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methods

Highly sensitive assay of HMG-CoA reductase activity by LC-ESI-MS/MS

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Abstract **We have developed a new sensitive and specific nonradioisotope assay method to measure the activity of HMG-CoA reductase, the rate-controlling enzyme in the cholesterol biosynthetic pathway. This method was based upon a stable isotope dilution technique by liquid chromatography-tandem mass spectrometry using electrospray ionization in positive mode. Mevalonic acid, the product of HMG-CoA reductase, was converted to mevalonolactone (MVL) in an incubation mixture, extracted by a salting-out procedure, derivatized into the mevalonyl-(2-pyrrolidin-1-yl-ethyl)-amide, and then purified using a disposable silica cartridge. The resulting mevalonylamide was quantified by selected reaction monitoring using the positive electrospray ionization mode. The detection limit of this mevalonylamide was found to be 240 amol (signal-to-noise ratio = 3), ~833 times more sensitive than that of MVL measured by a conventional radioisotope (RI) method (200 fmol). The variances between sample preparations and between measurements by this method were analyzed by one-way layout and calculated to be 3.2% and 1.8%, respectively. The recovery experiments were performed using incubation mixtures spiked with 0.77–2.31 nmol MVL/mg protein and were validated by a polynomial equation. These results showed that the estimated concentration within a 95% confidence limit was 0.47 ± 0.07 nmol/mg protein, which coincided completely with the observed X_0 nmol/mg protein with a mean recovery of 94.6%. This method made it possible to measure HMG-CoA reductase activity with a high degree of reproducibility and reliability, and especially with sensitivity superior to that of the conventional RI method.**—Honda, A., Y. Mizokami, Y. Matsuzaki, T. Ikegami, M. Doy, and H. Miyazaki. **Highly sensitive assay of HMG-CoA reductase activity by LC-ESI-MS/MS.** ***J. Lipid Res.*** **2007. 48: 1212–1220.**

Supplementary key words cholesterol biosynthesis • mevalonic acid • mevalonolactone • liquid chromatography-electrospray ionization-tandem mass spectrometry • 3-hydroxy-3-methylglutaryl-coenzyme A reductase

The activity of HMG-CoA reductase, the rate-limiting enzyme in the cholesterol biosynthetic pathway, is regulated at the levels of protein synthesis, degradation, and phosphorylation (1). Recent advances in molecular biological research have provided new insights into the regulation of HMG-CoA reductase activity. Sterol-regulatory element binding proteins (SREBPs) are positive transcription factors for the HMG-CoA reductase gene that are synthesized in the endoplasmic reticulum and released to the nucleus by proteolysis (2). This proteolysis is controlled by sterols and the SREBP processing system, which consists of SREBP cleavage-activating protein and insulin-induced genes (3). Although the methods for determining mRNA and protein expression levels in each regulatory step are well established, the only way to determine the overall consequence(s) of regulation by all factors is to measure HMG-CoA reductase activity.

The primary method for assaying HMG-CoA reductase activity is the radioisotope (RI) technique that measures the radioactivity in [^{14}C]mevalonic acid (MVA) produced from [^{14}C]HMG-CoA (4–6). This method is simple but requires the handling of radiolabeled materials. To overcome this disadvantage, in 1978, Miyazaki et al. (7) developed a new method using gas chromatography-chemical ionization-mass spectrometry (GC-CI-MS). This method was subsequently replaced by gas chromatography-electron ionization-mass spectrometry (GC-EI-MS) (8–10) because of the troublesome nature of GC-CI-MS operations, such as frequent cleaning of the CI ion source to maintain its high sensitivity. The GC-EI-MS methods are inferior in their sensitivity than the GC-CI-MS method but are still

Abbreviations: CI, chemical ionization; EI, electron ionization; GC, gas chromatography; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; MVA, mevalonic acid; MVL, mevalonolactone; MV-PLEA, mevalonyl-(2-pyrrolidin-1-yl-ethyl)-amide; P-ESI, electrospray ionization in positive mode; RI, radioisotope; S/N, signal-to-noise ratio; SREBP, sterol-regulatory element binding protein; SRM, selected reaction monitoring.

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more sensitive than the RI technique. In fact, GC-EI-MS methods have been used to quantify plasma (11) and urinary (12, 13) MVA that cannot be determined using the RI technique. However, contrary to our expectation, HMG-CoA reductase activity has not been assayed extensively using GC-EI-MS methods because of the laborious sample preparation and the need for a long analytical process to eliminate interfering peaks.

To analyze relatively polar compounds, such as MVA or mevalonolactone (MVL), liquid chromatography-mass spectrometry (LC-MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS) have been used more readily than GC-MS, because LC-MS and LC-MS/MS do not require a derivatization step. Park et al. (14) and Ndong-Akoume et al. (15) assayed HMG-CoA reductase activity by measuring MVL, the lactonized form of MVA, with LC-MS and LC-MS/MS, respectively, using the positive electrospray ionization (P-ESI) mode. Plasma and urinary MVA were quantified by LC-P-ESI-MS/MS after conversion into MVL (16) as well as directly by LC-negative ESI-MS/MS without lactonization (17, 18). However, there is no evidence that the detection limits of MVA or MVL by any LC-MS or LC-MS/MS method can surpass the detection limit of the RI technique using [^{14}C]MVL.

The aim of this study was to develop an LC-P-ESI-MS/MS method that was more sensitive and reliable than the conventional RI technique to measure HMG-CoA reductase activity.

MATERIALS AND METHODS

Chemicals

Unlabeled MVL and HMG-CoA were purchased from Sigma-Aldrich (St. Louis, MO), and 3,5-dihydroxy-[3-^{2}H$_3$]methyl-[4,4,5,5-^{2}H$_4$]valerolactone ([^{2}H$_7$]MVL) was from Merck Frosst Canada (Montreal, Canada). *RS*-[5-^{3}H]MVL (888.0 GBq/mmol) and 3-hydroxy-3-methyl-[3-^{14}C]glutaryl-CoA (2.15 GBq/mmol) were obtained from New England Nuclear (Boston, MA) and Amersham (Aylesbury, UK), respectively. 1-(2-Aminoethyl)piperidine, 1-(2-aminoethyl)piperazine, 4-(2-aminoethyl)morpholine, 1-(2-aminoethyl)pyrrolidine, 4-(2-aminoethyl)pyridine, *N,N*-dimethylethylenediamine, and 4-dimethylaminobenzylamine dihydrochloride were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Additional reagents and solvents were of analytical grade.

Procedure for the synthesis of mevalonyl-alkylamide derivatives

A 100 μl volume of toluene and 20 μl of each of the alkylamines were added to MVL, and the mixture was incubated at 75°C for 60 min. After evaporating the solvent by heating at 55–100°C under a stream of nitrogen, the residue was dissolved in methanol-water (1:1, v/v). 4-Dimethylaminobenzylamine was prepared from 4-dimethylaminobenzylamine dihydrochloride by the addition of an equimolar NaOH solution and extraction with diethyl ether.

Preparation of rat liver microsomes

Male Sprague-Dawley rats were purchased from Charles River Japan (Yokohama, Japan). They were euthanized between 1:00 and 2:00 PM under diethyl ether anesthesia. Livers were excised and frozen immediately in liquid N_2 and stored at −70°C until later use. The animal protocol was approved by the Institutional Animal Care and Use Committee. Microsomes were prepared from livers by differential ultracentrifugation (19), and the protein concentrations were determined by the method of Bradford (20).

Conventional method for the measurement of HMG-CoA reductase activity using RI

The conventional method for the measurement of microsomal HMG-CoA reductase activity using the RI technique was based on the methods of Shefer et al. (6) and Nguyen et al. (21) with some modifications. Microsomes (100 μg of protein) were incubated for 30 min at 37°C in a total 150 μl volume of 100 mM potassium phosphate buffer (pH 7.4) containing 0.1 mM EDTA, 50 mM KCl, 10 mM DTT, a NADPH generating system (34 mM NADPH, 30 mM glucose-6-phosphate, and 1 unit of glucose-6-phosphate dehydrogenase), and 30 nmol of [^{14}C]HMG-CoA (diluted with unlabeled HMG-CoA to give a specific activity of 30 dpm/pmol). The reaction was terminated with the addition of 20 μl of 6 N HCl, and the tubes were allowed to stand at room temperature for 10 min to ensure lactonization of the biosynthetic MVA at pH < 1. A 200 μl volume of ethanol containing 1 mg of [^{3}H]MVL (diluted with unlabeled MVL to give a specific activity of 40,000 dpm/mg), 1 ml of diethyl ether, 0.2 g of ammonium sulfate, and 50 μl of water was added to each tube in order. The tubes were vortexed for 1 min and centrifuged at 1,000 *g* for 2 min. The ether-phase supernatant was collected, and 1 ml of diethyl ether was again added to the residual fraction. After the same extraction procedure, the combined ether fraction was evaporated to dryness under nitrogen. The residue was redissolved in 50 μl of acetone, applied to a thin-layer chromatography plate, and developed with benzene-acetone (1:1, v/v). The MVL band was isolated from the plate, and the radioactivity was measured for 10 min by dual-label liquid scintillation counting.

