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Research Article

A Pharmacokinetic Model of Intravenously Administered Hyaluronan in Sheep

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Hyaluronan (HA; hyaluronic acid) is produced in the interstitium and reaches the blood circulation through the lymph. It is rapidly eliminated by means of specific receptors on liver endothelium. The elimination characteristics of intravenously administered HA were studied in 10 conscious sheep at the normal plasma HA concentration by injection of a ^{3}H-labeled tracer and at a very high concentration by an i.v. infusion of unlabeled HA and simultaneous injection of a tracer dose of ^{3}H-labeled HA. At a normal plasma HA concentration (0.12 ± 0.05 μg/ml; range, 0.072–0.228 μg/ml), the apparent $T_{1/2}$ of ^{3}H-HA was 5.3 ± 1.1 min (range, 3.3–6.5 min). At higher plasma concentrations (range, 1.83–3.35 μg/ml), the apparent $T_{1/2}$ was considerably prolonged (range, 18.2–43.5 min). A one-compartment, nonlinear model was fitted to data obtained from the bolus-infusion study of unlabeled HA. The Michaelis–Menten constant, K_m, was 0.12 ± 0.04 μg/ml, indicating that a deviation from linear kinetics will occur when the normal plasma concentration is exceeded. The V_{max} was 0.062 ± 0.009 μg/ml/min. Three-dimensional surface plots showed that the plasma HA concentration and the total hepatic plasma flow influence the apparent metabolic clearance, extraction ratio, turnover, and $T_{1/2}$ of intravenously injected hyaluronan. There was a high correlation between $T_{1/2}$ as measured by the injected ^{3}H-HA and $T_{1/2}$ calculated from the model (r = 0.96).

KEY WORDS: hyaluronan (hyaluronic acid); metabolism; Michaelis–Menten kinetics; extraction ratio; modeling; turnover; sheep.

INTRODUCTION

Hyaluronan (HA; hyaluronic acid), a major interstitial component, is synthesized in the tissues and is carried by lymph to the general circulation (1,2). The mean serum and plasma concentrations in healthy young and middle-aged people is 20–40 μg/liter; the value increases with age (3). HA is rapidly cleared from the blood by receptor-mediated uptake in the endothelial cells of the liver sinusoids (4–6), with a normal half-life of 2 to 5 min in the rabbit and man (7,8). A small proportion has also been reported to be eliminated via the kidneys (9), and some is eliminated in lymph nodes before entering the blood stream (10). For reviews on the turnover of HA, see Refs. 1 and 11.

An increased serum concentration of HA has been observed in patients with liver cirrhosis (12,13), presumably due to a decreased capacity to eliminate the polysaccharide in the liver, and in chronic inflammatory diseases such as rheumatoid arthritis (14,15), scleroderma (16), and psoriasis (17), most likely as a result of an increased production of HA in the tissues. An elevated serum concentration of HA has also been reported in patients with sepsis (18). Increased physical activity, which increases the lymph drainage, also leads to a temporary increase in the serum HA level (15). Furthermore, the increased use of concentrated HA in clinical practice, e.g., in eye surgery, may lead to elevated blood concentrations.

The above-mentioned observations have suggested that measurement of the serum HA concentration could be developed as a diagnostic tool for patients with liver diseases and with connective tissue disorders. In the individual patient, however, our present knowledge does not allow a distinction between increased synthesis and decreased elimination, or recognition of both, as the mechanism for an increased serum HA concentration.

This study was carried out in order to investigate further the kinetics of plasma HA. Previous data from rabbits have suggested that the elimination mechanism of HA may be saturated at a high plasma HA concentration (7), and the present study therefore included determinations of the elimination kinetics at normal as well as elevated plasma concentrations of HA.

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MATERIALS AND METHODS

Animals

Ten Merino ewes, weighing between 20 and 38 kg, were used. All animals were deprived of food and water for 12 hr prior to surgery.

Preparation of the Animals

The day before experimentation, the animals were lightly anesthetized with thiopental and Halothane. Venous and arterial catheters for infusion and blood sampling were inserted in the right jugular vein and the right carotid artery. At the time of experimentation the animals were fully awake and stood quietly throughout the period of observation without any signs of distress. Their cages permitted some movement.

