# Alpha benzene hexachloride inhibition of aflatoxin $B_1$-induced hepatocellular carcinoma. A preliminary report[1]

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*Summary.* Alpha benzene hexachloride protected against the development of liver carcinoma in male albino Fisher rats ingesting aflatoxin $B_1$.

The capacity of aflatoxin $B_1$ ($AFB_1$) to induce hepatocellular carcinoma in the rat[2] appears in some way to be related to its ability to inhibit nuclear DNA-dependent RNA-polymerases and thus RNA-synthesis[3-5]. Alpha benzene hexachloride ($\alpha$-BHC) has been shown to inhibit the development of liver carcinoma in male Fisher rats ingesting 3′ methyl-4-dimethylaminoazobenzene (3′ me-DAB) and DL-ethionine[6]. The present study was performed to determine whether $\alpha$-BHC would also be effective in preventing the induction of liver carcinoma by $AFB_1$.

*Methods.* 46 male albino Fisher rats (Animal Center, Faculty of Science, Mahidol University, Bangkok, Thailand) were used at b.wt 155–200 g. The animals were kept 2 in a cage in a temperature controlled room at 27 °C. 1 group of control was given only the basal diet, the 2nd group was given $AFB_1$ (Makor Co. Ltd, Jerusalem, Israel), the 3rd group was given $\alpha$-BHC (Tokyo Kasei Koaya Ltd, Tokyo, Japan) and the 4th group was given $\alpha$-BHC simultaneously with $AFB_1$. The $AFB_1$ was incorporated in the basal diet which contained 22% protein with other dietary components similar to those previously described[5]. $AFB_1$ was first dissolved in acetone and then in corn oil at a concentration of 1 ppm. The concentration of $\alpha$-BHC in the diet was 0.05%. All diets were given ad libitum continuously for 20 weeks and then replaced with standard laboratory chow pellets (Zuellig). 10 and 65 weeks after the onset of the experiment, the surviving animals were sacrified following 18 h of starvation. The livers were removed, weighed, fixed in 10% buffered formalin, sectioned and stained with hematoxylin and eosin.

*Results.* The b. liver wt and the diet consumed in the 10th week period are shown in table 1. The liver wt as percentage of the b.wt in the group receiving $\alpha$-BHC and $\alpha$-BHC + $AFB_1$ was significantly higher than the control and $AFB_1$ group. Histologically, there were no remarkable change in liver in any group, except for hypertrophy of hepatocytes in $\alpha$-BHC and $\alpha$-BHC + $AFB_1$ at the centrolobular region.

For the long-term experiment, animals in all groups showed an increase in b.wt during the first 15 weeks. The average b.wt, liver wt as well as percentage of b.wt are summarized in the table 2. 13 rats survived for the full 65 weeks. Hepatocellular carcinoma of the liver was found in the 3 surviving rats fed $AFB_1$, whereas the liver of the animals in the other 3 experimental groups showed no liver tumors.

Some of the animals (including controls) were affected with pulmonary diseases, e.g. bronchopneumonia was seen in all rats that died from the 20th week of feeding on the semisynthetic diet. The pulmonary infections were believed to be caused by a short period of overheating of the animal room during the 15th week of the experiment. The liver tumors were grossly seen in 2 of the $AFB_1$ group dead in the 56th and the 59th week. In addition, the neoplastic nodules were found in 2 of the same group dead in the 44th and the 46th week. One animal of this group developed hyperplastic nodules in the 33rd week but not in the 20th week. There was no remarkable sign of preneoplastic changes of the livers in other rats that died during the experiment.

*Discussion.* The present data suggest that $\alpha$-BHC prevents the induction of liver tumors by $AFB_1$ in rats similar to that observed in animals exposed to ethionine and 3′ me-DAB[6]. $\alpha$-BHC has been shown to induce microsomal enzymes[7] and the proliferation of smooth endoplasmic reticulum[6], as well as to reduce the narcotic effect of hexobarbital[8]. Thus it may be that $\alpha$-BHC induces the formation of liver hydroxylation enzymes which metabolize $AFB_1$ to a non-

