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Concentrations and lipid peroxidation in tissues and toxicity of para-aminobenzoic acid fed to rats in drinking water

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This study was designed to determine the accumulation and toxicity of para-*aminobenzoic acid (PABA) in rats. PABA was provided at 0, 0.1, 0.5 and 1% in the drinking water for 4 weeks, during which the rats were fed a standard laboratory diet. The results showed that PABA affected neither the body weight gain nor the organ (liver, kidney, and spleen) weights during the 4-week period. The contents of PABA in the liver, kidney, and blood were increased in rats given 0.5% and 1% PABA in the water, whereas 0.1% PABA had little or no effect. The highest level of PABA achieved was 13 μg/g (95 nmol/g) and 5.1 μg/mL (37 nmol/mL) in the liver and blood, respectively, at week 4, and was 11 μg/g (80 nmol/g) in the kidney at week 2 after feeding began. Plasma aspartate aminotransferase activities in rats given 0.5% and 1% PABA were significantly lower than that of control rats at week 2, but not at week 1 or 4. Plasma alanine aminotransferase activities were not significantly affected by PABA, although a trend existed toward decreased enzyme activity by PABA. PABA, at 1% in the water, significantly decreased in vivo and in vitro (*t*-butylhydroperoxide-induced) lipid peroxidation in the liver but not in the kidney. This study demonstrated that PABA was accumulated to a limited extent in rat liver, kidney, and blood and that PABA did not lead to overt toxicity to rats even at 1% in the drinking water. In addition, PABA was a weak membrane antioxidant against lipid peroxidation.* (J. Nutr. Biochem. 7:408–413, 1996.)

Keywords: para-aminobenzoic acid; tissue content, toxicity; plasma GOT; GPT; lipid peroxidation

Introduction

Para-Aminobenzoic acid (PABA), once known as vitamin B_x, is recognized for its essential role in the production of folic acid in many species of bacteria[1] and for its strong absorption in the ultraviolet B (UVB) region (280 to 320 nM),[1,2] for which the compound has been used in many sun-blocking creams (commonly contain 2 to 10% PABA).[3,4] In addition, PABA is known to be an antifibrotic agent[5,6] and to protect against skin cancer induced by UV irradiation in hairless mice,[2,4,7–9] ozone toxicity in vitro,[10] and nephrotoxicity of *cis*-diamminedichloroplatinum(II) in rats without compromising the antitumor activity of the latter compound.[11] Some of these effects of PABA may be attributed to its ability to scavenge certain reactive oxygen species in vitro.[12]

Despite many important functions of PABA, little is known about its absorption, tissue distribution, metabolism, and toxicity. In animals PABA is derived from the diet and not biosynthesized. Clinically, oral N-benzoyl-L-tyrosyl-*p*-aminobenzoic acid (BT-PABA) has been used as a pancreatic function test.[13,14] After specific digestion of BT-PABA by chymotrypsin, the PABA moiety is rapidly absorbed by the intestinal epithelium through a passive process and almost completely excreted in the urine in acetylated form. Oral PABA may be used for testing liver function because the ability of liver to metabolize PABA correlates with severe and lethal disease of the organ where PABA is me-

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tabolized to *para*-aminohippuric acid and to *para*-acetamidobenzoic acid and *para*-acetamidohippuric acid via a monomorphic N-acetyltransferase.[15] PABA is generally considered nontoxic, and many PABA-containing (generally from 25 to 100 mg/tablet) vitamin complexes are available as food supplements. However, a few side effects of PABA such as nausea and skin rash[16,17] have been described. A case report shows that oral PABA (12 g/day) for 2 months led to hepatotoxicity in a patient with scleroderma, as evidenced by elevated serum alanine aminotransferase and aspartate aminotransferase.[18] Topical PABA effectively prevented peripheral granulocytosis and enlarged spleen in nude mice exposed to UV but led to slightly decreased spleen weight, suggesting a systemic toxic effect of PABA.[19]

This study was aimed to determine the accumulation in blood, liver, and kidney and the overt toxicity of PABA in rats. In addition, the effect of PABA on in vivo and in vitro lipid peroxidation was studied because we showed that the compound effectively scavenged certain reactive oxygen species in vitro.[12] PABA was provided in drinking water rather than in the diet in order to prevent microbial utilization of PABA for folate biosynthesis in the diet.

