Regression of Aflatoxin B_1–Induced Hepatocellular Carcinomas by Reduced Glutathione

Abstract. *Reduced glutathione administered to rats bearing aflatoxin B_1–induced liver tumors caused regression of tumor growth and resulted in survival of the animals. Since glutathione is a harmless natural product, it merits further investigation as a potential antitumor drug for humans.*

In recent years there have been a number of reports concerning the ability of antioxidants to inhibit chemical carcinogenesis when the antioxidants are administered either prior to or at the same time as the carcinogen (*1*). I report now that reduced glutathione (GSH), a harmless natural antioxidant (*2*), produces regression of aflatoxin B_1 (AFB_1)–induced liver tumors when administered at the late stages of tumor progression.

Female rats (*3*) of the Wistar strain were obtained as weanlings and were maintained in conventional cages with free access to food (Altromin) and water. When they weighed 100 g (44 days of age), the administration with AFB_1 was started. The rats were transferred to screen-bottomed cages, three to a cage, and housed in an air-conditioned room with a 12-hour light-dark cycle. Water and food were continued without restriction, and the rats were weighed once a week. Each rat received, by stomach intubation, 25 μg of AFB_1 (Makor Chemicals, Jerusalem) in 2.5 μl of dimethylformamide (DMF) for five consecutive days weekly for eight consecutive weeks. Control animals received only DMF. Both solutions were made up to 1 ml with distilled water just prior to intubation of each rat.

This AFB_1 dosing regimen induces hepatocellular carcinomas in 100 percent of rats after 1 year (*4*). It does not affect the consumption of food or gain in weight of the rats for approximately 16 months after discontinuation of the carcinogen (*5*). Shortly after this time, the liver tumors become palpable, and the animals undergo a progressive loss of body weight and eventually die of liver tumors. Consequently, 16 months after the carcinogen treatment was discontinued, the group of AFB_1-treated rats and of DMF-treated control rats were each divided into two groups: one group was started on treatment with GSH (Fluka; 100 mg per rat per day in 2.5 ml of distilled water) by stomach intubation, and the other received no further treatment. In order to avoid traumatic lesions of the rat livers, in the present study no attempt was made to investigate the liver tumors by palpation.

All rats treated only with AFB_1 died of liver tumors within 20 months after discontinuing the carcinogen treatment, whereas 81 percent of the AFB_1-treated rats that were also treated with GSH were still alive and apparently healthy 4 months later (Table 1). At this time, the experiment was terminated and the animals were autopsied. No liver tumors developed in animals that had not received the carcinogen (Table 1). At autopsy, there were striking differences between the gross appearance of the livers of rats that had received only AFB_1 (Fig. 1, a to c) and those of rats that received AFB_1 and GSH (Fig. 1d). The absence of tumorous changes characterized the livers of the latter group of rats, and there was a rounding of the lobe edges, partial fusion of the liver lobes, and zonal thickening of the liver capsule. These macroscopic changes were highly suggestive of a remodeling of the liver.

Table 1. Survival data for rats treated with aflatoxin B_1 and reduced glutathione. Wistar female rats (44 days old) were treated with aflatoxin B_1 (AFB_1) in dimethylformamide (DMF) by stomach intubation for 8 weeks. Treatment with reduced glutathione (GSH), also administered by stomach intubation, was started 16 months later. Body weight is expressed in grams ± standard deviation.

Groups	Start of GSH treatment (16 months after AFB_1 treatment)		Mortality during GSH treatment (17 to 20 months after AFB_1 treatment)		Survival at end of experiment (24 months after AFB_1 treatment)	
	Rats (No.)	Body weight (g)	Rats (number dead/ number living)	Body weight at death (g)	Rats surviving (number and percentage)	Body weight when killed (g)
DMF	32	320 ± 23	0/32		32 (100)	345 ± 28
DMF + GSH	15	300 ± 25	0/15		15 (100)	340 ± 26
AFB_1	33*	275 ± 16	33/0	250 ± 15	0 (0)	
AFB_1 + GSH	21*	285 ± 32	4/17	252 ± 9	17 (81)	290 ± 29

*The unequal numbers of animals in the two groups is casual.

