ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2011, p. 925–928 Vol. 55, No. 2
0066-4804/11/$12.00 doi:10.1128/AAC.00949-10

Human Tissue Distribution of Voriconazole▽

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Received 13 July 2010/Returned for modification 4 October 2010/Accepted 7 November 2010

Voriconazole concentrations were determined in autopsy samples of eight patients who had been treated for a median of 7 days (interquartile range [IQR], 5 days). Voriconazole penetrates well into various tissues, with median levels of 6.26 μg/g ((interquartile range [IQR], 7.87 μg/g) in the lung, 3.41 μg/g (IQR, 16.72 μg/g) in the brain, 6.89 μg/g (IQR, 24.16 μg/g) in the liver, 6.47 μg/g (IQR, 6.19 μg/g) in the kidneys, 5.60 μg/g (IQR, 11.49 μg/g) in the spleen, and 7.55 μg/g (IQR, 16.91 μg/g) in the myocardium.

Voriconazole (VRC) is an expanded-spectrum triazole with a broad antimycotic spectrum, including *Aspergillus* and non-*albicans Candida* species. It is the drug of choice for treatment of invasive aspergillosis (12, 27). The bioavailability after oral intake of VRC is reported to be almost complete (96%) in healthy volunteers, but lower and variable in patients (23.7 to 63.3%) (11). Since data on VRC distribution into human tissues have been sparse so far, we determined concentrations in autopsy samples.

The study was approved by the local ethics committee. Consent was granted by the patients' relatives. Tissue samples were obtained during routine autopsy from eight patients who had died at the medical intensive care unit (ICU) during VRC treatment. The patients' characteristics and data on VRC therapy are displayed in Table 1; routine laboratory values are shown in Table 2.

VRC (Vfend; Pfizer) had been administered for possible, probable, or proven invasive aspergillosis intravenously (six patients) or as an oral suspension (two patients) at the standard dose. Aliquots (~7 g) were taken from lung, brain, liver, spleen, kidneys, and myocardium and were stored at −80°C. After thawing, samples were homogenized (Ultraturrax T25; Germany) and purified by C_{18} solid-phase extraction. VRC was quantified by the high-performance liquid chromatography method by Khoschsorur et al. (14), with some modifications, using a Zorbax 300SB-C_{18} analytical column, UV detection ($\lambda = 255$ nm), and a mixture of sodium dihydrogen phosphate buffer, acetonitrile, and methanol (35:45:20 [vol/vol]) as the mobile phase. The detection limit was 0.25 μg/g. The intraday precision, interday precision, and accuracy (mean ± standard deviation) were 4.2% ± 3.7%, 8.8% ± 5.2%, and 7.4% ± 9.6%, respectively. The concentrations were assessed by means of a linear standard curve ($R = 0.999$), obtained by external standards comprising respective bovine tissues spiked with VRC. The mean VRC recovery was 80%.

Table 3 displays the VRC concentrations in the different tissue samples. VRC was shown to penetrate well into tissues, with high variability. In patient 1, VRC was detectable even after a low single dose in all tissues but brain and myocardium. Tissue drug levels of all the other patients exceeded those achieved in patient 1. In patient 7 (daily dose of 600 mg for 6 days of VRC treatment), the highest concentrations were achieved in most tissue samples. He also exhibited the highest values in liver and renal function tests. No difference in VRC levels was found between different areas of the lung, nor were there discrepancies between cerebral cortex, hippocampus, nucleus caudatus, medulla oblongata, and cerebellum.

After multiple dosing, VRC levels in the liver correlated with the daily doses ($R = 0.79$, $P = 0.03$; in the other organs, R was between 0.38 and 0.64, $P > 0.05$), but not with the cumulative dose or the interval between the last administration and death. There was no significant difference in tissue concentrations between patients on and off renal replacement therapy. No signs of VRC toxicity could be observed (Table 2).

Invasive aspergillosis most frequently affects the respiratory tract and the central nervous system. The myocardium, the liver, and the spleen are further target organs of invasive fungal infections. We found mean VRC tissue concentrations that exceed therapeutic plasma levels (~2.0 to 5.5 μg/ml) (19, 22) and the MICs for *Aspergillus* species (~0.25 to 2 μg/ml) (15). However, the significance of *in vivo* target site concentrations in relation to *in vitro* MICs is controversial considering clinical efficacy.