Methods and materials

All chemicals used were of reagent or higher grade. Major reagents used were purchased from Sigma Chemical Co. (St. Louis, MO USA) including potassium *para*-aminobenzoate (K-PABA), thiobarbituric acid (TBA), *t*-butylhydroperoxide (BHP) and N-1-naphthylethylenediamine dihydrochloride (NNED).

Animals and diet

Fifty-one male Sprague-Dawley rats (5-week old, weighing ca. 150 g) were housed in stainless-steel cages (two per cage) and fed a standard laboratory diet (Purina Rat Chow 5001, Purina Mills, St. Louis, MO USA) with food and water provided ad libitum. PABA (potassium salt) was provided in drinking (deionized) water at 0%, 0.1%, 0.5%, and 1%, with each group having 12 rats. The pH values of the water containing various levels of PABA were between 7.5 and 7.7, and the water was changed every day to prevent microbial growth. The standard laboratory diet contained approximately 22 μg/g or 0.002% PABA, as determined using a photometric method as described below. Consumption of water was measured every day. Body weights were measured once a week during the 4-week study period.

Three rats from each group at week 1 and 2 and the remaining six rats from each group at week four were killed by decapitation and blood was collected in a beaker containing EDTA (approx. 2 mg/mL of blood). Tissues were removed immediately, rinsed in cold phosphate-buffered saline, and blotted on a filter paper before weighing. A portion of the blood was centrifuged (3,000 × g, 10 min at 4°) immediately to obtain plasma. Plasma, liver, and kidney tissues and remaining blood samples were quickly frozen in liquid nitrogen and stored at −35° before use.

Analysis of PABA

PABA was determined photometrically based on the coupling of PABA with NNED,[20] as described for feeds[21,22] and human serum[13] and plasma.[14] Liver and kidney tissues were homogenized in deionized water (10%, w/v) and centrifuged at 9,000 × g for 10 min at 4°. The supernatant was then deproteinized using TCA (final 5%) and hydrolyzed in a steam bath for 25 min. Blood samples were diluted 5 fold in deionized water before deproteinization and hydrolysis, as described. After diazotization and coupling with NNED the absorbance of the red complex was read at 545 nm. The method measures aromatic amines and is, thus, not specific for PABA. However, tryptophan, a major aromatic amine in tissues, has no apparent coupling with NNED.[21] The method also does not measure the PABA moiety of folic acid because the cleavage of folic acid requires strong oxidizing agents such as potassium permanganate.[23] The relatively large dilution of samples before deproteinization was to minimize interferences. The method was sensitive to submicromolar level with no apparent detection limit. When taken as twice the highest tissue blank value ($A_{545\ nm}$ = 0.012 for the kidney), a sensitivity of 0.8 μM PABA was obtained.

Enzyme assays

Plasma aspartate aminotransferase (glutamic oxaloacetic transaminase, GOT) and alanine aminotransferase (glutamic pyruvic transaminase, GPT) were determined based on coupled reactions as described elsewhere.[24]

Measurement of lipid-peroxidation

In vivo lipid peroxidation was determined in liver and kidney homogenates (10%, w/v) as TBA reactive substances (TBARS) as described previously.[25,26] In vitro lipid peroxidation was measured after incubation of liver and kidney homogenates with 0.75 mM BHP for 1 hr at 37°. The selection of BHP concentration was based on a induction of approximately twice the amounts of TBARS produced without BHP using the liver from control rats. Protein was measured by a dye-binding method (BioRad, Richmond, CA USA).

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) with Duncan's multiple range test for group mean comparison using a computerized program (SAS Institute, Cary, NC USA). A P value <0.05 was considered statistically significant.

Results

Changes in body and organ weights

PABA in the drinking water did not affect the growth of rats as indicated by the similar body weight gain during the 4-week feeding period (*Table 1*). Diet consumption was not

Table 1 Body weight gains of rats given varying amounts of PABA in the drinking water for 4 weeks

	Body weight gain (g)[1]		
PABA	Week 1	Week 2	Week4
Control	72 ± 6	112 ± 11	170 ± 13
0.1%	76 ± 7	121 ± 8	185 ± 12
0.5%	72 ± 7	113 ± 9	172 ± 11
1.0%	70 ± 10	112 ± 12	174 ± 15
P[2]	N.S.	N.S.	N.S.

