SIX-MONTH REPEATED ORAL TOXICITY STUDY OF NK-104 IN RATS

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ABSTRACT — NK-104 is a novel potent inhibitor of the rate-limiting enzyme in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, and has been shown to be a highly effective agent in lowering blood cholesterol. In the present study, NK-104 was orally administered to Wistar rats at a dose of 0.3, 1, 3 or 10 mg/kg for 6 months for examination of toxicity. Additional recovery groups of 8 rats each of both sexes receiving 0 and 10 mg/kg were maintained without treatment for 1 month in order to assess recovery. As a result, no toxicological changes were observed in general signs, body weight, food intake, ophthalmological examination, urinalysis, hematological and blood chemical examinations or organ weights. An autopsy revealed thickening of the forestomach mucosa in both sexes at a dose of 1 mg/kg or more. This change was microscopically recognized as hyperkeratosis and hypertrophy of the spinous layer associated with both cell infiltration of the mucosal propria and edema of submucosa in the forestomach in both sexes at doses of 3 and 10 mg/kg. Forestomach changes were not observed in any cases after 1 month cessation of drug treatment. The non-toxic dose of NK-104 in the 6-month repeated oral toxicity study in rats is estimated to be 1 mg/kg/day.

KEY WORDS: NK-104, Hypolipidemic agent, HMG-CoA reductase inhibitor, Chronic toxicity, Rat

INTRODUCTION

NK-104 is currently under investigation as an oral hypolipidemic agent. It is a potent inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), the rate-limiting enzyme in cholesterol biosynthesis (Aoki *et al.*, 1997).

As a part of safety assessment, we performed a 6-month repeated oral toxicity study in rats.

MATERIALS AND METHODS

Test materials

The chemical name of NK-104 is monocalcium bis [(3R, 5S, 6E)-7- [2-cyclo-propyl-4-(4-fluorophenyl)-3-quinoliny]-3, 5-dihydroxy-6-heptenoate] (Fig. 1) and the molecular weight is 881.00. NK-104 is a white or faintly yellow-brownish crystalline or crystalline powder with no odor and is almost insoluble in

water.

The content is 99.3%. The material used in this study was synthesized by Nissan Chemical Industries, Ltd. and is confirmed to be stable for 33 months in amber bottles protected from light under 4°C.

Fig. 1. Chemical structure of NK-104.

Test animals

Specific pathogen-free Wistar male and female rats aged 4 weeks were obtained from Japan SLC, Inc. (Hamamatsu, Japan). After acclimation, 76 male and female rats each aged 6 weeks without abnormal general signs were randomly assigned to groups as described in Table 1 based on the body weight on the final acclimation day. Then the rats were housed individually in animal rooms controlled at a temperature of $23 \pm 1^\circ\text{C}$, relative humidity of $55 \pm 10\%$ and ventilated 14 to 18 times an hour with a 12 hr light cycle. A pelleted diet (CE-2: CLEA JAPAN, Inc., Tokyo, Japan) sterilized by autoclaving and tap water UV-sterilized were provided *ad libitum*.

Experimental design

The dosages of NK-104 were set at 0.3, 1, 3 or 10 mg/kg from the results of a 28-day repeated oral toxicity study in rats (Akiba *et al.*, 1994).

The test compound was suspended with 0.5% carboxymethylcellulose solution (0.5% CMC) and followed by appropriate dilution with 0.5% CMC to obtain a constant volume of 2 ml/kg for every dosage group. These suspensions were orally administered daily by gavage to groups of 12 male and 12 female rats for 182 days. The control and 10 mg/kg treated groups in both sexes were composed of 20 males and females each. The control group was given the vehicle, 0.5% CMC solution.

One of the 2 groups each treated with the vehicle and 10 mg/kg of NK-104 for 6 months was maintained without treatment for 1 month after the 6-month treatment in order to investigate the reversibility of toxic changes (Table 1).

