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Toxicological Studies of Antioxidants, Butylated Hydroxytoluene(BHT) and Butylated Hydroxyanisole(BHA)

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항산화제 BHT와 BHA의 안전성

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Introduction

Butylated hydroxytoluene(BHT) and bulylated hydroxyanisole (BHA)(Fig. 1) are hindered phenolic compounds which are widely used as food antioxidants. These antioxidants help to maintain the quality of many food products by preventing oxidation of labile lipid components through donating a hydrogen to free radical. Food products usually are allowed to contain a total of 0.02% of BHT and BHA based upon the fat content of the food.

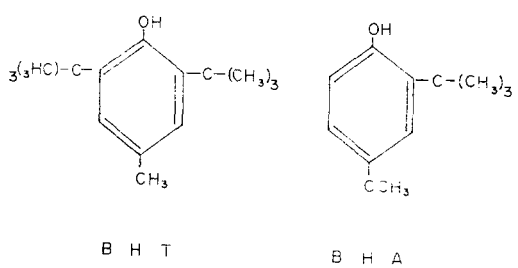

Fig. 1. Structure of butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA)

Both BHT and BHA are generally considered as safe for addition to food productat that dose: however, because of the widespread usage of them, it is essential that the safety of these antioxidants be evaluated critically.

Most toxicological studies with BHT and BHA have been conducted at the dose of 50 to 500 *mg/kg* /day through gastric intubation or 0.01 to 1.0% in diets. Although these doses are hundreds times higher than normal dietary intake of man, various toxic effects have been reported in liver, kidney, lung, blood system, and reproduction system in many experimental animals. The effects appear to have species and sex-differences as a result of differences which exist in the metabolism and excretion of BHT and BHA by those experimental animals. The purpose of this article is to review subacute

Table 1. Effects of BHT and BHA on hepatic enzyme activities in rats

BHT		BHA	
Enzyme Activity	Ref.	Enzyme Activity	Ref.
Incresae		Increase	
Glucose-6-phosphate dehydrogenase	5	Epoxide hydratase	20
ATPase	8	Aniline hydroxylase	21
Thymidine kinase	14	UDP-glucuronyl transferase	21
UDP-glucuronosyl transferase	15	NADPH cytochrome C reductaseo	21
P-450 content	6	G-6-P dH_2	21
Biphenyl-4-hydroxylase	4		
4-methoxybiphenyldemethylase	4		
Aminopyrine demethylase	2, 3, 6		
Hexobarbitone oxidase	2		
BHT-oxidase	16		
Nitroanisole demethylase	2, 6		
Decrease		Decrease	
Glucose-6-phosphatase	5	Benzopyrene hydroxylase	18
Acid phosphatase	17	UDP-glucuronly transferase	15
Succinic acid dehydrogenase	17	Gluose-6-phosphatase	5
GOT	17		
GPT	17		
Lactic acid dehydrogenase	17		
Cytochrome oxidase	15		
NADPH cytochrome C reductase	15		
Benzopyrene hydroxylase	15, 18, 19		

toxicological effects of BHT and BHA on various organ systems.

Effects on liver

One of the most striking effects of BHT and BHA is that they cause enlargement of liver in experimental animals. This effect is less pronounced with BHA than with BHT in rats. Dietary intake of 500 *mg/kg* BHA results in moderate liver hypertrophy[1,2], whereas BHT results in pronounced liver hepatomegary[1~9] and proliferation of smooth endoplasmic reticulum[1~3,7,8]. However, BHA results in more severe effect in monkeys. When infant and juvenile monkeys were given 500 *mg/kg*/day for 30 days, BHA resulted in pronounced increase in relative liver weight and marked proliferation of the smooth endoplasmic reticulum, but BHT rsulted in no increase in liver weight and only moderate proliferation of the smooth endoplasmic reticulum[10].

In addition, these antioxidants affect the activities of various enzymes located in the liver. Their effects on rodents are listed in Table 1. Generally, BHT produces more severe effects than BHA in rodents. In primate, meanwhile, BHA exerts greater effects. When fed with 500 *mg/kg*/day for 28 days, BHA resulted in pronounced decrease in microsomal glucose-6-phosphatase, succinic dehydrogenase and increased nitroanisole demethylase activity, but BHT had no change in glucose-6-phosphatase and moderate increase in hepatic nitroanisole demethylase [10,11].

