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# Effects of Neurotropin on Rat Liver Microsomes and Lysosomes, and *in Vitro* Lipid Peroxidation

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The effects of Neurotropin (NSP) on lysosomal and microsomal enzyme activities, antioxidant levels and fatty acid composition of microsomal phospholipids in rat liver were studied before and after $CCl_4$ intoxication. The effect on *in vitro* lipid peroxidation was also determined. NSP post-treatment (60 mg/kg/d for 3 and 7 d after $CCl_4$) did not produce a significant recovery of the enzyme activities, while NSP pretreatment (60 mg/kg/d for 7 d before $CCl_4$) partly prevented the decrease in lysosomal enzyme activities after $CCl_4$ intoxication and increased the level of water-soluble antioxidants as compared with that of $CCl_4$-treated rats. NSP significantly decreased ascorbate-induced lipid peroxidation *in vitro*, by 20% at the concentration of 1 mg/ml, without affecting the reduced nicotinamide adenine dinucleotide phosphate (NADPH)-induced lipid peroxidation. Further, NSP significantly enhanced, by 20—23%, the protecting effect of inhibitors ($CoCl_2$ and aniline) of lipid peroxidation. Thus, NSP pretreatment appears to have a partial protecting effect against $CCl_4$ intoxication and accompanying lipid peroxidation.

**Keywords**——neurotropin; carbon tetrachloride-intoxication; lipid peroxidation; lysosome; microsome; enzyme activity

Neurotropin (NSP), a neurosedative, is an extract containing many conjugated polysaccharides isolated from the skin or tissues of rabbits inoculated with living *cowpox virus*. It has been demonstrated that NSP has analgesic effects,[1,2] antihypertensive action in spontaneously hypertensive rats (SHR),[3] antiulcerogenic effects on restraint-plus-water-immersion ulcers and histamine-induced duodenal ulcers[2] and antiinflammatroy effects against allergic reactions of Types I—III.[4]

Ishii *et al.* reported an almost complete suppressive effect of NSP on the hepato-cirrhosis induced by carbon tetrachloride ($CCl_4$) and the hepatoma mediated by 3′-methyl diethylaminoazobenzene.[5] However, little is known of the effects of NSP on the lysosomes and microsomes of an injured liver. There is a fair amount of evidence that lysosomes are involved in the process of intracellular digestion and in various physiological and pathological phenomena of autolysis and necrosis.[6] Further, changes in the activities of the microsomal enzyme system affect the pharmacological action and toxicity of drugs.[7]

In order to clarify the action of NSP on the function of lysosomes and microsomes in liver and the protecting effect of NSP against $CCl_4$ introxication, the effects of NSP on enzyme activities of both organelles, hepatic antioxidant levels and fatty acid composition of microsomal phospholipids were studied before and after $CCl_4$ intoxication. In addition, the effect of NSP on the *in vitro* lipid peroxidation of microsomes mediated by enzymatic or non-enzymatic processes was investigated.

## Experimental

**Materials**——NSP (10 mg/ml) solution was obtained from Nippon Zoki Seiyaku Co., Ltd. Nicotinamide ade-

nine dinucleotide phosphate (NADP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma Chemical Co. Glucose-6-phosphate (G-6-P) dehydrogenase [EC 1.1.1.49] and G-6-P were obtained from Oriental Yeast Co., Ltd. Standard fatty acid methyl esters (GLC grade) were purchased from Nippon Gaskuro Kogyo. Polyvinyl alcohol (PVA)-117 was a gift from Kurashiki Rayon Co., Ltd. Other chemicals used were of special or analytical grade.

**Animals**——Male Wistar rats weighing about 120—130 g, maintained on MF diet (Oriental Yeast Co., Ltd.) for 3—4 d prior to the experiment, were used throughout. The animals were divided at random into 3 groups, each containing 4—6 rats.

(A) Post-treatment with NSP: a) Controls were treated for 2 d with daily *s.c.* injections of 0.15 ml of olive oil per 100 g of body weight, followed by a daily intraperitoneal (*i.p.*) injection of saline for 3 or 7 d. b) Animals were treated for 2 d with daily *s.c.* injections of 0.15 ml of $CCl_4$ and 0.15 ml of olive oil per 100 g of body weihgt, followed by a daily *i.p.* injection of NSP (6 mg/100 g weight) for 3 or 7 d ($CCl_4$-NSP-rats). c) Animals were treated as described in b), except that saline was given instead of NSP solution ($CCl_4$-rats). The animals were sacrificed by decapitation 2 h after the final administration of saline or NSP on the final day.

