

# Mixed-Lipid Storage Disorder Induced in Macrophages and Fibroblasts by Oritavancin (LY333328), a New Glycopeptide Antibiotic with Exceptional Cellular Accumulation

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Received 1 September 2004/Returned for modification 2 December 2004/Accepted 12 January 2005

**Oritavancin, a semisynthetic derivative of vancomycin endowed with a cationic amphiphilic character, accumulates to large extent in the lysosomes of eukaryotic cells (F. Van Bambeke, S. Carryn, C. Seral, H. Chanteux, D. Tyteca, M. P. Mingeot-Leclercq, and P. M. Tulkens, *Antimicrob. Agents Chemother.* 48:2853–2860, 2004). In the present study, we examined whether this accumulation could cause cell alterations in phagocytic (J774 mouse macrophages) and nonphagocytic (rat embryo fibroblasts) cells exposed to clinically meaningful (0- to 40-mg/liter) concentrations of oritavancin. Optical and electronic microscopy evidenced conspicuous alterations of the vacuolar apparatus in both cell types, characterized by the deposition of concentric lamellar structures, finely granular material, or other less-defined osmiophilic material, often deposited in giant vesicles. Biochemical studies showed an accumulation of phospholipids (1.5× control values) and free and esterified cholesterol (3 to 4× control values for total cholesterol). Accumulation of these lipids was in close relation to that of oritavancin (excess phospholipid/oritavancin and excess cholesterol/oritavancin molar ratios of 2 to 3 and 3 to 5, respectively). Cholesterol accumulation was rapid and reversible, and that of phospholipids was slower and poorly reversible. Vancomycin and teicoplanin, used as controls (50 and 100 mg/liter, respectively), did not cause any significant change in the lipid content of fibroblasts. The data therefore suggest that oritavancin has the potential to cause a mixed-lipid storage disorder in eukaryotic cells.**

There is now a growing need for agents directed against multiresistant gram-positive organisms such as enterococci and *Staphylococcus aureus* (27). Oritavancin, originally discovered in the Eli Lilly Research Laboratories (4), is a new glycopeptide antibiotic that shows highly concentration-dependent bactericidal activity towards both extracellular and intracellular forms of enterococci and *S. aureus* (1, 2, 21). In a previous study, we showed that oritavancin (see its structure in reference 26) accumulates in very large amounts in macrophages and other cultured cells, reaching apparent cellular concentrations as high as 350-fold the extracellular concentrations after 24 h of incubation (26). We also showed that oritavancin is not homogeneously distributed in cells but is predominantly, if not exclusively, localized in lysosomes (26). This specific tropism is probably the reason for its high activity against *S. aureus*, which is believed to take refuge and to thrive within these organelles. However, such a huge and specific accumulation also raises issues regarding the potential for cytotoxicity of oritavancin. We therefore decided to examine the morphology of cells exposed to clinically meaningful concentrations of oritavancin, using both phagocytic (J774 macrophages) and nonphagocytic (fibroblasts) cell lines. We found, and report here, that oritavancin causes in both cell types conspicuous morphological alterations of the lysosomes and related vacuoles that are as-

sociated with a marked accumulation of cholesterol and phospholipids.

## MATERIALS AND METHODS

**Cells, cell culture conditions, uptake and efflux studies, and assessment of cell viability.** J774 mouse macrophages and rat embryo fibroblasts were obtained and cultured exactly as previously described (26). Uptake and efflux studies, cell collection, and assessment of cell viability were also performed according to published methods (24, 25).

**Optic and electron microscopy.** For observations in the optic microscope ("plastic sections" technique), sections that were approximately 0.5  $\mu\text{m}$  thick were obtained with a glass knife, stained with toluidine blue, mounted in Spur, and examined with a Zeiss microscope under oil immersion at a lens objective of 63×. For electron microscopy, we followed exactly the methods described earlier (25).

**Biochemical and antibiotic assays.** Collected cells were disrupted by sonication, and the lysates were then used to determine the oritavancin, phospholipid, cholesterol, and protein contents. Oritavancin was measured by scintillation counting (using  $^{14}\text{C}$  radiolabeled drug; this assay has been validated previously in comparison with a bioassay [26]). The apparent cellular-to-extracellular concentration ratio of oritavancin was calculated assuming a cell volume of 5  $\mu\text{l}/\text{mg}$  of protein (26). Phospholipids were extracted and total lipid phosphorus measured as in our previous publication (25). Total cholesterol was assayed with a ready-to-use commercial kit (Sigma-Aldrich, St. Louis, MO). Free cholesterol was determined using the same procedure except that cholesterol esterase was omitted when preparing the reagent (all other components were present at the same concentration as in the ready-to-use kit). The cholesteryl ester content was then calculated by subtracting the free cholesterol content from the total cholesterol content. All experimental values were expressed as nanomoles of lipid phosphorus or cholesterol per mg of cell protein, by comparison with standards of phosphorus or cholesterol. Proteins were assayed by the Folin-Ciocalteu/biuret method (11).