Experimental Material

Unlabeled HA. Two different batches of Healon (Pharmacia AB, Uppsala, Sweden, Lot Nos. MF 47892 and LE 46544, accepted for clinical use) were subjected to heat degradation by autoclaving at 120°C for 1 hr. This procedure is known to reduce the weight-average molecular weight (MW) of Healon from 3500 to approximately 500 kD. This was confirmed by calibrated gel chromatography as described previously (19).

^{3}H-Labeled HA. ^{3}H-HA was synthesized *in vitro* by stimulated human synovial cells as described earlier (7). The ^{3}H-labeled HA was subjected to heat degradation by autoclaving at 120°C for 1 hr, whereafter the MW was determined as described previously (19). Two different preparations were used: 85-3HAH (sp act, 0.65×10^6 dpm/μg HA; MW 550 kD) and 87-2HAH (sp act, 1.1×10^6 dpm/μg HA; MW 100 kD).

Experimental Procedure

The procedure is outlined in Fig. 1a. The experiment was conducted in two stages:

(I) estimation of the apparent half-life under normal conditions, i.e., at a normal plasma concentration of HA, using the ^{3}H-labeled HA as a tracer; and

(II) estimation of the kinetics at elevated plasma HA concentrations, i.e., during bolus injection + infusion of unlabeled HA and with a concomitant bolus tracer dose of ^{3}H-HA.

Stage I. A 20-μg i.v. tracer dose of ^{3}H-labeled HA was given and arterial blood samples were drawn at baseline and 2, 4, 6, 8, 10, 15, 20, and 30 min after the injection.

Stage II. Three to four hours after stage I, a 2-mg i.v. bolus dose of unlabeled HA was given, immediately fol-

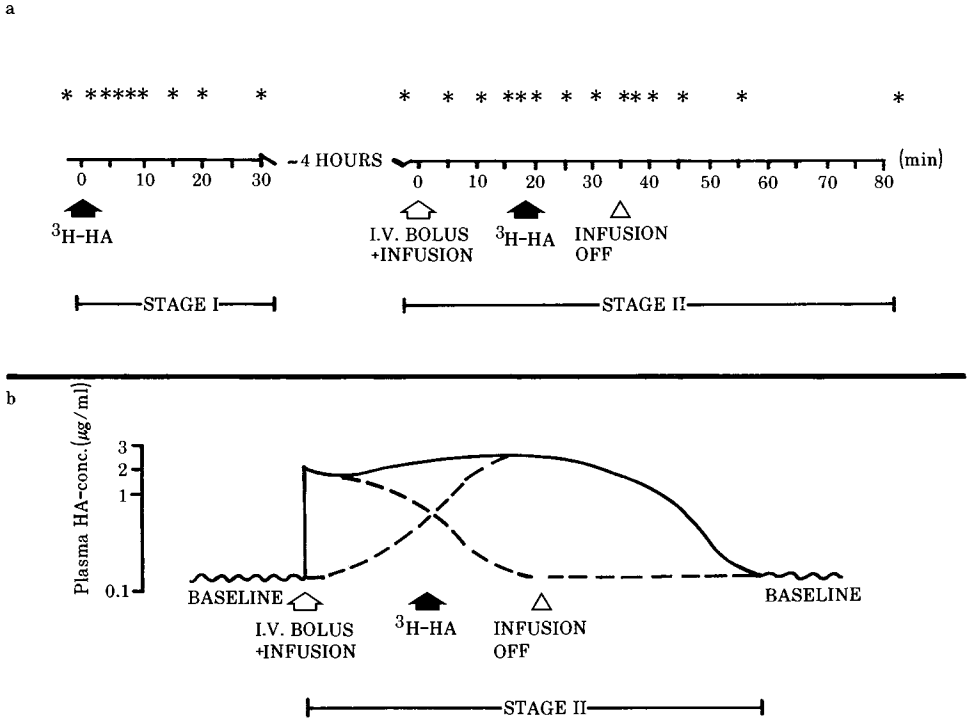

Fig. 1. (a) Experimental design. Sampling times in relation to times of injection of ^{3}H-labeled HA and infusion of unlabeled HA, during stages I and II. (*) Blood sampling; (●) injection of tracer dose of ^{3}H-HA; (○) injection of bolus dose of HA and start of continuous i.v. HA infusion; (△) end of HA infusion. (b) Schematic illustration of the design of stage II. (○) Injection of the HA bolus dose and start of the HA infusion; (△) end of HA infusion; (●) injection of ^{3}H-HA tracer. (– – – –) The hypothetical decline in the plasma HA concentration subsequent to injection of the HA bolus dose and the increase which would be attributable to HA infusion if each were given alone; (——) the desired HA concentration.

lowed by a 40-min i.v. infusion, at a rate of approximately 125 μg/min (2 ml/min) (Fig. 1b). The bolus dose and infusion rate were estimated a priori to give a steady-state plasma concentration of approximately 1–2 μg/ml.