Observations and examinations

General signs were observed twice daily, before and after dosing during the dosing period and once dai-

ly during the cessation period of treatment. Body weight was measured weekly during the first 3 months and thereafter at least once every 4 weeks throughout the treatment period. In the cessation period, body weight was measured weekly and on 4 consecutive days from the final dosing day. Food intake was measured weekly during the first 3 months and thereafter at least once every 4 weeks throughout the treatment period and once during the cessation period. Urinalysis was performed twice during the treatment period and once during the cessation period with a clinical ion-meter (CIM-102, SHIMADZU Co., Ltd., Kyoto, Japan). Parameters examined included urinary volume, specific gravity, Na, K, Cl conc. and excretion, pH, glucose, protein, ketone body, occult blood, urobilinogen, bilirubin and sediment. Ophthalmological examination was performed once each during the treatment and cessation periods with ophthalmoscopy (OS-1, Kowa Co., Ltd., Nagoya, Japan) and a fundus-camera (RC-2, Kowa Co., Ltd., Nagoya, Japan).

Necropsy was performed approximately 24 hr after the final dosing and on the final day of the cessation period. Hematological tests were performed for red blood cell count (RBC), white blood cell count (WBC), platelet, hematocrit, hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte, prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen and white blood cell differential count (differential WBC) using an Automatic Blood Cell Counter (K-2000, SYSMEX Co., Ltd., Kobe, Japan) and a Microcoagulometer (KC-10A, Amelung GmbH, Lemgo, Germany). Blood chemistry testing was performed using an Automatic Clinical Analyzer (7050, HITACHI Ltd., Tokyo, Japan) and a clinical ion-meter (CIM-102, SHIMADZU Co., Ltd., Kyoto, Japan). Blood chemistry parameters are as follows: glu-

Table 1. Group composition.

Group	Drug	Dose	Volume	Concentration	No. of animals	
		mg/kg	ml/kg	w/v%	Male	Female
1	Control 0.5% CMC	0	2	0	12(8)	12(8)
2	NK-104	0.3	2	0.015	12	12
3	NK-104	1	2	0.05	12	12
4	NK-104	3	2	0.15	12	12
5	NK-104	10	2	0.5	12(8)	12(8)

( ): No. of animals in recovery group

cose(glucokinase·G-6PDH), total protein(Biuret), albumin(BCG), A/G ratio(calculation), blood urea nitrogen(BUN) (urease GLDH), creatinine(Jaffe), Ca(OCPC), Na, K, Cl(I.S.E.), glutamic oxaloacetic transaminase(GOT)(Karmen), glutamic pyruvic transaminase(GPT) (Wroblewski-La Due), lactate dehydrogenase(LDH)(Wroblewski-La Due), creatine phosphokinase(CPK)(GSCC), alkaline phosphatase (ALP)(P-NPP), choline esterase (Ch. E.)(DTNB), triglyceride(GOP·DAOS), total cholesterol(Total choles.)(COD·DAOS) and phospholipid(ChOD·DAOS).

Major organs including the brain, heart, lung, liver, kidney, spleen, thymus, prostate, pancreas, adrenal, hypophysis, thyroid, testis, submaxillary gland, seminal vesicle, uterus and ovary were weighed, and a standard set of tissues collected and then fixed in 10% neutral buffered formalin (except the eye, which was fixed in a mixed solution of formaldehyde-glutaraldehyde before formalin fixation) for histopathological examination. The fixed organ specimens were embedded in paraffin, thin-sectioned and stained with hematoxylin and eosin(H.E.).

Statistical analysis

Bartlett's test (Gad and Weil, 1982) was applied to testing for homogeneity of variance between groups. If no significant heterogeneity was detected, one-way analysis of variance was used. Those parameters found to be significant in one-way analysis of variance were tested by Dunnett's multiple comparison, if the number of animals in each group was equal, or Scheffe's multiple comparison if the number of animals in each group was different. If significant heterogeneity of variance was detected, Kruskal-Wallis's test (Kruskal and Wallis, 1952) was performed. The parameters found to be significant in Kruskal-Wallis's test were tested by the non-parametric equivalent of Dunnett's or Scheffe's multiple comparison. P values less than 0.05 were considered significant.

RESULTS

Neither death nor treatment-related abnormalities in general signs, body weight (Fig. 2, 3), food intake, ophthalmological examination, urinalysis, hematological test (Table 2) or organ weights (Table 3) were observed in any groups throughout the experimental period.

Blood chemistry testing showed an increase in total cholesterol at 10 mg/kg in the female dose

group(Table 4, 5). However, this change was not seen after 1-month withdrawal. An increase in GOT was observed in the recovery group of female rats at 10 mg/kg. At the termination of dosing, macroscopic examination showed thickening of the forestomach mucosa in a range from 1mg/kg to 10 mg/kg in both sexes.