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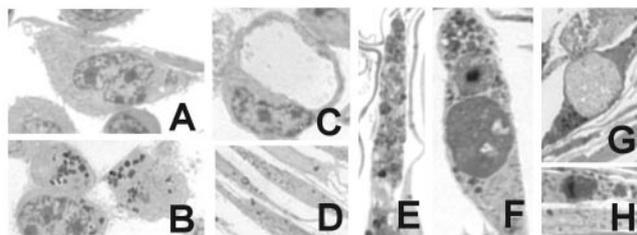


FIG. 1. Appearance of macrophages and fibroblasts under optic microscopy (plastic sections technique); all pictures were taken at an objective magnification of 63 $\times$ . (A) Control macrophages; (B and C) macrophages exposed for 24 h to 20 mg/liter of oritavancin and showing evidence of accumulation of heavily stained material in granules (B) or the appearance of large electron lucent vacuoles (C); (D) control fibroblasts; (E through H) fibroblasts exposed for 72 h to 20 mg/liter of oritavancin and showing evidence of accumulation of heterogeneous, heavily stained material in small granules (E and F), the appearance of large, heterogeneously stained structures (F and H), or structures filled with a finely granular, moderately homogeneously stained material (G).

**Materials.** Oritavancin (LY333328, supplied as diphosphate salt fully hydrated; potency, 80.6%) and [ $^{14}\text{C}$ ]oritavancin (3.5  $\mu\text{Ci}/\text{mg}$ ) were obtained from Eli Lilly & Co., Indianapolis, IN. The labeled drug was mixed with unlabeled oritavancin to obtain a specific activity of 0.6  $\mu\text{Ci}/\text{mg}$ . Vancomycin and teicoplanin were procured as Vancocin and Targocid (the commercial products registered for clinical use in Belgium and supplied by Glaxo-SmithKline Belgium [on behalf of Eli Lilly Benelux] and Aventis Belgium, respectively). Cell culture media and fetal calf serum were purchased from Gibco Biocult (Paisley, Scotland). Unless stated otherwise, all other reagents were of analytical grade and were purchased from E. Merck AG (Darmstadt, Germany) or from Sigma-Aldrich (St. Louis, MO).

## RESULTS

**Determination of conditions causing gross cytotoxicity.** In a first series of studies, we systematically examined which conditions of incubation would cause gross cell toxicity, as assessed by the release of lactate dehydrogenase in the culture medium. Using a release threshold corresponding to twice the amount found in control cells at the beginning of the experiments (16), we observed that macrophages could not be maintained for more than 24 h with 25 mg/liter of oritavancin and that fibroblasts could not be maintained for more than 72 h with 40 mg/liter of oritavancin. All experiments described hereunder used, therefore, incubation times and drug concentrations that did not exceed these values.

**Morphological studies.** Macrophages and fibroblasts incubated with 20 mg/liter of oritavancin and examined under a microscope revealed conspicuous modifications, which are illustrated in Fig. 1 and 2. These consisted of the appearance not only of small, heavily stained granules containing membranous materials, but also of large electron-lucent (macrophages) or heterogeneously stained structures suggestive of the formation of some sort of giant digestive vacuole. Other organelles (in particular, endoplasmic reticulum and mitochondria) seemed unaffected.

**Biochemical analyses and determination of cell accumulation of oritavancin.** Since part of the material accumulated by cells was structurally evocative of what is commonly observed in the tissues of patients suffering from disorders of the lysosomal catabolism of polar lipids, we examined the influence of