Measurement of HMG-CoA reductase activity by LC-P-ESI-MS/MS

The present method for the measurement of HMG-CoA reductase activity by LC-P-ESI-MS/MS was carried out as follows. The incubation of microsomes and extraction of biosynthetic MVA were performed by the same method described above for the RI assay, except that unlabeled HMG-CoA and [^{2}H$_7$]MVL (10 ng) were used instead of [^{14}C]HMG-CoA and [^{3}H]MVL, respectively. A 100 μl volume of toluene and 20 μl of 1-(2-aminoethyl)-pyrrolidine were added to the residue of the ether extract and incubated at 55°C for 60 min. After the addition of 2 ml of toluene, the mixture was applied to a Bond Elut SI cartridge (100 mg; Varian, Harbor City, CA) preconditioned with 1 ml of toluene. The cartridge was washed with 2 ml of toluene-ethyl acetate (1:1, v/v), and the mevalonyl-(2-pyrrolidin-1-yl-ethyl)-amide (MV-PLEA) was eluted with 2 ml of ethyl acetate-methanol (4:1, v/v). After evaporation, the residue was redissolved in 100 μl of methanol-water (5:95, v/v) containing 0.1% acetic acid, and an aliquot (1 μl) was injected into the LC-P-ESI-MS/MS system described below.

The system consisted of a TSQ Quantum Ultra quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an H-ESI probe and a Nanospace SI-2 HPLC system (Shiseido, Tokyo, Japan). Chromatographic separation of MV-PLEA was performed using a Hypersil GOLD aQ column (150 × 2.1 mm, 3 μm; Thermo Fisher Scientific) maintained at 40°C. The mobile phase consisted of methanol-water (5:95, v/v) containing 0.1% acetic acid and was used at a flow rate of 200 μl/min. The general LC-MS/MS conditions were as follows: spray voltage, 1,000 V; vaporizer temperature, 350°C; sheath gas (nitrogen) pressure, 50 p.s.i.; auxiliary gas (nitrogen) flow, 50 arbitrary units; ion transfer capil-

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lary temperature, 350°C; collision gas (argon) pressure, 1.5 mTorr; collision energy, 13 V; and ion polarity, positive. Selected reaction monitoring (SRM) was conducted using *m/z* 245 → *m/z* 227 for the MV-PLEA and *m/z* 252 → *m/z* 234 for the $^2H_7$ variant.

Evaluation of the extraction and derivatization processes

The absolute recovery of MVL in the salting-out extraction process was confirmed by adding 10 ng of MVL to the assay mixture without incubation. After diethyl ether extraction, followed by the addition of [$^2H_7$]MVL as an internal standard, MVL and its $^2H_7$ variant were derivatized, purified, and quantified by LC-P-ESI-SRM.

The recovery of MV-PLEA from the Bond Elut SI cartridge was determined by the addition of MV-PLEA (10 ng) to the cartridge with a derivatizing mixture, which was prepared from the assay mixture without incubation. After the purification steps, [$^2H_7$]MV-PLEA (10 ng) was added to the eluate as an internal standard, and both MV-PLEA and its $^2H_7$ variant were quantified by LC-P-ESI-SRM.

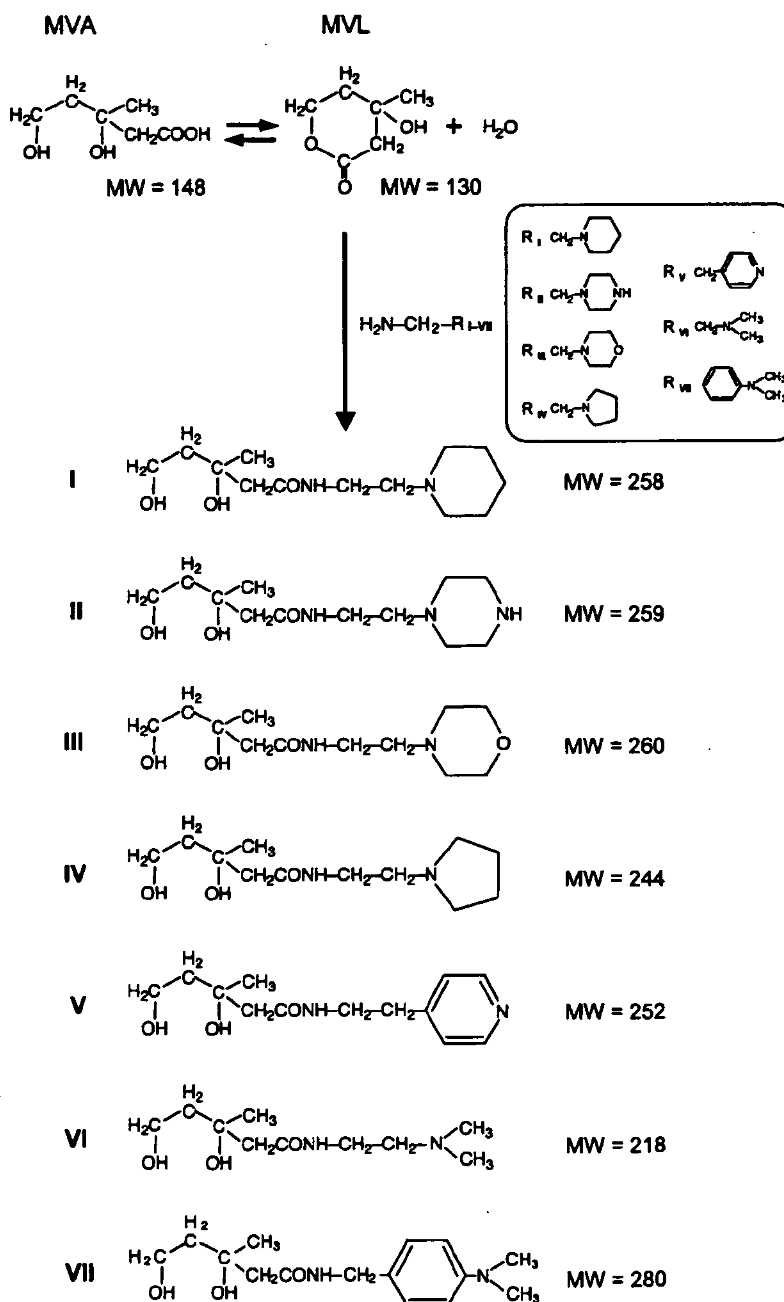

Fig. 1. Structures of mevalonic acid (MVA), mevalonolactone (MVL), and the alkylamide derivatives used in this study: I, mevalonyl-(2-piperidin-1-yl-ethyl)-amide; II, mevalonyl-(2-piperazin-1-yl-ethyl)-amide; III, mevalonyl-(2-morpholin-4-yl-ethyl)-amide; IV, mevalonyl-(2-pyrrolidin-1-yl-ethyl)-amide (MV-PLEA); V, mevalonyl-(2-pyridyl-4-yl-ethyl)-amide; VI, mevalonyl-(2-dimethylamino-ethyl)-amide; VII, mevalonyl-dimethylamino-benzylamide. MW, molecular weight.

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The effects of incubation temperature and duration on the (2-pyrrolidin-1-yl-ethyl)-amidation of MVL were examined. A 4 μg aliquot of MVL was incubated with 20 μl of 1-(2-aminoethyl)-pyrrolidine in 100 μl of toluene. After the addition of [$^{2}H_{7}$]MV-PLEA as an internal standard and purification by a Bond Elut SI cartridge, MV-PLEA and its $^{2}H_{7}$ variant were quantified by LC-P-ESI-SRM.

Statistics

Data are reported as means ± SD. Reproducibility was analyzed by one-way layout (JMP software; SAS Institute, Inc., Cary, NC). Recovery was analyzed using a polynomial equation (22). Linearity of the calibration curve, correlation between the amount of microsomal protein and the formation of MVL, and correlation between enzyme activities determined by LC-P-ESI-SRM and the RI methods were analyzed by simple linear regression. Regression analysis was also used to calculate the estimated amount ± 95% confidence limit in the recovery study. For all analyses, significance was accepted at $P < 0.05$.

RESULTS

Selection of the best derivative for the quantification of MVL

To find the best derivative for MVL, we synthesized seven kinds of mevalonyl-alkylamides (**Fig. 1**), and their P-ESI mass spectral and tandem mass spectral data were compared. As shown in **Table 1**, all mevalonyl-alkylamide derivatives exhibited protonated molecular ions ($[M+H]^+$) as the base peaks. The fragmentation pattern of the protonated molecular ion of each derivative under various levels of collision energy was examined, and the most abundant product ion was selected as a monitoring ion for SRM. The signal-to-noise ratios (S/Ns) of these derivatives determined by injecting 100 fg of each derivative were as follows: VI (20) > V (15) > IV (10) = III (10) > I (5) = II (5) = VII (5). However, additional factors, such as the lower boiling point of the remaining alkylamines, the convenience of the solid-phase purification procedure, and an improved symmetric shape of the peak in the chromatogram finally determined that MV-PLEA (derivative IV) was the best derivative for the quantification of MVL in the incubation mixture. The representative P-ESI mass spectrum and MS/MS spectrum of the MV-PLEA are shown in **Fig. 2**.

Evaluation of the extraction and derivatization processes

The absolute recovery of MVL from the incubation mixture by the salting-out extraction procedure was calculated as 88.1 ± 2.8% (n = 4). The recovery of MV-PLEA from the Bond Elut SI cartridge was found to be 98.1 ± 3.1% (n = 4). The derivatization of MVL to MV-PLEA achieved a maximum efficiency at 55°C for 60 min. Higher incubation temperature or longer incubation time did not result in increased formation of MV-PLEA. When the derivatizing mixture was incubated at room temperature (20°C) for 5, 10, 30, and 60 min, the derivatization was 8, 14, 81, and 94%, respectively.

