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Research Paper

Influence of Dyslipidemia on Moxidectin Distribution in Plasma Lipoproteins and on its Pharmacokinetics

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Purpose. We studied the influence of dyslipemia on the distribution of moxidectin, a potent antiparasitic drug of the macrocyclic lactone (ML) family, in plasma lipoproteins and on its pharmacokinetic behaviour.
Materials and Methods. Plasma samples from normolipidemic or dyslipidemic subjects were spiked with moxidectin (20 ng/ml). Rabbits fed with standard (n = 5) or cholesterol-enriched diet (n = 5) were injected subcutaneously with moxidectin (300 μg/kg) and blood samples were collected over 32 days. Lipoproteins were separated from plasma samples by ultracentrifugation on density gradients. Moxidectin and lipids were measured in plasma and in lipoproteins and the pharmacokinetic parameters calculated.
Results. In normolipidemic subjects or rabbits, the drug bound preferentially to HDL. In hyperlipidemic samples, moxidectin shifted to the VLDL–LDL fraction. In addition, hyperlipidemic rabbits had a 2.8-fold higher area under the plasma concentration *versus* time curve (AUC) and a lower clearance and volume of distribution when compared with controls.
Conclusion. Dyslipidemia led to major changes in moxidectin plasma distribution and in drug disposition. Therefore, a high variability in moxidectin disposition might be expected in humans or animals liable to develop dyslipidemia, with a possible impact on the efficacy and safety of this class of drugs.

KEY WORDS: antiparasitic; hyperlipidemia; lipoproteins; macrocyclic lactones; moxidectin; pharmacokinetics.

INTRODUCTION

Milbemycins and avermectins are structurally related macrocyclic lactones (MLs) active against a broad spectrum of parasites (1). Moxidectin, from the milbemycin family (Fig. 1), is a semi-synthetic derivative of nemadectin, the fermentation product of *Streptomyces cyanogriseus*, and is widely used for the control of nematode and arthropod parasites in cattle, sheep and companion animals. Notably, moxidectin is effective against filaria at different stages of development. Prophylactic treatment with moxidectin prevents the development of the adult worm infection by *Onchocerca ochengi* in cattle (2) and of dirofiliarosis in dogs (3,4). Since 1980, ivermectin (Mectizan®), a macrocyclic lactone from the avermectin family, has been given orally (5) to several million humans to prevent the blindness that is a major consequence of onchocerciasis (6), a parasitic infection by *Onchocerca volvulus*. This disease represents a major public health problem in Africa, in Central and South America and in Yemen. Due to the need for efficient antiparasitic control in onchocerciasis, moxidectin is currently under development for use in humans (7).

Moxidectin is attractive, because being highly lipophilic, it has a longer residence time and a higher volume of distribution in the organism, which consequently gives a longer exposure of the parasite to the drug when compared with ivermectin (8). This results in a long-lasting efficacy, as the antiparasitic activity of the MLs depends on the presence of an effective concentration for a suitable length of time in the systemic circulation. Furthermore, moxidectin is extensively associated with plasma lipoproteins *in vivo* and *in vitro* in mammals including humans (9) and drug availability in the body is strongly influenced by its storage in fat tissue (10,11). In addition, a fatty meal or a lipid-based formulation substantially improves the overall exposure to moxidectin when it is administered orally to humans (7) or to rabbits (12), emphasizing the involvement of lipids in its intestinal absorption.

We have previously reported that in several pathophysiological conditions, such as fasting (13) or heavy parasite infection (14), there are changes in the disposition of MLs that are associated with lipid turnover. There are also differences between species (8). Since lipophilic compounds mainly distribute into plasma lipoproteins, changes in plasma

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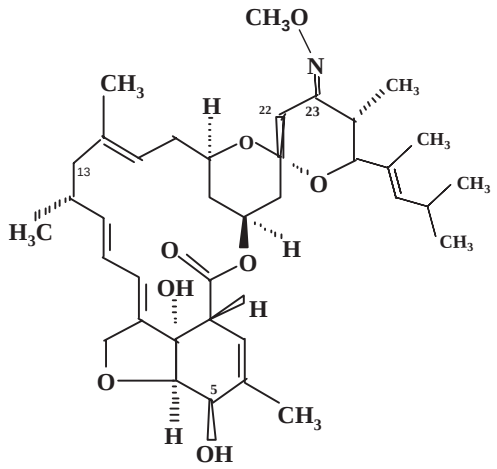

Fig. 1. Chemical structure of moxidectin.

lipid concentrations or turnover might not only affect the plasma distribution of these compounds, but may also have a bearing on their pharmacokinetics, pharmacodynamics and toxicity (15). This has been clearly demonstrated for cyclosporine A (16), halofantrine (17,18) and amphotericin B (19).

