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Posaconazole Concentrations in Human Tissues after Allogeneic Stem Cell Transplantation

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Few data have been published regarding posaconazole tissue concentrations in humans. We analyzed tissue concentrations in biopsy specimens taken at autopsy from seven patients who received posaconazole prophylaxis because of graft-versus-host disease. The results were compared to plasma concentrations collected before death. Tissue concentrations suggestive of an accumulation of posaconazole were found in the heart, lung, liver, and kidney but not in the brain.

Invasive fungal diseases (IFD) continue to be major complications after allogeneic hematopoietic stem cell transplantation (HSCT). Prophylaxis with posaconazole, a broad-spectrum oral triazole with *in vitro* activity against most *Candida* and *Aspergillus* species, has been shown to significantly reduce IFD in patients with graft-versus-host disease (GVHD) after HSCT and during induction therapy for acute myeloid leukemia (1–3). Data supporting a dose-exposure relationship for posaconazole have been published, and since the extent of posaconazole absorption from the intestinal tract is highly variable (4), routine therapeutic drug monitoring (TDM) is recommended in international guidelines (5–7). However, in the prophylactic setting, plasma concentrations are only surrogate markers for concentrations in tissues of importance, such as lung tissue, in the establishment of a mold infection. Published data regarding this relationship are scarce.

Tissue concentrations of posaconazole were investigated in human biopsy specimens taken at autopsy from seven patients who received posaconazole prophylaxis because of GVHD, and they were compared to plasma concentrations collected before death. This study was approved by the regional ethics committee in Stockholm, Sweden (DNR 2010/1611–31/4). All the patients were men (median age, 52 years; range, 37 to 64 years), and all of them were severely ill with very limited oral intake (median intake, 130 kcal during the last 7 days before death; range, 0 to 520 kcal) (see Table S1 in the supplemental material). Blood samples were collected during the posaconazole treatment period and stored at −80°C until analysis. The concentrations of posaconazole in the plasma samples were determined using a routine liquid chromatography mass spectrometry (LC-MS)-based method at the clinical pharmacology department of Karolinska University Hospital in Stockholm (see below). Multiple biopsy specimens from the brain, heart (not performed in one patient), lung, liver, and kidney were taken during a routine autopsy and stored at −80°C before analysis. Calibration curves were based on posaconazole (Schering-Plough, Kirkland, Canada) and internal standard (United Kingdom-115794, Janssen, Buckinghamshire, United Kingdom)-spiked homogenates of C57BL/6N mouse brain, heart, lung, liver, and kidney. Sample preparation was performed by tissue sonication in sodium chloride (0.9% [wt/vol]), followed by protein precipitation with 2 volumes of acetonitrile and injection of the supernatant onto an LC-MS system consisting of an Agilent 1100 MSD and Agilent 1100 LC (Santa Clara, CA, USA) with a C_{18} column (Phenomenex Kinetex, Torrance, CA, USA). Performance of the method was monitored with quality control samples at two levels. Chromatography was performed using a gradient based on aqueous 25 mM formic acid as solvent A and 100% acetonitrile as solvent B. Mass spectrometry was operated in selective ion monitoring using electrospray ionization in positive mode at *m*/*z* 351 for posaconazole and *m*/*z* 348 for the internal standard. The calibration curve was linear over a concentration range of 500 to 10,000 ng/ml for all organs. The quantification analysis was based on the ratio of the peak height for the analyte to the peak height of the internal standard and equal weighting by linear regression analysis. Quantifications were done using Agilent LC/MSD ChemStation software Rev. B.04.02.SP (Santa Clara, CA, USA). When comparing the individual plasma concentrations with tissue concentrations, the density of plasma was approximated to 1.0 g/ml.

Posaconazole dosages, plasma concentrations, and tissue concentrations are shown in Tables 1 and 2. The relationships between posaconazole concentrations in the tissues analyzed are presented in Fig. S1 in the supplemental material, and the relationships between the concentrations in plasma and in lung tissues are presented in Fig. S2 in the supplemental material. Since the dosage and duration of posaconazole treatment varied, as did the time elapsed between the administered doses and measurement of the plasma concentrations, no conclusion could be drawn regarding the exact relationship between plasma and tissue posaconazole concentrations. However, the tissue concentrations in

Received 5 May 2014 Returned for modification 20 May 2014
Accepted 25 May 2014
Published ahead of print 2 June 2014
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Supplemental material for this article may be found at http://dx.doi.org/10.1128/AAC.03252-14.