[1]The body weight gain (means ± SD) was obtained by subtracting the body weights from the beginning weights of individual rats.
[2]P values >0.05 are considered non-significant (N.S.) statistically.

measured but there was no difference in water consumption (data not shown). PABA had no significant effects on organ (liver, kidney, and spleen) weights, although the spleen weight was somewhat higher in all groups of rats fed PABA than that of control rats (*Table 2*).

Contents of PABA in Tissues

Figure 1 shows that PABA given at 0.1% in the water did not increase PABA contents in the liver. At weeks 1 and 2 only the rats given 1% PABA had significantly more liver PABA than rats of the other groups (*Figure 1*). At week 4 the liver PABA content (8.4 μg/g or 61 nmol/g) of rats given 0.5% PABA was significantly higher than those of control rats and of rats given 0.1% PABA, but was lower ($P < 0.05$) than that (13 μg/g or 95 nmol/g) of rats given 1% PABA. The liver PABA contents of the control rats fluctuated somewhat during the study period and was slightly higher ($P > 0.05$) at week 4 (7 μg/g) than at week zero (5 μg/g). The reason for such a fluctuation is unknown.

As in the liver, PABA at 0.1% in the water was unable to raise kidney PABA contents. Unlike the liver, the highest level of PABA in the kidney (11 μg/g or 80 nmol/g) was found at week 2 in rats given 1% PABA, which then decreased sharply at week 4 (*Figure 2*). At week 4 only the rats given 1% PABA had a significantly higher content of PABA than that of control rats.

The PABA content (0.6 μg/mL) of the blood was small before feeding began but rose with increasing PABA levels in the water and reached 5.1 μg/mL or 37 nmol/mL in rats given 1% PABA for 4 weeks (*Figure 3*). As in the liver and kidney, PABA at 0.1% in the water had little or no effect on the blood PABA contents.

Activities of GOT and GPT in plasma

Plasma GOT activities of rats given 0.5 and 1% PABA were significantly lower than that of control rats at week 2 (*Figure 4A*). However, the activities of GOT were all within normal ranges (0 to 40 IU/L), and the difference in activities became diminished and were not significant ($P > 0.05$) among the groups at week 4. PABA in the drinking water did not significantly affect plasma GPT activities, but a trend toward decreased GPT activities with increasing PABA levels existed during the 4-week study period (*Figure 4B*).

Table 2 Organ weights of rats given varying amounts of PABA in the drinking water for 4 weeks

PABA	Organ weights (g/100 g body weight)[1]		
	Liver	Kidney	Spleen
Control	3.30 ± 0.31	0.85 ± 0.04	0.23 ± 0.10
0.1%	3.41 ± 0.23	0.82 ± 0.04	0.29 ± 0.09
0.5%	3.26 ± 0.04	0.82 ± 0.04	0.27 ± 0.14
1.0%	3.44 ± 0.12	0.87 ± 0.06	0.26 ± 0.06
P	N.S.	N.S.	N.S.

[1]Values are means ± SD for six rats; *P* values >0.05 are considered non-significant (NS) statistically.