BHT and BHA also affect on hepatic lipid and cholesterol levels. There was a 33% decrease in hepatic neutral fat in rat when fed diet containing 0.1~0.5% BHT[12]. In monkeys, daily doses of 50

Table 2. Effect of BHT treatment of liver weight, bile flow and BSP biliary excretion of rats[a]

Treatment (% BHT)	Days of treatment	Liver weight (% body weight)	Bile flow (*mg*/min/*g* liver)	BSP biliary excretion (% dose/60 min)
0		3.38±0.17	2.05±0.09	59.2±0.87
	2	3.87±0.09	2.90±0.10[b]	65.7±2.10[b]
0.1	4	3.53±0.15	2.71±0.19[b]	71.9±2.72[b]
	10	3.77±0.24	3.31±0.23[b]	75.7±2.87[b]
	2	4.10±0.17[b]	2.98±0.24[b]	71.1±1.92[b]
0.25	4	4.14±0.14[b]	2.97±0.17[b]	66.3±0.98[b]
	10	4.00±0.21[b]	3.10±0.30[b]	74.4±2.90[b]
	2	4.06±0.09[b]	3.31±0.22[b]	67.3±8.36
0.75	4	4.57±0.15[b]	2.87±0.11[b]	71.7±2.60[b]
	10	4.43±0.17[b]	2.94±0.14[b]	71.5±1.22[b]

[a] Values are expressed as the mean±S.E.
[b] Indicates values significantly different from ccontrol($p<0.05$).

or 500 *mg/kg* BHA for 28 days significantly reduced total cholesterol level [11].

The administration of BHT results in an alteration of hepatic excretory function[13]. When rats were fed 0.1 to 0.75% BHT, plasma disappearance and biliary excretion of injected bromosulfophthalein (BSP) were greatly enhanced from 2 days after treatment (Table 2). Bile flow was also increased in BHT-treated rats.

Effects on lung

In mice, the pathogenesis of toxic lung damage by BHT was first described by Marino and Mitchell [22] and has been intensively studied by Witschi and collegues [23~26]. BHT, administered ip at doses above 200 *mg/kg*, causes distortion and necrosis of type I alveolar cells within 24 hours. This is followed by a dramatic enlargement of the lung due to hyperplasia, hypertrophy and edema. The proliferating type II alveolar stem cells undergo a morphological differentiation into type I cells to replace the damaged epithelial lining. Lung enlargement is maximal at 4~7 days and within 10 days the lung returns to an apparently normal size and morphology. Total lung weight and total DNA per lung almost double within this time and are accompanied by dose-dependent increases in the *in vivo* incorporation of thymidine into DNA and leucine into protein. The activities of several enzymes such as thymidine kinase, DNA polymerse, uridine kinase, glucose-6-phosphate dehydrogenase, and 5′-nucleotidase increase substantially after BHT. Whole lung tissue enzyme activities of glutathione peroxidase, glutathione reductase, and superoxide dismutase were also increased. The increased enzyme activities corresponded to inflamnatory and proliferative pulmonary changes resulting from acute lung cell injury and necrosis. Treatment with BHA had no changes in the lung enzyme activities [27]. The toxic effects of BHT in lung are not due to metabolic activation of the antioxidant but might result from a direct interaction of the BHT with cellular constituents.

Protein phosphorylation, which is altered in response to changes in hormone levels, cell-cell interactions, cell proliferation, and cell differentiation, was increased by injecting ip with 400 *mg/kg* of BHT. These changes in phosphorylation correlated in time with the transient lung enlargement by BHT and were dependent upon the dose of BHT. BHT also enhanced urethane tumorigenesis in mouse. Mice were treated with 1 *mg* urethane/*g* before, during or after 300 *mg* BHT/*kg*-stimulated cell growth in the lung. The number of pulmonary tumors found 13~15 weeks later was not different in BHT-treated mice compared to that in controls. On the other hand, repeated stimulation (beginning 7 days

later, weekly injections of 250 *mg* BHT/*kg*, ip) of cell growth after urethan treatment significantly enhanced tumorigenesis[26, 28].

Effects on kidney

Treatment of BHT and BHA causes renal dysfunction in experimental animals. When fed high dose of BHT (1% in diet, w/w for 1, 2 or 4 weeks), kidney weight was increased and adrenals were enlarged in rats. At lower dose(500 *mg/kg*/day, 6 days), both BHT and BHA increased urinary ascorbic acid output and urine volume. Urinary sodium and potassium concentration and osmolarity of urine were reduced[29~31].

In primary cultures of monkey kidney cells, addition of 30 ppm BHT caused a 40~90% decrease in cell replication and inhibited protein, RNA, and DNA synthesis within the first 30 min of exposure. A rapid depression of cellular metabolism was also noticed. Similar to the liver, several kidney enzyme activities were also affected by BHT. Renal organic acid transport was reduced with BHT or BHA treatment as determined by p-aminohippurate accumulation in renal cortical slices[17,32,33].

Effects on blood system

When daily doses of 1 *g* of BHT or BHA were given to rabbits for 7 days, there was marked decrease in serum potassium level. The K level fell to about half the normal value[34]. BHT also significantly depressed the frequency and amplitude of contraction of isolated artrial preparation and that of perfused hearts. A dose-related increase in creatin phosphokinase leakage into the perfusion solution was noticed in concentrations from 1 to 500 *μg*/liter of BHT [35].