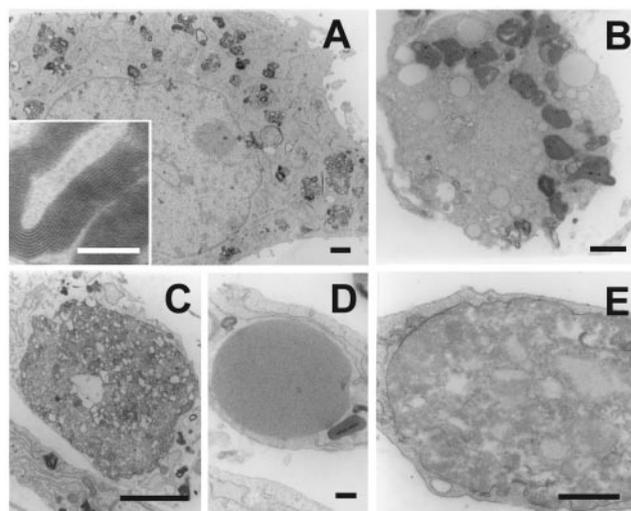


FIG. 2. Appearance under electron microscopy of cultured cells exposed to oritavancin at an extracellular concentration of 20 mg/liter. Panels illustrate the main alterations seen. (A) Mouse macrophages (24 h of incubation); (B through E) rat embryo fibroblasts (72 h of incubation). Both cell types show an abundance of electron-dense structures of various sizes and shapes (A and B) which, upon high magnification, display a clearly lamellar aspect (illustrated for macrophages in the inset of panel A). Treated fibroblasts also showed large structures, sometimes with a highly heterogeneous appearance (C) but also with a moderately osmiophilic granular content (D) or a mixture of electron-lucent and amorphous material (E). Bars, 1  $\mu\text{m}$  (100 nm for the inset of panel A).

oritavancin on the cell content of total phospholipids and cholesterol. For this purpose, cells were incubated with increasing concentrations of oritavancin for 1 day (macrophages) or 3 days (fibroblasts). Figure 3 shows (i) that phospholipids accumulated in a concentration-dependent fashion in both types of cells, reaching a content of about 150% that of controls at the highest concentrations tested (25 mg/liter and 40 mg/liter for macrophages and fibroblasts, respectively), and (ii) that accumulation of cholesterol was even more extensive, reaching values about 3.5 times those of controls under the same conditions. In control cells, cholesterol was found mostly in its free form (about 85% of the total content) (Fig. 3, middle panels). In macrophages exposed to oritavancin, both free and esterified cholesterol increased roughly in parallel. In fibroblasts, similar simultaneous increases were seen up to an extracellular concentration of 20 mg/liter, after which an increase was seen almost exclusively in the esterified form. In parallel, we measured the cell accumulation of oritavancin (lower panels of Fig. 3). As previously described (26), the accumulation of oritavancin occurred in a cooperative fashion with respect to its extracellular concentration. Based on the data obtained at the largest extracellular concentration tested, we calculated that the apparent cellular concentrations of oritavancin reached values about 400-fold (macrophages) and 600-fold (fibroblasts) higher than in the extracellular medium.

The kinetics of the accumulation of phospholipids and cholesterol were then examined in fibroblasts maintained for up to 5 days in the presence of 20 mg/liter of oritavancin. At the same time, we examined the reversibility of this accumulation by transferring cells incubated for 3 days in the presence of 20

