NOTES

MONACOLIN K, A NEW HYPOCHOLESTEROLEMIC AGENT THAT SPECIFICALLY INHIBITS 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE

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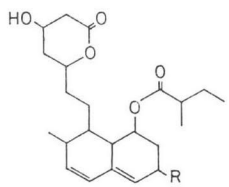
The fungal metabolite monacolin K has been isolated from cultures of *Monascus ruber* as a new hypocholesterolemic agent in this laboratory[1]. Chemical structure of this compound (Details of structural studies will be published elsewhere) is closely related to that of ML-236B (compactin) (Fig. 1), a potent inhibitor of 3-hydroxy-3-methylglutaryl(HMG)-CoA reductase (EC 1.1.1.34)[2,3], the rate-limiting enzyme in cholesterol synthetic pathway. The latter compound has been shown to have a potent hypocholesterolemic activity in several animal species as well as human subjects[4~8].

In the present paper, an inhibitory effect of monacolin K on nonsaponifiable lipid synthesis in a cell-free system and its hypocholesterolemic effects in Triton WR-1339-treated rats are reported. The results indicate that monacolin K is a specific competitive inhibitor of HMG-CoA reductase, and that it is 4~5 times more active than ML-236B in both *in vitro* and *in vivo* systems.

Materials and Methods

[^{14}C]Acetate, D,L-[3-^{14}C]HMG-CoA and D,L-[2-^{14}C]mevalonolactone were purchased from New England Nuclear. Lactone forms of monacolin K and ML-236B were isolated as described previously[1,2]. Acid forms of these agents were prepared by saponification of their respective lactone forms in 0.1 N NaOH at 50°C for 2 hours. Other chemicals were of the best grade commercially available.

Fig. 1. Structures of monacolin K and ML-236B (lactone forms).



Monacolin K R: $-CH_3$
ML-236B R: $-H$

Nonsaponifiable lipid synthesis

Rat liver microsomes and cytosolic enzyme fraction were isolated as described previously[9]. The reaction mixture (0.2 ml) contained 1 mM ATP, 10 mM glucose-1-phosphate, 6 mM glutathione, 6 mM $MgCl_2$, 0.04 mM CoA, 0.25 mM NAD, 0.25 mM NADP, 100 mM potassium phosphate buffer (pH 7.4), 0.15 mg protein of microsomes, 1.5 mg protein of cytosolic enzyme fraction and 1 mM [^{14}C]acetate (1.2 Ci/mol). Where indicated, [^{14}C]acetate was replaced by 0.15 mM D,L-[^{14}C]HMG-CoA (6.9 Ci/mol) or 0.5 mM D,L-[^{14}C]mevalonate (1.6 Ci/mol). After incubation at 37°C for 60 minutes, the reaction was terminated by adding 1 ml of 15% alcoholic KOH. The nonsaponifiable lipids formed were isolated and counted as described previously[9].

HMG-CoA reductase assay

HMG-CoA reductase was solubilized from rat liver microsomes (obtained as described above) by the method of HELLER and GOULD[10] and partially purified by the fractionation with ammonium sulfate. The fraction precipitated by 35~50% saturation was used after dialysis for 3 hours against 40 mM potassium phosphate buffer (pH 7.4) containing 100 mM sucrose, 50 mM KCl, 30 mM EDTA and 1 mM dithiothreitol. The reaction mixture (50 μl) contained: 100 mM potassium phosphate buffer (pH 7.4), 10 mM EDTA, 10mM dithiothreitol, 5 mM NADP, 0.1 mM D,L-[^{14}C]HMG-CoA (2.8 Ci/mol) and 1~2 μg of enzyme protein. After incubation at 37°C for 20 minutes, the reaction was terminated by adding 20 μl of 2 N HCl, and the mevalonolactone formed was isolated and counted as described previously[9].

Protein was determined by the method of LOWRY *et al.*[11]

Animal Experiments

Male Wistar-Imamichi rats (220~260 g) were

Fig. 2. Inhibition of nonsaponifiable lipid synthesis from [^{14}C]acetate by the lactone forms of monacolin K and ML-236B.

Experimental conditions are described in Materials and Methods. The results are % of control (without inhibitor). The value for control was 5,800 dpm/hour.