Treatment-related microscopic changes in the forestomach mucosa are shown in Table 6, 7. Slight or mild hyperkeratosis was observed in 6 males and females and slight hypertrophy of the spinous layer was observed in 3 males and females at 1 mg/kg. At 3 and 10 mg/kg, the above-mentioned changes developed more severely than those associated with cell infiltration of the mucosal propria and edema of the submucosa. However, these findings disappeared after 1-month withdrawal.

DISCUSSION

In the present study, only a plasma cholesterol increase in the female dose group at 10 mg/kg was observed in all examinations except for pathological examination as treatment-related changes. However, abnormalities of the liver associated with the plasma cholesterol increase were not observed in blood chemistry or histopathological examinations in any animals. A similar increase in plasma cholesterol with no toxicological finding in blood chemistry and histopathological examinations of liver has been reported in a toxicity study of plavastatin, an analogous drug, in rats (Kimura *et al.*, 1987). It has been reported that immunoelectron microscopy of high concentrations of HMG-CoA reductase on SER (smooth endoplasmic reticulum) membranes induced by lovastatin analogous to NK-104, are strongly suggested to be caused by the mechanism-based effect of HMG-CoA reductase inhibitors (Liscum *et al.*, 1983, Singer *et al.*, 1988). On the other hand, Kojima *et al.* (1994) showed that C_{max} and AUC (area under concentration time curve) of NK-104 in female rats are higher than those of male rats. From the above, the plasma cholesterol increase in female rats was due to high concentration, and the mechanism-based effect of NK-104 was then thought to be non-toxic. The increase in GOT at 10 mg/kg in the recovery female group was independent of GPT and Ch.E., and there were no changes in the related organs. Therefore, there was no toxicological significance.

Hyperkeratosis and hypertrophy of the spinous layer in the forestomach mucosa were observed at 1 mg/kg or more. However, these findings disappeared

Fig. 2. Body weight changes in male rats during 6-month oral administration of NK-104 and withdrawal period of 1 month

Fig. 3. Body weight changes in female rats during 6-month oral administration of NK-104 and withdrawal period of 1 month.

Subacute toxicity of NK-104 in rats.

Table 2. Summary of hematology testing in rats treated orally with NK-104 for 6 months.

Dose(mg/kg)		Treatment					Recovery	
		0(vehicle)	0.3	1	3	10	0(vehicle)	10
No. of rats		12	12	12	12	12	8	8
Male	RBC (10 ⁴ /mm ³)	962±34	967±47	962±39	971±41	942±36	916±26	928±37
	Hb (g/dl)	16.4±0.4	16.3±0.7	16.4±0.5	16.6±0.7	16.2±0.4	15.8±0.5	15.8±0.6
	Platelet (10 ⁴ /mm ³)	2±6	79±8	74±5	73±5	73±7	70±5	66±7
	WBC (10 ² /mm ³)	24±7	22±6	23±8	21±5	22±12	22±4	21±3
	PT (sec)	14.4±0.8	14.2±0.5	14.5±0.3	14.1±0.4	14.3±0.7	16.1±0.5	14.8±0.4***
	APTT (sec)	22.4±3.6	22.9±3.9	21.4±3.2	21.9±3.1	23.4±4.7	19.8±1.2	20.5±0.9
No. of rats		12	12	12	12	12	8	8
Female	RBC (10 ⁴ /mm ³)	881±18	845±30*	841±32**	853±60	860±32	954±35	861±40
	Hb (g/dl)	16.2±0.4	15.7±0.6	15.6±0.6	15.8±0.9	16.0±0.6	15.8±0.5	15.9±0.6
	Platelet (10 ⁴ /mm ³)	75±6	74±9	71±8	75±8	71±7	71±4	67±6
	WBC (10 ² /mm ³)	19±6	17±6	20±6	17±4	18±4	14±2	15±4
	PT (sec)	14.3±0.7	13.9±0.5	14.2±0.7	14.7±1.4	13.7±0.3	12.6±1.7	12.8±0.9
	APTT (sec)	19.2±2.7	21.4±5.6	19.2±1.6	18.8±1.0	18.1±1.7	20.1±5.3	18.2±2.4

Mean±S.D.

Significantly different from 0(vehicle) *P<0.05, **P<0.01, ***P<0.001(Dunnett's test)

Table 3. Summary of absolute and relative organ weights in rats treated orally with NK-104 for 6 months.