Arterial blood samples were drawn at baseline and 5, 10, and 15 min after the HA bolus dose. Twenty micrograms of ^{3}H-labeled HA was then given and arterial blood samples were taken 2, 5, 10, 15, and 20 min later. The HA infusion was then stopped and blood samples were taken after another 1, 5, 10, 20, and 40 min in order to follow the washout kinetics of the unlabeled HA (Fig. 1).

Handling of Samples

The blood samples were collected in EDTA-treated vials and centrifuged at 3000 rpm for 15 min. The plasma was stored at −70°C until analyzed.

Determination of Hyaluronan

The concentrations of unlabeled HA in plasma and chromatographic eluates were measured by means of a specific radiometric assay (20) (Pharmacia AB, Uppsala, Sweden). The concentrations of ^{3}H-labeled HA in plasma samples and chromatographic eluates were measured by liquid scintillation after subtraction of appropriate backgrounds (7).

Pharmacokinetic Analysis

A one-compartment model with Michaelis–Menten elimination kinetics was fitted to all the concentration–time data, i.e., those obtained during i.v. bolus and infusion of unlabeled HA and after cessation of the infusion (stage II), using the PCNONLIN program (21,22). The iterative reweighted least-squares method was used (21,23). The relative residual vs predicted concentrations and the sum of weighted squared residuals were used as goodness-of-fit criteria. The apparent volume of distribution, V_d (ml), the maximum metabolic rate, V'_{max} (μg/ml/min) or V_{max} (μg/min), and the Michaelis–Menten constant, K_m (μg/ml), were estimated.

In order to illustrate better the effect of changes in the total plasma concentration of HA (C_p) and the total hepatic plasma flow (Q_H) on the kinetics of HA to the reader, the extraction ratio, apparent plasma clearance, apparent biological half-life, and turnover were determined from the model parameters in Eqs. (1)–(5) and are presented as three-dimensional surface plots of the respective variable vs continuous ranges of C_p and Q_H.

The well-stirred model of hepatic elimination (24) was chosen on the basis of the assumption that there is instantaneous mixing of hyaluronan in the afferent and efferent blood from the liver.

The apparent plasma clearance (Cl_{app}) was determined from the relationship between the Q_H and the intrinsic clearance (Cl_{int}) according to Eqs. (1) and (2) (24).

$$Cl_{app} = \frac{Q_H \cdot Cl_{int}}{Q_H + Cl_{int}} \quad (1)$$

$$Cl_{int} = \frac{V_{max}}{K_m + C_p} \quad (2)$$

The apparent biological half-life ($T_{1/2}$) was calculated from the relationship (24)

$$T_{1/2} = \frac{\ln 2 \cdot V_d}{Cl_{app}} \quad (3)$$

The hepatic extraction ratio (E) was calculated as (24)

$$E = \frac{Cl_{int}}{Q_H + Cl_{int}} \quad (4)$$

The turnover was calculated from the relationship (24)

$$\text{Turnover} = Cl_{app} \cdot C_p \quad (5)$$

In applying Eqs. (1)–(5) the following assumptions were made.

(I) There is no significant binding of HA to plasma or blood compounds such as proteins or blood cells (25; Fraser *et al.*, unpublished observations).

(II) The volume of distribution of intravenously injected HA corresponds to the plasma space and the major part of the HA is eliminated by the liver (8).

To estimate the normal daily turnover and the apparent biological half-life (Fig. 4), the total hepatic plasma flow was estimated, assuming that the hepatic blood flow was 37% of a cardiac output of 76 ml · kg bw^{-1} (26) and using the body weight (Bw) and hematocrit (Hct) of the individual sheep:

$$Q_H = 0.37 \cdot (76 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg bw}^{-1}) \cdot \text{Bw} \cdot \left(1 - \frac{\text{Hct}}{100}\right) \quad (6)$$

For these calculations it was also assumed that the baseline plasma concentration, calculated as the mean of the endogenous HA concentrations during stage I in the individual animals, was constant. The plasma concentration during the plateau level of stage II was also considered to be constant and was calculated as the mean of the measured cold HA concentrations during the period from the injection of the ^{3}H-labeled HA to the end of the infusion.