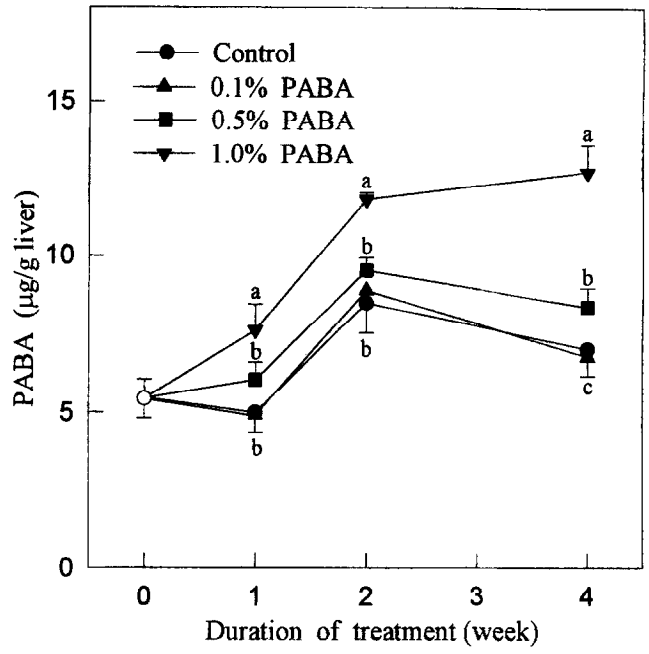

Figure 1 PABA contents in the liver of rats given varying amounts of PABA in drinking water for 4 weeks. Data are means ± SD of three (weeks 0, 1, and 2) or six (week 4) rats; values not sharing the same letters are significantly different ($P < 0.05$).

Lipid peroxidation in vivo and in vitro

Liver TBARS not induced by an exogenous oxidant was measured as in vivo lipid peroxidation (*Table 3*). Significantly decreased TBARS were found in the liver from rats

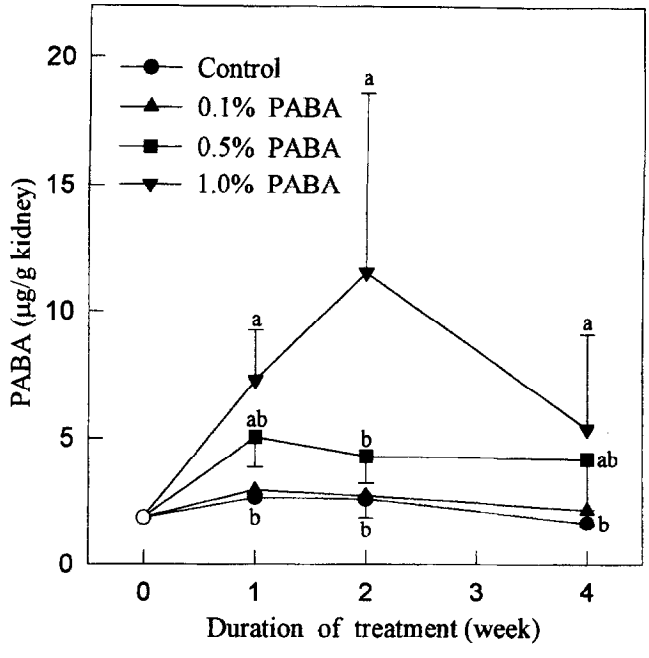

Figure 2 PABA contents in the kidney of rats given varying amounts of PABA in drinking water for 4 weeks. Data are means ± SD of three (weeks 0, 1, and 2) or six (week 4) rats; values not sharing the same letters are significantly different ($P < 0.05$).

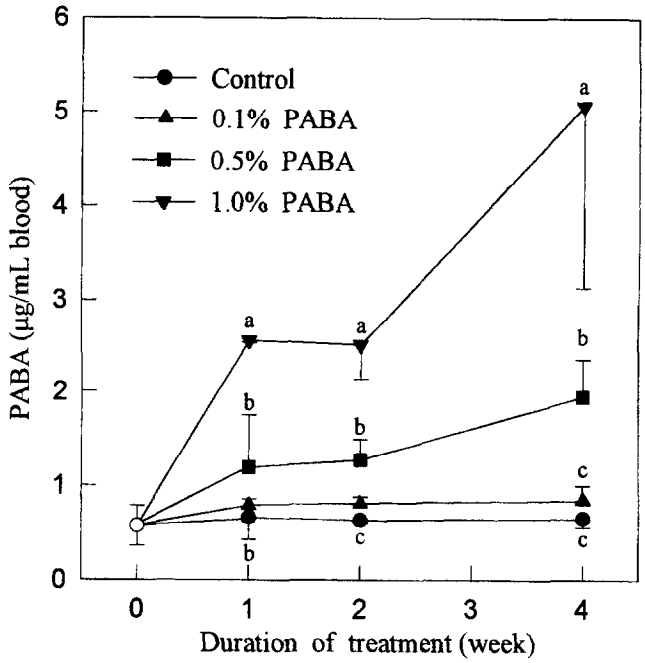

Figure 3 PABA contents in the blood of rats given varying amounts of PABA in drinking water for 4 weeks. Values are means ± SD of three (weeks 0, 1, and 2) or six (week 4) rats; values not sharing the same letters are significantly different ($P < 0.05$).