Sex	Organs		Treatment				Recovery		
			Dose(mg/kg)	0(vehicle)	0.3	1	3	10	0(vehicle)
Male	No. of rats		12	12	12	12	12	8	8
	Lung	(g)	1.16±0.07	1.21±0.07	1.20±0.11	1.22±0.09	1.19±0.09	1.24±0.07	1.25±0.05
		(%)	0.29±0.02	0.30±0.01	0.31±0.02*	0.30±0.01*	0.30±0.01	0.28±0.02	0.29±0.03
	Liver	(g)	13.35±1.11	13.52±0.76	12.84±0.94	13.25±1.22	13.14±1.28	14.33±0.98	14.55±1.06
		(%)	3.30±0.16	3.36±0.12	3.27±0.13	3.28±0.15	3.31±0.17	3.25±0.12	3.40±0.10*
	Kidney	(g)	2.53±0.14	2.55±0.15	2.51±0.23	2.54±0.17	2.56±0.22	2.77±0.15	2.71±0.12
		(%)	0.63±0.03	0.64±0.04	0.64±0.04	0.63±0.02	0.65±0.03	0.63±0.03	0.63±0.03
	Thymus	(g)	0.13±0.02	0.13±0.02	0.13±0.01	0.12±0.02	0.12±0.03	0.13±0.02	0.14±0.03
		(%)	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01
	Thyroid	(mg)	32±7	31±9	25±6	31±6	28±6	22±2	20±3
(mg%)		8±2	8±2	6±2	8±2	7±1	5±0	5±1	
Female	No. of rats		12	12	12	12	12	8	8
	Lung	(g)	0.90±0.08	0.90±0.06	0.89±0.04	0.90±0.06	0.90±0.05	0.88±0.07	0.92±0.08
		(%)	0.41±0.03	0.41±0.03	0.41±0.03	0.41±0.02	0.40±0.02	0.38±0.01	0.40±0.02
	Liver	(g)	7.25±0.67	7.44±0.50	7.05±0.66	7.08±0.53	7.34±0.49	7.60±0.62	7.60±0.63
		(%)	3.32±0.22	3.36±0.15	3.23±0.16	3.26±0.17	3.29±0.19	3.30±0.18	3.28±0.19
	Kidney	(g)	1.64±0.23	1.58±0.11	1.60±0.08	1.55±0.10	1.61±0.11	1.66±0.11	1.67±0.14
		(%)	0.75±0.09	0.71±0.04	0.73±0.05	0.72±0.04	0.72±0.03	0.72±0.04	0.72±0.03
	Thymus	(g)	0.13±0.03	0.12±0.04	0.12±0.02	0.11±0.03	0.11±0.03	0.11±0.01	0.10±0.02
		(%)	0.06±0.01	0.05±0.02	0.06±0.01	0.05±0.01	0.05±0.01	0.05±0.00	0.04±0.01
	Thyroid	(mg)	23±7	19±3	18±3	18±3	19±3	14±5	19±5 *
(mg%)		11±3	9±2	8±2	8±1	8±1	6±2	8±2 *	

Mean±S.D.

Significantly different from 0(Vehicle) *P<0.05 (Dunnett's test)

Table 4. Summary of blood chemical testing in male rats treated orally with NK-104 for 6 months.

Male	Dose(mg/kg)	Treatment					Recovery	
		0(vehicle)	0.3	1	3	10	0(vehicle)	10
No. of rats		12	12	12	12	12	8	8
T.protein	(mg/dl)	5.39 ±0.15	5.48 ±0.18	5.42 ±0.13	5.35 ±0.13	5.36 ±0.26	5.38 ±0.08	5.32 ±0.09
BUN	(mg/dl)	20.0 ± 2.1	20.4 ± 1.9	20.1 ± 2.2	20.5 ± 2.8	21.0 ± 2.1	22.2 ± 1.6	21.6 ± 1.1
Creatinine	(mg/dl)	0.66 ±0.07	0.67 ±0.06	0.65 ±0.07	0.67 ±0.08	0.69 ±0.06	0.64 ±0.07	0.62 ±0.03
GOT	(mU/ml)	145 ± 52	132 ± 32	123 ± 35	117 ± 29	142 ± 49	236 ±132	192 ± 83
GPT	(mU/ml)	79 ± 25	67 ± 19	61 ± 19	58 ± 17	71 ± 28	115 ± 56	99 ± 39
CPK	(mU/ml)	669 ±776	699 ±607	597 ±603	656 ±257	517 ±389	877 ±595	763 ±384
Ch.E.	(mU/ml)	118 ± 24	105 ± 16	103 ± 22	113 ± 17	119 ± 22	116 ± 24	103 ± 19
Triglyceride	(mg/dl)	83 ± 18	78 ± 25	85 ± 29	90 ± 35	75 ± 12	89 ± 16	94 ± 21
T.choles.	(mg/dl)	68 ± 7	65 ± 8	64 ± 6	64 ± 7	66 ± 7	69 ± 15	61 ± 4