Calibration curve

A calibration curve was established for MVL (**Fig. 3**). Different quantities (0.04, 0.1, 0.4, 1, 4, 10, 40, and 100 ng) of authentic MVL were each mixed with 10 ng of [$^{2}H_{7}$]MVL, derivatized to the MV-PLEA, and purified using the Bond Elut SI cartridge, as described in Materials and Methods. The weight ratio of MVL, relative to the corresponding deuterated internal standard, was compared with the peak area ratio of the MV-PLEA to the $^{2}H_{7}$ variant measured by LC-P-ESI-SRM. The linearity of the standard curve, as determined by simple linear regression, was excellent

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TABLE 1. Positive ESI-MS, MS/MS, and SRM data of the mevalonyl-alkylamides

Derivatives[a]	MS Data $[M+H]^+$ (Relative Intensity)	MS/MS Data[b] (Collision Energy at Maximum Intensity)				SRM Data[c] Collision Energy	SRM Data[c] Precursor to Product	S/N[d]
	m/z (%)	m/z (V)				V	m/z	
I	259 (100)	241 (13)	174 (18)	86 (23)	112 (17)	13	259→241	5
II	260 (100)	224 (13)	113 (20)	86 (21)	138 (22)	13	260→224	5
III	261 (100)	243 (13)	114 (18)	138 (20)	86 (21)	13	261→243	10
IV	245 (100)	227 (13)	98 (19)	86 (20)	138 (24)	13	245→227	10
V	253 (100)	106 (30)	112 (15)	77 (51)	–[e]	30	253→106	15
VI	219 (100)	91 (31)	202 (13)	86 (20)	–[e]	31	219→91	20
VII	281 (100)	134 (29)	118 (48)	91 (54)	–[e]	29	281→134	5

ESI, electrospray ionization; MS, mass spectrometry; MS/MS, tandem mass spectrometry; S/N, signal-to-noise ratio; SRM, selected reaction monitoring.

[a] Mevalonolactone (MVL) was derivatized to I, mevalonyl-(2-piperidin-1-yl-ethyl)-amide; II, mevalonyl-(2-piperazin-1-yl-ethyl)-amide; III, mevalonyl-(2-morpholin-4-yl-ethyl)-amide; IV, mevalonyl-(2-pyrrolidin-1-yl-ethyl)-amide (MV-PLEA); V, mevalonyl-(2-pyridyl-4-yl-ethyl)-amide; VI, mevalonyl-(2-dimethylamino-ethyl)-amide; and VII, mevalonyl-dimethylamino-benzylamide.

[b] $[M+H]^+$ was used as a precursor ion for each MS/MS analysis. Major product ions were arranged in the order of abundance from left to right.

[c] The same HPLC column and flow rate described in Materials and Methods were used, but the following gradient system was used for the elution of all mevalonyl-alkylamides from the column: for the first 2 min, the mobile phase consisted of methanol-water (1:9, v/v) containing 0.1% acetic acid; then it was programmed in a linear manner to methanol-water (1:1, v/v) containing 0.1% acetic acid over 4 min. The final mobile phase was kept constant for 2 additional min. The retention times of derivatives I–VII under this gradient HPLC condition were 2.9 min (II), 2.9 min (III), 3.0 min (IV), 3.2 min (V), 3.8 min (I), 4.6 min (VI), and 7.0 min (VII).

[d] S/Ns were determined by injecting 100 fg of each derivative.

[e] Intense ion (>5% of base peak) was not observed.

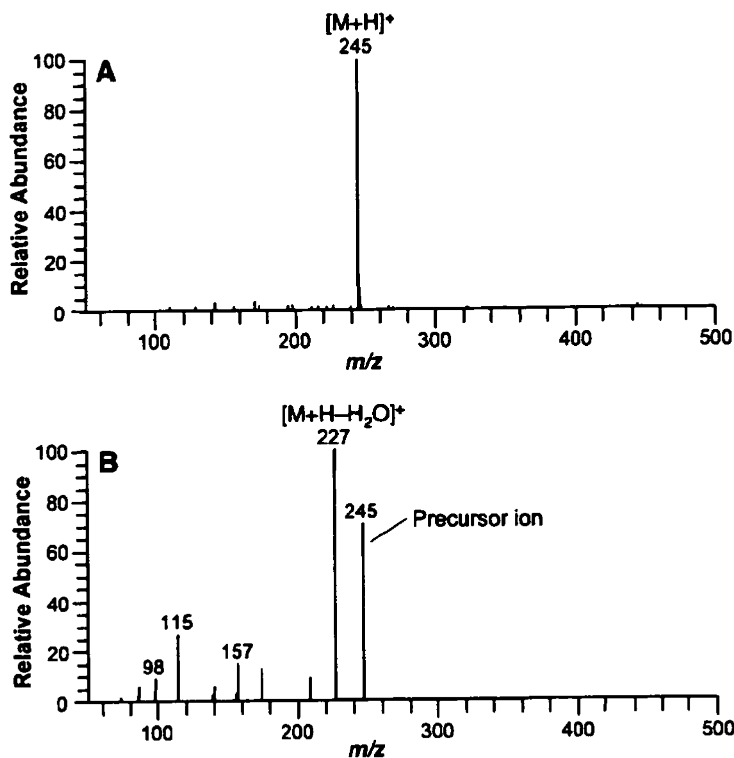

Fig. 2. Typical electrospray ionization in positive mode (P-ESI) mass spectrum (A) and product ion mass spectrum using *m/z* 245 as a precursor ion (B) of the MV-PLEA. The general liquid chromatography-tandem mass spectrometry (LC-MS/MS) conditions were as follows: introducing solvent, methanol-water (5:95, v/v) containing 0.1% acetic acid; flow rate, 200 μl/min; spray voltage, 1,000 V; collision energy, 13 V.

for weight ratios between 0.004 and 10 ($n = 8$, $r = 1.000$, $P < 0.0001$).

Representative recordings of LC- P-ESI-SRM

Figure 4 presents typical LC-P-ESI-SRM chromatograms obtained by analysis of a standard incubation mixture

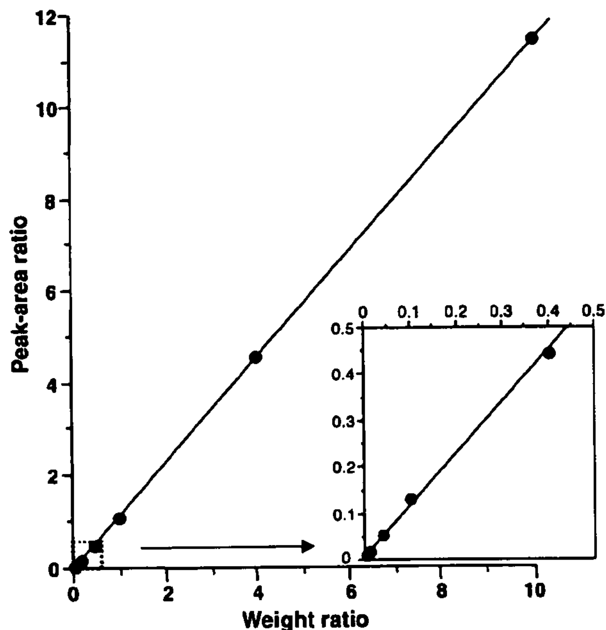

Fig. 3. Calibration curve for the weight ratio of MVL to the corresponding deuterated internal standard. Linearity was checked by simple linear regression, and the equation for the line of best fit was $y = 1.144x - 0.004$ ($n = 8$, $r = 1.000$, $P < 0.0001$).

using 100 μg of protein of microsomes from normal rat liver. The peak for MV-PLEA at *m/z* 245 → *m/z* 227 in Fig. 4B reflects the amount of MVL after incubation and corresponds to ~90 pg (~23 pmol/min/mg protein). As shown in Fig. 4A, endogenous MV-PLEA was detected at zero time, but the amount did not significantly affect the assay of the enzyme activity.

Precision and accuracy of this method

The precision and accuracy of our method was determined using the same microsomes obtained from a normal rat liver. Reproducibility was investigated by analyzing four samples in triplicate by LC-P-ESI-SRM (**Tables 2, 3**). The results were analyzed by one-way layout, in which the analytical errors were divided into two sources: sample preparation and LC-P-ESI-SRM measurement. The variances were considered to be attributable to the measurement, because the errors during sample preparation were negligible. The interassay coefficients of variation for the between- and within-sample variations were 3.2% and 1.8%, respectively.

For the recovery experiment (**Table 4**), known quantities of MVL (10–30 ng, 0.77–2.31 nmol/mg protein) were spiked into the mixture at the end of the incubation. After the extraction and clean-up procedures, LC-P-ESI-SRM was conducted in triplicate for each sample. The recoveries of the known spiked amounts of MVL ranged from 92.6% to 96.2%, with a mean of 94.6%. In addition, the amount of MVL found in the unspiked incubation mixture was within the 95% confidence limit for the estimated amount of MVL, as calculated by linear regression analysis; this also constituted an index for the precision and accuracy of the method.

