THE EFFECT OF CHLORAMPHENICOL ON THE BIOGENESIS OF MITOCHONDRIA OF RAT LIVER *IN VIVO*

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1. Introduction

It is well known that chloramphenicol strongly inhibits protein synthesis in isolated mitochondria (see ref. [1]). From the work of Linnane and coworkers [2, 3] with yeast it is clear that the presence of chloramphenicol in culture media gives rise to a profound alteration in the relative amounts or activities of several mitochondrial enzymes. Cytochromes *b*, c_1 and aa_3 are no longer detectable in the cytochrome spectrum whereas the amount of cytochrome *c* is not altered. Succinate dehydrogenase, fumarate hydratase and some other mitochondrial enzymes remain present if chloramphenicol is added to the culture medium, albeit at a lower specific activity. Using beating rat-heart cells we have shown earlier [4] that the chloramphenicol inhibition of aminoacid incorporation by isolated mitochondria is reflected in these cells by a complete block in the formation of functionally active cytochrome *c* oxidase. By spectral analysis Firkin and Linnane [5] have confirmed this observation for HeLa cells. Because chloramphenicol may have severe toxic side effects in man [6], we were interested to see whether it would be possible to relate chloramphenicol treatment of rats *in vivo* to changes in mitochondriogenesis. For this purpose we have investigated the changes in cytochrome *c* oxidase activity in regenerating liver from control and chloramphenicol-treated rats on the second and third day after partial hepatectomy. This paper describes the experiments which led us to conclude that chloramphenicol can indeed be a potent inhibitor of the formation of fully equipped and functionally active mitochondria in higher animals. Part of these experiments has been discussed before [7–9].

2. Methods

Male Wistar rats, weighing about 200 g, were partially hepatectomized exactly as described by Higgins and Anderson [10]. As the narcotic we used 0.5 ml of a solution containing 0.02% phentanyl and 1% fluanisone (Hypnorm, Philips-Duphar, The Netherlands) per kg body weight.

Chloramphenicol was given twice a day (early in the morning and late in the afternoon) subcutaneously in a dialysis bag, containing 2 ml of chloramphenicol succinate in 0.15 M NaCl, the total dose of chloramphenicol per day being 500 mg per kg body weight. The dialysis bags were applied via a small cross-incision in the neck. The control animals were treated in the same way with omission of the chloramphenicol succinate from the salt solution in the dialysis bags.

Cytochrome *c* oxidase (E.C. 1.9.3.1) activity was measured by following the decrease in absorbance of reduced cytochrome *c* at 550 nm in a Zeiss PMQ II spectrophotometer as described previously [11]. Protein was measured by a biuret method [12]. Chloramphenicol concentration in rat sera was determined by a microbiological method [13].

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Table 1
Effect of chloramphenicol on total cytochrome *c* oxidase activity in homogenates of regenerating rat liver.

Time elapsed since the operation (hr)	Cytochrome *c* oxidase activity (expressed as percentage of the activity of the total liver before hepatectomy)					
	Total activity				Increase in activity	
	Control animals		Chloramphenicol-treated animals		Control animals	Chloramphenicol-treated animals
	No. of experiments	% ± S.E.	No. of experiments	% ± S.E.		
0	–	30	–	30	–	–
46	8	51 ± 3	7	31 ± 3	21	1
70	4	62 ± 6	5	36 ± 1	32	6

The experimental conditions are described under methods and in the legend to fig. 1.

3. Results and discussion

In early experiments we observed no effect of chloramphenicol on liver regeneration if the drug was administered by injection [14]. Measurements of the concentration of active chloramphenicol in the serum by a microbiological method showed, however, that following injection the chloramphenicol level fell very rapidly. It was clear, therefore, that chloramphenicol is detoxified extremely efficient in the experimental animals, even if 70% of the liver and, therefore, also 70% of the total detoxification capacity are removed.

To obtain any effect of chloramphenicol in rat liver *in vivo* it was necessary continuously to keep the level of active chloramphenicol in the blood of the experimental animals at about 5–10 μg per ml. In our experiments this could not be achieved with injections not even if given at 4-hour intervals day and night. We therefore developed a method to assure a *continuous* level of 5 μg or more chloramphenicol per ml blood. We found that subcutaneous administration of chloramphenicol in the way described under methods could guarantee this minimal concentration at least during the first three days after partial hepatectomy. Fig. 1 gives the data of a series of such experiments. It can be seen that liver regeneration as such was not hampered by the chloramphenicol treatment, since there were no significant differences in total wet-weight or total protein between control and chloramphenicol treated animals. Table 1 gives the total cytochrome *c* oxidase activity of the livers on the second and third day after partial hepatectomy. As shown in this table, during the first two days there is *no* increase in the cytochrome *c* oxidase content of the livers during treatment with chloramphenicol, whereas also during the third day after operation the increase in cytochrome *c* oxidase is still strongly inhibited as compared to the control animals.

From these experiments we conclude that chloramphenicol may prevent the synthesis of functionally active cytochrome *c* oxidase in the intact animal. A

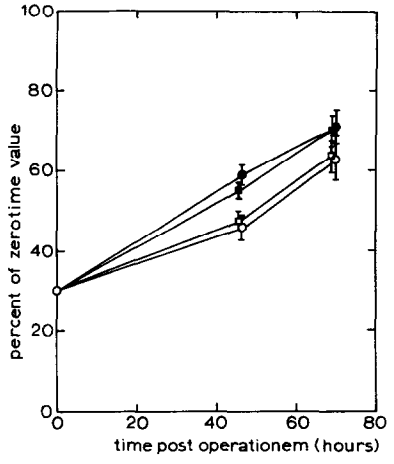

Fig. 1. Increase of wet-weight and total protein content of rat livers after partial hepatectomy with and without chloramphenicol treatment. The values for the part of the livers excised during partial hepatectomy (zero time) are taken as 70% [10]. Standard errors are indicated by the vertical bars. Each point is the average of 3 to 8 experiments. ●—● wet-weight chloramphenicol-treated animals, ■—■ wet-weight control animals, ○—○ total protein chloramphenicol-treated animals, □—□ total protein control animals.

prerequisite for such an inhibitory effect *in vivo* appears to be that the serum levels do not fall below 5 μg/ml for long periods during the experiment. Once its mitochondria become deficient in enzymes of the respiratory or phosphorylating chains, it is feasible that the cell may lack a sufficient supply of ATP to support its energy requiring activities. If many cell divisions occur, active mitochondria may be diluted out in such a way that this deficiency leads to a complete cessation of further division and differentiation of these cells. If this hypothesis is correct it follows that bone marrow and immunologically active tissue during antigenic stimulation are specially prone to chloramphenicol inhibition. We therefore suggest that most cases of bone marrow depression [15] and immunosuppression [16] induced by chloramphenicol have to be ascribed to the inhibition of mitochondrial protein synthesis. As we have pointed out elsewhere [9, 17] we do not think that the inhibition of cytochrome *c* oxidase formation does necessarily mean, that this enzyme is synthesized within the mitochondria and coded for by mitochondrial DNA as suggested by Firkin and Linnane [5].

Further studies are in progress to directly relate the *in vivo* chloramphenicol effects to their apparent target tissues such as bone marrow.

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