(B) Pretreatment with NSP: a) Controls were treated for 7 d with daily *i.p.* injection of saline, followed by a single *s.c.* injection of 0.2 ml of olive oil per 100 g of body weight 2 h after the last saline injection. b) Animals were treated for 7 d with daily *i.p.* injections of NSP (6 mg/100 g weight), followed by a single *s.c.* injection of $CCl_4$ (0.2 ml $CCl_4$ and 0.2 ml olive oil/100 g weight) 2 h after the last NSP injection (NSP-$CCl_4$-rats). c) Animals were treated as described in b), except that saline was administered instead of NSP solution ($CCl_4$-rats). The animals were sacrificed 24 h after olive oil or $CCl_4$ administration.

**Preparation of Liver and Microsomal Fractions**——The lysosomal fractions (heavy lysosomal fractions) were prepared by a modification of the method of Tanaka and Iizuka,[8] as described previously.[9] The microsomes were fractionated according to the procedure of Omura and Sato.[10]

**Assays of Enzymes**——Aspartate aminotransferase (GOT) [EC 2.6.1.1] activity in serum was assayed according to the method described by Babson *et al.*[11] Aniline *p*-hydroxylase [EC 1.14.1.1] activity was measured at 37 °C accoridng to the method of Ikeda.[12] The demethylation of aminopyrine was assayed at 37 °C by the method of Ariyoshi and Takabatake,[13] and formaldehyde formed was determined by the method of Nash.[14] The concentration of cytochrome P-450 (P-450) was determined at room temperature according to the method of Omura and Sato.[10] The lysosomal fractions were frozen and thawed 6 times and centrifuged for 20 min at 20000 × *g*. The supernatant was used for the assay of each enzyme. Acid phosphatase [EC 3.1.3.2] and β-glucuronidase [EC 3.2.1.31] activities were assayed at 37 °C by the procedure of Symons *et al.*[15] and Fishman *et al.*,[16] respectively. For the assay of lipolytic enzyme [EC 3.1.1.3] activity at 37 °C, the incubation mixture consisted of 1 ml each of 25% (v/v) olive oil–2% (w/v) PVA 117 emulsion and 0.1 M phosphate buffer, pH 7.2, and 0.5 ml each of 4% bovine serum albumin and enzyme solution. The determination of fatty acids liberated was done by the method of Dole.[17]

**Separation of Lipids and Fatty Acid Analyses**——The separation of the neutral lipids and phospholipids, and extraction of microsomal membrane lipids were done by the method of Colbeau *et al.*,[18] using a column of silicic acid–Celite 545 (2 : 1, by weight). The fatty acids of phospholipids were methylated with $BCl_3$–$CH_3OH$.[19] The methyl esters were analyzed by gas-liquid chromatography (GLC) on a Shimadzu gas chromatograph, model GC-4BM, with a hydrogen flame ionization detector (15% polyethylene glycol succinate polyester on 60—80 mesh Chromosorb W, 3 mm × 2 m).

***In Vitro* Lipid Peroxidation and Estimation of Peroxidation**——Ascorbate-induced and NADPH-induced lipid peroxidations were carried out for 1 h at 37 °C, using 0.3 ml of microsomal fraction (5 mg protein/ml), by the method described by Devasagayam *et al.*[20] The malondialdehyde formed was estimated immediately by using the thiobarbituric acid (TBA) (0.5% 2-thiobarbituric acid–10% trichloroacetic acid–2 mM ethylenediaminetetraacetic acid (EDTA)–0.63 M hydrochloric acid) reaction.[21,22]

**Measurement of Antioxidants**——The determination of fat-soluble and water-soluble antioxidants in liver homogenates was done by the procedure of Glavind.[23]