The lack of therapeutic VRC monitoring precluded a comparison with plasma drug levels. The limited number of patients, differences in underlying diseases, including hepatic and renal function, as well as varying cumulative VRC doses and variable intervals between the last VRC administration and death of the patient are further limitations of our study. Agonal or postmortem changes in VRC tissue concentrations cannot be ruled out completely, although VRC was found to be stable in tissue at 4°C for at least 72 h. Between the tissue drug concentrations and death-to-sampling interval, no correlation was observed. Free VRC and protein-bound VRC were not separated by our assay. The tissue samples consisted of various compartments that are potential targets of fungal invasion, such as different cells, extracellular matrix, and blood vessels

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▽ Published ahead of print on 15 November 2010.

TABLE 1. Demographic and clinical characteristics of patients

Patient[a]	Sex	Age (yr)	VRC administration[b]	Dose[c]			Therapy day	Interval between last VRC administration and death (h)	CRRT[d]	IFI definition[e]	Diagnosis[f]	Interval between death and sampling (h)	Cause of death
				Daily (mg)	By wt (mg/kg)	Cumulative (mg)							
1	Female	58	i.v.	200 (single)	3.33 (single)	200	1	36.0	−	Proven	Pancreatitis, liver failure, COPD, CMP, and Candida sepsis	13	Liver failure, pancreatitis
2	Male	52	os	300 q24h	1.85 q24h	2,500	6	18.0	+	Possible	Sepsis, pneumonia, ARF, and ALL	12	Septic shock
3	Female	57	i.v.	300 q12h	2.63 q12h	4,800	8	8.5	+	Probable	Sepsis, ARF, GvHD, and AML	21	Septic shock
4	Male	35	os	200 q12h	2.94 q12h	3,800	10	12.0	−	Possible	Sepsis, pneumonia, DLBCL, and St. p. alloSCT	62	Septic shock
5	Male	67	i.v.	300 q12h	3.70 q12h	2,500	4	41.7	+	Probable	Sepsis, ARF, LF, DM, CVD	10	Septic shock
6	Male	47	i.v.	200 q12h	3.77 q12h	10,000	25	85.0	+	Possible	Sepsis, pneumonia, ARF, and multiple myeloma	60	Septic shock, multiorgan failure
7	Male	24	i.v.	300 q12h	4.00 q12h	3,500	6	5.5	−	Possible	Sepsis, pneumonia, and Hodgkin's disease	66	Respiratory failure
8	Male	75	i.v.	350 q12h	4.17 q12h	7,500	10	25.0	+	Probable	Sepsis, pneumonia, ARF, and oropharynx cancer	26	Septic shock
Median		55		300	3.52	3,650	7	21.5				23.5	
Interquartile range		22		100	1.1	3,650	5	28.6				48.5	

[a] Patient 1 received only a single-dose of VRC. Patient 2 received VRC every 24 h because of hepatic impairment. In patient 3 (body weight, 114 kg; body mass index, 40.4), the dosage was guided by the adjusted body weight.

[b] i.v., intravenous; os, oral suspension.

[c] q12h, dose administered every 12; q24h, dose administered every 24 h.

[d] CRRT, continuous renal replacement therapy. +, patient on CRRT; −, patient off CRRT.

[e] IFI, invasive fungal infection. The definition of IFI is based on EORTC-MSG criteria, including the following clinical mycological criteria and host factors (7): microbiology for patient 1, *Candida glabrata* from central venous line and *Candida tropicalis* in bile; patient 3, *A. fumigatus* in bronchoalveolar lavage; patient 5, *A. fumigatus* in bronchial aspirate and *C. glabrata* from central venous line; patient 8, mycelium (and *C. glabrata*) in bronchoalveolar lavage; patients 2, 4, 6, and 7, no microbiological or histopathological findings of a fungal infection. There was postmortem histopathological confirmation of proven or probable IFI in patients 1 (lung, kidney, and pancreas), 3 (lung), and 5 (lung). All detected pathogens were VRC sensitive: the MIC for *A. fumigatus* was <4 μg/ml, and the MIC for *C. glabrata* was <2 μg/ml.