Mean ± S.D.

Table 5. Summary of blood chemical testing in female rats treated orally with NK-104 for 6 months.

Female	Dose(mg/kg)	Treatment					Recovery	
		0(vehicle)	0.3	1	3	10	0(vehicle)	10
No. of rats		12	12	12	12	12	8	8
T.protein	(mg/dl)	5.56 ±0.33	5.62 ±0.24	5.58 ±0.30	5.69 ±0.23	5.57 ±0.26	5.75 ±0.25	5.55 ±0.35
BUN	(mg/dl)	21.1 ±2.9	20.2 ±2.5	19.3 ±2.9	19.9 ±2.2	20.4 ±1.7	18.6 ± 1.1	18.0 ± 2.4
Creatinine	(mg/dl)	0.67 ±0.06	0.66 ±0.07	0.65 ±0.06	0.67 ±0.06	0.69 ±0.07	0.61 ±0.04	0.64 ±0.06
GOT	(mU/ml)	107 ±37	115 ±50	119 ±23	92 ±27	97 ±31	106 ±14	164 ±45 **
GPT	(mU/ml)	52 ±21	53 ±22	55 ±14	39 ±14	40 ±14	58 ±13	74 ±24
CPK	(mU/ml)	634 ±421	596 ±244	906 ±385	770 ±433	914 ±582	758 ±446	518 ±278
Ch.E.	(mU/ml)	1227 ±149	1272 ±133	1236 ±113	1279 ±115	1194 ±100	1231 ± 72	1188 ±104
Triglyceride	(mg/dl)	58 ±28	55 ±7	45 ± 14	46 ± 15	42 ±8	63 ±25	50 ±17
T.choles.	(mg/dl)	95 ± 7	96 ±12	96 ± 9	104 ±17	113 *** ±10	98 ±7	95 ±6

Significantly different from 0(vehicle) ** P<0.01, *** P<0.001 (Dunnett's test).

Mean ± S.D.

Subacute toxicity of NK-104 in rats.

Table 6. Summary of microscopical testing in male rats treated orally with NK-104 for 6 months
——— Number of animals showing a finding ———

Dose mg/kg	No. of rats	Forestomach			
		Hyperkeratosis	Hypertrophy of stratum spinosum	Cell infiltration of mucosal propria	Edema of submucosa
Male	0	—	12	12	12
		±			
		+			
		++			
	0.3	—	12	12	12
		±			
		+			
		++			
	1	—	6	9	12
		±	6	3	
		+			
		++			
3	—		11	11	
	±	1	1	1	
	+	7	11		
	++	4			
10	—		5	10	
	±	1	5	2	
	+	5	6		
	++	6	6		

—: no change, ±: slight change, +: mild change, ++: moderate change,
Each figure represents the number of animals with each finding.

Table 7. Summary of microscopical testing in female rats treated orally with NK-104 for 6 months
——— Number of animals showing a finding ———

Number of animals showing a finding						
	Dose mg/kg	No.of rats	Hyperkeratosis	Forestomach Hypertrophy of stratum spinosum	Cell infiltration of mucosal propria	Edema of submucosa
Female	0	12	—	12	12	12
			±			
			+			
			++			
	0.3	12	—	12	12	12
			±			
			+			
			++			
	1	12	—	6	9	12
			±	5	3	
			+	1		
			++			
	3	12	—		10	12
			±		2	
			+	7	9	
			++	5	1	
	10	12	—		9	12
			±		3	
			+	4	8	
			++	8	4	

—: no change, ±: slight change, +: mild change, ++: moderate change,
Each figure represents the number of animals with each finding.