Table 1. Changes in body and liver weight in rat treated with $AFB_1$ and $\alpha$-BHC with average amount of diet, $AFB_1$ and $\alpha$-BHC for 10 weeks

| Experimental group | No. of rat | B.wt (g) Initial | Final | Gain | Liver wt g | B.wt (%) | Consumed/day Diet (g) | Chemicals (mg) |
|---|---|---|---|---|---|---|---|---|
| Control | 3 | 175 | 285 | 110 | 7.3 | 2.56 ± 0.07* | 18.1 | |
| $AFB_1$ | 3 | 168 | 255 | 87 | 6.6 | 2.61 ± 0.00 | 15.0 | 0.015 |
| $\alpha$-BHC | 3 | 183 | 295 | 112 | 12.2 | 4.19 ± 0.18 | 19.0 | 9.5 |
| $\alpha$-BHC + $AFB_1$ | 3 | 167 | 276 | 109 | 12.5 | 4.49 ± 0.04 | 27.8 | 13.90<br>0.027 |

* Mean ± SD. Probability of table 1 of liver wt as percentage of b.wt between 2 groups: control vs $AFB_1$; $p<0.005$; control vs $\alpha$-BHC, $p<0.005$; control vs $AFB_1$ + $\alpha$-BHC, $p<0.0005$; $AFB_1$ vs $\alpha$-BHC, $p<0.0005$; $AFB_1$ vs $\alpha$-BHC + $AFB_1$, $p<0.0005$; $\alpha$-BHC vs $\alpha$-BHC + $AFB_1$, $p<0.10$.

Table 2. Effect of $\alpha$-BHC on liver tumor incidence, body and liver weight, average amount of diet, in rats fed with $AFB_1$ and $\alpha$-BHC for long-term experiments

| Experimental | No. of rats (start/finish) | B.wt (g) Initial | Final | Gain | Liver wt g | B.wt (%) | Consumed/day Diet (g) | Chemicals (mg) | Tumor Incidence | Percent |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 6/3 | 191 | 353 | 162 | 7.2 | 2.02 ± 0.17* | 20.7 | | 0/3 | 0 |
| $AFB_1$ | 9/3 | 181 | 313 | 132 | 10.5 | 3.26 ± 1.79 | 16.5 | 0.016 | 5/5** | 100 |
| $\alpha$-BHC | 9/5 | 176 | 350 | 174 | 7.8 | 2.23 ± 0.22 | 15.8 | 7.9 | 0/5 | 0 |
| $\alpha$-BHC + $AFB_1$ | 13/8 | 171 | 393 | 222 | 9.4 | 2.39 ± 0.01 | 23.4 | 11.7<br>0.023 | 0/8 | 0 |

* Mean ± SD. ** This includes 2 rats that died in the 56th and the 59th week.

carcinogenic product. We postulate from previous[6] as well as from the present study that $\alpha$-BHC induces microsomal enzymes that participate in the inhibition of 3′ me-DAB, DL-ethionine and $AFB_1$ carcinogenesis in a similar hepatotoxic pathway. This antagonistic effect of $\alpha$-BHC on the carcinogenic activity of several chemical agents is of interest, and further investigation is needed to clarify the mechanisms involved.

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2 G.N. Wogan and P.M. Newberne, Cancer Res. *27*, 2370 (1967).
3 J.I. Clifford and K.R. Rees, Nature *209*, 312 (1966).
4 H.V. Gelboin, J.S. Wortham, R.G. Wilson, M. Friedman and G.N. Wogan, Science *154*, 1205 (1966).
5 Y. Moule and C. Frayssinet, Nature *218*, 93 (1968).
6 W. Thamavit, Y. Hiasa, N. Ito and N. Bhamarapravati, Cancer Res. *34*, 337 (1974).
7 W. Koransky, J. Porting, H.W. Vohland and I. Klempau, Archs exp. Path. Pharmak. *274*, 61 (1964).
8 W. Koransky, J. Portig, H.W. Vohland and I. Klempau, Archs exp. Path. Pharmak. *274*, 49 (1964).

## Ultrastructural differences in mitochondria of skeletal muscle in the prerigor and rigor states[1]

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*Summary.* Loss of cristae and matrix occur in the mitochondria of skeletal muscles prior to any observable changes in myofibrillar proteins during the development of rigor mortis. Care must be observed because ultrastructural changes in mitochondria in some studies may be attributed to a specific trauma, whereas the changes may be due to the lower pH in postmortem muscle.