[f] CMP, cardiomyopathy; COPD, chronic obstructive pulmonary disease; DLBCL, diffuse large B-cell lymphoma; St. p. allo SCT, status post-allogeneic stem cell transplantation; ARF, acute renal failure; LF, liver failure; DM, diabetes mellitus; CVD, cardiovascular disease; ALL, acute lymphoblastic leukemia; GvHD, graft versus host disease; AML, acute myeloid leukemia.

TABLE 2. Routine laboratory results for patients

Patient	Laboratory result[a]									
	Creatinine (mg/dl)	Urea (mg/dl)	Bilirubin (mg/dl)	AST (U/liter)	ALT (U/liter)	GGT (U/liter)	PT (%)	WBC (10^9 cells/liter)	Hb (g/liter)	PLT (10^9 cells/liter)
Baseline testing										
1	0.40	46.6	6.12	55	40	1,317	86	27.0	95	185
2	0.88	69.7	11.00	60	149	686	70	10.6	77	106
3	0.73	60.8	9.75	61	50	148	50	11.8	97	24
4	1.47	83.2	0.95	20	40	480	90	7.1	99	21
5	1.12	76.1	8.63	235	214	2,534	72	9.1	79	136
6	0.68	81.1	1.14	33	22	232	76	4.5	89	21
7	0.64	47.1	0.28	15	9	35	90	16.9	91	241
8	0.92	62.0	0.93	50	40	433	77	5.2	91	296
Median	0.81	65.9	3.63	53	40	457	77	9.9	91	121*
Interquartile range	0.36	24.7	8.25	34	69	812	17	8.2	12	191
Final testing										
1	0.40	46.6	6.12	55	40	1,317	86	27.0	95	185
2	1.99	106.7	9.29	221	109	1,129	67	13.5	84	15
3	0.62	80.5	15.33	107	75	180	51	9.5	101	12
4	0.98	57.1	3.45	43	66	338	89	7.0	74	18
5	0.89	60.5	7.93	205	186	1,827	70	6.2	87	112
6	1.53	183.1	0.46	31	20	209	108	3.1	73	28
7	3.07	250.7	0.36	1,079	667	102	25	39.9	108	108
8	2.45	100.5	1.00	56	29	656	82	6.6	88	177
Median	1.26	90.5	4.79	82	71	497	76	8.25	88	82*
Interquartile range	1.47	86.1	7.88	164	113	1,029	29	13.9	19	128

[a] The results from routine laboratory tests on the first day of VRC treatment (baseline testing) and final routine laboratory test results are shown as follows: serum creatinine, normal range, 0.51 to 1.17 mg/dl; urea, normal range 18 to 55 mg/dl; plasma bilirubin, normal range, 0.00 to 1.29 mg/dl; aspartate aminotransferase (AST), normal range, 10 to 50 U/liter; alanine aminotransferase (ALT), normal range, 10 to 50 U/liter; γ-glutamyl transferase (GGT), normal range, 6 to 71 U/liter; prothrombin time (PT), normal range 70 to 130%; white blood cell (WBC) count, normal range, 4.0×10^9 to 10.0×10^9 cells/liter; hemoglobin (Hb), normal range, 120 to 177 g/liter; and platelet (PTL) count, normal range, 150×10^9 to 380×10^9 cells/liter. *, significant differences between the first and last testings (Wilcoxon matched-pairs test). No significant increases in liver and renal function tests were observed during VRC therapy. Since all patients were on sedoanalgesia, neurotoxicity of VRC could not be assessed.

(18). However, with our method we could not study the VRC distribution on a cellular level.

In pulmonary epithelial lining fluid (ELF) of lung transplant recipients, VRC levels were between 0.3 and 83.3 μg/ml (3). In ELF of healthy volunteers, mean levels amounted to 10.1 to 48.3 μg/ml; in alveolar macrophages, mean levels were 10.3 to 20.6 μg/ml (5). Thus, the mean VRC concentration we found in lung tissue was somewhat lower than that in ELF. *In vitro* incubation of polymorphonuclear leukocytes with 2 μg/ml of VRC resulted in intracellular levels of ~15 μg/ml (1). Relatively small amounts of VRC (0.7 to 4.4 μg/ml) were recovered from pleural fluid (17, 21, 23). VRC penetration into cerebro-