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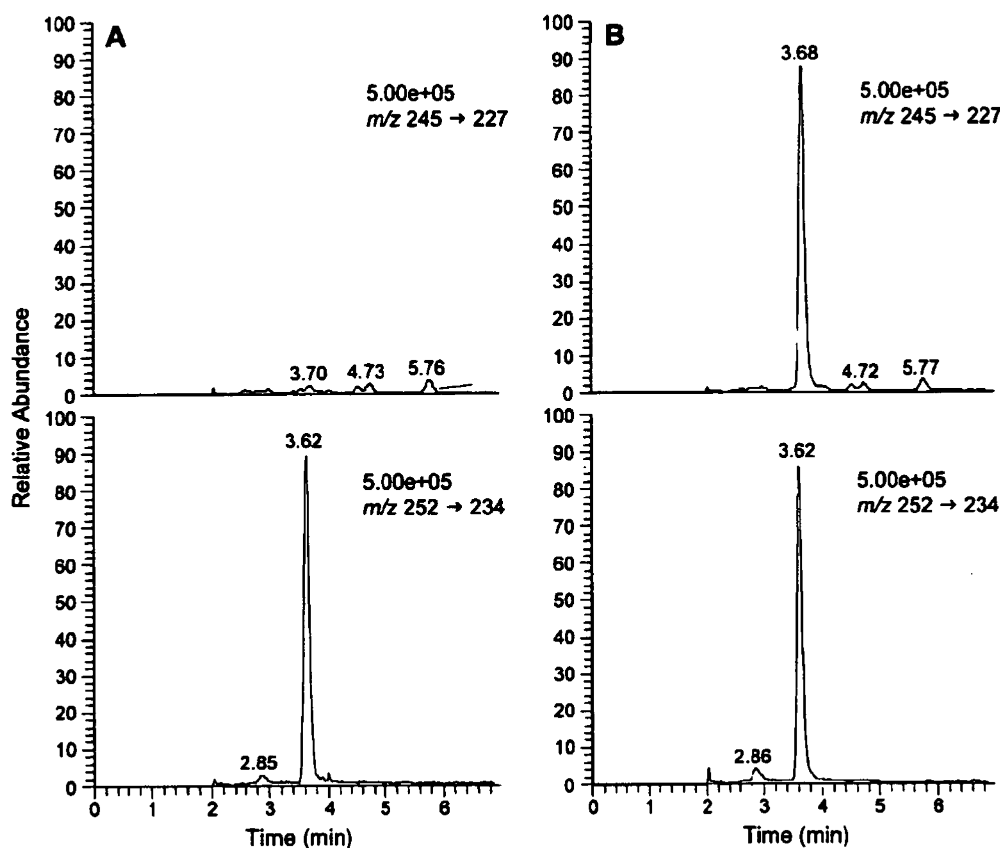

Fig. 4. Representative LC-selected reaction monitoring (SRM) chromatograms of MV-PLEA and its $^2H_7$ variant (internal standard) in extracts obtained from the standard incubation mixture. A: Zero time. B: Incubated with 100 μg of protein of rat liver microsomes for 30 min. The peak of MV-PLEA in B corresponds to ~90 pg (~23 pmol/min/mg protein).

Application to a microscale assay

We studied the effect of reduced levels of microsomal protein on the quantification of MVL amount and enzyme activity. Different quantities (5, 10, 25, 50, and 100 μg of protein) of normal rat liver microsomes were used for the enzyme assay, and extraction and quantification of MVL were conducted as described in Materials and Methods. As shown in **Fig. 5**, proportionality was observed when the quantity of microsomal protein ranged from 5 μg up to at least 100 μg for the production of MVL.

Correlation between our method and the RI method

Hepatic HMG-CoA reductase activities were measured by both RI and positive LC-P-ESI-SRM methods in 11 normal rats. The HMG-CoA reductase activities obtained by this method correlated well with those obtained by the RI method ($r = 0.930$, $P < 0.0001$) (**Fig. 6**).

DISCUSSION

We have developed and optimized various derivatization methods that are appropriate for enhancing the sensitivity

TABLE 2. Reproducibility in the measurement of HMG-CoA reductase activity: analytical data

Sample	Individual Values			Mean ± SD
	pmol/min/mg protein			
A	15.7	16.1	15.2	15.7 ± 0.4
B	16.0	15.9	16.0	16.0 ± 0.1
C	15.3	15.4	15.3	15.4 ± 0.1
D	15.3	15.0	15.7	15.4 ± 0.4
Mean ± SD				15.6 ± 0.4

HMG-CoA reductase activity was measured using 100 μg of protein of rat microsomes.

TABLE 3. Reproducibility in the measurement of HMG-CoA reductase activity: ANOVA

Source	S	f	V	F_0	Relative SD
					%
Sample preparation	0.742	3	0.247	3.04	3.2
Error (SRM)	0.650	8	0.081		1.8
Total	1.392	11			
			$F(3,8,0.05) = 4.07$		

S, residual sum of squares; f, number of degrees of freedom; f_1, $f_{sample\ preparation}$; f_2, f_{error}; V, unbiased variance; F_0, observed value after F distribution variance ratio ($V_{sample\ preparation}/V_{error}$); $F(f_1, f_2, \alpha)$, density function of F distribution with f_1 and f_2 degrees of freedom.

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TABLE 4. Recovery of MVL from the incubation mixture

Sample (X_0 + na) (n = 0,1,2,3)	Amount Added	Amount Found			Recovery[a] (mean ± SD)	Estimated Amount ± 95% Confidence Limit[b]
		nmol/mg protein			%	*nmol/mg protein*
X_0	0	$\overline{X}_0$ ± SD = 0.47 ± 0.01[c]				0.47 ± 0.07
X_0 + a	0.77	1.24	1.23	1.20		
X_0 + a	0.77	1.19	1.20	1.19	96.2 ± 2.6	
X_0 + 2a	1.54	1.89	1.88	1.91		
X_0 + 2a	1.54	1.86	1.88	1.94	92.6 ± 1.7	
X_0 + 3a	2.31	2.70	2.76	2.60		
X_0 + 3a	2.31	2.70	2.65	2.55	95.1 ± 3.3	

Known amounts of MVL were spiked into the mixture at the end of the incubation.
[a] Recovery (%) = (amount found − $\overline{X}_0$)/amount added × 100.
[b] The estimated amount was calculated by a polynomial equation.
[c] This value was obtained from Table 2.

of the microanalysis of endogenous substances in biological specimens by GC-MS. As described in our previous investigations (7, 10, 11), MVA was lactonized easily to MVL and the resulting MVL was converted to the corresponding alkylamides with the primary alkylamines under mild conditions without any catalysts. This amidation via MVL from MVA was a characteristic reaction for γ-hydroxy fatty acids such as MVA, and the resulting alkylamides were further converted to the final derivatives suitable for the detection of ionization. Thus, these derivatives provided excellent GC-MS properties in their separation, specificity, and sensitivity and enabled us to quantify trace amounts of MVA in biological specimens.

LC-MS methods, using ESI or atmospheric pressure CI, have been used to quantify trace amounts of biologically important fatty acids after enhancing their detection sensitivity through derivatization, such as pentafluorobenzylation (23–25). Although several methods for quantifying MVA or MVL in biological specimens by LC-ESI-MS or LC-ESI-MS/MS have been described, there have been no methods to enhance their detection sensitivity through derivatization. The negative and positive modes for ESI have been used for the quantification of MVA and MVL, respectively. We used the positive mode for this quantification because it provides more abundant ions than the negative mode (26). To enhance the sensitivity of detection, the amidation reaction described above, which is characteristic for MVA, was conducted using seven kinds of primary alkylamines with a tertiary amine moiety to promote protonation. Thus, the mevalonyl-alkylamides synthesized in this study were all versatile derivatives. The resulting mass spectra exhibited $[M+H]^+$ as the base peaks, which is suitable for the highly sensitive detection of MVL using LC-P-ESI-MS/MS. Collision of $[M+H]^+$ for derivatives I–IV under relatively low energy (<14 V) produced the characteristic product ions related to the

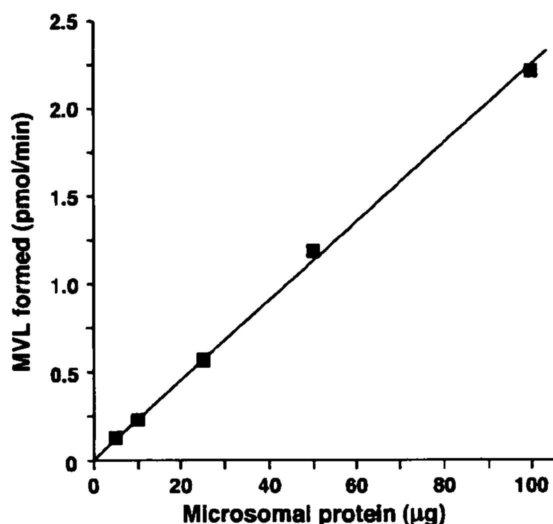

Fig. 5. Effects of reduced microsomal protein on the determination of HMG-CoA reductase activity. Linearity was checked by simple linear regression, and the equation for the line of best fit was y = 0.022x + 0.026 (n = 5, r = 0.999, P < 0.0001).

