EFFECT OF DIISOPROPYL 1,3-DITHIOL-2-YLIDENEMALONATE ON MICROSOMAL ELECTRON TRANSPORT SYSTEM IN RAT LIVER

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Effect of diisopropyl 1,3-dithiol-2-ylidenemalonate (NKK-105) on the components of rat liver microsomal electron transport system was investigated by comparison with those of phenobarbital, 3-methylcholanthrene and polychlorinated biphenyl. When NKK-105 was administered to rats at a dose of 250 mg/kg/day for 7 days, cytochrome b_5 content and NADPH-cytochrome c reductase activity were significantly increased but cytochrome P-450 content to the lesser extent. Three inducers of the drug metabolizing enzymes remarkably increased cytochrome P-450 content but increased cytochrome b_5 content to a lesser extent.

Keywords—diisopropyl 1,3-dithiol-2-ylidenemalonate; NKK-105; cytochrome b_5 ; enzyme induction; microsomal electron transport system; cytochrome P-450; NADPH-cytochrome c reductase

Diisopropyl 1,3-dithiol-2-ylidenemalonate (NKK-105) (Chart 1) has been reported to have several pharmacologic actions in rats,¹⁻³⁾ and to induce the microsomal drug metabolizing enzymes in rat liver.⁴⁾

CHART 1. Chemical Structure of NKK-105

The present study was, therefore, undertaken to determine the effect of NKK-105 on microsomal electron transport system associated with cytochrome P-450-mediated drug metabolism.

Male Sprague-Dawley rats weighing approximately 180 g were used. They were treated with NKK-105 in olive oil at a dose of 250 mg/kg/day for 7 days. Phenobarbital (PB), 3-methylcholanthrene (MC) and polychlorinated biphenyl (KC-400, PCB), which were dissolved in olive oil were intraperitoneally administered at a dose of 40 mg/kg/day for 4 days. Control rats received equal volume of the solvent for 4 days. Twenty-four hr after the final administration of the drugs, the rats were sacrificed by cervical dislocation. They were fasted for 18 hr prior to sacrifice, but had free access to tap water. Livers were rapidly removed, weighed, perfused with 1.15% KCl solution and homogenized in 4 vols. of the same solution. After centrifugation at 9000 g for 20 min, the microsomal pellet was obtained by further centrifugation at 105000 g for 1 hr. After washing once with the KCl solution, the pellet was suspended in 0.1 M Na/K phosphate buffer (pH 7.5) in a protein concentration of approx-

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TABLE I. *Effect of NKK-105, PB, MC and PCB on the Components of Microsomal Electron Transport System in Rat Liver*

Treatment	Body weight (g)	Microsomal Cyt. P-450 protein (mg/g liver)	Cyt. b ₅ (μmol/g liver)	Fp ₁ (μmol/min/g liver)	Fp ₂ (μmol/min/g liver)	<i>p</i> -Nitroanisole O-demethylase (μmol/h/g liver)		
Control	190±4	3.2±0.1	28.1±3.6	24.1±1.2	8.0±0.3	127±11	3.75±0.34	0.85±0.01
NKK-105	188±9	3.7±0.1*	32.3±1.9	37.0±2.3**	18.2±1.0**	185±15*	9.03±0.69***	1.22±0.12**
PB	195±8	3.7±0.1**	31.6±0.2	63.0±4.7***	10.0±0.9	168±10	9.54±0.74***	2.06±0.16***
MC	188±4	4.1±0.2***	27.6±1.3	56.2±4.2***	13.6±0.9**	139±8	3.65±0.19	4.18±0.25***
PCB	190±6	3.6±0.1**	29.3±1.4	59.5±5.3***	16.0±1.3**	173±23	6.22±0.54**	3.30±0.36***

Each value is expressed as the mean $\pm$ S.E. from 4 rats.

a) Relative liver weight ratio (g/100 g).

NKK-105 was administered orally at a dose of 250 mg/kg/day for 7 days and PB, MC and PCB were administered intraperitoneally at a dose of 40 mg/kg/day for 4 days.

* $p < 0.05$ vs. control; ** $p < 0.01$ vs. control; *** $p < 0.001$ vs. control (Student's *t*-test).

imately 10 mg/ml. Cytochrome P-450,⁵⁾ cytochrome b_5 ,⁵⁾ NADH-cytochrome b_5 reductase (fp₁),⁶⁾ NADPH-cytochrome c reductase (fp₂)⁷⁾ and *p*-nitroanisole O-demethylase⁸⁾ were assayed according to the methods previously reported. Microsomal protein content was determined by Lowry's method.⁹⁾

The effect of NKK-105 on the components of microsomal electron transport system in rat liver was compared to those in PB-, MC- and PCB-pretreated rats. As shown in Table I, administration of NKK-105 caused a remarkable increase in both cytochrome b_5 content and fp₂ activity, and cytochrome P-450 content and *p*-nitroanisole O-demethylase activity were also increased to a lesser extent. MC and PCB increased cytochrome P-450 content rather than cytochrome b_5 , however, PB caused no increase in cytochrome b_5 content. The ratios of cytochrome P-450 to cytochrome b_5 were 3.0 in control, 2.0 in NKK-105-treated and 3.7–6.3 in other inducer-treated rats, respectively. Griseofulvin¹⁰⁾ and AF-2¹¹⁾ were known to increase cytochrome b_5 content and, conversely, decreased cytochrome P-450 content. Recently, ethoxyquin,¹²⁾ an antioxidant, has been reported to enhance both cytochrome b_5 and cytochrome P-450 content to the same extent. In comparison with the changes in cytochrome b_5 and cytochrome P-450 contents by three chemical agents, NKK-105 was characterized

to increase cytochrome b_5 more significantly, as compared to the increase in cytochrome P-450 content in rats.

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