Changes in the size and ultrastructural components of mitochondria have been observed in a variety of metabolic, developmental and pathological states. Of particular interest is the effect of various stresses that produce atrophy or degeneration of the mitochondria. These stresses would include: nutritional[2-4], tenotomy[5] and denervation[6] of skeletal muscles, malignancy[7], ecdysis[8], aging[9,10] and in ischaemic[11,12], degenerating[13] and autolysing[14] cardiac muscle cells. The tissues in the above studies, though undergoing atrophy, do not present a uniform pattern of changes in mitochondrial ultrastructure. For example, damage to the myofibrillar components, especially the I-band and Z-line, occurred before mitochondrial damage[11,12] in ischaemic dog myocardium. However, in degenerating cardiac muscle cells from humans with cardiac hypertrophy, some severely degenerated cells had few or no myofibrils, but these cells were virtually filled with intact mitochondria[13]. Proposed mechanisms for the degradation of the mitochondria vary. Mitochondrial disintegration in sperm after fertilization has been described as a process of self-autolysis[15]. An explanation for the increased membrane junctions in mitochondria from heart tissue that were stored after isolation was attributed to a lowering in pH[16]. Lower pH-values would be expected for stored tissue because the cells become anaerobic with time, and glycolysis would produce hydrogen ions. The effect of lowering pH on mitochondrial ultrastructure has been demonstrated recently in autolysing dog heart muscle[14]. pH-values 6.0–6.2 at 3 h postmortem produced marked mitochondrial swelling, loss of matrix density and disorganization of the cristae[14]. Some skeletal muscles may attain the ultimate pH (approximately 5.6) very rapidly, depending on the extent of physical activity of the muscle before death[17]. Therefore, it is of practical significance to determine the effect of postmortem changes on the ultrastructure of mitochondria from skeletal muscles lest ultrastructural changes in some studies are attributed to effects other than the postmortem effect produced by the lower pH-values.

*Methods.* 3 adult turkeys were killed by exsanguination. The semitendinosus muscle was removed immediately postmortem and processed for electron microscopy by a procedure outlined elsewhere[18]. The semitendinosus muscle on the other limb was allowed to enter rigor mortis at 20 °C. Rigor mortis, based on ATP, pH and response to electrical stimulation, occurs after 6–8 h postmortem. The semitendinosus muscle of the turkey was chosen because it is a red muscle[19], and therefore, high in mitochondrial density.

*Results.* Normal mitochondria in prerigor muscle can be observed in longitudinal and transverse sections (figure, a, b). After rigor development, the matrix has disappeared, and intramitochondrial dense inclusions are observed (figure, c, d). These dense inclusions develop after relatively small changes in pH, and before lactate ions are present in sufficient quantity to exert their swelling effect on the mitochondria[14]. The pH of the skeletal muscles in rigor mortis in this study was 5.5–5.7. The observed effect in the figure, a–d, was uniform in all sections examined. Therefore, the effect of low pH may be a factor in gross alteration in mitochondrial ultrastructure. However, a perusal of electron micrographs in our laboratory indicated that similar ultrastructural changes occurred in the biceps brachii muscle of the mouse[20]. The ultimate pH of this muscle in rigor mortis is about 6.4[21]. It would be wrong to infer from these statements that pH decrease, per se, is the sole factor responsible for the ultrastructural changes observed in the muscles. Many biochemical changes occur in postmortem skeletal muscles[17]. An interesting mechanism of muscle necrosis in various muscle diseases has been proposed recently, and similar events may occur in postmortem muscle. This involves an increased net influx of calcium into cells which triggers a 'vicious cycle' of mitochondrial calcium overloading, energy depletion and structural damage to the mitochondria.

It should be noted that the weakest component in the myofibrillar ultrastructure is at the Z-line-I-band junction[22]. The Z-line is the first myofibrillar component to show signs of destruction in the postmortem degradation of skeletal muscle. The electron micrographs in the figure, c–d, indicate that the mitochondrial changes occurred prior to any significant myofibrillar degradation.

It is apparent, therefore, that structural changes occur in the mitochondria of postmortem skeletal muscles prior to any observable changes in the myofibrillar proteins. Concomitant with these structural changes is a decrease in muscle