**Protein Determination**——Protein was estimated by the method described by Lowry *et al.*[24]

## Results

### Effect of NSP Post-treatment on Lysosomal and Microsomal Enzyme and GOT Activities in $CCl_4$-Intoxicated Rats

The enzyme activities of lysosomes and microsomes in rat liver as well as the GOT activity were measured after exposure of rats to $CCl_4$, followed by NSP treatment. As shown in Table I, a single daily dose of $CCl_4$ for 2 d significantly decreased the microsomal drug-metabolizing enzyme activities and markedly enhanced serum GOT level on day 3, while $CCl_4$ administration had little effect on the activities of lysosomal enzymes, acid phosphatase and β-

Table I. Effect of NSP Post-treatment of Activities of Lysosomal and Microsomal Enzyme and GOT in $CCl_4$-Intoxicated Rats

| Enzymes | | Control | $CCl_4$ | | $CCl_4$–NSP | |
|---|---|---|---|---|---|---|
| | | | Saline (3 d) | Saline (7 d) | NSP (3 d) | NSP (7 d) |
| Relative liver weight[a] | | 4.46±0.36 | 5.86±1.46 | 4.92±0.54 | 4.96±0.20 | 5.03±0.46 |
| Serum GOT[b] | | 0.249±0.015 | 0.958±0.440[f] | 0.289±0.043 | 0.731±0.220[g] | 0.305±0.055 |
| MS | Aniline hydroxylase[c] | 0.379±0.057 | 0.115±0.096[h] | 0.325±0.062 | 0.073±0.011[i] | 0.343±0.056 |
| MS | Aminipyrine N-demethylase[c] | 2.45±0.35 | 1.29±0.23[i] | 2.10±0.44 | 1.07±0.07[i] | 2.34±0.23 |
| MS | Cyt. P-450[d] | 0.752±0.073 | 0.350±0.107[i] | 0.674±0.060 | 0.371±0.038[i] | 0.706±0.029 |
| LS | Acid phosphatase[e] | 0.346±0.030 | 0.310±0.077 | 0.372±0.031 | 0.362±0.044 | 0.403±0.062 |
| LS | β-Glucuronidase[e] | 50.2±7.3 | 55.8±10.5[f] | 50.1±1.8 | 75.1±10.3[g] | 61.1±2.0[f,i] |

Animals were treated for 2 d with daily *s.c.* injections of 0.3 ml of $CCl_4$–olive oil mixture (1 : 1, v/v) per 100 g, followed by daily *i.p.* injections of NSP (60 mg/kg, *i.p.*) for 3 or 7 d.

*a*) g/100 g of body weight. *b*) μmol of oxaloacetic acid formed/ml of serum/min. *c*) nmol of product/mg of microsomal protein/min. *d*) nmol of P-450/mg of microsomal protein. *e*) μmol of product/mg of lysosomal protein/min. MS, microsomes; LS, lysosomes. *f*) $p<0.05$ in control *vs.* $CCl_4$–saline 3, control *vs.* $CCl_4$–NSP 7 and $CCl_4$–saline 3 *vs.* $CCl_4$–NSP 3. *g*) $p<0.025$ in control *vs.* $CCl_4$–NSP 3. *h*) $p<0.01$ in control *vs.* $CCl_4$–saline 3. *i*) $p<0.005$ in control *vs.* $CCl_4$–saline 3, control *vs.* $CCl_4$–NSP 3 and $CCl_4$–saline 7 *vs.* $CCl_4$–NSP 7.

Table II. Effect of NSP Pretreatment on Lysosomal and Microsomal Enzyme Activities in $CCl_4$-Intoxicated Rats

| Enzymes | | Control | $CCl_4$ | NSP–$CCl_4$ |
|---|---|---|---|---|
| Relative liver weight | | 4.92±0.31 | 5.60±0.51 | 5.37±0.49 |
| Serum GOT | | 0.405±0.025 | 0.655±0.202[c] | 0.464±0.030[e] |
| MS | Aniline hydroxylase | 0.376±0.048 | 0.123±0.035[a] | 0.128±0.024[d] |
| MS | Aminopyrine N-demethylase | 2.59±0.36 | 1.58±0.43[c] | 1.68±0.31[f] |
| MS | Cyt. P-450 | 0.728±0.152 | 0.365±0.067[b] | 0.456±0.085 |
| LS | Acid phosphatase | 0.392±0.038 | 0.265±0.028[a] | 0.372±0.024[g] |
| LS | β-Glucuronidase | 59.6±7.0 | 46.6±6.1 | 57.3±4.3 |
| LS | Lipase | 96.2±16.5 | 37.8±11.1[a] | 54.3±12.1[g] |

Animals were treated for 7 d with daily *i.p.* injections of saline or NSP (60 mg/kg) followed by a single injection of $CCl_4$ (0.2 ml/100 g, *s.c.*). The activities of enzymes and the content of P-450 are expressed in the same ways as in Table I.