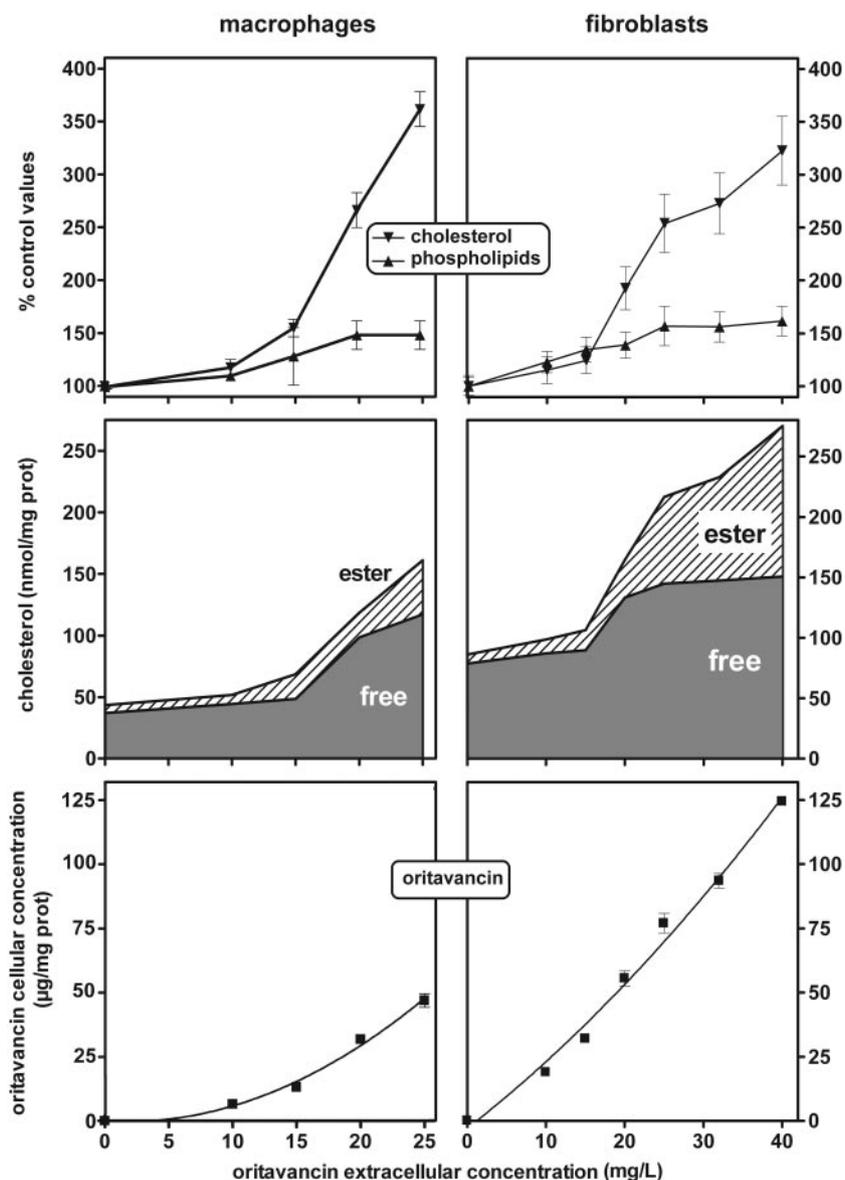


FIG. 3. Accumulation of total cholesterol and phospholipids (top panel), free and esterified cholesterol (middle panels), and oritavancin (lower panels) in mouse J774 macrophages (left panels) after 24 h of incubation or in rat embryo fibroblasts (right panels) after 72 h of incubation with increasing extracellular concentrations of oritavancin. All data are means  $\pm$  the standard deviations of three independent experiments (error bars that are not visible are included in the symbols). Data for phospholipids and total cholesterol are expressed as percentages of the original content of control cells collected at the same time ( $160.0 \pm 5.3$  nmol/mg of protein for phospholipids and  $40.2 \pm 2.6$  nmol/mg of protein for cholesterol in macrophages;  $259.9 \pm 21.7$  nmol/mg of protein for phospholipids and  $86.1 \pm 8.8$  nmol/mg of protein for cholesterol in fibroblasts).

mg/liter of oritavancin to drug-free medium for 3 additional days. (This design was adopted after preliminary experiments had disclosed that confluent fibroblasts exposed to 20 mg/liter of oritavancin could not be maintained for more than a total of 6 days in culture. These experiments also showed that confluent macrophages could not be maintained viable in culture for more than 24 to 36 h.) As shown in Fig. 4 (upper panel), the cell phospholipid content increased continuously as a function of the time of incubation and was only partially reversible upon transfer of the cells to drug-free medium. In contrast with phospholipids, the accumulation of cholesterol reached its maximum after only 1 day of incubation and slightly decreased

thereafter (middle panel). The accumulation of cholesterol was largely reversible upon transfer of the cells to drug-free medium. The lower panel of Fig. 4 shows that the accumulation of oritavancin proceeded continuously during the period of exposure, with, however, a trend towards a plateau at the 5th day (at an apparent cellular-to-extracellular concentration ratio of about 700). Efflux of oritavancin from fibroblasts proceeded at a rate apparently higher than its uptake for the first 24 h but remained thereafter largely incomplete, since about half of the cell-associated drug was still present after 3 days of efflux.