given 1% PABA as compared with the individual controls at weeks 2 and 4. As expected, levels of TBARS were raised in the liver incubated with BHP (37°, 1 hr). This magnification of lipid peroxidation also led to the same basic findings, i.e., only the highest PABA (1%) was sufficient to significantly lower lipid peroxidation.

In the kidney PABA had no significant effects on either in vivo or in vitro (i.e., BHP-induced) lipid peroxidation, although there was a trend toward decreased TBARS with increasing PABA contents in rats of the same ages (*Table 4*). Similar results were found when lipid peroxidation was expressed based on weights, i.e., nmol TBARS/g kidney (data not shown).

Discussion

Several points were demonstrated in this investigation. Firstly, PABA can be accumulated in tissues and blood, but only to a relatively limited extent. PABA at 0.1% in the drinking water barely increased PABA contents in the liver, kidney, and blood. The liver and kidney of rats given 1% PABA in the water (equivalent to 57 mM as the potassium salt) for 4 weeks contained only approximately twice the amounts of PABA in the liver and kidney of control rats. The highest amounts of PABA achieved in the liver and kidney were 13 μg/g (95 nmol/g) and 11 μg/g (80 nmol/g), respectively, with the liver generally accumulating more PABA than the kidney. Blood PABA concentrations increased markedly from 0.6 μg/mL (4.4 nmol/mL) in the control rats to 5.1 μg/mL (37 nmol/mL) in rats given 1% PABA for 4 weeks. The relatively limited accumulation of PABA is consistent with the fact that PABA in its free form

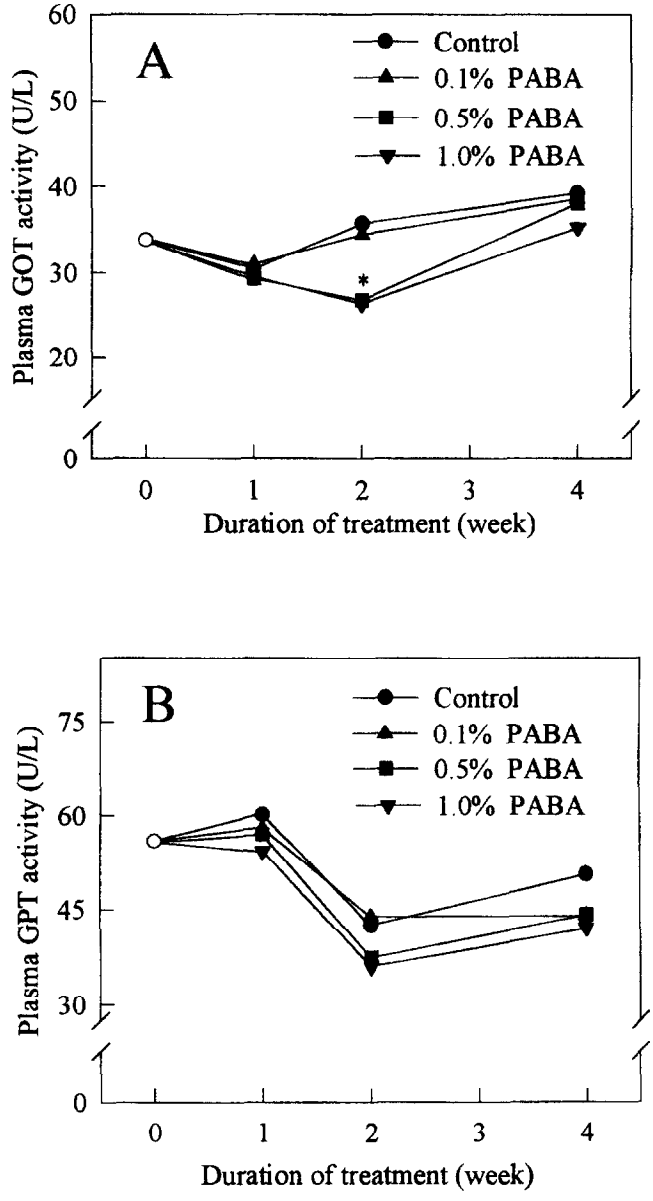

Figure 4 Activities of GOT (A) and GPT (B) in the plasma of rats given varying amounts of PABA in drinking water for 4 weeks. Values are means ± SD of three (weeks 0, 1, and 2) or six (week 4) rats. *Significantly different ($P < 0.05$) from the control at week 2.