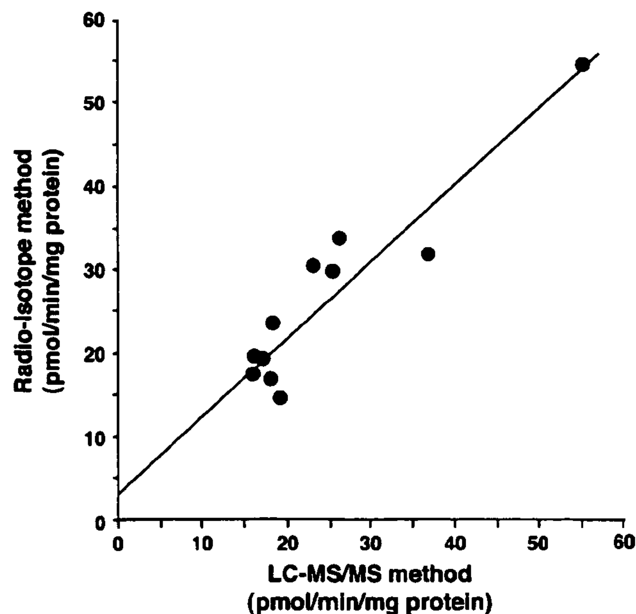

Fig. 6. Correlation between our LC-MS/MS method and the conventional radioisotope method for the assay of HMG-CoA reductase activity. Linearity was checked by simple linear regression, and the equation for the line of best fit was y = 0.905x + 3.899 (n = 11, r = 0.930, P < 0.0001).

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$[M+H-H_2O]^+$ or $[M+H-2H_2O]^+$. In contrast, the increase of collision energy (>14 V) resulted in the formation of product ions with low mass numbers from all seven derivatives.

All of these seven derivatives exhibited excellent LC-P-ESI-MS/MS properties with extremely high sensitivity, as shown in Table 1. However, MV-PLEA was selected as the best derivative for our purpose for the following reasons. First, the boiling point of 1-(2-aminoethyl)pyrrolidine is the second lowest, after *N,N*-dimethylethylenediamine, which is advantageous for evaporating contaminated alkylamines after solid-phase purification. Second, mevalonyl-2-(dimethylamino)ethylamide (VI) exhibited a better S/N peak than MV-PLEA, but the peak shape of derivative VI was considerably broader compared with that of derivative IV (0.3 vs. 0.7 min). Third, MV-PLEA was successfully purified from the derivatizing mixture with almost complete elimination of excess 1-(2-aminoethyl)pyrrolidine using a small Bond Elut SI cartridge. Because 1-(2-aminoethyl)pyrrolidine can be evaporated by heating at 55°C under a nitrogen stream, the solid-phase extraction step was not necessary to quantify MV-PLEA by LC-SRM. However, this step was successful not only in eliminating the excess alkylamine but also for cleaning up the extracts with diethyl ether, which reduced the load on the LC-MS/MS system.

In the conventional RI method, the specific activity of radiolabeled HMG-CoA was 30 dpm/pmol, which was equivalent to 33.3 fmol/dpm. When the standard deviation of background noise is 2 dpm, the signal would be 6 dpm when the S/N = 3. Therefore, the detection limit of the conventional RI method was calculated to be 200 fmol (26 pg) (S/N = 3). Although it may be not practical, if carrier-free [^{14}C]HMG-CoA without dilution with unlabeled HMG-CoA were used, the minimum detection limit of the RI method would be 46.5 fmol (6.0 pg) (S/N = 3), because the specific activity of the carrier-free [^{14}C]HMG-CoA is 2.15 GBq/mmol = 2.15 Bq/pmol = 129 dpm/pmol = 7.75 fmol/dpm. Clearly, the measurement of HMG-CoA reductase activity by LC-MS (14) and LC-MS/MS (15) is more specific than by the RI method, in which radioactivities are counted on thin-layer chromatography plates. However, the detection limit of underivatized MVL by LC-MS was 50 fmol (6.5 pg) (S/N = 3) (14), which was ~100,000 times more sensitive than that obtained by HPLC-ultraviolet (5.7 nmol [740 ng]) (27) but did not apparently exceed that obtained by the RI method. In comparison, the present LC-P-ESI-MS/MS method made it possible to detect only 240 amol (31 fg) (S/N = 3) of MVL. Therefore, even if a sufficient quantity of microsomes were not available, this method would still enable us to measure HMG-CoA reductase activity (Fig. 5). In addition to the high sensitivity, the use of [$^2H_7$]MVL as an ideal internal standard contributed to the development of this highly reproducible method.

In this method, we followed the incubation and extraction procedures of the conventional RI method, with the exception that unlabeled HMG-CoA and [$^2H_7$]MVL were used instead of [^{14}C]HMG-CoA and [^{3}H]MVL, respectively. The subsequent derivatization and purification steps are very simple, and the HPLC system operates isocratically. Therefore, it would be easy to switch from the conventional RI method to the present non-RI method if an LC-MS/MS system were available. In addition, the data obtained by this non-RI method are comparable with previous data obtained by the RI method, because both methods obtained very similar results (Fig. 6). Furthermore, this method can be used as a high-throughput screening method for HMG-CoA reductase inhibitors.

In summary, we developed a very sensitive and specific non-RI method for the measurement of HMG-CoA reductase activity. Derivatization of MVL into the MV-PLEA allowed it to be quantified by LC-P-ESI-MS/MS with higher sensitivity than in the RI method. Reproducibility and recovery experiments verified that this method resulted in HMG-CoA reductase activities with high reliability and reproducibility.

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ウイルス肝炎の日常管理

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ウイルス肝炎の日常管理

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はじめに●

ウイルス肝炎の日常管理は病状により異なる．軽い慢性肝炎から，前肝硬変(pre-chirrhosis)，肝硬変(LC)までさまざまである．B型，C型肝炎ウイルス(HCV)による慢性肝疾患の日常管理について本稿ではまとめてみる．食事の項は別途あるのでそちらに譲るとし，慢性ウイルス肝炎患者さんの，運動，それに付随するこむら返り(有痛性筋肉けいれん)，飲酒，喫煙，薬物，健康食品摂取，日常感染対策予防について，慢性肝炎から肝硬変までを視野に入れ述べる．

ウイルス肝炎患者における運動●

1. 運動の基本

慢性肝疾患における運動のポイント

ウイルス肝炎に罹患した場合(C型にしろB型でも)，慢性肝炎から限りなく肝硬変に近い慢性肝炎，慢性肝炎と境の肝硬変などさまざまな病態がある．まず大事なことは，自分がどの程度の肝機能障害なのかを理解させることである．肝臓が悪い患者さんの場合，肝臓の悪さの程度で運動の種類，時間，程度も異なるのである．一般的には，慢性肝炎の方や肝硬変でも軽度の方は，あまり運動の制限はしない．ただ，AST, ALTが100 IU以上の場合や，AST, ALTの上下がある方は肝機能が悪化しているときは，無理な運動をしないように指導する．自分で疲労感を感じるような運動はなるべく控えたほうがよいだろう．さらに食後すぐに動かず，20～30分くらいゆっくりするといいといわれる．科学的根拠(EBM)ははっきりしないが肝血流分布の問題と昔からいわれている．慢性肝炎の進行された方や，肝硬変の軽い方は，心地よい疲労感が得られる程度の散歩などをして，筋肉が萎縮しないようにすることも大切である．

2. 慢性肝炎と軽度肝硬変患者の運動

AST, ALTが落ち着いており，血小板なども10万以上ある慢性肝炎で安定している場合，さらに肝硬変でも落ち着いている，初期の方などは，QOLを考え，日常生活の管理さえ可能であれば，運動制限は行わず，ふつうの人と同じように生活してもらう．

3. 中等度以上の肝硬変患者の運動

肝硬変でも，黄疸，腹水，下腿浮腫，消化管出血などが併発している場合，安静が基本になる．軽く汗をかく程度の20分程度の散歩を，ゆっくりと，食後2～3時間後に行う．夏場の炎天下での散歩は控える．散歩が終わったら30分ほどは安静にした方がよい．黄疸，腹水などがある場合は，無理をせず，安静を基本とし，座っていてもできる，ストレッチ体操程度を行うことで筋肉の萎縮を避けるために重要である．

4. 通勤や食後，風呂

中等度以上の肝硬変患者さんの場合，通勤ラッシュアワーにおいては，立ちっぱなしでいることは避け，座るようにした方がよい．また，食後寝たほうがよいといわれるが，肝疾患の方誰もが必ず寝られるわけではない．中等度以上の肝硬変の方は，食後30分～1時間ほどは，横臥した方がよい．しかし慢性肝炎と軽度肝硬変患者さんの場合は，食後すぐに動くのではなく，30～40分は安静にして，リラックスをする時間をとるとよいとされる．風呂も中等度以上の肝硬変の患者さんは，ぬるめのお湯につかり，熱い湯で長湯をしないことが望ましい．運動疲労と同じことが起こるからである．

5. 無症候性キャリアの患者さんの場合

いわゆる，肝炎ウイルスのみを持っていて，肝障害のない方の場合は，原則運動制限はしなくてよい．

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●患者さんに自分の肝機能の重症度を理解させる.
●軽度肝障害の患者さんの運動制限は特にいらない.
●中等度以上の肝硬変の患者さんは，筋肉の萎縮が起こらぬよう軽い運動をする.