The data generated by these studies were then used to ex-

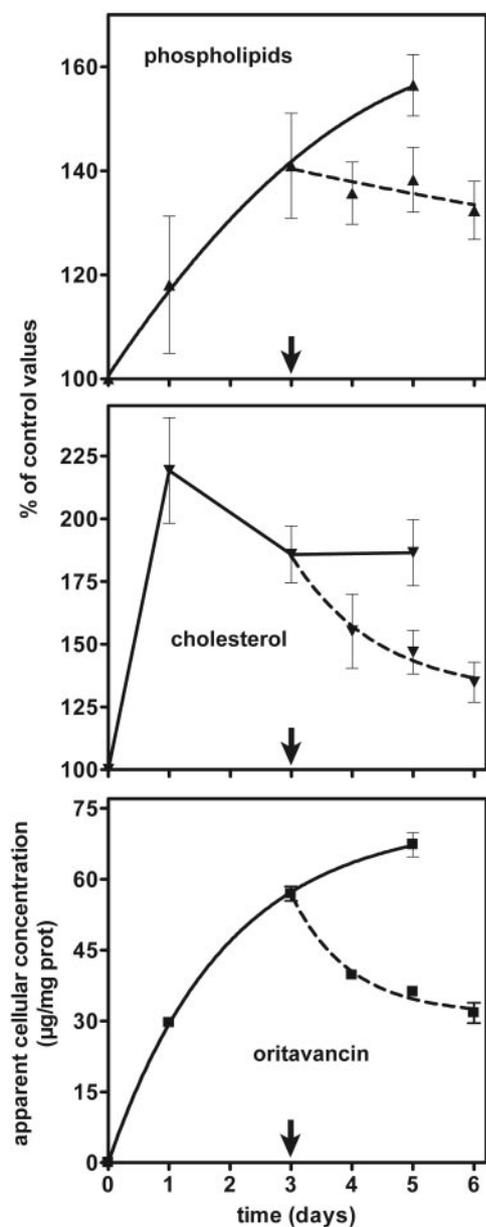


FIG. 4. Kinetics of accumulation and loss of phospholipids (upper panel), total cholesterol (middle panel), and oritavancin (lower panel) in fibroblasts exposed to 20 mg/liter of oritavancin. All cells were incubated in the presence of 20 mg/liter of oritavancin for a first period of 3 days. At this time point (indicated by a vertical arrow), cultures were either maintained in the continuing presence of oritavancin (solid lines) or washed and reincubated in drug-free medium (broken lines). All data are means  $\pm$  the standard deviations of three independent experiments (error bars that are not visible are included in the symbols). Data for phospholipids and cholesterol are expressed as percentages of the content of control cells at the same day (values were  $221.3 \pm 28.2$  nmol/mg of protein for phospholipids and  $58.5 \pm 6.7$  nmol/mg of protein for cholesterol at day 0, and values increased during the culture to reach  $252.9 \pm 11.3$  nmol/mg of protein [prot] for phospholipids and  $94.9 \pm 4.1$  nmol/mg of protein for cholesterol at day 5).

amine whether constant molecular relationships could be deduced from our observations. For this purpose, all phospholipids, cholesterol, and oritavancin accumulation data were converted into nmol per mg protein, and for phospholipids and

TABLE 1. Accumulation of phospholipids and cholesterol in rat fibroblasts incubated for 3 days with glycopeptides

Glycopeptide	Extracellular concn (mg/liter)	% of control value for:	
		Phospholipids	Cholesterol
Vancomycin	50	$93 \pm 7$	$105 \pm 26$
Teicoplanin	100	$109 \pm 10$	$127 \pm 28$
Oritavancin	25	$136 \pm 10$	$272 \pm 47$

cholesterol, data were expressed as amounts in excess of control values. Figure 5 shows a highly significant correlation between the excess of total cholesterol and the cell accumulation of oritavancin. The correlation was less significant for phospholipids, partly due to their lack of disappearance upon drug efflux. The correlation between the molar excess of cholesterol and that of phospholipids was also only partial, but the slope of the correlation suggested a constant molar ratio close to 1, disregarding the type of cells studied.

**Specificity versus other glycopeptides.** The specificity of the effects of oritavancin on lipid accumulation in fibroblasts was tested by repeating these experiments with vancomycin and teicoplanin (at 50 and 100 mg/liter, respectively). Table 1 shows that neither vancomycin nor teicoplanin significantly affected the cholesterol and phospholipid content of cells.