after 1-month withdrawal. It has been reported that HMG-CoA reductase inhibitors except for pravastatin (Kimura *et al.*, 1987) can induce hyperkeratosis and hypertrophy of the spinous layer in the forestomach mucosa associated with cell infiltration and edema in rodents (Gerson *et al.*, 1990). Moreover, Kloss *et al.* (1991) reported that these are dependent on the potency of the inhibitory activity of HMG-CoA reductase inhibitors. These reports suggest that sustained inhibition of HMG-CoA reductase in rat forestomach keratinocytes induces accumulation of HMG-CoA reductase (from immunostaining), and hyperkeratosis and hyperplasia by inhibiting sterol synthesis, assembly of lamellar bodies and formation of intercellular lipid sheets (from electron microscopy) (Singer *et al.*, 1991). However, it is considered that the developed changes that were followed by cell infiltration and edema at 3 and 10 mg/kg can't be toxicologically disregarded.

It is concluded that NOAEL (No Observed Adverse Effect Level: non-toxic dose) in the 6-month repeated oral toxicity study of NK-104 in rats is estimated to be 1 mg/kg/day. In addition, NOEL (No Observed Effect Level) is estimated to be 0.3 mg/kg/day.

REFERENCES

- Akiba, T., Okubo, M., Asanuma, A., Koga, T., Tanaka, M. and Takimoto, M. (1994): 28-DAY REPEATED ORAL TOXICITY STUDY OF NK-104 IN RATS. Unpublished
- Aoki, T., Nishimura, H., Nakagawa, S., Kojima, J., Suzuki, H., Tamaki, T., Wada, Y., Yokoo, N., Sato, F., Kimata, H., Kitahara, M., Toyoda, K., Sakashita, M. and Saito, Y. (1997): Pharmacological Profile of a Novel Synthetic Inhibitor of 3-Hydroxy-3-methylglutaryl-coenzyme A Reductase. *Arzneim.-Forsch./Drug Res.*, **47**, 904-909.
- Gad, S. C. and Weil, C. S. (1984): Statistics for toxicologist, In Principles and methods of toxicology (haayes, A. W. ed.), Student ed., 273-320 Raven Press, New York.
- Gerson, R. J., MacDonald, J. S., Lankas, G. R., Jensen, R. G., Bokelman, D. L., Usui, Y., Otiai, K. and Kemi, M. (1990): Toxicity Study of Simvastatin (2): A 53-Week Oral Toxicity Study in Rats with A 27-Week Interim Necropsy, *Oyo Yakuri/Pharmacometrics*, **39**, 103-126.
- Kimura, K., Miyakoshi, K., Yamashita, K., Ohohashi, Y., Matsumoto, E., Okada, T., Matsunuma, N. and Masuda, H. (1987): Toxicological Studies of Pravastatin Sodium (II)—Thirteen Weeks Oral Toxicity Studies in Rats —*Jpn. Pharmacol. Ther.*, **15**, 4957-4973.
- Kloss, M. W., Patrick, D. H. and MacDonald, J. S. (1991): Studies on the effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors on the rodent forestomach. *Fd. Chem. Toxic.*, **29**, 621-628.
- Kojima, J. and Kimata, H. (1994): Unpublished.
- Kruskal, W. H. and Wallis, W. A. (1952): Use of ranks in one-criterion variance analysis. *J. Am. Statist. Assoc.*, **47**, 583-621.
- Liscum, L., Luskey, K. L., Chin, D. J., Ho, Y. K., Goldstein, J. L. and Brown, M. S. (1983): Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase and its mRNA in rat liver as studied with a monoclonal antibody and a cDNA probe. *J. Biol. Chem.*, **258**, 8450-8455.
- Singer, I. I., Scott, S., Kazazis, D. M. and Huff, J. W. (1988): Lovastatin, an inhibitor of cholesterol synthesis, induces hydroxymethylglutaryl-coenzyme A reductase directly on membranes of expanded smooth endoplasmic reticulum in rat hepatocytes. *Proc. Natl. Acad. Sci. USA*, **85**, 5264-5268.
- Singer, I. I., Kawka, D. W., Scott, S., Bailey, P., Kloss, M. W., Majka, J. and MacDonald, J. S. (1991): Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A Reductase induce reductase accumulation and altered lamellar bodies in rat forestomach keratinocytes. *Arteriosclerosis and Thrombosis*, **11**, 1156-1165.