In this study, we first evaluated the influence of a lipid disorder on the distribution of moxidectin in plasma lipoproteins from patients with different types of lipidemic profiles. Then, using the cholesterol-fed rabbit as an experimental model, we have investigated the influence of hyperlipidemia on the association of moxidectin with plasma lipoproteins and the consequences on the pharmacokinetics.

MATERIALS AND METHODS

Patients and Plasma Drug Spiking

Normolipidemic plasma samples were obtained and pooled from three fasted volunteer donors. Dyslipidemic plasma samples from seven fasted patients, selected according to their lipoprotein plasma parameters, were individually processed (Lipoprotein Laboratory, INSERM U563, CHU Purpan, France). The procedure for blood withdrawal and plasma use followed the tenets of the Declaration of Helsinki promulgated in 1964 and was approved by the local human experimental committee with the informed consent of the participants. Drug-free plasma samples (1.1–1.5 ml) were spiked with moxidectin solutions in acetonitrile and incubated for 1 h at 37°C under gentle shaking. The final concentration of moxidectin was 20 ng/ml in 0.2% final acetonitrile.

Animal Model and Dietary Protocol

A total of ten male New Zealand white rabbits (INRA, SELAP, Auzeville, France) weighing 2.0–2.5 kg were used. The rabbits were housed in standard rabbit cages at a room temperature of 20°C and a 12-h light:12-h dark cycle, with free access to water. During an acclimatization period of 1 week, all rabbits were given 120 g/day of a standard commercial rabbit diet (Lapin Regal, France). The diet content was (as percent of wet weight): 15% protein, 15% cellulose, 2.5% fat, 11.5% minerals. Rabbits were randomly divided into two experimental groups according to the diet to be administered (40 g/kg day). The control group (n = 5) received the standard diet. The dyslipidemic rabbits (cholesterol-fed, n = 5) received the standard diet supplemented with 2.5% (w/v) coconut oil and 1% (w/v) cholesterol for 21 days prior to the experiments and for 15 days thereafter. All procedures adhered to the "Principles of Laboratory Animal Care" (NIH publication no. 85-23, 1985).

Study Design

Cholesterol-fed and control rabbits were given a single subcutaneous dose of moxidectin in the right flank as a 1% Cydectine® injection for cattle (Fort-Dodge International, France) at the recommended dose of 300 μg/kg body weight.

Blood samples were collected from the marginal ear vein in lithium–heparin tubes at 21 and 15 days prior to the injection in order to verify the lipidemia, and over 32 days after drug administration at the following time intervals: 0 (pre-treatment), 1.0, 2.0, 4.0, 8.0, 12, 24, 36 h and then days 2, 3, 4, 5, 6, 8, 10, 15, 20, 25 and 32. An additional blood sampling was performed at 5 h post-treatment on three rabbits. Blood sampling for lipid analysis were performed after overnight fasting. Blood samples were centrifuged at 1,500 × g for 15 min and the plasma stored at −20°C until analysis.

Plasma Lipoprotein Separation

Lipoproteins were isolated from normolipidemic or dyslipidemic drug spiked human plasma or rabbit plasma collected 5 h after moxidectin administration, on the basis of their hydrated density with a single-step procedure using ultracentrifugation on a potassium bromide (KBr) gradient as previously described by Terpstra *et al.* (20). In brief, plasma samples (1–2 ml) were adjusted to a density of 1.25 g/ml by adding KBr (770 mg) and sucrose (50 mg) and gently mixed. A sample of buffered saline spiked with moxidectin was run in parallel on a KBr gradient in order to verify that the drug did not float at any density corresponding to lipoprotein. Samples were carefully overlayered with solutions of decreasing density as follows: 2 ml of 1.225 g/ml density solution (315.54 and 11.42 mg/ml KBr and NaCl); 4 ml of 1.10 g/ml density solution (133.48 and 11.42 mg/ml KBr and NaCl); 4 ml of distilled water that was the final and upper layer. Ultracentrifugation was performed in a Kontron T1170 ultracentrifuge (Kontron, Italy) using a SW-41Ti swinging bucket rotor at 39,000 × g at 10°C for 22 h. Twenty-four fractions of 0.5 ml were collected from the top to the bottom of the tubes and the KBr density and cholesterol were measured in each fraction. Salt density was measured in standard KBr solutions, plasma samples or lipoprotein fractions with a refractometer (Sopelem, France). Based on the cholesterol content and density, the fractions were then pooled into four main fractions corresponding to the three different classes of plasma lipoproteins: very low (VLDL), low (LDL) and high (HDL) density lipoproteins and a lipoprotein-deficient fraction (LPDF). The fractions collected were stored at −20°C until further analysis.