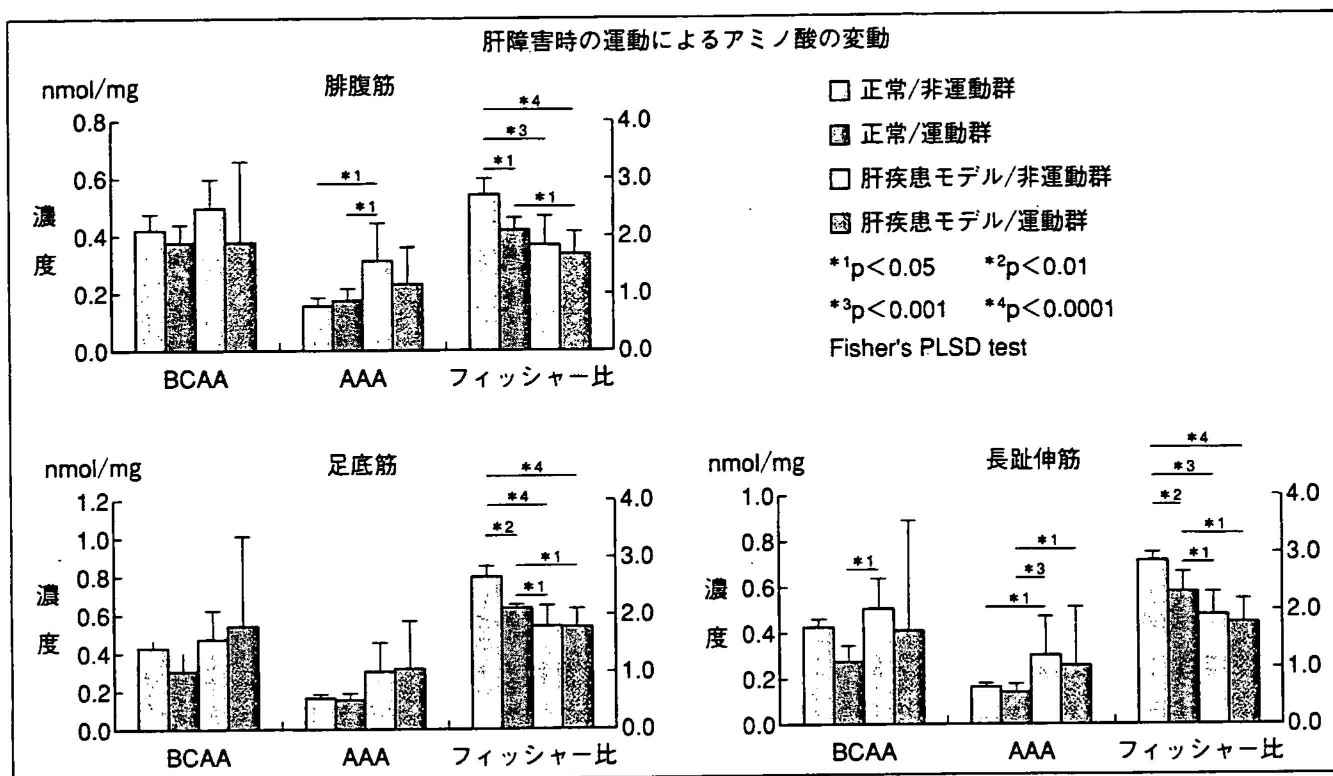


図1 正常・肝疾患時における生体組織BCAA濃度に及ぼす運動の影響(文献1)より)

6. 慢性肝疾患における運動の論理

この問題に関するEBMは実証されていない. 経験や，動物実験成績などから，考えられることをまとめてみる.

a. 肝疾患と運動

基本的に筋肉疲労が起こる機序は，運動をするとエネルギー産生により，乳酸とアンモニアが産生される. 筋肉内にこれらが蓄積すると内部が酸性となり，弱アルカリ性で働くエネルギー産生に必要な酵素活性が低下する. 十分な運動エネルギー産生ができなくなり，筋肉運動が低下し，疲労感が増すとされる. 肝硬変が進行すると(肝性脳症など起こすような患者さん)肝臓内での，アンモニアを代謝する尿素回路の働きが低下し，筋肉内でその代わりをするようになる. 筋肉が十分にこの働きをできないと，脳症になったり，脱力感が強くなったりする.

b. 動物実験から

肝障害と運動時の分岐鎖アミノ酸(BCAA)の関係につき，ラットを用いて検討をした成績を提示する. 人間でいう，中等度以上の肝硬変を作製し，こむら返りが頻発する，下腿骨格筋のBCAA濃度を検討した(図1)[1].

LCモデルの骨格筋のBCAAは増加傾向を示した. さらに，芳香族アミノ酸(AAA)においても同様に増加傾向を示していることから血中から骨格筋へのアミノ酸全体の取りこみが促進していることが示唆される. これは，LC時には，肝臓におけるグルコースの貯蔵が減少しエネルギー産生が低下するため，骨格筋のBCAAからアラニンを合成し，グルコース-アラニン回路による糖新生で代償しているためである. さらに，LCの

⑬筋肉疲労が起こると筋肉内に乳酸，アンモニアが蓄積する．
⑭高度肝硬変では，筋肉内で尿素回路が働きアンモニアの処理をする．
⑮肝硬変モデルラットでは，Fischer 比は下腿骨格筋において低下する．

肝組織では，肝細胞の減少，シャント(副血行路)の形成により，肝での尿素サイクルによるアンモニアの処理能が低下し，その結果として高アンモニア血症が生じる．この際，骨格筋内の BCAA から生成されるグルタミン酸がアンモニアを解毒同化しグルタミンに固定するという作用が働く．骨格筋内で盛んにアンモニアの処理が亢進していることが示唆された．肝臓をはじめ各組織から放出された BCAA は骨格筋内に取りこまれ，肝臓代謝能低下に対する骨格筋での代償に BCAA が用いられているためであろう．

肝硬変ラットでは，肝臓の運動負荷をかけた場合，下腿骨格筋中の BCAA が減少する．LC 時には各組織におけるアミノ酸インバランスに伴う全身的な蛋白異化が亢進していることが示唆された．動物実験であるがヒトの体内動態をある程度推測することが可能であろう．

慢性肝疾患におけるこむら返り●

肝硬変の患者さんにおいてはこむら返りがしばしば起こる方がいる．ふくらはぎに起こることが多い．ひどい場合は，手がつってしまい，箸がもてないなどということも起こる．肝硬変の患者さんの 70% ぐらいの方に起こるといわれている．肝臓が悪い患者さんの場合，肝臓の悪さの程度で運動の種類，持続時間，程度も異なる．一般的には，慢性肝炎の方や肝硬変でも軽度の方は，起こる回数は少ない．ただ，中等度以上の肝硬変の方や，慢性肝炎でも線維化が強い方の場合，しばしば起こることがある．どのようなきっかけで起こるかよく自分で覚えておくことが大切である．なるべくそのきっかけをつくらないようにすることが肝要である．筋肉疲労が強いと起こることが多いので，無理をさせないようにする．

1. 診療管理のポイント

a. 肝疾患とこむら返りの原因

基本的にこむら返りが起こる機序は，高ナトリウム血症，低カリウム血症，低カルシウム血症などの電解質代謝異常，多量の汗をかいたとき，下痢による脱水，タウリン，ビタミン B_1 欠乏状態などにより，神経や筋肉が興奮しやすくなるといわれている．運動をするとエネルギー産生により，乳酸とアンモニアが産生される．筋肉内にこれらが蓄積すると内部が酸性となり，筋肉細胞や，神経と筋肉の接合部での膜の不安定性が起こり，膜の興奮性が増すとも考えられている．しかし，いまだ確実な発症機序は不明である．

b. 予防法

まず，予防法は筋肉を冷やさないように，また過度に運動負荷をかけないようにする．肝硬変の中等度以上の患者さんの場合は，散歩のあとやゲートボールの試合の後などに生じる場合が多いようである．寝るまえに少しマッサージやストレッチをするのもよい．肝臓病が原因である場合，タウリンの不足も関係するとされ，タウリンを服用することでこむら返りが起こらなくなる．タウリンを多く含む，貝類，甲殻類などを食べることも予防策のひとつとされる．

c. 対処法

起きたら筋肉を伸展させるようにするとよい．痛いときに行うのはつらいが，我慢をして行うと以外と早く治まる．足の親指を引っ張るようにし，ふくらはぎを伸ばすことがこつである．カルシウムの静注も効果があると古くから知られている．また，前述のように，タウリンを服用すると，効果が早く出る方もおられる．さらに，漢方薬である芍薬甘草湯が急性期に効果がある．これは，長期服用は副作用の出現(偽アルドステロン症，心不全など)があるので，注意を要する．あくま

◎慢性肝疾患特に肝硬変症において，こむら返りの併発が多い.
◎こむら返りの発症機序はまだ明らかでない.
◎こむら返りには筋肉疲労の回避，タウリン，芍薬甘草湯などが効果がある.