is rapidly absorbed by the intestinal epithelium but is almost completely excreted in the urine in acetylated form.[13] Thus, whether PABA is provided in the diet or through drinking water may not result in marked difference in tissue accumulation.

Even in rats given 1% PABA in the water for 4 weeks, there was no overt toxicity, i.e., no significant effects on body and organ (liver, kidney, and spleen) weights or on plasma GOT and GPT activities. Indeed, PABA is generally considered nontoxic except for a few minor side effects such as nausea and skin rash.[16,17] However, a case report[18] showed that oral PABA (12 g/d) for 2 months for treatment of lichen sclerosus et atrophicus of a 64-year-old female led to hepatotoxicity, as evidenced by elevated serum GOT

Table 3 In vivo and in vitro lipid peroxidation in the liver of rats given varying amounts of PABA[1]

	TBARS (nmol/mg protein)[2] × 10^2		
	Week 1	Week 2	Week 4
In vivo (non-induced)			
Control	2.8 ± 0.2	3.9 ± 0.9[a]	3.3 ± 0.5[a]
0.1%	2.6 ± 0.4	3.7 ± 0.6[b]	3.4 ± 0.5[a]
0.5%	2.9 ± 0.3	3.2 ± 0.4[ab]	3.0 ± 0.1[a]
1.0%	2.5 ± 0.3	2.7 ± 0.1[b]	2.5 ± 0.4[b]
P	N.S.	≤0.05	≤0.05
In vitro (induced by 0.75 mM BHP) (37°, 1 hr)			
Control	5.8 ± 0.3[a]	10.1 ± 0.4[a]	10.2 ± 1.0[a]
0.1%	4.5 ± 0.4[b]	10.0 ± 0.5[a]	9.5 ± 1.0[ab]
0.5%	5.5 ± 0.4[a]	10.6 ± 1.7[a]	10.5 ± 1.7[a]
1.0%	4.7 ± 0.5[b]	6.5 ± 0.8[b]	8.6 ± 1.0[b]
P	≤0.05	≤0.05	≤0.05

[1]Lipid peroxidation was measured as TBA reactive substances either directly from the liver (in vivo) or after incubation with BHP for 1 hr (in vitro).
[2]Values are means ± SD (n = 3 for weeks 1 and 2; n = 6 for week 4). Values in a column (i.e., the same week) with the same superscripts are not significantly different (N.S., $P > 0.05$).

(600 IU/L) and GPT (912 IU/L). Our studies showed no marked changes in these plasma enzymes in rats given PABA in the drinking water. In fact, plasma GOT in rats given 0.5 and 1% PABA was significantly lower than that in the control rats after 2 weeks of feeding, and PABA tended to decrease plasma GPT activities during the 4-week study period. Although direct comparison between humans and rats may seem unwarranted, a crude calculation could provide useful information. Thus, 1% PABA in the water would be equivalent to 0.5 g PABA per day for a 250-g rat (our rats grew from 150 to 330 g during the study) based on an average daily water intake of 50 mL. For a 60-Kg person, this would be equivalent to 120 g PABA per day. Hence, the hepatotoxicity of the patient described in the case report may not be attributed solely to PABA. It cannot be ruled out that the 4-week feeding period in our study is not long enough to reveal the toxicity of PABA.