All values are presented as the mean ± SD unless otherwise stated.

RESULTS

Stage I

The normal endogenous HA plasma concentration was 0.12 ± 0.05 μg/ml (range, 0.07–0.23 μg/ml). The elimination of ^{3}H-labeled HA occurred with a $T_{1/2}$ of 5.3 ± 1.1 min (range, 3.3–6.5 min). The elimination curve obtained fitted well to a linear, one-compartment, kinetics model (Fig. 2).

Stage II

The mean plasma concentration of HA at the plateau level was 2.42 ± 0.48 μg/ml (range, 1.83–3.35 μg/ml). From the HA bolus + infusion data the mean V_d was calculated to be 54.6 ± 5.2 ml/kg bw (range, 49.0–64.5 ml/kg bw). The plasma volume (V_p) calculated from the weight and hematocrit, assuming a blood volume of 66.4 ml/kg bw (27), was found to be 47.4 ± 1.7 ml/kg bw (range, 42.8–48.5 ml/kg bw).

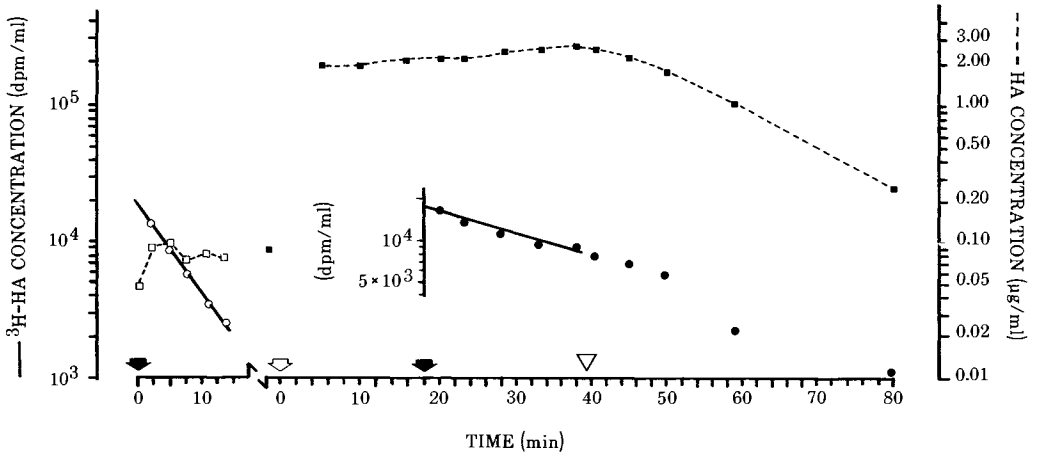

Fig. 2. Concentration of unlabeled plasma HA in one sheep before (□- - -□; stage I) and after ■- - -■; stage II) infusion of HA. The open arrowhead indicates the start and the open triangle the end of the HA infusion. Tracer doses of ^{3}H-labeled HA (85-3HAH) were injected at the start of the experiment (○——○) and as indicated by the filled arrowhead during the infusion experiments (●——●), and the radioactivity was followed as a function of time.

V'_{max} and K_m were 0.062 ± 0.009 μg/ml/min (range, 0.047–0.076 μg/ml/min) and 0.12 ± 0.04 μg/ml (range, 0.08–0.24 μg/ml), respectively (Table I). The maximal metabolic rate, V_{max}, was calculated to be 3.32 ± 0.30 μg/min/kg bw (range, 2.85–3.73 μg/min/kg bw).

Under the assumption that the well-stirred model could be used for prediction of the kinetics of HA, three-dimensional surface plots of E, Cl_{app}, $T_{1/2}$, and turnover versus C_p and Q_H were constructed and are shown in Figs. 3a–d, respectively. Figures 3a, b, and d show the nonlinear effect of changes in the plasma HA concentration and total hepatic blood flow on the extraction ratio, apparent clearance, and turnover of HA. Note the impact of a change in perfusion on, for example, the extraction ratio as the hepatic plasma flow approaches zero (Fig. 3a). The plot of half-life vs plasma concentration (Fig. 3c) shows a linear relationship; the higher the concentration, the longer the half-life.

