4. What bioanalytical methods are used to assess concentrations?

See the response to section IV.F.1 above.

V. LABELING RECOMMENDATIONS

Please refer to Appendix A, Annotated Label.

39 pages redacted from this section of the approval package consisted of draft labeling

Appendix B. Individual Study Reviews

An investigation of the potential for daptomycin to inhibit cytochrome P450 1A2, 2A6, 2C9, 2C19, 2D6, 2E1, and 3A4 in cryopreserved human hepatocytes (ADME Report #12)

OBJECTIVE:
The purpose of the study was to assess the potential of daptomycin to inhibit hepatic cytochrome P450 mediated metabolism via CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP 3A4.

FORMULATION:
Daptomycin (Lot No. X800384)

STUDY DESIGN:
The *in vitro* evaluation of daptomycin as an inhibitor of human cytochrome P450 (CYP P450) isozymes was performed using daptomycin concentrations of 2.5, 10, and 40 µg/mL. Human hepatocytes were pooled from five adult male (all Caucasian) and five adult female (4 Caucasian, 1 Hispanic) subjects. Hepatocytes were incubated in protein-free Krebs-Henseleit buffer (KHB) containing 0.1 M $CaCl_2$.

Isolated hepatocytes were diluted with suspension media (Dulbecco's modified Eagle medium) to determine viability using Tryptan Blue. Cells were then centrifuged and diluted with incubation medium (KHB) to prepare a cell suspension (2×10^6 cells/mL).

The CYP form-specific activities were evaluated using the following probe substrates: 50 µM phenacetin (CYP1A2), 50 µM coumarin (CYP2A6), 75 µM tolbutamide (CYP2C9), 50 µM S-mephenytoin (CYP2C19), 8 µM dextromethorphan (CYP2D6), 50 µM chlorzoxazone (CYP2E1), and 50 µM testosterone (CYP3A4). A negative control consisted of incubation media alone (KHB buffer). Positive control inhibitors consisted of 10 µM furafylline (CYP1A2), 50 µM diethyldithiocarbamate (CYP2A6), 1 µM sulfaphenazole (CYP2C9), 10 µM omeprazole (CYP2C19), 1 µM quinidine (CYP2D6), 100 µM 4-methylpyrazole (CYP2E1), and 1 µM ketoconazole (CYP3A4).

The rate of enzyme activity was assessed by the rate of formation of acetaminophen (CYP1A2), 7-hydroxycoumarin (CYP2A6), 4-hydroxytolbutamide (CYP2C9), 4-hydroxymephenytoin (CYP2C19), dextrorphan (CYP2D6), 6-hydroxychlorzoxazone (CYP2E1), and 6β-hydroxytestosterone (CYP3A4). The percentage of the activity remaining was calculated as the ratio of enzyme activity in the presence of daptomycin relative to the enzyme activity in the presence of negative control. The impact of the positive control was calculated using the vehicle control (incubation medium + 0.1% DMSO) instead of the negative control.

RESULTS:
The viability of hepatocytes in suspension media using Tryptan blue was 73%.

The positive controls yielded greater than 50% inhibition of enzyme activity and adequately inhibited the activity of the isoforms.

The rate of enzyme activity (pmol/million cells/min) in the presence of negative control, positive control, and daptomycin is shown in Table 1. The percent of the activity remaining for each P450 isozyme is shown in Table 2.

Daptomycin, at concentrations of 2.5, 10, and 40 µg/mL, did not appreciably inhibit of the activity of CYP1A2, CYP2A6, CYP2C9, CYP2D6, and CYP3A4 in human hepatocytes. The activity of CYP2E1

was reduced in the presence of daptomycin (67.5% to 73.2%), although the inhibition was less than 50% of the positive control (4-methylpyrazole).

Table 1. Effects of daptomycin on *in vitro* metabolism rates of major human CYP P450 specific activities

CYP P450	Probe Substrate	Enzyme activity (pmol/million cells/min)				
		(-) Control	(+) Control	2.5 µg/mL	10 µg/mL	40 µg/mL
1A2	Phenacetin	13.5 ± 0.3	1.02 ± 0.06	14.1 ± 0.2	14.4 ± 0.3	14.1 ± 0.2
2A6	Coumarin	3.64 ± 0.17	0.49 ± 0.04	2.94 ± 0.13	3.43 ± 0.30	3.12 ± 0.10
2C9	Tolbutamide	10.2 ± 0.3	1.98 ± 0.04	10.4 ± 0.3	10.4 ± 0.2	10.9 ± 0.3
2C19	S-Mephenytoin	8.69 ± 0.43	2.02 ± 0.15	8.05 ± 0.41	8.41 ± 0.15	8.53 ± 0.31
2D6	Dextromethorphan	10.0 ± 0.3	1.15 ± 0.16	11.7 ± 0.4	12.4 ± 0.2	12.4 ± 0.3
2E1	Chlorzoxazone	6.61 ± 0.25	0.49 ± 0.04	4.84 ± 0.15	4.60 ± 0.23	4.46 ± 0.52
3A4	Testosterone	20.1 ± 3.2	0.77 ± 0.15	20.7 ± 3.5	20.6 ± 2.6	32.6 ± 13.6

Table 2. Percentage of activity remaining of major human CYP P450 isoenzymes in the presence of daptomycin

CYP P450	Probe Substrate	Activity remaining (%)			
		Positive Control	2.5 µg/mL	10 µg/mL	40 µg/mL
1A2	Phenacetin	8.2%	104.4%	106.7%	104.4%
2A6	Coumarin	13.7%	80.8%	94.2%	85.7%
2C9	Tolbutamide	21.1%	102.0%	102.0%	106.9%
2C19	S-Mephenytoin	25.3%	92.6%	96.8%	98.2%
2D6	Dextromethorphan	10.2%	117.0%	124.0%	124.0%
2E1	Chlorzoxazone	17.2%	73.2%	69.6%	67.5%
3A4	Testosterone	5.1%	103.0%	102.5%	162.2%

Following the administration of daptomycin IV 4 mg/kg q24h, anticipated peak plasma concentrations of daptomycin are approximately 50 µg/ml (total) and 5 µg/mL (unbound). Thus, the maximum concentration of daptomycin assessed in the study exceed the anticipated peak plasma concentrations of daptomycin by approximately 8-fold.

CONCLUSIONS:
Based on the *in vitro* results, daptomycin IV 4 mg/kg is unlikely to inhibit the metabolism of drugs dependent on P450 isoforms CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4.

COMMENTS:
Although the sponsor states that the activities of all substrates were analyzed using proprietary methods, assay validation data were not provided with the study report. The sponsor is encouraged to provide the full assay validation data with the study report in the future.

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