Lipid Parameters Analysis

Total cholesterol and triglycerides were determined in human plasma samples with enzymatic reagents in an automated analyser (Roche Diagnostics-Hitachi, Meylan, France). HDL-cholesterol was assayed likewise, by a direct method, using polyanions/dextran as a masking reagent. LDL-cholesterol was calculated according to the Friedwald's formula, or by direct measurement on KBr density gradients after ultracentrifugation, for patients with major dyslipidemia (patients 5 and 6). Apoproteins A-I and B were measured by immunoturbidimetry, using specific antisera (Roche Diagnostics).

In rabbit, triglycerides were measured in plasma using a commercial enzyme-based colorimetric assay (Triglycerides Reagent, ThermoTrace, Australia). Total cholesterol was measured in the plasma and in the lipoprotein fractions by an enzymatic method (21) using a cholesterol reagent kit (Reactif Cholesterol Infinity™; Sigma Diagnostics, St. Louis, MO, USA).

Moxidectin Analysis

The plasma and lipoprotein fraction samples were analyzed for moxidectin by HPLC after automated solid-phase extraction and with fluorescence detection, using a method adapted from Alvinerie *et al.* (22). Briefly, 1 ml of acetonitrile was added to 1 ml of plasma or lipoprotein fractions, mixed and centrifuged at 2,000 × g for 2 min. The supernatants were transferred into tubes which were placed in the appropriate rack of a Benchmate II apparatus (Zymarck, Hopkinton, MA, USA). The eluates were evaporated to dryness and the residues dissolved in 100 µl of a *N*-methylimidazole (Aldrich, Milwaukee, USA) solution in acetonitrile (50%, v/v) and the derivatization was initiated with 150 µl of a trifluoroacetic anhydride (Aldrich) solution in acetonitrile (33%, v/v). An aliquot (100 µl) was injected into the HPLC system with fluorescence detection (Excitation 355 nm Emission 465 nm model FP-920; Jasco, Tokyo, Japan). The separation was carried out on a stainless steel analytical column (150 mm long, 4.6 mm i.d., 5 µm, Supelcosil LC18; Supelco, Bellefonte, PA, USA). The mobile phase of acetic acid (0.2% in water)/methanol/acetonitrile (4/40/56, v/v/v) was pumped at a flow rate of 1.5 ml/min. The analytical procedure for moxidectin was validated for lipoprotein fractions (9), for triglyceride-rich lymph (23) and for dyslipidemic plasma samples. Similar validation parameters were obtained for all these samples with a quantification limit of 0.05 ng/ml, linearity for concentrations ranging from 0.05 to 50 ng/ml, an extraction efficiency of 90% and a coefficient of variation of 2.5%. In order to fit within the linear concentration range, samples with high drug concentrations were diluted prior to assay.

Pharmacokinetic and Statistical Analysis

Data were analyzed using a compartmental approach with version 4.2 of the Kinetica™ computer program (InnaPhase, Philadelphia, USA). The resulting bi-exponential equation was fitted to the plasma concentration *versus* time data using a program for non-linear progression analysis adapted from Multi (24) based on Akaike's information criterion (25). A bi-exponential equation resulted in the best-fit concentration–time curves and was used to describe the plasma availability kinetics of moxidectin (mono-compartmental model). The terminal elimination half-time ($T_{1/2\beta}$) and absorption half-time ($T_{1/2ka}$) were calculated as ln2 β and ln2 ka, respectively. The areas under the plasma concentration–time curves from time zero to the last time with a measurable concentration (AUC_{0-last}) were calculated using the linear trapezoidal rule. The mean residence time (MRT) was calculated using the linear trapezoidal rule without extrapolation to infinity, using the formula:

$$MRT = AUMC/AUC$$

where AUMC is the area under the momentum curve and AUC the area under the plasma concentration *versus* time curves, as previously defined. The peak concentration (C_{max}) and time of the peak concentration (t_{max}) were read from the plotted concentration *versus* time curve plotted for each animal. The plasma clearance (Cl/*F*) was calculated from the ratio of the administered dose divided by the AUC. The apparent volume of distribution volume (V_d/F) was the product of Cl/*F* and MRT. The pharmacokinetic parameters are given as means ± standard deviation.

Statistical comparisons of the means were performed using a two-tailed Student test for paired or unpaired data according to the experimental design. $p < 0.05$ was considered as statistically significant. Pearson correlation coefficients were evaluated using a two-sided Student test ($n - 2$ degrees of freedom). The correlation was considered significant when $p < 0.05$.