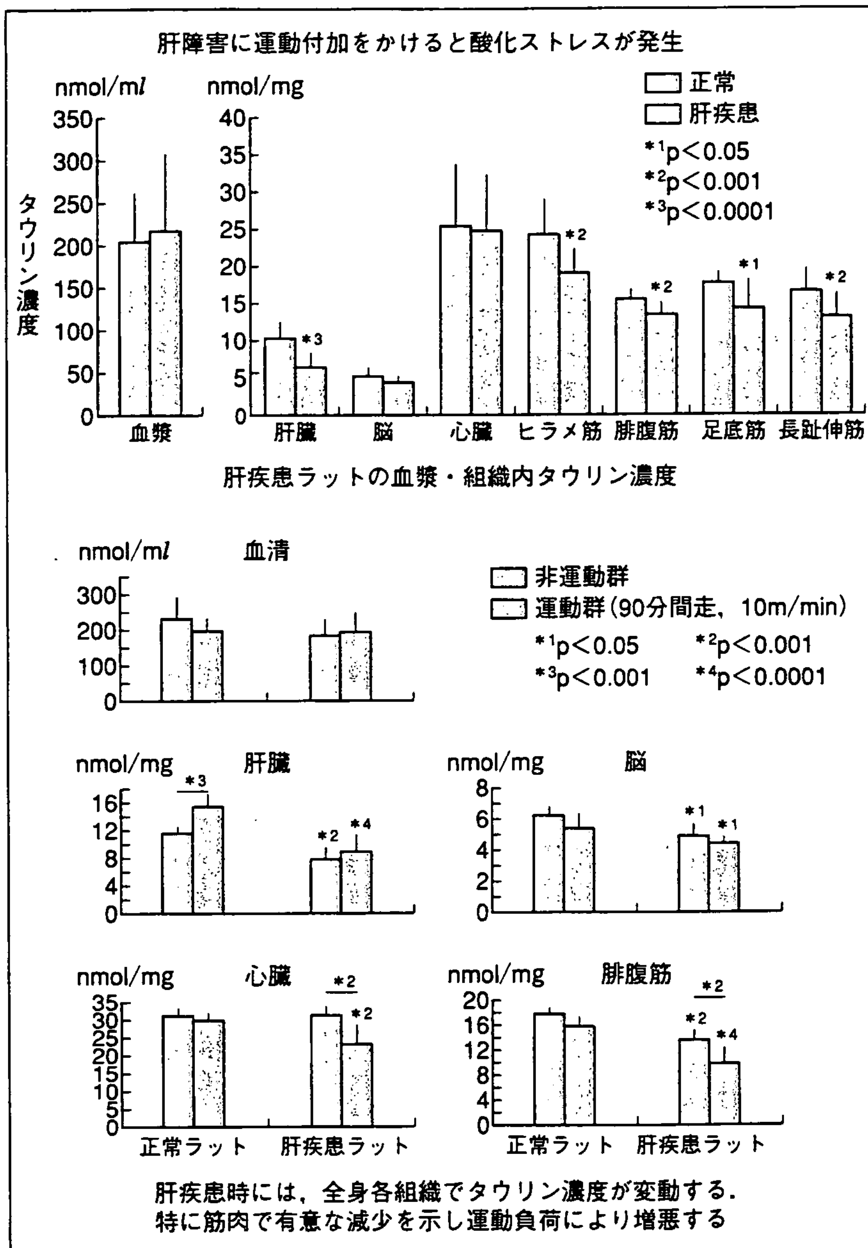


図2 肝疾患時における運動の組織タウリン濃度への影響
(文献2)より)

で，こむら返りがひどいときの頓服薬としての使用を指導する.

2. タウリンがなぜこむら返りに有効か？

肝障害と運動時のタウリン，BCAAの関係につき，ラットを用いて検討をした成績を提示する．ヒトでいう，中等度以上の肝硬変を作製し，こむら返りが頻発する，下腿骨格筋のタウリン，BCAA濃度を検討した．肝硬変ラットでは，運動負荷をかけた場合，下腿骨格筋中の，BCAAやタウリンが減少する(図1, 2)[1,2]．ヒトでは証明されていないが，これらアミノ酸の，こむら返りに対する効用を考えると，傍証かもしれない.

◎肝疾患モデルラットにおいては，全身各臓器のタウリン濃度が変動する．
◎肝疾患モデルラットにおいては，骨格筋でタウリンは有意に減少する．
◎肝疾患モデルラットにおいては，運動負荷をかけるとBCAA，タウリンが減少する．

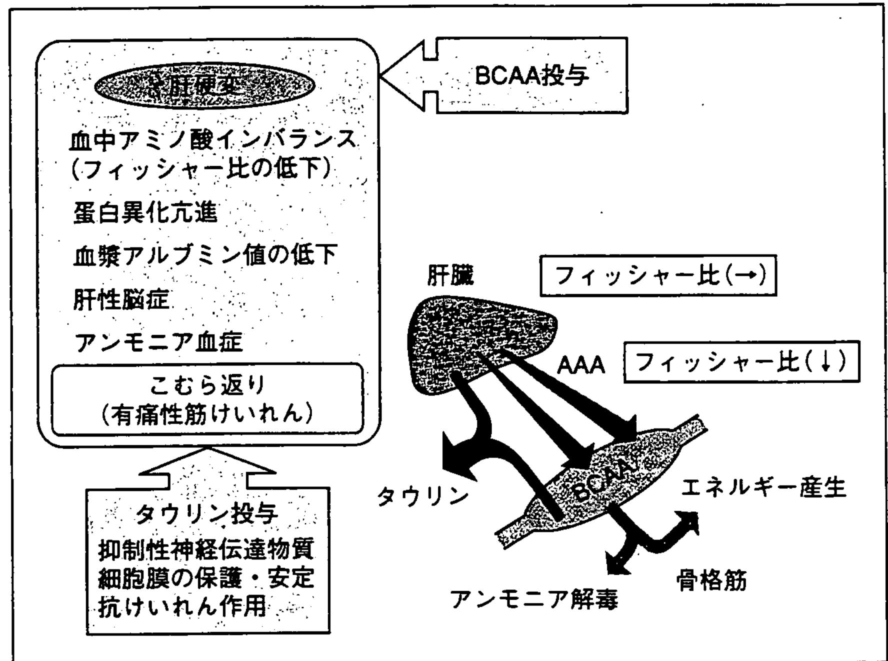


図3 肝硬変におけるタウリン，BCAAの役割の仮説
(文献15)より)

タウリンの最も確立された作用は胆汁酸抱合であるが，そのほかに，膜安定化・保護作用[3]，血圧降下作用[4]，浸透圧調節作用[5]，抗酸化作用[6]，抗炎症作用[7]，抑制性神経伝達物質作用[8]，抗不整脈作用[9]などが報告されている．しかしながら，肝疾患時における諸症状に対するタウリンの作用については，心疾患や網膜疾患などの他の疾患と比べ研究報告が乏しい．

われわれは，こむら返りを併発している肝硬変患者12人に対して，6ヵ月間，1日6gのタウリン製剤の経口投与を試みた．その結果，投与1ヵ月後で8人(66.7%)の患者において大きな副作用もなくこむら返りが消失し，残りの4人においても期間内での症状の改善が認められたことを報告した[10,11]．

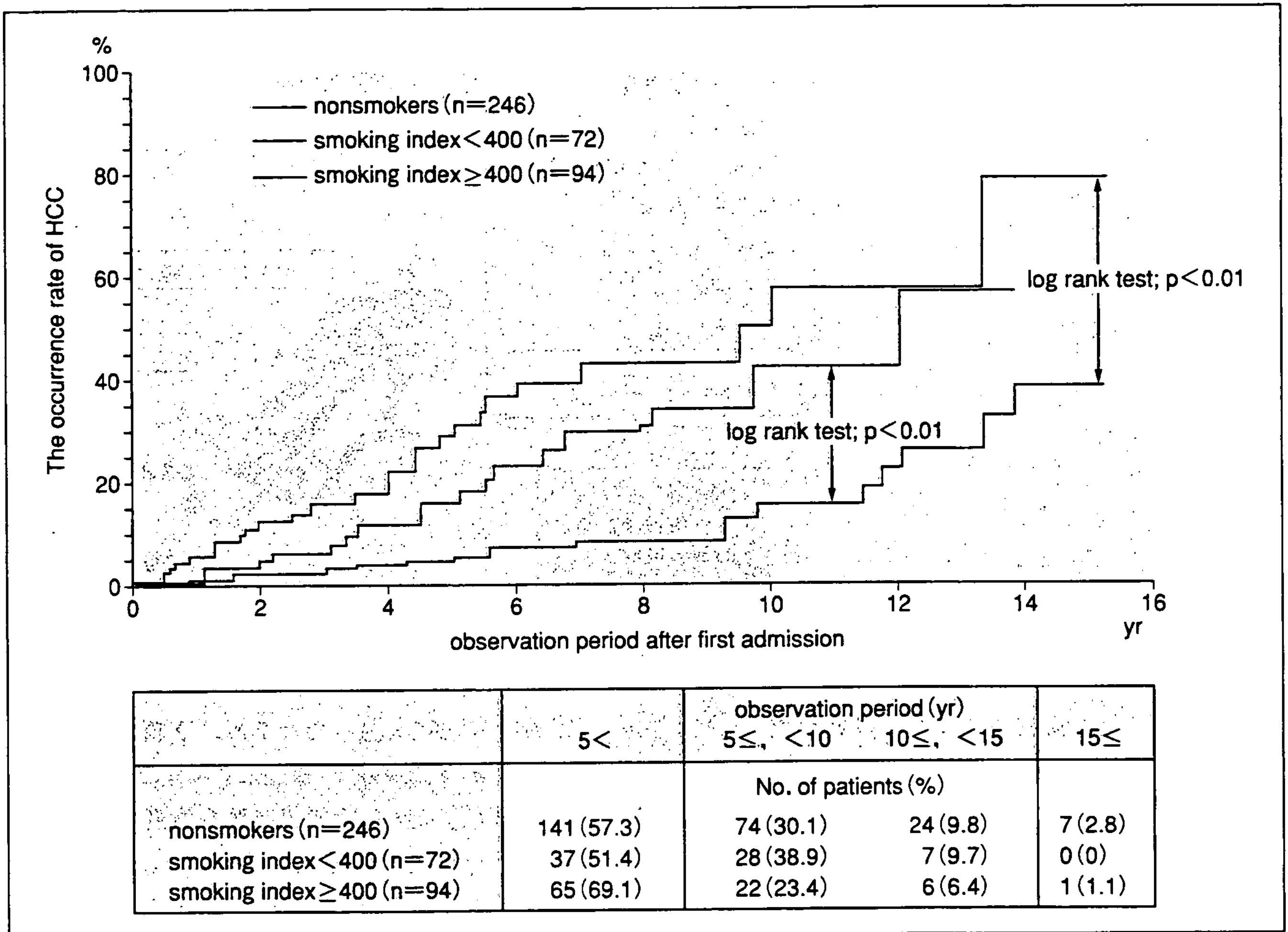
そこで，肝硬変時のこむら返りとタウリンとの関連性について検討することを目的に，実験動物にて以下の検証を行った．まず，こむら返りは運動疲労時に高頻度で発症するため，正常ラットに走運動負荷試験を施したところ，下腿骨格筋タウリン濃度が運動時間の増加に伴い減少傾向を示し，疲労困憊に至る時間で有意な濃度減少がみられた[12]．一般的に骨格筋においては，遅筋が速筋に比べタウリン濃度が高く[13]，本研究では運動負荷により速筋のタウリン濃度が著しく減少することも明らかとなり，疲労しやすい速筋の特性はタウリン濃度変化と何らかの関連がある可能性が示唆された[12]．さらに，経口タウリン投与により，運動負荷による骨格筋タウリン濃度の減少を抑制し，さらに運動能の向上をもたらした[14]．

