals from each group (exposed) and control both, were maintained on minimal vitamin C (2 - 10 milligrams vitamin C) and eight animals remaining received adequate vitamin C (50 milligrams per day. From these groups 18 were sacrificed for electron microscopy over a period of four months to 1.5 years post-exposure and were distributed among the groups as follows: vinyl chloride low vitamin C (VC/2mg C) n=4: VC/50mg C n=7, filtered air 2C (air/2mg C) n=1 (#301, the single guinea pig that was the basis of investigating intracisternal layered granules in alveolar type II cells - a huge outlier is reported elsewhere) : air/50mg C n=6. Animals were sacrificed after Nembutal anesthesia and tissues were minced in cold fixative, post fixed in osmium tetroxide, dehydrated and embedded in Plastic. One micron sections were stained with toluidine blue for light microscopy and ultrathin sections were placed on naked copper grids, stained with uranyl acetate and lead citrate and examined with an Elmiskop 1A.

Micrographs for morphometry were obtained at 5000 x mag at the scope using pole piece V (50%) and enlarged 4 x =10,000 x total. Minimum of six type II cells from each animal were photographed in an unbiased manner. The lamellar bodies (more than 700) from type II cells were digitized, and areas, perimeters, and surface/area ratios obtained and analyzed.

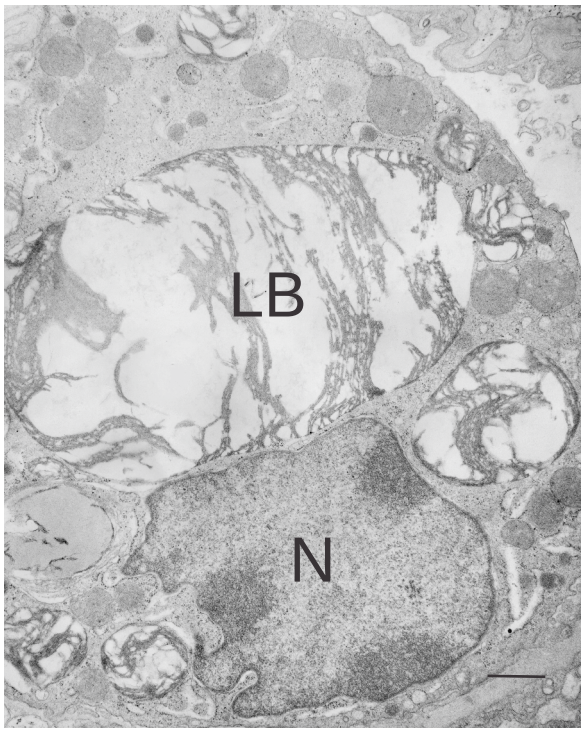
Results

Of the 18 animals of the 160 animals total, selected randomly, were examined for tumors at autopsy, and also used for electron microscopy. No tumors found in non-VC exposed animals. No tumors were observed in the liver. When tumors were found in lung, the architecture was papillary, and in most cases, histologically, cells were cuboidal to columnar and were all differentiated type II alveolar cells (i.e. they contained lamellar bodies, multivesicular bodies, mitochondria, RER and golgi and nuclei that were perhaps less dense with heterochromatin, suggesting some hypertrophy. But not all tumor cells contained lamellar bodies. Those without were usually retained their cuboidal shape and areas of glycogen and a blunted microvillar surface. There was no increase in the number of surfactant protein granules (dilated RER with highly layered protein content).

Examination of the remaining lung revealed diffuse changes in the type II alveolar cells. In three experimental groups, particularly in the two groups which received VC (with 2mg or 50mg vitamin C) some lamellar bodies were very enlarged even becoming as large as the profiles of the nuclei (Figure 1). These changes were visible with the light microscope, and this increase in size was not found in the vitamin C only group. Although fibrosis was common in guinea pig lungs (animals were 2.5 years old) calcium deposits in the basement membranes of the alveoli occurred in all groups, with no difference between the controls and the experimental. Large mitochondria which were neither necrotic nor pale were found in two animals from the highest exposure group, but a measure of the volume density of mitochondria overall was not made (Figure. 2)