## DISCUSSION

While accumulation of antibiotics in cells and tissue may give cells and tissue a potential for activity against intracellular bacteria, it may also lead to the development of cellular alterations. This is well exemplified here by the behavior of oritavancin if the present data are compared with data dealing with intracellular activity (21, 26). We know that oritavancin accumulates in lysosomes of cultured cells (26), which strongly suggests that the alterations seen here are related to some sort of drug-induced storage disorder at the level of these organelles. One of the key functions of lysosomes is to degrade, through the concerted action of a large array of acid hydrolases, both endogenous and exogenous materials that reach them through the processes of autophagy and heterophagy (5). The highly heterogeneous aspect of the material seen in cells incubated with oritavancin suggests that several lysosomal catabolic pathways are affected. Our images are indeed reminiscent of two situations in which the activities of several lysosomal enzymes are simultaneously decreased, namely, (i) mucopolipidosis type II (I-cell disease [6, 9]), a situation in which newly synthesized lysosomal enzymes are diverted to the extracellular milieu (18) so as to result in a multiple lysosomal enzyme defect (10), and (ii) the impairment of the activities of several lysosomal hydrolases by means of antibodies (23). We may, however, also envisage that oritavancin, as a cationic amphiphile (4), binds to membranes and, indirectly, causes the alterations seen. Cationic amphiphilic drugs indeed form complexes with membranes (12) but usually tend to induce a specific accumulation of phospholipids (7). Accumulation of cholesterol has, however, been reported for some agents (14, 19) and could be related to a stimulation of its synthesis and/or an inhibition of its transport. Future studies will need to further examine these non-mutually exclusive possibilities.

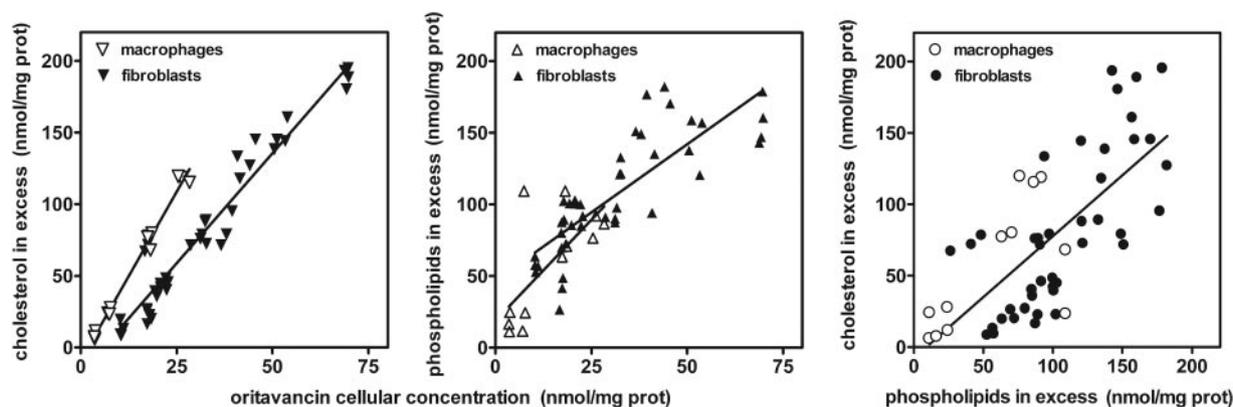


FIG. 5. Correlation between the cell accumulation of oritavancin and the excess of total cholesterol (left panel) and between the excess of cholesterol and the excess of phospholipids (right panel) in macrophages (open symbols) and fibroblasts (closed symbols) exposed to oritavancin. The data used to construct this figure are those of Fig. 3 and 4, but all data are expressed in molar amounts (for cholesterol and phospholipids, the values found in the matched control cells have been subtracted). Regression parameters: cholesterol versus oritavancin,  $R^2 = 0.987$  and slope =  $4.73 \pm 0.17$  in macrophages and  $R^2 = 0.930$  and slope =  $3.07 \pm 0.13$  in fibroblasts; phospholipids versus oritavancin,  $R^2 = 0.488$  and slope =  $2.82 \pm 0.91$  in macrophages and  $R^2 = 0.663$  and slope =  $1.90 \pm 0.21$  in fibroblasts; cholesterol versus phospholipids,  $R^2 = 0.507$  and slope =  $0.85 \pm 0.11$ . prot, protein.