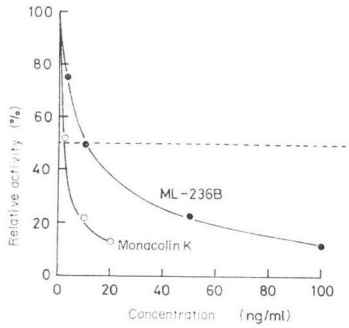
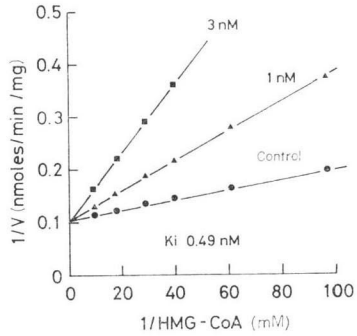


Fig. 3. Double reciprocal plots of the inhibition of HMG-CoA reductase by monacolin K sodium salt.

Experiments were carried out as described in Materials and Methods, except that concentrations of HMG-CoA were varied as indicated. The reaction mixtures contained the indicated concentrations of monacolin K sodium salt.



maintained on commercial rats chow *ad libitum* for 5 days before use. The detergent Triton WR-1339, dissolved in saline at a concentration of 100 mg/ml was injected to the animals intravenously through a tail vein at a dose of 400 mg/kg. Rat chow was withdrawn at time of Triton injection and fasting was continued during experimental periods. Lactone forms of monacolin K and ML-236B, suspended in saline, were administered to rats by gastric incubation at 0, 6, 12 and 18 hours after Triton injection (4 times) at 1, 5 or 25 mg/kg. Thus total doses were 4, 20 or 100 mg/kg. Control animals were given Triton and saline alone. The animals were killed by cardiac puncture 24 hours after Triton injection and the liver and plasma were pooled and assayed for total cholesterol and triglyceride as described previously[7].

Results and Discussion

As shown in Fig. 2, monacolin K lactone was found to be more inhibitory than ML-236B lactone in the [^{14}C]acetate incorporation into nonsaponifiable lipids. Concentrations required for 50% inhibition of the lipid synthesis were 5 nM (2 ng/ml) for monacolin K lactone and 24 nM (10 ng/ml) for ML-236B lactone. The inhibitory potency was approximately doubled by the conversion of lactone forms to their respective acid forms (sodium salt).

Conversion of [^{14}C]acetate and D,L-[^{14}C] HMG-CoA into lipids was inhibited by monacolin K to similar extent. However, D,L-[^{14}C] mevalonate conversion into lipids was not affected by both forms of monacolin K at concentrations up to 10 nM, indicating that this compound inhibits specifically the enzymic step for the conversion of HMG-CoA to mevalonate catalyzed by HMG-CoA reductase.

The inhibition of HMG-CoA reductase by monacolin K was competitive with respect to HMG-CoA (Fig. 3) and noncompetitive with respect to NADPH. The *Ki* value for the sodium salt of monacolin K was 0.49 nM. Under the same conditions, *Ki* value for ML-236B sodium salt and *Km* value for HMG-CoA were 1.2 nM and 9.1 μM, respectively.

As shown in Table 1, monacolin K lactone produced a significant reduction of both plasma and liver cholesterol levels in Triton-treated rats at doses of 4 and 20 mg/kg, while ML-236B lactone showed no detectable activity at 4 mg/kg but reduced cholesterol levels of both plasma and liver at 20 and 100 mg/kg. Thus, the experiments show that monacolin K lactone is substantially more active in reducing cholesterol concentrations than ML-236B lactone. Plasma triglyceride levels were not significantly affected by both monacolin K and ML-236B in these experiments.

The results obtained in the present study clearly indicate that monacolin K is approximately 5 times more active than ML-236B in the inhibition of HMG-CoA reductase activity

Table 1. Hypolipidemic effects of monacolin K and ML-236B lactones in Triton WR-1339-treated rats. Experimental conditions are described in Materials and Methods.

Drug	Total dose mg/kg	Plasma lipids, mg/dl		Liver cholesterol mg/g
		Cholesterol	Triglyceride	
Control		631±19*	3,861±165	4.14±0.1
Monacolin K	4	490±16 (−22.3%, P<0.01)**	4,020±178	3.40±0.11 (−17.5%, P<0.01)
	20	422±18 (−33.1%, P<0.01)	3,856±231	3.19±0.14 (−22.8%, P<0.01)
ML-236B	4	640±24	3,765±220	4.00±0.12
	20	512±18 (−18.9%, P<0.02)	4,003±187	3.43±0.10 (−16.7%, P<0.01)
	100	408±15 (−35.3%, P<0.001)	3,960±135	3.16±0.29 (−23.3%, P<0.01)

* Mean ± standard error for 5 rats.

** Figures in parentheses represent % decrease from control and P value.

as well as in the hypocholesterolemic effects in Triton-treated rats.

Acknowledgements

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