The elimination of the ^{3}H-labeled HA at the plateau level occurred with a $T_{1/2}$ of 26.9 ± 7.0 min (range, 18.2–43.5 min). Again, the elimination curve fitted well to a linear, one-compartment kinetics model (Fig. 2).

The apparent biological half-lives estimated by injection of tritium-labeled HA at different plasma concentrations during stages I and II correlated well with the apparent biological half-lives calculated with the Michaelis–Menten model, for corresponding plasma concentrations, calculated as described under Materials and Methods and assuming normal total hepatic plasma flows in the individual sheep as defined in Eq. (6) (Fig. 4).

DISCUSSION

The normal plasma concentration of HA found in this study in sheep was distinctly higher, 0.12 ± 0.05 μg/ml, than has previously been reported for humans, 0.02–0.04 μg/ml, but similar to values previously found in sheep (3). The half-life noted at normal plasma concentrations in the sheep was also slightly higher (range, 3.3–6.5 min) than the values previously reported for humans (8).

From our data we estimated that the mean K_m was approximately 0.12 μg/ml, which means that in sheep the kinetics are already nonlinear at a normal baseline plasma con-

Table I. Body Weight (Bw), Hematocrit (Hct), Plasma Volume (V_p), Infusion Rate (R_{inf}), Apparent Volume of Distribution (V_d), Maximum Metabolic Rate (V'_{max}) and Michaelis–Menten Constant (K_m) in the Individual Sheep [SE = Standard Error as Calculated in the PCNONLIN Program (21,22)]

Sheep No.	Bw (kg)	Hct (%)	V_p (ml/kg bw)	R_{inf} (μg/min)	V_d ± SE (ml/kg bw)	V'_{max} ± SE (μg/ml/min)	K_m ± SE (μg/ml)
1	27.0	28	48.1	135	61.7 ± 4.3	0.056 ± 0.003	0.093 ± 0.009
2	27.5	29	47.1	140	50.0 ± 2.0	0.070 ± 0.002	0.085 ± 0.003
3	38.0	27	48.4	150	52.8 ± 4.5	0.066 ± 0.005	0.244 ± 0.025
4	28.5	27	48.5	125	64.5 ± 3.3	0.047 ± 0.002	0.093 ± 0.006
5	20.0	35	42.8	128	55.6 ± 4.8	0.065 ± 0.006	0.120 ± 0.020
6	24.0	30	46.8	102	49.0 ± 10.1	0.076 ± 0.019	0.083 ± 0.176
7	26.0	28	47.8	113	51.9 ± 3.3	0.055 ± 0.001	0.120 ± 0.106
8	20.0	27	48.4	106	57.6 ± 3.2	0.055 ± 0.003	0.122 ± 0.016
9	27.0	27	48.4	122	50.7 ± 2.4	0.057 ± 0.002	0.102 ± 0.009
10	27.0	26	47.3	138	52.1 ± 5.6	0.070 ± 0.005	0.138 ± 0.015
Mean ± SD	26.5 ± 4.8	28 ± 2	47.4 ± 1.7	126 ± 16	54.6 ± 5.2	0.062 ± 0.009	0.120 ± 0.045

