EFFECT OF MITOMYCIN C AND RUBOMYCIN C ON NUCLEIC ACID METABOLISM IN THE RAT LIVER

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The dynamics of changes in the content of RNA and DNA and in the activity of acid ribonuclease (RNase) and deoxyribonuclease (DNase) in the liver of rats was studied at various times after administration of LD_{50} of mitomycin C and rubomycin C (daunomycin). Both antibiotics sharply reduced the RNA content in the liver within a few hours of their administration. The DNA content remained virtually unchanged throughout the experiment (for 96 h). No correlation could be found between changes in the RNA content in the liver of the experimental animals and acid RNase activity. It is suggested that an important role in the disturbance of nucleic acid metabolism produced by both mitomycin C and rubomycin C is played by inhibition of RNA synthesis.

The antibiotics mitomycin C and rubomycin C (daunomycin) have been shown to possess marked antitumor activity. The mechanism of their action is claimed to be by the formation of a complex between these substances and DNA, with a consequent disturbance of nucleic acid metabolism in the cells [3, 6, 7, 9].

Mitomycin C has been shown to inhibit DNA and RNA synthesis, and at the same time to intensify DNA breakdown. The mechanism of this effect has not yet been explained: some workers attribute the increased DNA breakdown to activation of DNase, while others have found no increase in the activity of this enzyme [7, 10].

The object of this investigation was to study changes in the DNA and RNA content in the rat liver during administration of mitomycin C and rubomycin C to the animals and also to study the activity of acid DNase and RNase.

EXPERIMENTAL METHOD

Growing male Wistar rats weighing initially about 40 g were used. The animals received an intraperitoneal injection of a solution of mitomycin C (2.5 mg/kg body weight) or of rubomycin C (20 mg/kg body weight). The doses chosen correspond to LD_{50} for rats [1, 8]. The control rats received the equivalent volume of physiological saline. The animals were decapitated 1, 3, 6, 12, 24, 48, 72, and 96 h after receiving the antibiotics.

Pieces of liver were fixed in acetone and Carnoy's fluid and embedded in paraffin wax. From a general survey of the histological changes sections of the liver were stained with hematoxylin-eosin. DNA and RNA were detected by luminescence microscopy in sections stained with acridine orange by von Bertalanffy's method [5] and RNA was also detected by Brachet's method. As a control some sections

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TABLE 1. RNA Content in Liver of Rats Receiving Mitomycin C and Rubomycin C (in conventional units)

Time after administration (in h)	Mitomycin C	Rubomycin C
Control	48,6±0,70	49,7±1,51
1	39,5±0,51	25,7±1,70
3	22,5±4,30	20,3±0,83
6	28,0±0,70	25,2±1,31
12	21,2±3,31	18,5±1,33
24	20,6±0,84	18,5±0,69
48	21,0±0,70	26,4±1,40
72	24,1±1,00	26,1±0,97
96	25,2±1,11	39,7±1,42

Note. Mean results of seven experiments ($\bar{X} \pm S_{\bar{X}}$) are given; $P < 0.001$.

were treated with RNase. Sections stained with acridine orange were investigated cytophotometrically, using a photometric attachment to the luminescence microscope [4]. To measure the intensity of luminescence of RNA and DNA, interference filters with a transmission maximum at 600 and 520 nm respectively were used. The content of RNA and DNA was expressed in conventional units reflecting the intensity of luminescence of the sections. Activity of acid DNase and RNase was determined in liver homogenates by spectrophotometric micromethods based on the use of the ultramicrosystem of biochemical analysis developed by Pokrovskii et al. [2]. Activity of the enzymes was expressed in micromoles of substrate converted by 1 g protein per minute.

Material for electron-microscopic investigation was fixed in buffered osmium tetrachloride solution and embedded in Araldite.

EXPERIMENTAL RESULTS

The toxic action of mitomycin C and rubomycin C was manifested on the 2nd day after administration of the antibiotics and took the form of increasing adynamia and loss of weight of the experimental groups of animals. The greatest decrease in weight (by 30-40%) was observed at the end of the experiment (72-96 h), when it was combined with a marked decrease in volume of the animals' liver.

Macroscopically and in histological sections stained with hematoxylin-eosin, no changes were observed in the liver of the rats receiving mitomycin C or rubomycin C. However, cytophotometric estimation of the RNA content in the rats' liver revealed a marked decrease in RNA after administration of mitomycin C and rubomycin C (Table 1).

Histological examination of the sections stained by Brachet's method showed that the RNA content was reduced equally in all zones of the hepatic lobule after administration of mitomycin C.