生体内とくに組織内のタウリン濃度を高めることが，生体内のタウリン濃度の減少防止や維持が肝疾患におけるさまざまな症状の改善に重要であることが考えられる(図3)[15]．

飲酒の問題●

ウイルス肝炎をもっている患者さんの場合，肝機能が落ち着いているからと安心し，大量飲酒

- タウリンは細胞膜の保護や，安定性を保つ働きがある．
- 正常ラットに運動負荷をかけると，下腿骨格筋のタウリン濃度が減少する．
- タウリンを投与しておくと疲労困憊したラットは，運動能の向上をもたらす．



	observation period (yr) 5<	5≤, <10	10≤, <15	15≤
	No. of patients (%)			
nonsmokers (n=246)	141 (57.3)	74 (30.1)	24 (9.8)	7 (2.8)
smoking index<400 (n=72)	37 (51.4)	28 (38.9)	7 (9.7)	0 (0)
smoking index≥400 (n=94)	65 (69.1)	22 (23.4)	6 (6.4)	1 (1.1)

図4　HCV 陽性慢性肝疾患患者における喫煙指数と累積肝細胞癌発癌率

や，毎日晩酌など常習飲酒を行うと肝炎が悪化する場合があるので十分注意を要する．HCV キャリアで週に 210～560 g のエタノール摂取で 2.33 倍の危険率で肝硬変が早まることが明らかとなっている．C 型慢性肝炎の患者さんの場合，飲酒量が増加するに従い肝癌の合併率が増加するとされている．われわれのデータでも，C 型肝硬変の方で，飲酒をしている時点で多変量多重解析を行うと，毎日 80 g 純エタノールの 5 年以上飲酒は，80 g 以下の飲酒者と比べ 3.27 倍の危険率で肝癌の発癌因子として有意な危険因子となる（図4）[16]．

このように HCV 関連肝疾患患者さんにおいては禁酒をさせるべきである．さらにインターフェロン治療中など効果の判定に苦慮する場合や，効果が悪くなる場合なども考えられ治療中は禁酒を勧めるべきである．

喫煙の問題●

喫煙と発癌の問題は各臓器でいわれている．肝癌発生に関しても疫学データがでてきている．わが国で喫煙者は非喫煙者の 1.5 倍の危険率で肝癌

❸ C 型慢性肝炎患者においては，大酒により肝硬変への進行が早くなる．
❹ C 型慢性肝炎患者においては，飲酒量の増加で肝癌の合併率が増加する．
❺連日の大酒は肝癌の発癌因子の一つになりうる．

表1 多変量多重解析による HCV 陽性慢性肝疾患患者の肝細胞癌発癌危険因子

factor	beta	standard error	p value	Odds ratio (95% CI)
intercept	1.4785	0.5183	0.0043	
sex	−1.4345	0.4320	0.0009	
male				4.20 (1.80〜9.78)
female				1.00
habitual drinking	−1.1846	0.4096	0.0038	
≧80g pure alcohol/day for 5 years or more				3.27 (1.46〜7.30)
< 80 g pure alcohol/day for 5 years				1.00
anti-HBV[a]	−0.6975	0.3507	0.0467	
positive				2.01 (1.01〜3.99)
negative				1.00
age (years)	−0.7227	0.3676	0.0493	
> 60				2.06 (1.00〜4.23)
≦60				1.00

[a] anti-HBs and/or anti-HBc

（文献18）より）

発癌がありうることが報告されている[17]．われわれの prospective データでも，C 型肝炎ウイルス陽性慢性肝疾患の方においては，Cox 比例ハザード解析を行うと，喫煙指数が 400 以上の患者さんは非喫煙者に比べ 2.46 倍の危険率で肝癌の発癌率は増加する（表 1）[18]．同じような成績が台湾，韓国，フランスなどでウイルス性肝炎患者さんにおいて出ている[19]．

たばこは百害あって一利なしであり，ウイルス性慢性肝疾患の患者さんは，発癌や肝硬変の進行などを考慮し，禁酒同様に禁煙も必要であろう．

薬物の使用に関する問題（健康食品含む）●

一般的に肝硬変の患者さんの場合は，肝内での薬物代謝が悪くなり副作用も起こりやすくなるので注意を要する．慢性肝炎の場合は障害の程度でさまざまである．

ただウイルス肝炎の場合注意をする必要のあることがある．それは，B 型肝炎患者さんにステロイドを使用する場合である．B 型肝炎ウイルス（HBV）のゲノムにはステロイド感受性部位があり，ステロイド投与によりウイルスの増殖を起こし急性増悪を起こすことがある．したがって，偶然の投与を避けるように指導しておく必要がある．最近では耳鼻科や内科で花粉症にステロイドを使用することがある．眼科などでも使用するので，これらの専門科を受診しステロイドを使う場合，肝臓専門医と連携をとれるように患者に指導することが望ましい．

肝硬変になっている患者さんの場合の非ステロイド系鎮痛解熱剤（NSAIDs）の使用が問題となることがある．肝硬変の場合腹水や浮腫があるとき，プロスタグランディン（PG）を利用し腎血管を代償的に拡張する方向へ体内は変化している．そこへ PG 合成阻害剤である NSAIDs を使用すると腎血管が収縮し肝腎症候群を起こすことがある

- C型慢性肝炎患者においては，過剰喫煙は肝癌の発癌率を増加させる．
- C型慢性肝炎患者においては，B型肝炎の感染の既往も発癌因子となる．
- ウイルス肝炎患者さんに対する薬物投与には注意が必要である．

ので注意を要する．また，同様な肝硬変の場合点滴にNaが多く含まれる輸液を使用すると浮腫などが悪化することがあるので注意する．また肝硬変の進行した患者さんの場合，門脈圧亢進胃粘膜障害が起こり消化管出血を起こしやすい状態となっている．そこにNSAIDsを使用するとNSAIDs潰瘍や急性胃粘膜障害(AGML)を起こすことがあるので使用には十分注意を要する．proton pomp阻害剤などを併用するなどが必要である．

水虫の内服薬は胆汁うっ滞を健常者でも起こす．慢性肝炎，肝硬変の患者さんへの使用は注意せねばならない．肝臓専門医と皮膚科医との連携が望まれる．

最後に健康食品の問題である．現在多くの健康食品をさまざまな患者さん方が使用されていることが多い．含有されている成分に反応する方がおられるので，使用には注意を要する．肝機能が悪化するウイルス肝炎の慢性肝疾患の患者さんには，健康食品の使用を丹念に聞き出すことも必要である．鉄分の含有が多いドリンク剤や健康食品を服用しているC型肝炎の患者さんもみられるので注意を要する[20]．

毛染めについては現在のところ肝機能を悪化させるとの報告はない．

日常生活からの感染予防対策●

1. 感染場所

一般に家庭内，保育所，学校，介護施設などの集団生活の場でHCV，HBVの感染が起こることはないとされている．ごく常識的な日常生活習慣を守っている限り，HBV, HCVの持続感染者であっても，集団生活の場で他人に肝炎ウイルス感染を起こすことはまずないといえる．ただ，ごくまれに保育所でのHBV感染の事例報告があるので，集団生活の場では注意を要す．

2. 予防法

1) B型，C型肝炎両者を通して，血液や分泌物がついたものは，むき出しにならぬようにしっかりくるみ廃棄するか，流水でよく洗い流す．

2) 外傷，皮膚炎，鼻血，生理時の血液などなるべく自分で対処し，手当や介助を受ける場合は，介助者が血液，分泌物で汚染しないよう注意する．

3) カミソリ，歯ブラシ，など日用品は個人専用とし他人に貸さないようにまた借りないようにする．

4) 乳幼児に，口移しで食べ物を与えないようにする．

5) トイレを使用した後は流水で手洗いする．

3. 疫学的検証

広島の地域保健対策協議会によると，HBV集団感染について，ある介護，福祉施設の入所者703名を4年間観察したところ，新規にB型肝炎に感染した人はいなかったという結果が出されている．この703名の中には，18名のHBVキャリアが特別な扱いを受けずに他の方々とともに入所していたことが明らかとなっている．

また，同時にC型肝炎についても検討をしており，新規のC型肝炎の発症はなかったとしている．この703名の中には，25名のHCV感染者が特定されないまま，入所していたことが判明している．

以上の事実より，上記に示すごとく，通常の注意をした上での日常生活においては，集団生活での肝炎感染はきわめてまれであるということとなる．

ただ，予防の項に示した注意をあくまで遵守することにより予防可能である．