*a*) $p<0.005$. *b*) $p<0.025$. *c*) $p<0.05$ in control *vs.* $CCl_4$. *d*) $p<0.005$. *e*) $p<0.025$. *f*) $p<0.05$ in control *vs.* NSP–$CCl_4$. *g*) $p<0.05$ in $CCl_4$ *vs.* NSP–$CCl_4$.

glucuronidase. The negligible effect of $CCl_4$ on the lysosomal enzymes is probably the reason for the rapid recovery of lysosomal function in 3 d. Administration of NSP for 7 d to $CCl_4$-poisoned rats tended to increase all enzyme activities in lysosomes and microsomes as compared with the activities in $CCl_4$-rats, but the effects were not statistically significant, except for β-glucuronidase activity. In appearance, the liver of rats given NSP was similar to that of the control, whereas the internal organs of $CCl_4$-rats were all whitish in color.

**Effect of NSP Pretreatment on the Activities of Lysosomal Enzymes, Microsomal Enzymes and GOT in $CCl_4$-Intoxicated Rats**

The most marked decrease among the lysosomal enzyme activities was in lipase activity. This may be due to the relatively high sensitivity of this enzyme to lipid peroxides, as reported previously.[9]

The NSP pretreatment (for 7 d) did not have a significant protecting effect on microsomal

TABLE III. Effect of NSP Pretreatment on Fatty Acid Composition of Microsomal Phospholipids in $CCl_4$-Intoxicated Rats

| Fatty acid | Composition (%) | | |
|---|---|---|---|
| | Control | $CCl_4$ | NSP–$CCl_4$ |
| 16:0 | 18.81±0.99 | 20.88±1.53 | 20.52±1.14 |
| 18:0 | 28.76±0.93 | 29.08±1.92 | 28.96±1.57 |
| 18:2 | 11.40±1.47 | 13.98±3.54 | 14.11±1.30[a] |
| 20:4 | 30.49±2.45 | 24.87±2.66 | 26.07±1.42[b] |
| 22:6 | 10.55±0.43 | 11.20±1.40 | 10.36±0.88 |

Animals were pretreated with NSP (60 mg/kg/d) or saline for 3 d and $CCl_4$ (0.2 ml/100 g) was given 2 h after the last administration of NSP. Each value represents the mean±S.D. of 4 rats.
*a*) $p<0.05$. *b*) $p<0.025$ compared with control.

TABLE IV. Effect of NSP Pretreatment on Hepatic Antioxidant Levels in $CCl_4$-Intoxicated Rats

| Treatment | Water-soluble (μeq/g liver) | Fat-soluble (μeq/g liver) |
|---|---|---|
| Control | 14.16±2.05 | 0.060±0.002 |
| $CCl_4$ | 18.71±2.51[a] | 0.081±0.002 |
| NSP–$CCl_4$ | 23.85±3.56[b] | 0.083±0.002 |

Animals were pretreated with NSP (60 mg/kg/d, *i.p.*) or saline, and $CCl_4$ (0.2 ml/100 g, *s.c.*) was given 2 h after the last administration of NSP. Each value represents the mean±S.D. of 4 rats.
*a*) $p<0.005$. *b*) $p<0.05$ compared with control.

enzyme activities in $CCl_4$-intoxicated rats, though some lysosomal enzyme activities were slightly but significantly higher than those of $CCl_4$-rats ($p<0.05$), as shown in Table II. GOT activity of NSP–$CCl_4$-rats was almost at the control level. This suggests that NSP is able partly to prevent the decrease in hepatic lysosomal enzyme activities in $CCl_4$-intoxicated rats.