Table 4 In vivo and in vitro lipid peroxidation in the kidney of rats given varying amounts of PABA[1]

	TBARS (nmol/mg protein)[2] × 10^2		
	Week 1	Week 2	Week 4
In vivo (non-induced)			
Control	13.1 ± 1.6	15.2 ± 1.5	12.8 ± 4.0
0.1%	14.0 ± 1.4	15.8 ± 2.3	14.3 ± 2.4
0.5%	13.2 ± 1.4	14.7 ± 2.6	12.2 ± 2.7
1.0%	12.7 ± 0.4	13.1 ± 1.1	12.6 ± 1.3
P	N.S.	N.S.	N.S.
In vitro (induced by 0.75 mM BHP) (37°, 1hr)			
Control	16.3 ± 0.9	21.4 ± 1.9	25.4 ± 4.5
0.1%	16.0 ± 2.9	22.7 ± 3.1	27.1 ± 4.1
0.5%	16.0 ± 0.8	19.5 ± 3.0	23.8 ± 2.9
1.0%	14.7 ± 1.1	17.0 ± 4.2	22.6 ± 2.9
P	N.S.	N.S.	N.S.

[1]Lipid peroxidation was measured as TBA reactive substances either directly from the kidney (in vivo) or after incubation with BHP for 1 hr (in vitro).
[2]Values are means ± SD (n = 3 for week 1 and 2; n = 6 for week 4). Values in a column (i.e., the same week) were compared; N.S.: not significantly different (N.S., $P > 0.05$).

Flindt-Hansen and Ebbesen[19] showed that female nude mice exposed to UV for 2 months developed peripheral granulocytosis and enlarged spleen, both of which were effectively prevented by topical PABA (5% lotion). However, the UV-irradiated and PABA-treated (full-time protected) mice had a slightly decreased mean spleen weight compared with that of the controls (0.15 vs. 0.17 g, $P < 0.001$). Based on this, they suggested that PABA may have a systemic toxic effect. In our study, we noted that the spleen weights of rats given PABA (irrespective of the levels of PABA in the water) for 4 weeks appeared to be higher ($P > 0.05$), but not lower, than those of the control rats. In the study by Flindt-Hansen and Ebbesen[19] there was no PABA control group, i.e., mice treated with PABA without UV exposure. Thus, whether or not PABA may change spleen weights awaits further investigations.

PABA was a weak membrane antioxidant since only rats given 1% PABA in the water had decreased in vivo and in vitro lipid peroxidation in the liver (and tended to do so in the kidney). This finding is consistent with our observations that exogenous PABA only weakly protected liver microsomes and red blood cell membranes against in vitro lipid peroxidation (induced by H_2O_2, BHP or Fe^{3+}/ascorbate) (unpublished data). The results imply that PABA may be most relevant in topical use for skin protection because the compound strongly absorbs UVB[1,2] and effectively scavenges hydroxyl radicals and singlet oxygen.[12] Future studies may also be directed to determining the distribution of PABA in other tissues and cells such as polymorphonuclear leukocytes where its metabolism[27] or reaction with hypochlorous acid[12] may be responsible for the antiinflammatory activity of PABA.

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References

1 Combs, G.F., Jr. (1991). *The vitamins: fundamental aspects in nutrition and health,* p. 426, Academic Press, New York, NY USA
2 Flindt-Hansen, H., Thune, P. and Larsen, T.E. (1990). The inhibiting effect of PABA on photocarcinogenesis. *Acta Dermatol. Res.* **282,** 38–41
3 Kumar, K.G. and Indrasenan, P. (1989). Titrimetric determination of para-aminobenzoic acid using N-bromophthalimide and N-bromosaccharin. *J. Pharmaceu. Biomed. Anal.* **7,** 627–631
4 Flindt-Hansen, H., Thune, P., and Eeg-Larsen, T. (1990). The effect of short-term application of PABA on photocarcinogenesis. *Acta Derm. Venereol.* **70,** 72–75
5 Zarafonetis, C.J.D. (1964). Antifibrotic therapy with potaba. *Am. J. Med. Sci.* **248,** 550–561
6 Torres, M.A. and Furst, D.E. (1990). Treatment of generalized systemic sclerosis. *Rheum. Dis. Clin. North Am.* **16,** 217–241
7 Knox, J.M., Griffin, A.C., and Hakim, R.E. (1960). Protection from ultraviolet carcinogenesis. *J. Invest. Dermatol.* **31,** 51–58
8 Flindt-Hansen, H. (1992). Photocarcinogenesis in mice and the effect

of a sunscreen, para-aminobenzoic acid. *Dan. Med. Bull.* **39,** 355–362

9 Snyder, D.S. and May, M. (1975). Ability of PABA to protect mammalian skin from ultraviolet-light induced skin tumors and actinic damage. *J. Invest. Dermatol.* **65,** 543–546