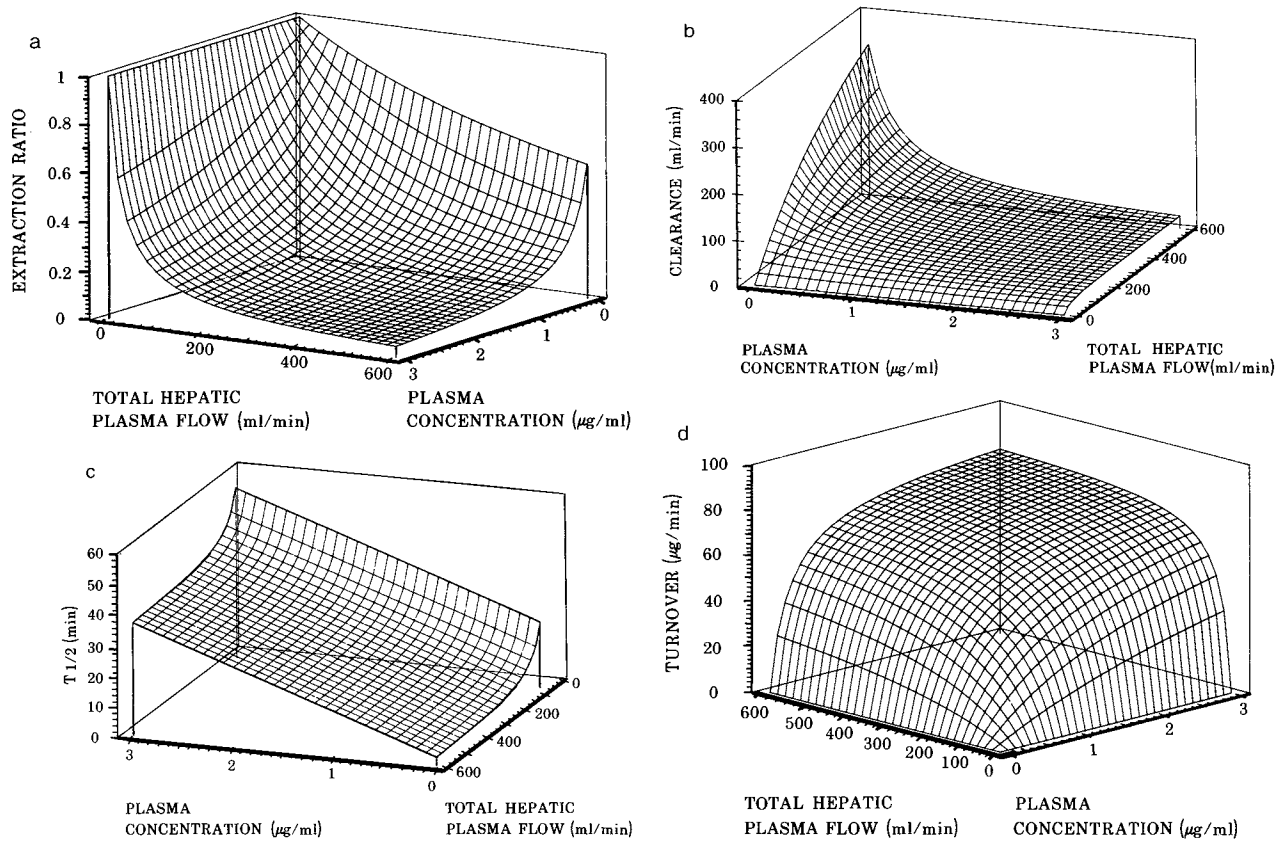

Fig. 3. The plasma concentration of HA and the total hepatic plasma flow plotted against the individual kinetic parameters as calculated by Eqs. (1), (3), (4), and (5) using the mean K_m and V_{max} obtained with the PCNONLIN program. (a) Extraction ratio vs HA plasma concentration and total hepatic plasma flow. (b) Apparent plasma clearance vs HA plasma concentration and total hepatic plasma flow. (c) Biological half-life ($T_{1/2}$) vs HA plasma concentration and total hepatic plasma flow. (d) Turnover vs HA plasma concentration and total hepatic plasma flow.

centration of HA. As the maximum metabolic rate was found to be 3.32 ± 0.30 μg/min/kg bw, the maximal total daily turnover of HA can be estimated to be 4.8 ± 0.4 mg/kg bw. Using the average baseline concentration of HA (stage I) and the total hepatic plasma flow calculated as in Eq. (6), the normal apparent clearance was calculated to be 215 ± 47 ml/min. The normal daily turnover of HA can be calculated to be approximately 1.4 mg/kg bw. This value is of the same order of magnitude as has been reported for man (8).

In a previous study we found that the weight-average molecular weight of the HA in lung lymph during sepsis or during elevation of the transvascular hydrostatic pressure was in the range of 20–100 kD (19). The MW in sheep lymph of different origins has previously been reported to be in the range of 160–2000 kD. The molecular polydispersity, however, is very large (28). In this study, all preparations of HA used had a MW of between 100 and 550 kD, thus comparable to that of HA in sheep lymph, but also to the MW of HA found in human serum, which is approximately 200 kD (28). No marked differences were found among the $T_{1/2}$ derived from the different HA preparations used in this study.

In earlier studies in man in which the half-life of HA was measured with a tracer dose of ^{3}H-labeled HA under normal and pathological conditions, i.e., at normal and elevated HA concentrations, a linear, one-compartment, pharmacokinetic model gave the best fit of the experimental data (8,12). This can be explained by the fact that Michaelis–Menten kinetics is impossible to detect within a narrow concentration range, i.e., when the "substrate" concentration, here the plasma concentration of HA, is virtually unchanged during the experiment.