RESULTS

Influence of Dyslipidemia on the Distribution of Moxidectin in Human Plasma Components

Seven patients were selected according to their lipidemia and were compared with plasma lipid profiles obtained from healthy volunteers as normolipidemic reference (Table I). These plasma samples were spiked *in vitro* with moxidectin at 20 ng/ml which is an average concentration measured in plasma after therapeutic treatment. Ultracentrifugation of the plasma samples on a KBr density gradient enabled the plasma cholesterol distribution to be determined (Fig. 2). Fractions 1–4, with a density less than 1.006 g/ml contained the VLDL, fractions 5–10, in the density range of 1.006–1.063 g/ml contained the LDL and, finally, fractions 11–17, with the density between 1.063 and 1.21 g/ml, corresponded to the HDL. The fractions with a density above 1.21 g/ml density were the lipoprotein deficient fraction (LPDF). Figure 2 and Table I show the plasma parameters and the plasma cholesterol distribution in normolipidemic subjects and in typical dyslipidemic profiles: hypercholesterolemia (patient 1), hypertriglyceridemia with normal cholesterol (patients 2 and 3), mixed hyperlipidemia with increases in both cholesterol and triglycerides (patients 5 and 6) and hypocholesterolemia (patient 7).

In normolipidemic plasma samples, with 60% of the circulating cholesterol in LDL, 35% in HDL and the remaining in VLDL, moxidectin was mainly bound to the HDL (68%), 22% was associated with the LDL fraction, only 4.8% with

VLDL and 5.2% with the lipoprotein deficient fraction (LPDF).

The dyslipidemic plasma 1 was characterized by an increase in total and LDL-cholesterol concentrations, the latter being two-fold higher than in normolipidemic plasma samples, but normal triglyceride and HDL-cholesterol (Table I). In this sample, we observed a reduction in the percentage of moxidectin association with HDL (51%) in favour of a higher association with LDL (38%) and VLDL (7%), when compared with normolipidemic plasma (Fig. 3). Patients 2, 3, and 4 were all characterized by moderate hypertriglyceridemia, normal total plasma cholesterol but low HDL cholesterol. In these cases moxidectin accumulated more in LDL (36–42%) and VLDL (15–28%) and to a lesser extent in HDL (31–37%).

Patients 5 and 6 were selected for mixed hyperlipidemia, with cholesterol and triglyceride levels two and ten-fold above normolipidemic values and low HDL levels. Patient 5 presented a massive accumulation of both VLDL and LDL while in patient 6, hyperlipidemia was more associated with an accumulation of triglyceride-rich lipoproteins of density <1.006 g/ml. In both patients, the major part of total plasma moxidectin was associated with LDL (45%) and VLDL (35%) while only 15% was in the HDL fraction.

In patient 7, with a clear hypolipoproteinemia, low triglyceride and apoA1 and B concentrations, the majority of the drug was bound to HDL (55%) and 20% with LDL.

A significant correlation existed between the cholesterol concentration in the HDL and the percentage of moxidectin in this fraction when the hypocholesterolemic patient (7°N) was excluded ($r = 0.83$, $n = 7$). In addition, when considering all patients, triglyceridemia significantly correlated with the percentage of moxidectin in VLDL ($r = 0.86$, $n = 8$). Similarly, the cholesterol concentration in the VLDL–LDL fraction was predictive of the percentage of moxidectin associated to these two fractions ($r = 0.79$, $n = 8$) and was inversely correlated with the percentage of moxidectin in HDL ($r = 0.71$, $n = 8$).

Influence of Hyperlipidemia on the Distribution of Moxidectin in Rabbit Plasma Components

Rabbits were fed a cholesterol-enriched diet for 20 days prior to drug administration and during the following 10 days. The mean weight of the cholesterol-fed rabbits was not significantly different from that of the standard diet-fed rabbits prior to drug administration (2.6 ± 0.1 *versus* 2.7 ± 0.1 kg) and at the end of experiment (3.2 ± 0.1 *versus* 3.5 ± 0.3 kg).

On the seventh day after the beginning of the cholesterol-rich diet, hypercholesterolemia started and was maximal on the 20th day of the diet, corresponding to the time of drug administration (Table II). Cholesterol remained at high concentrations throughout the experiment and at the time of drug administration the triglyceridemia was 0.9 ± 0.3 mM for the control rabbits and 3.7 ± 0.5 mM for the hyperlipidemic ones. Plasma fractionation was performed 5 h after moxidectin administration. In the control rabbits, most of the plasma cholesterol was in the HDL fraction (57%) while only 40% was in LDL and 6% in VLDL. In dyslipidemic rabbits, the LDL and VLDL fractions contained 75 and 19%, respectively, of the total plasma cholesterol with only 6% being in the HDL (Table II).

The distribution of moxidectin in the plasma was determined at 5 h following drug administration, which was the time closest to the maximum moxidectin concentration. In control rabbits, plasma moxidectin was essentially recovered in lipoproteins (92.8%) with the majority being in association with HDL (84.6%), only 10% in LDL and a minimal amount in the VLDL (2%). Following its administration to hyperlipidemic rabbits, the percentage of moxidectin associated with lipoproteins was significantly higher (99.1%, $p < 0.001$). The bulk of the drug was recovered in the LDL and VLDL fractions (60.9 and 15.0%, respectively) with a lower proportion in HDL (22.2%) and in the LPDF fraction (0.9%) when compared with the controls (Table II).