**Effect of NSP Pretreatment on Fatty Acid Composition of Microsomal Phospholipids in $CCl_4$-Intoxicated Rats**

The major fatty acid composition of microsomal phospholipids was estimated after NSP pretreatment for 3 d in $CCl_4$-intoxicated rats. The results are shown in Table III. After a dose of $CCl_4$, a clear decrease in arachidonic acid, which is the most unsaturated component of the fatty acid spectrum of microsomal phospholipids, was seen, probably due to peroxidative decomposition of the unsaturated acid, whereas the percentages of linoleic and docosahexaenoic acids were not markedly changed. The pretreatment with NSP for 3 d partly prevented the decrease in the arachidonic acid, but the effect was not statistically significant.

**Effect of NSP Pretreatment on Hepatic Antioxidant Levels in $CCl_4$-Intoxicated Rats**

It is well known that antioxidants can act as free radical scavengers which decrease the lipid peroxidation following $CCl_4$ administration.[25] In order to clarify whether or not the protecting effect of NSP on hepatic enzymes can be ascribed to an increased amount of antioxidants, the levels of water-soluble and fat-soluble antioxidants in the liver of rats pretreated with NSP were determined after $CCl_4$ administration. As shown in Table IV, $CCl_4$ treatment significantly increased water-soluble antioxidant levels ($p<0.005$) but not fat-soluble antioxidant levels in the liver. NSP pretreatment further enhanced the levels of the water-soluble antioxidants, from 14.16 μeq/g liver in the control to 23.85 μeq/g liver in the

Table V. Effect of Pretreatment with NSP on Hepatic Antioxidant Levels in $CCl_4$-Intoxicated Rats

| | Control | $CCl_4$ | NSP–$CCl_4$ |
|---|---|---|---|
| Relative liver weight[a] | 4.47±0.33 | 5.23±0.15[c] | 5.10±0.46[e] |
| α-Tocopherol[b] | 0.160±0.041 | 0.216±0.025 | 0.221±0.033[e] |
| Ascorbic acid[b] | 0.731±0.248 | 0.986±0.127 | 1.096±0.189[e] |
| Reduced glutathione[b] | 3.866±0.178 | 6.374±0.968[d] | 6.260±1.263[d] |

Animals were treated for 3 d with daily *i.p.* injections of NSP (60 mg/kg), followed by a single injection of $CCl_4$ (0.2 m/100 g, *s.c.*). The animals were sacrificed 12 h after $CCl_4$ administration.
*a*) g/100 g of body weight. *b*) μg/mg protein. *c*) $p<0.01$. *d*) $p<0.02$. *e*) $p<0.05$ compared with control.

Table VI. Effect of NSP on *in Vitro* Lipid Peroxidations

| NSP concentration (mg/ml) | Lipid peroxidation | | | |
|---|---|---|---|---|
| | Ascorbate-induced | | NADPH-induced | |
| | TBA value | Inhibition (%) | TBA value | Inhibition (%) |
| None | 1.713±0.164 | | 1.156±0.204 | |
| 1.00 | 1.371±0.179[a] | 20.0 | 1.070±0.175 | 7.2 |
| 0.33 | 1.579±0.142[b] | 7.7 | 1.022±0.151 | 11.6 |

TBA value is expressed in optical density ($\times 10^3$)/mg protein.
*a*) $p<0.01$ in none *vs.* NSP. *b*) $p<0.05$ between NSP.

NSP group, although NSP had no significant effect on the content of fat-soluble antioxidants.

**Effect of NSP Pretreatment on Hepatic Ascorbic Acid, Reduced Glutathione and α-Tocopherol Levels in $CCl_4$-Intoxicated Rats**

Since NSP pretreatment increased the antioxidant levels in the liver, as mentioned above, various factors related to lipid peroxidation were estimated following NSP pretreatment of $CCl_4$-intoxicated rats. As shown in Table V, the contents of the antioxidants were slightly increased following $CCl_4$ administration. NSP pretreatment enhanced the content slightly (not statistically significant) as compared with that of $CCl_4$-rats; the increased level of glutathione, a water-soluble antioxidant, was the greatest in $CCl_4$-intoxicated rats. This result is reasonably consistent with those for hepatic antioxidants shown in Table IV.