A tracer dose will decline exponentially if the concentration of cold material is constant. The kinetic parameters, e.g., $T_{1/2}$, of the tracer dose will therefore reflect the corresponding parameters for the unlabeled compound at the actual steady-state concentration. In the present study we did not achieve a true steady state during stage II, since our initially estimated infusion rate of HA (125 μg/min) exceeded the V_{max} predicted from the model (90 μg/min), resulting in a continuous small increase in the cold HA concentration during the decline of the ^{3}H-HA. This small increase, however, did not seem to affect the exponential decline of the ^{3}H-HA to any great extent.

The close agreement between the half-lives estimated from the tritium-labeled HA injections and those calculated from the Michaelis–Menten model, using the individual means of the plasma concentrations obtained at baseline and at the plateau level (Fig. 4), appears to support the validity of this model, although it should still be regarded as preliminary.

In conclusion, the preliminary nonlinear model obtained in this study suggests that the kinetics of HA is more com-

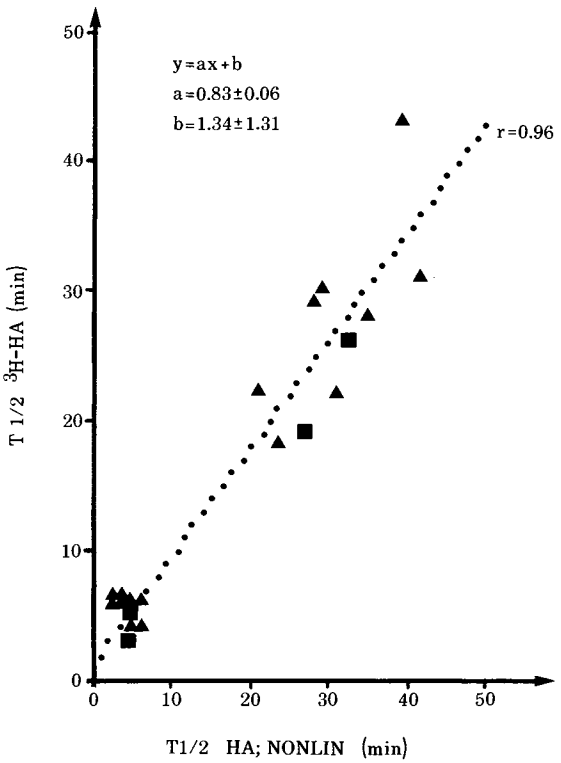

Fig. 4. Correlation between the biological half-life ($T_{1/2}$ ^{3}H-HA), estimated from the ^{3}H-labeled HA tracer dose at the individual plasma HA concentrations during each of stages I and II and the biological half-life ($T_{1/2}$ HA; NONLIN) derived from the model [Eq. (3)] for the corresponding plasma HA concentrations. (▲) $T_{1/2}$ ^{3}H-HA as measured by 85-3HAH; (■) $T_{1/2}$ ^{3}H-HA as measured by 87-2HAH.

plex than has previously been recognized, especially at moderate to high plasma concentrations of HA. It is evident from Figs. 3a and b that the extraction ratio and the apparent metabolic clearance of HA will decrease rapidly as the plasma concentration rises toward the levels observed in various pathological conditions in man (12–18). Figure 3 also shows that changes in the hepatic plasma flow may affect the kinetics, especially at low to moderate HA concentrations. These findings imply that a basic knowledge of the kinetics of this endogenous compound may be of great value when interpreting clinical data on HA concentrations.

Furthermore, in sheep the transition from linear to nonlinear elimination kinetics occurs already at normal plasma concentrations (i.e., K_m = 0.1 μg/ml). In human clinical practice serum HA values above 0.1 μg/ml are commonly seen in several conditions, e.g., in liver disorders (12,13), and determination of the Michaelis–Menten constants (V_{max} and K_m) may prove to be a valuable test of the liver function, possibly a specific test of the function of liver endothelial cells. Alternatively, it may also be possible to use the tritium-labeled HA for estimating an apparent biological $T_{1/2}$ in order to gain the same information. However, as there may be species differences in the elimination characteristics of HA, determinations of the pharmacokinetic parameters in man are required before these results can be generalized to human disease states.

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