doi:10.1128/AAC.03252-14

TABLE 1 Posaconazole dosage and plasma concentration

Patient no.	Time from initiation of treatment to death (days)	Dose (mg)[a]	Results of plasma concn determination closest to death					Interval between last dose and death (h)
			Time between dose and sampling (h)	Steady state reached	Concn (ng/ml)	Time from sampling to death (days)	Administered dosage (mg) (no. of doses) between sampling and death	
1	5	200 (2 doses 5 days before death, 1 dose 3 days before death)	21	No	30	2	None	81
2	4	400 (2 doses 4 days before death), 200 (2 doses 1 day before death)	57	No	10	1	200 (2)	16
3	31	200 (q12h, every second day)	33	Yes	40	0	None	65
4	42	200 (q12h, every second day)	9	Yes	70	0	None	24
5	196	200 (q12h, every second day)	34	Yes	50	4	200 (2)	79
6	19	200 (q12h, every second day)	33	Yes	330	3	200 (1)	58
7	22	200 (q8h), 200 (q12h the last 2 days before death)	15	Yes	390	0	None	19

[a] q12h, every 12 hours; q8h, every 8 hours.

heart, lung, liver, and kidney exceeded the plasma level in all the patients, while they were approximately equal in brain tissue and plasma. In two patients who received only a few doses of posaconazole before death, concentrations in the lung tissues were 200 and 160 ng/mg (Tables 1 and 2). The distribution of posaconazole was highest to liver tissue, followed by kidney, heart, and lung tissues (Table 2; see Fig. S1 in the supplemental material).

Posaconazole concentrations in all examined organs except the brain were higher than in plasma, suggesting accumulation in these tissues. However, no firm conclusion may be drawn regarding the relationship between plasma and tissue concentrations because of the variable dosages of posaconazole and the elapsed time between dose intake and plasma sampling. The main reasons for the very low dose of posaconazole (200 mg, two times daily every second day) given to four of the patients were very poor oral intake and fear of toxicity in these severely ill patients. However, the same profile of tissue accumulation was found in all the patients, regardless of dosage. An interesting finding was that three of the four patients who received 400 mg posaconazole every second day had lung tissue concentrations of ≥550 ng/mg. Similar findings were presented in a case report in which posaconazole accumulation was seen in the lung, liver, spleen, and kidney tissues of a renal transplant patient (8). Our finding was also in accordance with the findings that posaconazole was accumulated in alveolar macrophages and white blood cells in humans (9–11). A recently published study using an *in vitro* model of pulmonary epithelial cells found accumulation of posaconazole in cell membranes, especially in the endoplasmic reticulum, which persisted for at least 48 h after the removal of extracellular posaconazole (12, 13). When *Aspergillus fumigatus* conidia came in contact with the epithelial cells, the posaconazole was rapidly transferred to the conidia. The authors concluded that the accumulation of posaconazole in the cell membranes seemed to be an important mechanism in prophylaxis and that the accumulation was a possible explanation for the prolonged antifungal effect that was seen in the experiments (12, 13). Our observations of posaconazole accumulation in different organ tissues *in vivo* are in accordance with this hypothesis and may be an important explanation for the low breakthrough rates of IFD seen in clinical trials, despite relatively low serum levels (1, 12, 14, 15).

TABLE 2 Posaconazole tissue concentrations

Patient no.	Plasma concn (ng/ml)[a]	Posaconazole concn (ng/g) in:				
		Brain	Kidney	Liver	Heart	Lung
1	30	160	480	620	310	140
2	10	80	320	660	180	200
3	40	40	280	260	ND[b]	110
4	70	ND	510	1,000	510	550
5	50	60	330	500	260	670
6	330	260	4,600	7,460	1,790	4,530
7	390	320	1,550	2, 290	1, 730	890

[a] Reproduced from Table 1.
[b] ND, not determined.

ACKNOWLEDGMENTS

This work was supported by grants from the Karolinska Institutet, the Swedish Research Council (K2012-64X-22020-01-3), and the Children's Cancer Foundation (PROJ10/052).

We thank the staff at the Center for Allogeneic Stem Cell Transplantation for competent and compassionate care of the patients, Bruno Vanherberghen for critical reading of the manuscript, and Jens Gertow for help with figures.

O.B. and J.M. previously received unrestricted research grants from MSD. The other authors declare no competing financial interests.

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