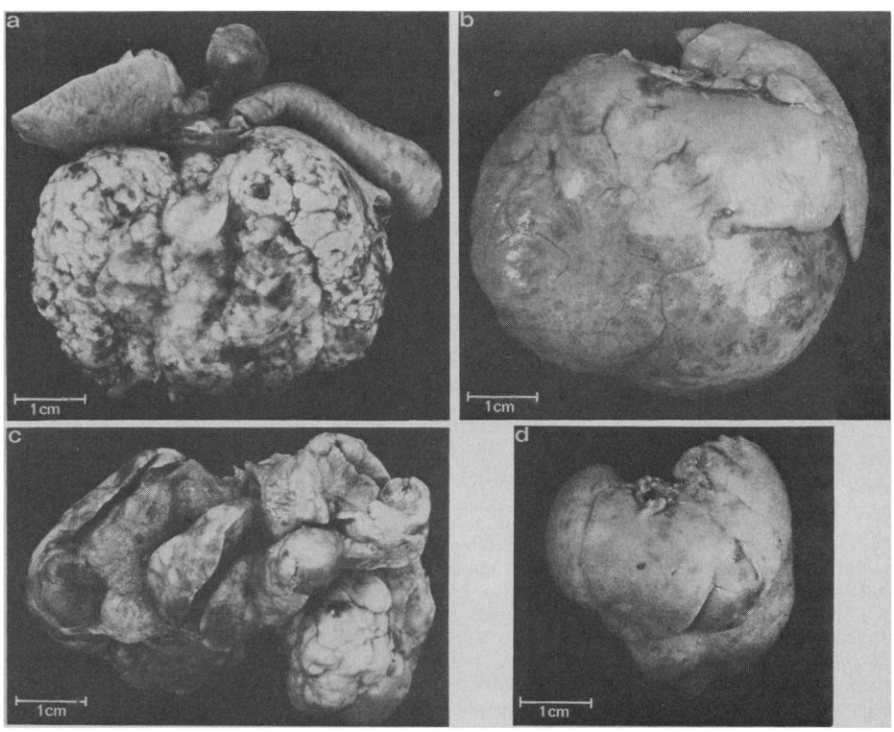

Fig. 1. Effect of glutathione (GSH) on the gross appearance of the livers of rats with AFB_1-induced tumors. (a to c) Livers of rats that died of liver tumors 17 to 20 months after the rats had been treated with AFB_1 for 8 weeks. (d) Typical liver of rats surviving 24 months after AFB_1 treatment, with GSH treatment for the last 8 months.

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The histological hallmark of the livers of rats that died of AFB_1-induced liver tumors was a conspicuous basophilia of the entire parenchyma, the areas with neoplasia staining more intensely than the adjacent liver; the hepatocellular carcinomas had primarily a trabecular pattern (Fig. 2a). Electron microscopic examination of sections of the malignant hepatocytes showed that large aggregates of rough endoplasmic reticulum occupied most of the cytoplasm, which was devoid of smooth endoplasmic reticulum, glycogen, and Golgi apparatus (Fig. 2b).

The livers of rats treated with AFB_1 and GSH were very different, both histologically and cytologically, from the livers of the AFB_1-treated rats. The liver parenchyma had become acidophilic, as shown by eosin staining. This was also true for the neoplastic areas; these areas, however, were seen in only three (18 percent) of the surviving rats in this group. The normal architecture of the liver was, for the most part, retained, although the hepatocytes within the liver lobules were arranged mainly in irregular linear patterns (Fig. 2c). Abundant smooth endoplasmic reticulum characterized the cytoplasm of these cells, which also showed a normal content of cytoplasmic organelles (Fig. 2d). The livers of rats treated with DMF alone and with DMF plus GSH showed no morphological changes when examined with either light or electron microscopy.

To my knowledge, the growth of fully transformed malignant cells being reversed by administration of a chemical compound has not been reported previously. An interesting question is whether the chemical responsible, GSH, induces regression of tumor growth by modifying the properties of malignant cells, for instance, by inducing differentiation, or by selectively killing the neoplastic cells and allowing normal cells to reconstitute the liver parenchyma. Some of the intermediate aspects of the observed ultramicroscopic changes, such as the reappearance of smooth endoplasmic reticulum within the neoplastic areas (*5*), suggest that the mechanism involves a reversion of malignancy. While it is now widely accepted that hyperbasophilia caused by the presence of RNA represents the final step toward neoplasia (*6*), the significance of the increased smooth endoplasmic reticulum induced by GSH is an intriguing question. Ultrastructural, biochemical, and pharmacological studies have shown that enhanced drug-metabolism activity is accompanied by increased hepatocellular smooth endoplasmic reticulum (*7*). The cytochrome P-450–dependent monooxygenase system is known to be involved in activation and detoxification of aflatoxin B_1 (*8*). The present results suggest that these enzymes may also be involved in the regulation of neoplastic growth. The effect of GSH, a harmless natural product, on carcinogen-induced hepatocellular carcinoma in rats strongly suggests that this antioxidant merits further investigation as a potential antitumor agent in humans.