The present work raises critical questions regarding the potential toxicity of oritavancin to patients. Our observations were made with concentrations of oritavancin close to the expected maximum concentration of drug in the sera of humans but were maintained continuously for several days, creating an area under the concentration-time curve considerably larger than what could be seen during conventional, discontinuous administration. Oritavancin accumulated by cells, however, leaks out only very slowly and partially, so that discontinuous administration will also eventually result in a sustained accumulation in the long term. Oritavancin is also reported to be highly protein bound in human serum (about 90%) (P. A. Rowe and T. J. Brown, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. A-2193, 2001), so that cell uptake *in vivo* could be much lower if related to free concentration only. We, unfortunately, lack data concerning the tissular accumulation of oritavancin in animals or humans. As discussed earlier (26), however, J774 macrophages incubated with 20 mg/liter oritavancin in the presence of 10% calf serum (as in the present conditions) accumulate roughly the same amount of drug as those maintained with 3 to 5 mg/liter oritavancin in the absence of serum. Extrapolation to human cells remains, however, largely uncertain. Yet, cellular accumulation seems to be a key event, since no significant cell alteration has been reported for vancomycin and teicoplanin, which accumulate to much lower amounts in cultured cells (13, 28). More importantly, perhaps, we do not know from the present study whether the lysosomal alterations induced by oritavancin will lead to organ dysfunction and toxicity. Drug-induced phospholipid accumulation and lysosomal alterations have been associated with renal, lung, and liver dysfunction for aminoglycosides (15), amiodarone (20), or diethylaminoethoxyhexestrol (29), respectively. Excesses of both free and esterified cellular cholesterol are toxic, as illustrated by their involvement in the pathogenesis of the Niemann-Pick disease type C (17, 19) or atherosclerosis (8, 22). In this respect, the screening of the lipidogenic capacity of drugs on cell culture models is nowadays considered to be an important aspect in the evaluation of

their safety profiles (3). The present observations may therefore trigger more-comprehensive studies aimed at better delineating the true toxic potential of oritavancin.

#### ACKNOWLEDGMENTS

M. C. Cambier and F. Renoird provided dedicated technical assistance.

F.V.B. is Chercheur Qualifié of the Belgian Fonds national de la Recherche Scientifique. We thank Eli Lilly (Indianapolis, IN) for the kind gift of oritavancin. This work was supported by the Fonds Spéciaux de Recherches (FSR) of the Université catholique de Louvain.

#### REFERENCES

1. al Nawas, B., and P. M. Shah. 1998. Intracellular activity of vancomycin and Ly333328, a new semisynthetic glycopeptide, against methicillin-resistant *Staphylococcus aureus*. *Infection* **26**:165–167.
2. al Nawas, B., J. Swantes, and P. M. Shah. 2000. *In vitro* activity of LY333328, a new glycopeptide, against extracellular and intracellular vancomycin-resistant enterococci. *Infection* **28**:214–218.
3. Casartelli, A., M. Bonato, P. Cristofori, F. Crivellente, G. Dal Negro, I. Masotto, C. Mutinelli, K. Valko, and V. Bonfante. 2003. A cell-based approach for the early assessment of the phospholipidogenic potential in pharmaceutical research and drug development. *Cell Biol. Toxicol.* **19**:161–176.
4. Cooper, R. D., N. J. Snyder, M. J. Zweifel, M. A. Staszak, S. C. Wilkie, T. I. Nicas, D. L. Mullen, T. F. Butler, M. J. Rodriguez, B. E. Huff, and R. C. Thompson. 1996. Reductive alkylation of glycopeptide antibiotics: synthesis and antibacterial activity. *J. Antibiot. (Tokyo)* **49**:575–581.
5. de Duve, C., and R. Wattiaux. 1966. Functions of lysosomes. *Annu. Rev. Physiol.* **28**:435–492.
6. Hanai, J., J. Leroy, and J. S. O'Brien. 1971. Ultrastructure of cultured fibroblasts in I-cell disease. *Am. J. Dis. Child.* **122**:34–38.
7. Kodavanti, U. P., and H. M. Mehendale. 1990. Cationic amphiphilic drugs and phospholipid storage disorder. *Pharmacol. Rev.* **42**:327–354.
8. Kruth, H. S. 2001. Lipoprotein cholesterol and atherosclerosis. *Curr. Mol. Med.* **1**:633–653.
9. Leroy, J. G., and J. W. Spranger. 1970. I-cell disease. *N. Engl. J. Med.* **283**:598–599.
10. Lightbody, J., U. Wiesmann, B. Hadorn, and N. Herschkowitz. 1971. I-cell disease: multiple lysosomal-enzyme defect. *Lancet* **i**:451.
11. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275.
12. Lullmann, H., R. Lullmann-Rauch, and O. Wassermann. 1978. Lipidosis induced by amphiphilic cationic drugs. *Biochem. Pharmacol.* **27**:1103–1108.
13. Maderazo, E. G., S. P. Breaux, C. L. Woronick, R. Quintiliani, and C. H. Nightingale. 1988. High teicoplanin uptake by human neutrophils. *Chemotherapy* **34**:248–255.
14. Matsuzawa, Y., and K. Y. Hostetler. 1980. Studies on drug-induced lipidosis: subcellular localization of phospholipid and cholesterol in the liver of rats