Pharmacokinetics of Moxidectin in Dyslipidemic Rabbits

Moxidectin was measured in plasma over 32 days after a single subcutaneous administration at 300 μg/kg. The logarithmic plots of the mean plasma moxidectin concentrations *versus* time are presented in Fig. 4. During the entire experiment, the plasma moxidectin concentrations were higher in cholesterol-fed rabbits than in the controls. While the moxidectin T_{max} and MRT were equivalent in the two groups, the maximum concentration (C_{max}) and the areas

Table I. Lipid and Apolipoprotein Concentrations in Human Plasma

	Total Cholesterol (mmol/l)	Total Triglyceride (mmol/l)	Lipoprotein Cholesterol			Apo A1 (g/l)	Apo B (g/l)
			VLDL (mmol/l)	LDL (mmol/l)	HDL (mmol/l)		
Normal range values	3.9 – 6.2	0.6 – 1.7	0.25 – 0.78	2.8 – 4.2	1.0 – 2.0	1.1–2.1	0.5–1.35
Normolipidemic ($n = 3$)	5.1 ± 0.4	1.3 ± 0.3	0.6 ± 0.1	2.6 ± 0.2	1.9 ± 0.4	1.9	0.8
Patient 1	7.6	1.9	0.9	5.4	1.3	*nd*	*nd*
Patient 2	6.0	3.0	1.4	3.9	0.7	1.0	1.3
Patient 3	5.0	2.0	0.9	3.4	0.6	0.8	*nd*
Patient 4	4.9	2.9	1.3	2.7	0.9	*nd*	*nd*
Patient 5	7.3	9.4	2.4	4.3	0.6	*nd*	*nd*
Patient 6	11.3	12.3	6.8	3.3	0.9	0.9	2.3
Patient 7	2.7	1.4	0.6	1.6	0.5	0.7	0.4

nd Not determined.

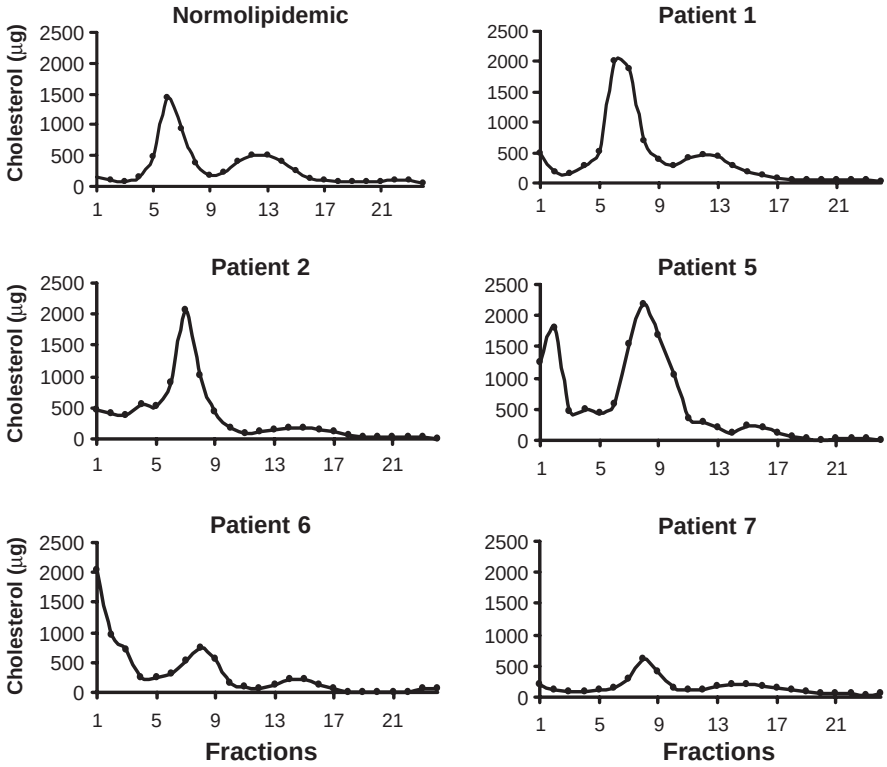

Fig. 2. Cholesterol profile in human plasma lipoproteins. Lipoproteins from normolipidemic or dyslipidemic human plasma samples were separated on a potassium bromide (*KBr*) density gradient by ultracentrifugation. Based on the cholesterol analysis four major fractions were identified: very low (*VLDL*), low (*LDL*), high (*HDL*) density lipoprotein and lipoprotein-deficient fraction (*LPDF*).

under the plasma concentration versus time curves (AUC) were both increased by 2.5 and 2.8-fold, respectively, in cholesterol-fed rabbits as compared with controls (Table III). The apparent clearance (Cl/*F*) and volume of distribution (V_d/F) were both significantly decreased in cholesterol-fed rabbits compared with controls (Table III).