Various inhibitory effects upon *in vitro* cell culture systems were also reported. BHT not only caused a shortening of the cycle in surviving cells and resulted in a dose-related decrease in cell survival in phytohemagglutinin-stimulated human leucocyte culture [36], but also inhibited excision repair synthesis in normal human peripheral lymphocytes damaged by UV light[37]. BHA inhibited the primary *in vitro* antibody response of C57 BL/6 spleen cell to a thymus-dependent antigen and a thymus-independent antigen [38].

Upon administration of these antioxidants activities of several enzymes were changed. In blood of rats, they decreased plasma catalase, peroxidase, and cholinesterase activities [39]. In monkey, 500 *mg/kg* of BHA decreased blood catalase and serum acid phosphatase [10]. Several investigators have reported that high dosage of BHT resulted in the increased serum cholesterol and phospholipids in rat [40~42]. BHT reduced activities of vitamin K-dependent clotting factors II, VII, IX and X. Administration of vitamin K_1, K_2, and K_3 corrected the prolongation of prothrombin time produced by BHT [43].

Effects on reproduction

BHT is excreted in high concentrations in rat-milk and known to cross the placental barrier. However, the influence of BHT and BHA on the reproduction capacity of animalsis still not clear. The earliest report of possible effects was that 3 of 30 liters had anophthalmic young when the parents had been fed 0.5% BHT in diet (w/w) for 5 months [44]. Recently, Meyer and Hansen [45] studied behavioral and developmental effects of BHT dosed to rat in utero and in lactation period. BHT 500 *mg/kg*/day was given in the diet to F_0-rats from 6 weeks of age to weaning of the F_1-generation(growth period to age 19 week, gestation and lactation period) and subsequent to F_1-animals until 21 days of age. Body weight and weight gain were significantly reduced in both F_0 and F_1. A parallel adverse effect on different developmental parameters was found in the F_1 generation. The effect on F_1 offspring occurred mainly in the lactation period, caused either by a decreased nutrition due to BHT's influence on the lactation of the F_0 females, BHT's influence on the sucking ability of the F_1 rats or both combination.

Meanwhile, many other investigators reported that BHT and BHA had no teratogenic effect in rodent[39,46], monkey[47], and rabbit [19].

Concluison

We reviewed the toxicological effects of BHT and BHA on various organs. Since high dosage was used in most experiments, it is uncertain these antioxidants certainly exert harmful effects in man at usual dietary dose. The joint FAO/WHO Expert Committee on Food Additives set an unconditionally acceptable daily intake of 0.5 *mg/kg* body weight for BHT plus BHA. Therefore, to examine the exact effect of them, long-term study must be carried out more extensively.

To study the interactions with other xenobiotics is necessary. Since these antioxidants are potent microsomal enzyme inducers, it is important to know whether their continuous feedings potentiate toxicity of carcinogens or other drugs. Cytochrome P-450 dependent monooxygenases in liver microsomes have an important role in controlling the duration and intensity of action of foreign chemicals. Three homogenous cytochrome P-450 isozymes (cytochrome P-450a, and P-450b and P-450c) have been separated and purified. Wheather the induction of microsomal cytochrome(s) P-450 will be beneficial or harmful to an organism depends upon both the specific cytochrome(s) P-450 that is/are induced and the chemical environment of the organism. To know which hemoproteins are induced by these antioxidants and to quantify the relative amounts of each of the cytochrome(s) P-450 in individual subjects will afford the clue in studying the effect of BHT and BHA on an individuals. It must be pointed out that we have no intention to raise any arguments about the current use of these antioxidants in food through this article.

요 약

식품에 항 산화제로 첨가시키는 BHT와 BHA의 독성에 대해 고찰하였다. BHT와 BHA는 실험동물에서 간, 폐, 신장, 순환계, 생식계 등에 여러 영향을 주며 이러한 영향은 동물의 종류와 성에 따라 상당한 차이를 나타내고 있다. 대부분 실험에 사용된 BHT와 BHA의 양이 인체가 매일 식품으로부터 흡수할 수 있는 양보다는 훨씬 많은 양을 사용하였기 때문에 동물실험의 결과로 인체에 유독성을 직접 판단하기는 어렵고 이들의 안전성에 대해서는 보다 체계적인 연구가 앞으로 요구된다. 미량의 BHT와 BHA를 장기간 복용하였을 때 생기는 부작용에 대한 연구가 필요하며 또한 이들 항 산화제는 약물대사에 관여하는 효소의 합성내지 활성도를 증가시키는 것으로 알려져 있기때문에 체내에서 다른 약물의 대사 및 독성에 어떤 상호작용을 가져오는 지에 대한 면밀한 연구가 요구되고 있다. 더불어 본논문은 현시점에서 이들 항산화제의 식품에 첨가여부를 논쟁하고자 하는 의도가 전혀 없음을 명백히 하는 바이다.

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