TABLE 3. Voriconazole concentrations in 128 tissue samples from eight patients

Patient	VRC concn (μg/ml) in[a]:					
	Lung	Brain	Liver	Spleen	Kidney	Myocardium
1[b]	0.74 (0.72–0.76)	<0.25	2.14 (1.86–2.42)	1.31 (1.29–1.34)	1.97 (1.69–2.26)	<0.25
2	4.44 (3.64–5.20)	1.67 (1.38–2.06)	6.28 (6.22–6.34)	5.93	6.05 (5.99–6.10)	2.04 (1.96–2.13)
3	6.57 (6.12–6.78)	6.54 (5.77–6.95)	19.83 (19.58–20.08)	5.72 (5.60–5.84)	4.88 (4.83–4.92)	7.55 (6.53–8.57)
4	1.98 (1.47–2.04)	3.36 (3.08–3.56)	4.21 (3.67–4.76)	2.95 (2.92–2.99)	6.89 (6.84–6.93)	2.44 (2.27–2.62)
5	5.94 (4.14–7.86)	2.27 (2.25–3.79)	8.69 (7.44–9.95)	2.22 (2.22–2.22)	2.89 (2.76–3.02)	3.17 (3.10–3.23)
6	6.59 (4.74–9.38)	2.72 (2.57–3.35)	2.98 (2.83–3.12)	6.10 (5.46–6.74)	8.89 (8.64–9.13)	13.47 (13.11–13.84)
7	20.26 (19.82–20.87)	27.72 (26.78–34.70)	35.53 (35.04–36.03)	18.73 (17.98–19.47)	13.58 (11.10–16.05)	25.79 (25.54–26.05)
8	13.68 (12.04–15.07)	20.09 (17.43–23.13	40.04 (37.77–42.31)	14.27 (13.71–14.83)	14.81 (13.55–16.08)	19.85 (19.53–20.18)
Median	6.26	3.41	6.89	5.60	6.47	7.55
Interquartile range	7.87	16.72	24.16	11.49	6.19	16.91

[a] Shown are median VRC concentrations (with ranges in parentheses). Multiple sampling was performed from different sites as follows: lung, four samples (upper and lower lobe, left and right); brain, five samples (cortex, hippocampus, nucleus caudatus, medulla oblongata, and cerebellum); liver, two samples from different sites; spleen, two samples from different sites; kidney, two samples (left and right); and myocardium, two samples (ventricular septum and anterior wall).

[b] Patient 1 received only a single dose of VRC.

spinal fluid appears to be variable, yielding levels of 0.08 to 3.93 μg/ml (6, 16). Cerebral VRC concentrations had been determined previously in autopsy samples from two patients where 11.8 and 58.5 μg/g were measured and, thus, which exceed the levels we measured in our study population (16). In a brain abscess, 1.2 to 1.4 μg/g was reached by oral intake of 4 mg/kg twice daily (8). In contrast, none of our patients presented with cerebral mycosis. Studies of chickens and horses revealed remarkable interspecies differences in tissue penetration of VRC (2, 4, 20). In rats, free extracellular lung concentrations reached ~2.5 μg/ml after a single dose (13).

Fluconazole, the ancestor drug of VRC, has been found to achieve high levels in various tissues, including the brain (25). In contrast, amphotericin B preparations accumulate in liver and spleen but achieve only low levels in brain and myocardium (26). Unlike with VRC, amphotericin B levels in lung tissue are much higher than in ELF (28). In comparison with amphotericin B, VRC therapy of invasive aspergillosis achieved a superior clinical outcome (12). Echinocandins displayed a tissue distribution similar to that of amphotericin B in animal studies (9, 10, 24).

In conclusion, treatment with VRC at standard doses yields concentrations above the MICs of relevant fungal pathogens in various frequently affected tissues. The significance of target site levels of antifungals for the clinical response should be addressed by adequately powered clinical trials.

We are indebted to Gregor Mikuz, Andrea Brunner, Christian Ensinger, Jens Krugmann, Hans Maier, Patrizia Moser, Ralf Rieker, Consolato Sergi, Irmgard Verdorfer, and Bettina Zelger, Institute of Pathology, Innsbruck Medical University, for providing the tissue samples.

We thank Pfizer, Austria, for financial support.

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