DISCUSSION

Since lipophilic drugs distribute predominantly in plasma lipoproteins, changes in plasma lipid metabolism could affect their pharmacokinetics and pharmacodynamics (15). The highly lipophilic moxidectin is extensively bound to

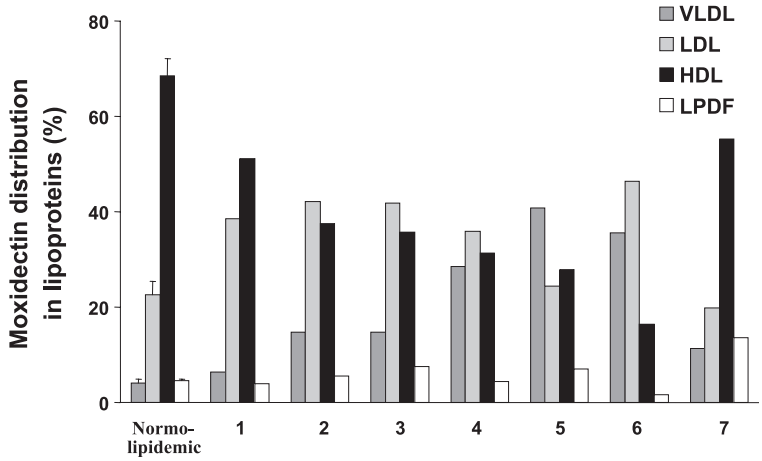

Fig. 3. Distribution of moxidectin in human plasma lipoproteins. Plasma from normolipidemic volunteers ($n = 3$) or from seven patients with different type of dyslipidemia were spiked with moxidectin (20 ng/ml) and moxidectin was measured in the lipoproteins by HPLC.

plasma lipoproteins and the distribution between fractions depends on the lipoprotein profile, characteristic of different species (9). We anticipated that in the pathology associated with dyslipidemia, the distribution of moxidectin in plasma and the pharmacokinetic behaviour of the drug would be modified.

In order to determine the influence of the lipid plasma profile on the distribution of moxidectin among plasma components, we selected several plasma samples from dyslipidemic patients. In normolipidemic plasma moxidectin was preferentially associated with HDL, while in hypercholesterolemia or hypertriglyceridemia, a shift of the drug towards the VLDL and LDL fractions was observed which was accentuated in major hyperlipidemia.

The increased association of the drug with the VLDL and LDL fractions is certainly due either to an increase in the particle number, or in the lipid mass of the VLDL or the LDL, occurring during hyperlipidemia. This is indicated by the correlation between the cholesterol concentration in VLDL–LDL and the percentage of moxidectin in both fractions. Similarly, an increase in plasma triglyceride, which is the consequence of the accumulation of triglyceride-rich particles, correlated with the increase of moxidectin associated with the VLDL fraction. A change in the drug affinity for HDL due to a change in the particle composition cannot be excluded. Alterations of the plasma distribution have been associated with changes in the lipoprotein profile for several lipophilic drugs such as cyclosporine A (26), halofantrine (18) and amphotericin B (19). The triglyceride enrichment of HDL leading to a decrease in affinity of the drug for HDL has also been suggested (26).

It is interesting to note that in hypocholesterolemic plasma with very low amounts of HDL, the moxidectin distribution was similar to that in normolipidemic plasma with a preferential association to HDL. This observation shows that at the therapeutic concentration used, the drug did not saturate the HDL, although their levels were very low in hypocholesterolemic plasma samples. This excludes the possibility that the low amounts of HDL observed in hyperlipidemia account for the shift of moxidectin to LDL.

These results clearly show that major changes in the distribution of moxidectin in plasma occurred during hyperlipidemia. The shift of moxidectin in favour of LDL in hyperlipidemia is certainly associated to a different distribution and disposition of the drug in the organism.