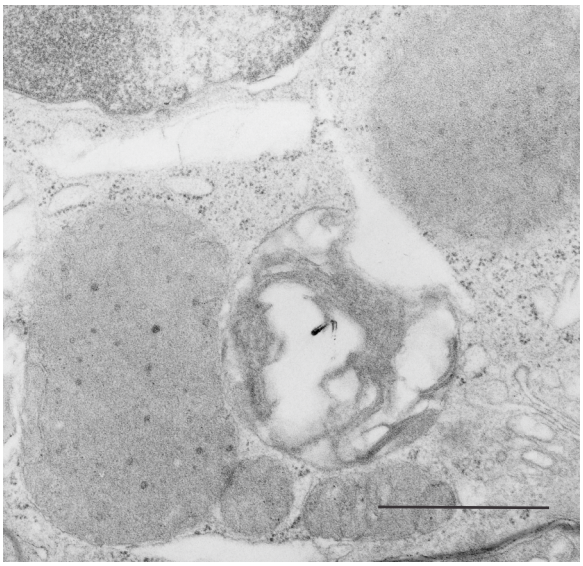
Table 1 Area of lamellar bodies in type II cells from lungs of individual animals
Areas are in square inches on enlarged micrographs (10,000 x)(stats in Figure 3)

VC /2mg C	VC/V50mg C	Air/50mg C
0.93+0.99	0.97+1.31	0.77+0.79
1.14+1.77	1.12+1.38	1.02+0.75
1.66+1.67	1.56+2.60	1.06+0.9
0.83+0.95	2.22+6.82	0.53+0.46
	0.84+0.63	0.45+0.35
	1.58+4.22	0.64+0.5
1.17+0.21	**1.381+0.2**	**0.75+0.1**
1.296		



n=6506, block M8039, guinea pig # 94, vinyl chloride/50mg vitamin C. Enlarged lamellar body, LB, nucleus N. magnification 10,000 x, bar=@ 1 micron.

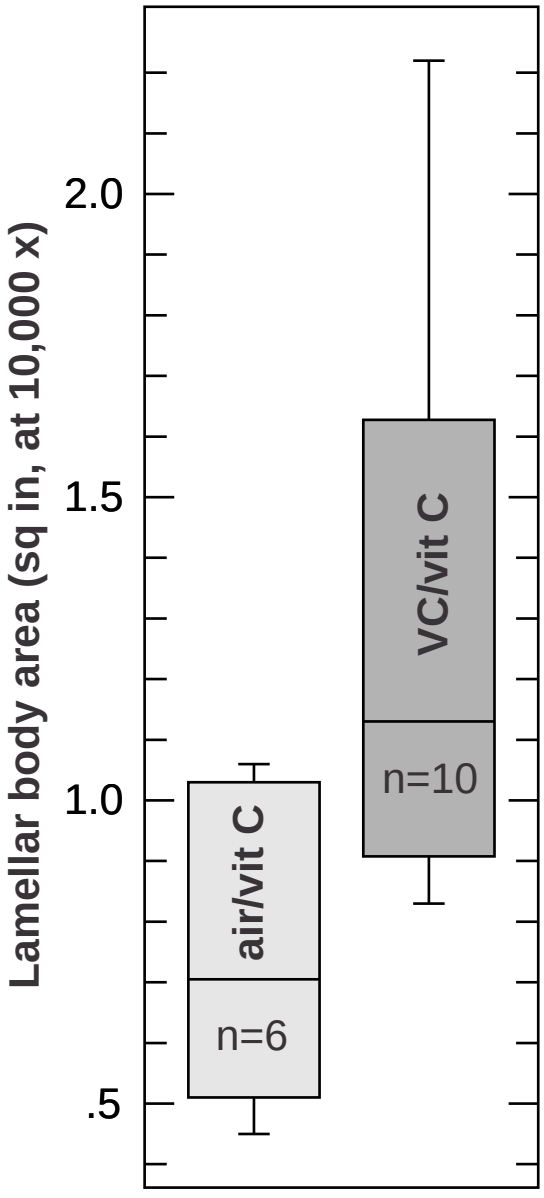
Figure 1



n=6524, block M8055, guinea pig # 121, vinyl chloride/50mg vitamin C. Enlarged lamellar body, LB, nucleus N. magnification 10,000 x, bar=@ 1 micron.

Figure 2

Figure 3



Student's t-Test: Results

The results of an unpaired t-test performed at 12:24 on 1-NOV-2016

t= -2.65
sdev= 0.403
degrees of freedom = 14 The probability of this result, assuming the null hypothesis, is **0.019**
Group A: Number of items= 6
0.450 0.530 0.640 0.770 1.02 1.06

Mean = 0.745
95% confidence interval for Mean: 0.3925 thru 1.098
Standard Deviation = 0.253
Hi = 1.06 Low = 0.450
Median = 0.705
Average Absolute Deviation from Median = 0.205
Group B: Number of items= 10
0.830 0.840 0.930 0.970 1.12 1.14 1.56 1.58 1.77 2.22

Mean = 1.30
95% confidence interval for Mean: 1.023 thru 1.569
Standard Deviation = 0.465
Hi = 2.22 Low = 0.830
Median = 1.13
Average Absolute Deviation from Median = 0.358

http://www.physics.csbsju.edu/cgi-bin/stats/t-test_paste.n.plot
http://vassarstats.net/tu.html