**Effect of NSP on *in Vitro* Lipid Peroxidation**

In the ascorbate-induced lipid peroxidation experiments, NSP (0.33 mg and 1.0 mg/ml incubation mixture) decreased the peroxidation by 7.7 and 20.0%, respectively (not statistically significant), as shown in Table VI. It appears that the extent of the inhibition is proportional to the concentration of NSP.

The NADPH-induced lipid peroxidation was only slightly inhibited by NSP (not statistically significant). These results suggest that NSP has a protecting effect against non-enzymatic, ascorbate-induced lipid peroxidation.

**Effects of NSP and Inhibitors on NADPH-Induced and Ascorbate-Induced Lipid Peroxidation**

To further examine the protecting effect of NSP against lipid peroxidation, the effects of NSP and various inhibitors on NADPH-induced and ascorbate-induced lipid peroxidations were evaluated. The results are shown in Fig. 1. NSP (1.0 mg/ml) significantly enhanced the

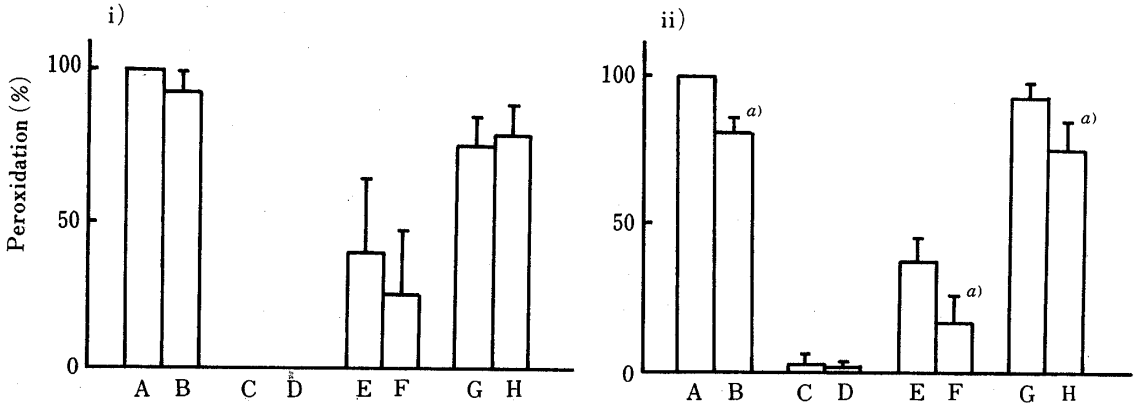


Fig. 1. Effect of NSP and Inhibitors on NADPH- and Ascorbate-Induced Lipid

i) NADPH-induced lipid peroxidation, ii) ascorbate-induced peroxidation. The concentration of NSP was 0.33 mg/ml mixture in i) and 1.0 mg/ml mixture in ii). The inhibitor concentration was 0.2 mM.

A, NADPH- or ascorbate-peroxidation; B, A+NSP; C, A+EDTA-$Na_2$; D, A+EDTA-$Na_2$+NSP; E, A+$CoCl_2$; F, A+$CoCl_2$+NSP; G, A+aniline; H, A+aniline+NSP.

*a*) $p<0.01$ in A *vs.* B, E *vs.* F and G *vs.* H.

protecting effect of various inhibitors ($p<0.01$), by about 21—23% except for the EDTA experiment (since EDTA produced almost complete inhibition, the effect of NSP could not be estimated). The inhibitory effect of NSP is similar to that (20.0%) shown in Table VI.

On the other hand, NSP did not produce additional inhibition of NADPH-induced lipid peroxidation, suggesting that NSP has no significant inhibitory effect on enzymatic peroxidation *in vitro*.

## Discussion

NSP has been proven to be clinically effective for various diseases, but the mechanism of its action has been only partially clarified. Thus, to elucidate the action of NSP on rat liver, some experiments were carried out *in vivo* and *in vitro*.