10 Verweij, H. and van Steveninck, J. (1981). Protective effects of semicarbazide and p-aminobenzoic acid against ozone toxicity. *Biochem Pharmacol.* **30,** 1033–1037

11 Esposito, M., Vannozzi, M.O., Viale, M., Fulco, R.A., Collecchi, P., Merlo, F., Cian, F.D., Zicca, A., Cadoni, A., and Poirier, M.C. (1993). Para-aminobenzoic acid suppression of cis-diamminedichloroplatinum(II) nephrotoxicity. *Carcinogenesis* **14,** 2595–2599

12 Hu, M.-L., Chen, Y.-K., Chen, L.C., and Sano, M. (1995). Para-aminobenzoic acid scavenges reactive oxygen species and protects DNA against UV and free radical damage. *J. Nutr. Biochem.* **6,** 504–508

13 Lang, C., Gyr, K., Tonko, I., Cohen, D. and Stalder, G.A. (1984). Value of serum PABA as a pancreatic function test. *Gut,* **25,** 508–512

14 Delchier, J-C. and Soule, J-C. (1983). BT-PABA test with plasma measurements: evaluation of sensitivity and specificity. *Gut* **24,** 318–325

15 Furuya, K.N., Roberts, E.A., Durie, P., Soldin, S., Yung-Jato, L., Verjee, Z., and Ellis, L. (1990). Measurement of para-aminobenzoic acid (PABA) and its metabolites as a test of liver function. *Hepatology* **12** (4 part 2), 1009 (Abstract #688)

16 Penneys, N.S. (1984). Treatment of lichen sclerosus with potassium para-aminobenzoate. *J. Am. Acad. Dermatol.* **10,** 1039–1042

17 Physicians' desk reference, 38th ed., p. 991, 1984, Medical Economics Co., Inc., Ordell, NJ, USA

18 Kantor, G.K. and Ratz, J.L. (1985). Liver toxicity from potassium para-aminobenzoate. *J. Am. Acad. Dermatol.* **13,** 671–672

19 Flindt-Hansen, H. and Ebbesen, P. (1991). Ultraviolet light induction of peripheral granulocytosis with splenomegaly: protection of mice with topical para-aminobenzoic acid (PABA). Br. J. Dermatol. **39,** 355–362.

20 Bratton, A.C. and Marshall, E.K., Jr. (1939). A new coupling component for sulfanilamide determination. *J. Biol. Chem.* **128,** 537–550

21 Merwin, R.T. (1962). *p*-Aminobenzoic acid in feeds. *J. Am. Osteopath. Assoc.* **45,** 289–291

22 A.O.A.C. Methods of Analysis, 12th ed., W. Horwitz, ed., p. 778, 1975

23 Hubbard, W.D., Hintz, M.E., and Libby, D.A. (1966). Chemical determination of folic acid in the presence of p-aminobenzoic acid or procaine hydrochloride. *J. Am. Osteopath. Assoc.* **49,** 804–806

24 Anonymous (1991/1992). Glutamic oxaloacetic transaminase and glutamic pyruvic transaminase. Calzyme Laboratories, Inc., San Luis Obispo, CA

25 Tappel, A.L. and Zalkin, H. (1959). Inhibition of lipid peroxidation in mitochondria by vitamin E. *Arch. Biochem. Biophys.* **80,** 333–336

26 Hu, M.-L., Frankel, E.N., Leibovitz, B.E., and Tappel, A.L. (1989). Effect of dietary lipids and vitamin E on in vitro lipid peroxidation in rat liver and kidney homogenates. *J. Nutr.* **119,** 1574–1582

27 Sagone, A.L., Jr., Husney, R.M., and Davis, W.B. (1993). Biotransformation of para-aminobenzoic acid and salicylic acid by PMN. *Free Radic. Biol. Med.* **14,** 27–35