In order to study the influence of alterations in plasma lipids on the systemic disposition of moxidectin, we have used New Zealand rabbits fed with a high cholesterol diet. Rabbits are the appropriate experimental animals to use when determining lipoprotein distribution because the behaviour and structure of the lipoproteins and the function of cholesteryl ester transfer protein (CETP) are close to those in humans (27). In addition, rabbits respond to high cholesterol feeding by developing hyperlipidemia without any alteration in kidney and liver function or haematological profile (28,29). As expected, in our experimental conditions, feeding a high cholesterol diet to rabbits induced hypertriglyceridemia and hypercholesterolemia associated with profound changes in the lipoprotein profile. While the HDL was the major cholesterol carrier in control animals, the LDL and VLDL fractions increased

Table II. Cholesterol and Moxidectin in Rabbit Plasma and Lipoproteins

Experimental Groups	Total Plasma Cholesterol (mmol/l)		Percent of Cholesterol and Moxidectin in Plasma Fractions (5 h after drug administration)							
			VLDL		LDL		HDL		LPDF	
	Before Experiment	21 days with Experimental Diet	Cholesterol	Moxidectin	Cholesterol	Moxidectin	Cholesterol	Moxidectin	Cholesterol	Moxidectin
Control ($n = 5$)	1.6 ± 0.5	1.3 ± 0.4	5.8 ± 1.2	1.0 ± 0.5	29.9 ± 4.2	8.2 ± 2.7	57.1 ± 4.6	84.2 ± 0.9	7.2 ± 0.8	6.6 ± 2.4
Cholesterol fed ($n = 5$)	1.8 ± 0.7	33.5 ± 3.6*	19.1 ± 0.9*	15.4 ± 3.5*	74.3 ± 1.3*	61.1 ± 9.4*	5.7 ± 0.9*	22.2 ± 6.1*	0.9 ± 0.4*	1.3 ± 0.3*

Cholesterol and moxidectin was measured in plasma and in lipoproteins of control and dyslipidemic rabbits 21 days before and 5 h after moxidectin administration (300 μg/kg). Data are expressed as percentage of the total plasma cholesterol or moxidectin concentrations. Results are mean ± standard deviation of five animals for total plasma and three animals for lipoprotein analysis.

VLDL Very low density lipoproteins, *LDL* low density lipoproteins, *HDL* high density lipoproteins, *LPDF* lipoprotein-deficient fraction.

*$p < 0.001$ when compared with the control group or with the same group before experiment.

in the dyslipidemic rabbits. In parallel, while moxidectin was mainly bound to HDL in normolipidemic rabbits, as in other species, when the drug was administered to dyslipidemic rabbits, a greater percentage of moxidectin was recovered in the VLDL and LDL fractions. These data are in complete agreement with those obtained in pat0ients with major hyperlipidemia.

In addition, there were major changes in the systemic disposition of moxidectin in the presence of hyperlipidemia. The areas under the plasma moxidectin concentration *versus* time curves (AUC_{0-last}) and C_{max} were increased 2.5-fold in dyslipidemic rabbits compared with their normolipidemic counterparts. These results reflect an increase in the overall systemic moxidectin in response to a lower apparent clearance (Cl/F) and volume of distribution (V_d/F) of the drug in dyslipidemic rabbits.

We suggest that with hyperlipidemia, the large increase in the LDL and VLDL fractions, together with their preferred affinity for the drug, may certainly be a determining factor in the body distribution of the moxidectin. LDL and VLDL become important mediators that contribute to trapping the drug in plasma, resulting in a lower distribution volume in dyslipidemic rabbits. The biochemical events induced in the plasma by a cholesterol rich-diet are complex and not fully understood. Among them, the chemico-structural changes due to the cholesterol enrichment of LDL or VLDL particles may contribute to a higher affinity of moxidectin for these particles. Also, CETP which is involved in the remodelling of LDL and HDL particles, has been shown to mediate amphotericin B or halofantrine transfer to the LDL or VLDL fractions (15,30) while it rather mediates cyclosporin A transfer to HDL (31). Because plasma CETP activity increases in hypercholesterolemia (32) we assume that this enzyme might also be involved in moxidectin exchange between lipoproteins and may contribute to the shift of moxidectin into the hyperlipidemic LDL fraction in rabbit as well as in patients. Further studies are required to clarify this view.

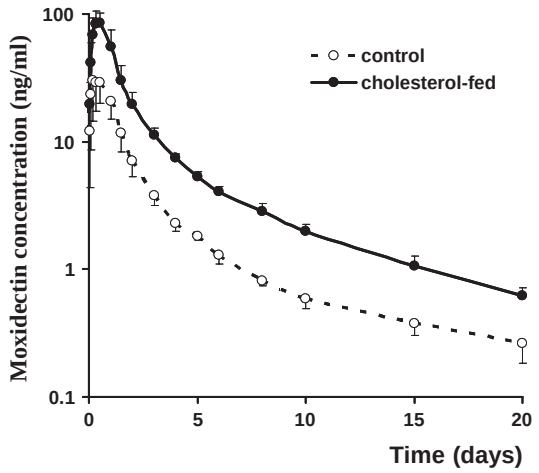

Fig. 4. Plasma concentration–time curve of control and hyperlipidemic rabbits. Moxidectin was measured in the plasma of control and hyperlipidemic rabbits at regular time intervals over 32 days after drug subcutaneous administration. Results are mean ± standard deviation of five animals.