In the present study, administration of $CCl_4$ for 2 d was found to decrease the microsomal drug-metabolizing enzyme activities in rat liver, as has been shown in many studies.[26-30] Administration of NSP for 3 or 7 d to $CCl_4$-poisoned rats did not significantly restore the decreased activities in microsomal fractions. On the other hand, pretreatment with NSP for 7 d before $CCl_4$ exposure significantly reduced the decrease in acid phosphatase and lipase activities ($p<0.05$), although $\beta$-glucuronidase activity was not diminished, in the lysosomal fractions. The results suggest that pretreatment with NSP may partly protect the liver from injury. The pretreatment with NSP in $CCl_4$-intoxicated rats had different effects on hepatic microsomes and lysosomes; NSP did not have a significant protecting effect on the microsomal enzyme activities, while the agent showed slight protection of lysosomal enzyme activities (Tables I and II). The difference may be due to a difference in uptake capacity for antioxidants, especially water-soluble antioxidants, since the membrane lipid of microsomes is able to catalyze preferentially the metabolism of a wide variety of fat-soluble compounds,[31] while lysosomes are concerned with intracellular digestion by phagocytosis.[32] Thus, the increase of water-soluble antioxidants by NSP in $CCl_4$-intoxicated rats may stabilize the lysosomal membranes to some degree.

The present study has clearly demonstrated that *i.p.* administration of NSP increased the water-soluble antioxidant formation in the liver associated with $CCl_4$ administration. Hepatic antioxidants were found to be mostly the water-soluble type with only a small amount of fat-soluble type in our study (Table IV). A similar result was obtained by Nakashima *et al.*[33]

Therefore, an increment in the water-soluble antioxidant levels is apparently involved in the protecting effect of NSP against lipid peroxidation. The water-soluble antioxidants, glutathione,[34,35] ascorbic acid[36] and other reactive substances,[23] may be essential for protecting cells against oxidative damage, although in some cases ascorbate has an opposite effect on microsomal lipid peroxidation.[35]

In the *in vitro* experiment on lipid peroxidation, NSP could partly prevent ascorbate-induced peroxidation (Table VI and Fig. 1). These results demonstrate that NSP has a slight but direct suppressive effect on the process of non-enzymatic lipid peroxidation, though the reason why NSP directly inhibits the peroxidation is not clear on the basis of the results obtained.

An abundance of recent evidence indicates that the properties of many tightly bound microsomal enzymes are intimately related to interactions with the microsomal phospholipids.[37,38] A dose of $CCl_4$ resulted in the clear decrease in arachidonic acid content in phospholipids. This result is consistent with the data reported previously.[39] The decrease in the unsaturated fatty acid can be interpreted on the basis of $CCl_4$-induced lipid peroxidation, indicating that the arachidonic acid in the microsomal phospholipids is far more sensitive to damage induced by free radical attack than other fatty acids. NSP appears to have only a slight protecting effect against the peroxidative decomposition of the acid.

The experiments on the effects of NSP on $CCl_4$ intoxication and *in vitro* lipid peroxidation lead to the conclusion that NSP increases the decreased lysosomal enzyme activities or partly prevents the decline and enhances water-soluble antioxidant levels in $CCl_4$-poisoned rat liver. The mechanisms of NSP action probably include direct inhibition of non-enzymatic lipid peroxidation and an increase in antioxidant levels.

