DEVELOPMENT OF TUMOR IN THE COURSE OF SPONTANEOUS RESTORATION OF CARBON TETRACHLORIDE INDUCED CIRRHOSIS OF THE LIVER IN RATS

HIROSHI KAWASAKI

*First Department of Pathology, Kurume University School of Medicine, Kurume, Japan*

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INTRODUCTION

The study of carbon tetrachloride (CCl₄) cirrhosis of the liver began with the report on rabbits by Midorikawa (1925) and followed a year later by Samson and Wing who published their study on dogs. However, as an extensive and systematic study, the experimental CCl₄ liver cirrhosis in rats by Cameron must be mentioned. For years, the author has followed Cameron’s experimental studies of CCl₄ liver cirrhosis in rats, chiefly for the survey purpose of examining changes in the structure of vessels occurring in the course of spontaneous healing of the disease in rats. In the course of this study the author happened to observe the development of a liver tumor during the period of recovery of the damaged liver. Since the literature contains no similar success in rats, although CCl₄ could produce a neoplasma under a different condition in mice, the finding seems to warrant this report.

MATERIALS AND METHODS

Wistar system male rats (350) weighing about 120 g were used in this study equivalent mixture of CCl₄ and olive oil repeatedly administered subcutaneously, in doses of 0.2-0.3 cc per 100 g weight twice a week, in all 50 times (39 rats) 60 (6 rats) and 70 (4 rats) respectively. Subsequently, the animals were left alone for a certain period of time to observe the spontaneous healing of cirrhotic changes.

Methods of investigation.

All the animals were sacrificed after stopping administration at intervals of 1, 3, 6, 12, and 18 months. Tumor tissues were found to have developed in two cases, one case which was given CCl₄ 50 times and then left alone for 6 months, and the other was a case administrated CCl₄ 60 times and left alone for 3 months. In both cases, the liver was perfused with physiological salt solution and acrylates sorted by color were injected into the portal vein, the hepatic artery and the hepatic vein. Moreover,
in latter case the relationship of blood at the site of the tumor was examined using cats, and the other histological observation was made by fixing tumor tissues in 10 per cent formol saline and staining paraffin sections in the usual manner with hematoxylin and eosin, PAS, Azan and by Gomori’s silver impregnation method.

RESULTS

Case 1. On the under surface the lobus sinister, a well-defined tumor about the size of a little finger’s head was found. As shown in on injection-corrosion casts, the portal branches were reduced in accordance with the site of tumor masses and a picture of defect of the adjacent sinusoids due to poor infusion was noted. On the contrary, the arterial branches were regionally enlarged and in adjacent areas a fine, irregular, cotton flower-like infusion was clearly observed. (Fig.5, 6) In the tumor tissue itself the branches of the hepatic vein were not conspicuous, its periphery and the peripheral branches were found to be not only strongly compressed but also congregated and intertwined around the tumor tissue as if to surround it. (Fig. 6)

Case 2. In the lower edge of the lobus medianus, there were two protuberant tumor masses about the size of a little finger’s head, penetrating through the surface and the reverse of the liver. (Fig. 7)

Macroscopically in the region of tumor the infusion of resins from the portal canals to the sinusoids met with a strong resistance. The branches of the hepatic vein, changed in various forms, were seen closely arranged around the tumor tissue but the infusion in it was only rudimentary.

Histological examination revealed that the hepatic cell cords were irregularly arranged, and at the site of tumor, the protoplasm of the cell was basophil, the nuclei of various sizes were polychromatophil and the nucleoli large and distinguishable. (Fig.8) Tumor cells forming large and small lamina were found in various groups, showing an adenomatous structure, but sometimes the arrangement of them in rows was also observable. (Fig.9,10) These cell groups were separated from each other by sinusoids covered with the endothelium. (Fig.10, 11)

COMMENT

Regarding the restoration of an experimental CCl₄ liver cirrhosis in rat there are several reports by Cameron, Shimokawa and others. According to Cameron, the disease presented is divided into two stages: a so-called precirrhotic reversible stage in which restoration occurs when the administration of CCl₄ is discontinued and a cirrhotic irreversible stage in which no restoration occurs. All the cases examined represent cases in which CCl₄ was given successively more than 50 times and thus correspond in quantity to the irreversible stage in the sense of Cameron.

As stated above, the two cases in which a tumor had developed were cases which were left alone for 6 and 3 months, respectively after 50 and 60 repeated administration of CCl₄. In the region, except for the tumor tissue hyperplastic nodules had become flat, and the interstitial tissue had been markedly narrowed in width, which obviously suggests the morphology of restoration. It is, indeed, a matter of much
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interest that unlike the hepatoma formation occurring during a period of administration of CCL4 in mice as described by Stowell and others, the development of tumor in both cases happened in the course of liver regeneration after stoppage of CCL4.

Histologically, the tumor presented findings of a hepatoma-like malignant tumor. However, the focus of metastasis was not detected and an extensive spread of pathological changes was also not observed. Thus, the degree of malignancy could not be determined. The angioarchitecture in the tumor is quite different from that seen in hyperplastic nodules. Namely, blood supply from the portal branch is scant, while the arterial blood is supplied in abundance, which is no doubt characteristic of tumor tissues.

CONCLUSION

Liver cirrhosis produced by prolonged, repeated administration of CCL4 (twice a week, in all over 50 times) may form, though rarely, a tumor in the course of its restoration. From their angioarchitecture and their histological findings, the tumor tissues examined are quite different from hyperplastic nodules. They are unmistakably a tumor itself. Investigation will be made in no distant future whether they are malignant or not.

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REFERENCES

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Explanation of figures

Fig. 1. Cast of a normal liver in rat.
There is regular interdigitation of portal veins (yellow) and hepatic veins (blue).

Fig. 2. Portion of the cast of a normal liver in rat.
The distal hepatic arteries (red) are finer than portal vein (yellow).

Fig. 3. Portion of a tumor in rat (Case 2).

Fig. 4. The injection-corrosion (before washing) of the liver in rat (Case 1).

Fig. 5. The same injection-corrosion cast (after washing) in Fig. 4 (Case 1).

Fig. 6. Photograph enlarged the portion of the tumor in Fig. 5.

Fig. 7. Macroscopic finding of tumor of the liver (Case 2).

Fig. 8, 9, 10. Histological findings of Case 2.
H.E. stain, ×295, ×320, ×295.

Fig. 11. Histological findings of Case 2.
Azan stain, ×295.

Fig. 12. Histological findings of Case 2.
Gomori’s silver impregnation method, ×295.