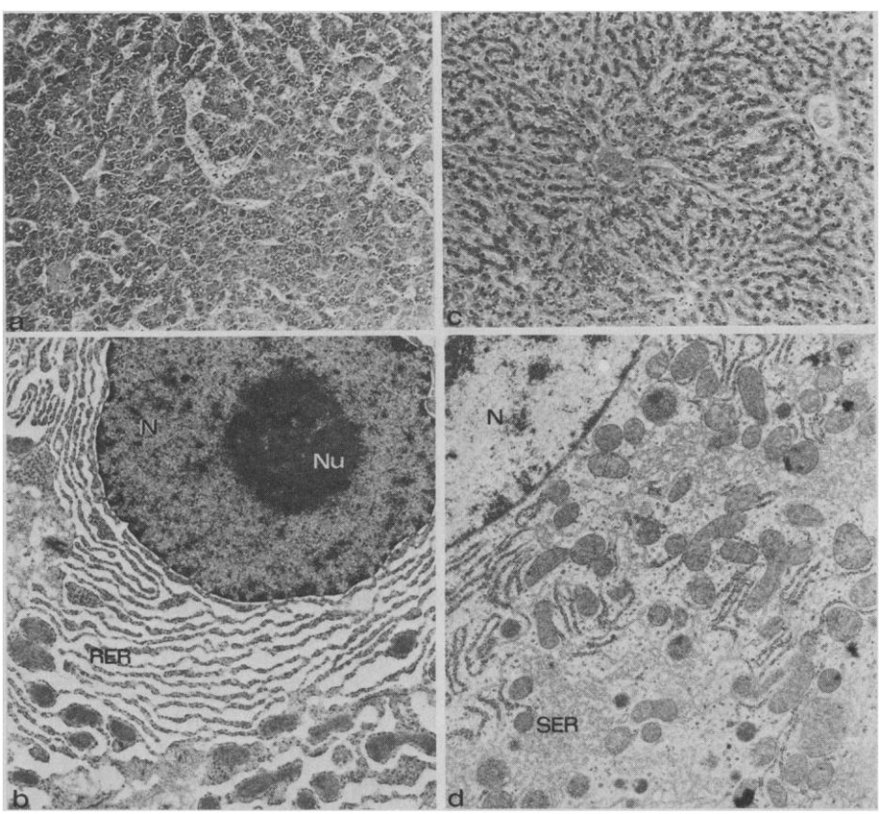

Fig. 2. Histological and subcellular changes induced in the livers of rats treated with AFB_1 and AFB_1 plus GSH. Liver specimens were fixed for electron microscopy with a mixture of cold 2.5 percent glutaraldehyde and 0.8 percent paraformaldehyde in 0.1*M* cacodylate buffer, *p*H 7.4, and further processed as previously described by Novi (*4*). (a) Paraffin section of liver of rat that died 17 months after end of treatment with AFB_1, showing hepatocellular carcinoma with trabecular arrangement of the tumor cells. Hematoxylin and eosin stain (×65). (b) Electron micrograph of a portion of the tumor cells shown in (a). Agglomerates of degenerating rough endoplasmic reticulum (*RER*) widely extend throughout the cytoplasm; nucleus (*N*) with prominent nucleolus (*Nu*) (×14,000). (c) Paraffin section of liver of surviving rat 24 months after end of AFB_1 treatment, with GSH added for the last 8 months of life. Note the irregular arrangement of the liver plates within the lobule and lack of tumorous changes. Hematoxylin and eosin stain (×65). (d) Electron micrograph of a portion of the hepatocytes shown in (c). Abundant smooth endoplasmic reticulum (*SER*) is surrounded by intact cytoplasmic organelles (×15,000).

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References and Notes

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