## References and Notes


1) T. Kita, T. Hata and R. Yoneda, *Nippon Yakurigaku Zasshi*, **72**, 573 (1976).
2) N. Tsuyama, T. Hara, R. Nibin, R. Mikami, K. Shigero, M. Takahashi and Y. Iwasaki, *Kiso To Rinsho*, **11**, 309 (1977).
3) T. Hata, T. Kita and R. Yoneda, *Nippon Yakurigaku Zasshi*, **72**, 879 (1976).
4) A. Koda, H. Nagai, Y. Kurimoto, T. Yamada, H. Mori, T. Nishiyori and N. Inagaki, *Nippon Yakurigaku Zasshi*, **78**, 319 (1981).
5) K. Ishii, N. Yamada, H. Shibata, H. Okabe, K. Sasaki and M. Okudaira, The 17th Nippon Kanzo Gakkai, Gifu, July, 1981.
6) C. de Duve and R. Wattiaux, *Annu. Rev. Physiol.*, **28**, 435 (1966).
7) H. Remmer, "Proceedings of the First International Phamacological Meeting," Vol. 6, ed. by B. B. Brodie and E. G. Erdös, Macmillan Co., New York, 1962, p. 235.
8) K. Tanaka and Y. Iizuka, *Biochem. Pharmacol.*, **17**, 2023 (1968).
9) T. Ogiso, T. Kobayashi and Y. Kato, *Jpn. J. Pharmacol.*, **25**, 401 (1975).
10) T. Omura and R. Sato, *J. Biol. Chem.*, **239**, 2370 (1964).
11) A. L. Babson, P. O. Shapiro, P. A. R. Williams and G. E. Phillips, *Clin. Chim. Acta*, **7**, 199 (1962).
12) M. Ikeda, *J. Biochem.* (Tokyo), **55**, 231 (1964).
13) T. Ariyoshi and E. Takabatake, *Life Sci.*, **9**, 361 (1970).
14) T. Nash, *Biochem. J.*, **55**, 416 (1953).
15) A. M. Symons, D. A. Lewis and R. J. Ancill, *Biochem. Pharmacol.*, **18**, 2581 (1969).
16) W. H. Fishman, B. Springer and R. Brunetti, *J. Biol. Chem.*, **173**, 449 (1948).
17) V. P. Dole, *J. Clin. Invest.*, **35**, 150 (1956).
18) A. Colbeau, J. Nachbaur and P. M. Vignais, *Biochim. Biophys. Acta*, **249**, 462 (1971).
19) L. D. Metcalfe, A. A. Schmitz and J. R. Pelka, *Anal. Chem.*, **38**, 514 (1966).
20) T. P. A. Devasagayam, C. K. Pushpendran and J. Eapen, *Biochim. Biophys. Acta*, **750**, 91 (1983).
21) F. E. Hunter, J. M. Gebicki, P. E. Hoffsten, J. Weinstein and A. Scott, *J. Biol. Chem.*, **238**, 828 (1963).
22) E. G. Mimnaugh, M. A. Trush, E. Ginsburg, Y. Hirokata and T. E. Gram, *Toxicol. Appl. Pharmacol.*, **61**, 313 (1981).
23) J. Glavind, *Acta Chem. Scand.*, **17**, 1635 (1963).
24) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

25) M. U. Dianzani and G. Ugazio, "Lipid Peroxidation. In Biochemical Mechanisms of Liver Injury," ed. by T. F. Slater, Academic Press, London, 1978, pp. 669—707.
26) C. H. Rouiller, "The Liver," Vol. II, ed. by C. H. Rouiller, Academic Press, New York, 1964, p. 335.
27) R. O. Recknagel, *Pharmacol. Rev.*, **19**, 145 (1967).
28) H. A. Sasame, I. A. Castro and J. Gillette, *Biochem. Pharmacol.*, **17**, 1759 (1968).
29) E. A. Smuckler, E. Arrenius and T. Hultin, *Biochem. J.*, **103**, 55 (1967).
30) T. Ogiso, T. Kobayashi, K. Kuhara and Y. Kato, *Jpn. J. Pharmacol.*, **25**, 411 (1975).
31) B. B. Brodie, "Enzymes and Drug Action," ed. by J. L. Monger and A. V. S. de Reuck, Churchill, London, 1962, p. 317.
32) C. de Duve, R. Wattiaux and M. Wibo, *Biochem. Pharmacol.*, **9**, 97 (1962).
33) T. Nakashima, T. Taniko and K. Kuriyama, *Jpn. J. Pharmacol.*, **32**, 583 (1982).
34) L. Flohe, "Oxygen Free Radicals and Tissue Damage," Ciba Found. Symp., 65, Excerpta Medica, Amsterdam, 1979, pp. 95—122.
35) J. R. Wright, H. D. Colby and P. R. Miles, *Arch. Biochem. Biophys.*, **206**, 296 (1981).
36) J. S. Bus and J. E. Gibson, "Reviews in Biochemical Toxicology," ed. by E. Hodgson, J. R. Bend and R. M. Philpot, Elsevier North Holland, New York, 1979, pp. 125—149.
37) D. A. Vessey and D. Zakim, *J. Biol. Chem.*, **247**, 3023 (1972).
38) A. Martonosi, J. Donley and R. A. Halpin, *J. Biol. Chem.*, **243**, 61 (1968).
39) T. Ogiso, K. Kuhara, T. Kobayashi, H. Masuda and Y. Kato, *Chem. Pharm. Bull.*, **25**, 87 (1977).