- treated with chloroquine or 4,4'-bis (diethylaminoethoxy)alpha, beta-diethylidiphenylethane. *J. Lipid Res.* **21**:202–214.
15. **Mingeot-Leclercq, M. P., and P. M. Tulkens.** 1999. Aminoglycosides: nephrotoxicity. *Antimicrob. Agents Chemother.* **43**:1003–1012.
  16. **Montenez, J. P., F. Van Bambeke, J. Piret, R. Brasseur, P. M. Tulkens, and M. P. Mingeot-Leclercq.** 1999. Interactions of macrolide antibiotics (erythromycin A, roxithromycin, erythromyclamine [dirithromycin], and azithromycin) with phospholipids: computer-aided conformational analysis and studies on acellular and cell culture models. *Toxicol. Appl. Pharmacol.* **156**:129–140.
  17. **Morris, J. A., and E. D. Carstea.** 1998. Niemann-Pick C disease: cholesterol handling gone awry. *Mol. Med. Today* **4**:525–531.
  18. **Neufeld, E. F., G. N. Sando, A. J. Garvin, and L. H. Rome.** 1977. The transport of lysosomal enzymes. *J. Supramol. Struct.* **6**:95–101.
  19. **Palmeri, S., C. Battisti, A. Malandrini, and A. Federico.** 1995. Amiodarone induced lipidoses similar to Niemann-Pick C disease. Biochemical and morphological study. *Life Sci.* **57**:1963–1971.
  20. **Reasor, M. J., and S. Kacew.** 1996. An evaluation of possible mechanisms underlying amiodarone-induced pulmonary toxicity. *Proc. Soc. Exp. Biol. Med.* **212**:297–304.
  21. **Seral, C., F. Van Bambeke, and P. M. Tulkens.** 2003. Quantitative analysis of gentamicin, azithromycin, telithromycin, ciprofloxacin, moxifloxacin, and oritavancin (LY333328) activities against intracellular *Staphylococcus aureus* in mouse J774 macrophages. *Antimicrob. Agents Chemother.* **47**:2283–2292.
  22. **Tabas, I.** 2002. Consequences of cellular cholesterol accumulation: basic concepts and physiological implications. *J. Clin. Investig.* **110**:905–911.
  23. **Tulkens, P., A. Trouet, and F. Van Hoof.** 1970. Immunological inhibition of lysosome function. *Nature* **228**:1282–1285.
  24. **Tyteca, D., S. P. Van Der, M. Mettlen, F. Van Bambeke, P. M. Tulkens, M. P. Mingeot-Leclercq, and P. J. Courttoy.** 2002. Azithromycin, a lysosomotropic antibiotic, has distinct effects on fluid-phase and receptor-mediated endocytosis, but does not impair phagocytosis in J774 macrophages. *Exp. Cell Res.* **281**:86–100.
  25. **Tyteca, D., S. P. Van Der, F. Van Bambeke, K. Leys, P. M. Tulkens, P. J. Courttoy, and M. P. Mingeot-Leclercq.** 2001. Azithromycin, a lysosomotropic antibiotic, impairs fluid-phase pinocytosis in cultured fibroblasts. *Eur. J. Cell Biol.* **80**:466–478.
  26. **Van Bambeke, F., S. Carryn, C. Seral, H. Chanteux, D. Tyteca, M. P. Mingeot-Leclercq, and P. M. Tulkens.** 2004. Cellular pharmacokinetics and pharmacodynamics of the glycopeptide antibiotic oritavancin (LY333328) in a model of J774 mouse macrophages. *Antimicrob. Agents Chemother.* **48**:2853–2860.
  27. **Van Bambeke, F., Y. Van Laethem, P. Courvalin, and P. M. Tulkens.** 2004. Glycopeptide antibiotics: from conventional molecules to new derivatives. Pharmacological properties and clinical use. *Drugs* **64**:913–936.
  28. **Van der Auwera, P., T. Matsumoto, and M. Husson.** 1988. Intraphagocytic penetration of antibiotics. *J. Antimicrob. Chemother.* **22**:185–192.
  29. **Yamamoto, A., S. Adachi, K. Ishikawa, T. Yokomura, and T. Kitani.** 1971. Studies on drug-induced lipidoses. 3. Lipid composition of the liver and some other tissues in clinical cases of “Niemann-Pick-like syndrome” induced by 4,4'-diethylaminoethoxyhexestrol. *J. Biochem. (Tokyo)* **70**:775–784.