Table III. Pharmacokinetic Parameters of Moxidectin in Control and Dyslipidemic Rabbits

Parameter	Control (n = 5)	Cholesterol-fed (n = 5)
$T_{1/2ka}$ (day)	0.10 ± 0.07	0.11 ± 0.04
$T_{1/2\beta}$ (day)	7.9 ± 3.7	6.41 ± 1.57
AUC_{0-last} (ng·day/ml)	59.1 ± 6.7	165.9 ± 26.1**
C_{max} (ng/ml)	33.1 ± 12.4	82.1 ± 18.1**
T_{max} (day)	0.32 ± 0.15	0.37 ± 0.09
Cl/F (l/day/kg)	5.13 ± 0.55	1.85 ± 0.33**
V_d/F (l/kg)	60.6 ± 33.7	16.9 ± 4.1*
MRT (day)	3.26 ± 1.07	3.45 ± 0.58

Moxidectin concentration was measured in rabbit plasma over 32 days after subcutaneous administration (300 μg/kg).
AUC_{0-last} Partial area under the plasma–concentration curve, C_{max} observed peak plasma concentration, T_{max} time to reach C_{max}, $T_{1/2ka}$ half-life of absorption, $T_{1/2\beta}$ half-life of elimination, MRT mean residence time, Cl/F subcutaneous clearance, V_d/F subcutaneous apparent volume of distribution. Values are mean ± standard deviation of five animals.
*$p < 0.05$ and **$p < 0.01$ when compared with the control group.

Major differences in moxidectin pharmacokinetics are observed between species (8) and given our results, we propose that these differences may be related to the specificity of the lipoprotein profile, characterizing species such as ruminants or rodents as "HDL-mammals" or humans as "LDL-mammals" (33). Indeed, in humans, the amount of moxidectin associated to VLDL–LDL was higher when compared with other species (9). In parallel, after oral administration of moxidectin, a high AUC (126 μg/day ml) and a long half-life (34 days) have been reported in humans (7), when compared with other species with lower moxidectin half-lives, for example, of 7, 12 and 21 days in rabbits, goats and sheep, respectively (8). We suggest that the LDL involved in the delivery of cholesterol to extrahepatic tissues may contribute to maintain higher moxidectin levels for a longer period of time in the body.

It is known that the pharmacokinetics and toxic effects of a number of drugs vary when administered to patients, compared with healthy controls. Previous studies in rabbits have reported that an increase in LDL-cholesterol generates a higher percentage of amphotericin B recovered in the LDL and VLDL fractions and modifies the drug disposition and its renal toxicity (19). Similarly, for cyclosporine A, the dose that is deemed non-toxic in healthy animals and humans is ineffective and toxic when administered to diseased patients (34,35). For halofantrine, postprandial hypertriglyceridemia is associated with a decrease in drug clearance (18) and lower efficacy *in vitro* (17). In the case of moxidectin, neurotoxic signs have been previously reported in humans with the experimental administration of high doses of moxidectin (7). An unpredictable increase in the drug concentration together with a preferential association of the drug to LDL when administered to dyslipidemic patients or animals, may lead to a modified drug distribution to tissues and subsequent change in the therapeutic index of the drug.

Parasite survival requires exogenous cholesterol because the parasites are unable to complete cholesterol synthesis (36,37). For worms, cholesterol is supplied by the host tissues,

or the mucus or blood on which they feed. Worms thus have the ability to absorb, transport and distribute cholesterol throughout their organism and into proteins similar to mammal apolipoproteins and LDL-receptors that have been identified in the nematode *Caenorhabditis elegans* (38). This suggests that the association of moxidectin with LDL would favour the incorporation of the drug into parasitic worms. However, whether or not the increase in LDL-cholesterol in hypercholesterolemia facilitates the targeting of moxidectin to parasites, remains to be determined.

In conclusion, we have shown that major changes in the distribution of moxidectin in lipoproteins occurred in hyperlipidemia, with a subsequent alteration in the pharmacokinetics. Our data reveal that high variability in moxidectin disposition might be expected in humans or animals liable to develop dyslipidemia with a possible impact on the efficacy and safety of this class of drugs.

Ivermectin is extensively used in emerging countries for the treatment of onchocerciasis and lymphatic filiaris in humans while moxidectin is currently under development. In Western countries, these compounds are extensively used in domestic animals and livestock, and due to the need for efficient antiparasitic control in humans, they will certainly be marketed for human therapy in the near future. Given that diseases associated with major dyslipidemia such as atherosclerosis, diabetes and obesity are common in humans and appear more and more frequently in dogs (39,40), this study shows that cholesterol and triglyceride plasma levels will have to be taken into account when using moxidectin and other MLs in humans and animals.

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