Cytoplasmic RNA consisted of fine particles distributed diffusely or concentrated in small groups near the nucleus and at the periphery of the cytoplasm. In the latter cases, because of the irregularity of distribution of the RNA particles and their weak luminescence the cytoplasm of the hepatocytes appeared optically empty in fluorescent preparations. In such cases only the cell membrane and nuclei were luminescent. The nucleoli were hardly visible, small in size, or oval or slightly rectangular in shape.

After administration of rubomycin C the distribution of RNA in liver cells was either as particles or as a fine dust. The particles irregularly dsitributed in the cytoplasm and concentrated mainly at the periphery of the cell, less frequently near the nucleus. In fluorescent sections the cytoplasm of these cells appeared optically empty, for the rare RNA particles, reddish in color, were only dimly luminescent. As a rule the nucleoli of these cells were invisible, but the nuclear membrane was slightly thickened and gave a bright whitish fluorescence. Particles of chromatin were clearly visible, especially in fluorescent sections, where they gave a greenish luminescence.

No significant changes were found in these sections of the liver on investigation of their DNA content.

The dynamics of changes in the activity of the acid nucleases of the rat liver under the influence of mitomycin C and rubomycin C is shown in Table 2. During the first few hours after administration of mitomycin C the change in nuclease activity was slight, and not until after 24 h were definite differences discovered in their behavior: activity of acid DNase fell progressively to reach 54% of the control level after 96 h, whereas acid RNase activity was unchanged at these times.

The changes in enzyme activity in the animals receiving rubomycin C were rather different in character. Some increases in the activity of both enzymes (by 23% in the case of acid RNase and by 18% for acid DNase) was observed 1 h after its injection. However, by the end of the first day of the experiment the activity of acid DNase and RNase showed a marked decrease (to 76 and 66% of the control level respectively). By the end of the period of observation their activity had returned to its original level.

The action of both mitomycin C and rubomycin C was thus to cause a sharp decrease in the RNA content in the liver (and, in particular, RNA of the ribosomes), while only slight changes were found in RNase

TABLE 2. Dynamics of Changes in Activity of Acid DNase and RNase in Liver of Rats Receiving Mitomycin C and Rubomycin C (in μmoles/min/g protein)

Group of animals	Time after injection (in h)	Acid DNase				Acid RNase			
		mitomycin C		rubomycin C		mitomycin C		rubomycin C	
		$\overline{X}\pm S_{\overline{x}}$	P	$\overline{X}\pm S_{\overline{x}}$	P	$\overline{X}\pm S_{\overline{x}}$	P	$\overline{X}\pm S_{\overline{x}}$	P
Control	6	1,20±0,11	—	1,33±0,05	—	5,15±0,14	—	6,30±0,18	—
	48	1,19±0,06	—	1,37±0,09	—	4,50±0,25	—	5,50±0,18	—
	96	1,21±0,06	—	1,29±0,09	—	4,85±0,22	—	5,00±0,25	—
Experimental	1	1,37±0,09	>0,05	1,57±0,10	<0,001	5,40±0,24	>0,05	7,76±0,18	<0,001
	3	0,88±0,05	<0,05	1,82±0,16	<0,001	5,18±0,27	>0,05	6,72±0,13	>0,05
	6	1,20±0,09	>0,05	1,52±0,08	>0,05	5,56±0,41	>0,05	5,31±0,18	<0,01
	12	1,23±0,03	>0,05	1,52±0,07	>0,05	5,35±0,21	<0,05	3,40±0,16	<0,001
	24	1,06±0,07	>0,05	1,05±0,09	<0,05	5,44±0,13	>0,05	3,61±0,34	<0,05
	48	0,95±0,06	<0,02	1,23±0,06	>0,05	4,71±0,25	>0,05	6,70±0,29	<0,01
	72	0,74±0,04	<0,001	1,20±0,04	>0,05	5,33±0,17	>0,05	5,12±0,23	>0,05
	96	0,65±0,04	<0,001	1,27±0,12	>0,05	4,58±0,12	>0,05	5,56±0,24	>0,05

Note. Mean results of seven experiments are shown.

activity. The DNA content remained virtually unchanged throughout the experiment. Acid DNase activity showed a marked tendency to decrease.

These investigations confirm results in the literature indicating that mitomycin C has a substantial effect on the metabolism of nucleic acids and, in particular, of RNA, the content of which falls sharply. Similar results were obtained in the present experiments also for the new Soviet antitumor antibiotic, rubomycin C, whose mechanism of action has not yet been explained.

It cannot be unequivocally concluded from the results of these experiments that the decrease in the RNA content in these experiments was due to activation of an enzyme participating in its intracellular catabolism: the changes in acid RNase activity were only slight in character. Evidently inhibition of RNA synthesis plays an important role in the disturbance of nuclei acid metabolism following administration of both mitomycin C and rubomycin C.

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