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Rifampin Concentrations in Various Compartments of the Human Brain: A Novel Method for Determining Drug Levels in the Cerebral Extracellular Space

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Antimicrobial therapy for brain infections is notoriously difficult because of the limited extent of knowledge about drug penetration into the brain. Therefore, we determined the penetration of rifampin into various compartments of the human brain, including the cerebral extracellular space (CES). Patients undergoing craniotomy for resection of primary brain tumors were given a standard dose of 600 mg of rifampin intravenously before the operation. A microdialysis probe (10 by 0.5 mm) was inserted into the cortex distantly from the resection and was perfused with two different rifampin solutions. Rifampin concentrations in the CES were calculated by the no-net-flux method. Intraoperatively, samples were taken from brain tumor tissue, perifocal tissue, and normal brain tissue in the case of pole resections. Rifampin concentrations in the various samples were determined by using a bioassay with *Sarcinea lutea*. In the various compartments, rifampin concentrations were highest within tumors (1.37 ± 1.34 μg/ml; $n = 8$), followed by the perifocal region (0.62 ± 0.67 μg/ml; $n = 8$), the CES (0.32 ± 0.11 μg/ml; $n = 6$), and normal brain tissue (0.29 ± 0.15 μg/ml; $n = 7$). Rifampin concentrations in brain tumors do not adequately reflect concentrations in normal brain tissue or in the CES. Rifampin concentrations in the CES, as determined by microdialysis, are the most reproducible, and the least scattered, of the values for all compartments evaluated. Rifampin concentrations in all compartments exceed the MIC for staphylococci and streptococci. However, CES concentrations may be below the MICs for some mycobacterial strains.

Dose recommendations for antibiotic treatment of bacterial infections of the brain are usually based on concentrations in cerebrospinal fluid (CSF). Alternatively, drug levels in brain tumors, rather than those in nontumorous brain tissue or the cerebral extracellular space (CES), are taken into consideration.

Rifampin is used for its bactericidal effect on mycobacteria and its excellent antistaphylococcal effect (16). Penetration of antibiotics into the central nervous system (CNS) has been determined only in the tissue of brain tumors, the CSF, and intracranial abscess formations (1, 2, 4, 13). In contrast to other antistaphylococcal drugs, rifampin penetrates well into the CSF (1, 13), and consequently it is used in the treatment of CSF shunt infections (7). Therefore, rifampin would be a good candidate for the treatment of staphylococcal or streptococcal brain abscesses and of infections after neurosurgery. However, there are no data on its penetration into nontumorous brain tissue or into other compartments of the brain, except from animal experiments (11).

Since brain infections spread mainly through the CES, we determined rifampin concentrations in the CES in comparison with those in other brain compartments. We examined tissue samples and used intraoperative microdialysis in patients undergoing craniotomy for tumor removal. The tissue samples were taken from the following sites: nontumorous brain tissue distant from a brain tumor, perifocal tissue in close proximity to a brain tumor, and brain tumor tissue. Rifampin concentrations in the CES were determined by using intraoperative microdialysis.

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MATERIALS AND METHODS

Patients. This prospective, open trial was started in February 1996. The study was approved by the Ethical Committee of the University Hospitals of Basel. Patients who were admitted to the Department of Neurosurgery for surgery of a primary brain tumor, and who had no previous antibiotic therapy within 5 days prior to surgery, were eligible for the trial provided that the expected duration of the operation exceeded 3 h. All patients gave written informed consent to participation in the trial. Each patient entering the trial received a single preoperative dose of 600 mg of rifampin infused over 3 h in 500 ml of physiological saline, starting 3 h before skin incision. No further antibiotics were administered.

Microdialysis. Prior to the insertion of the microdialysis probe into the brain and the commencement of microdialysis, the entire tubing and the probe of the microdialysis system were flushed with the rifampin solution of the perfusate, thus eliminating the problem of rifampin loss due to its adherence to plastic surfaces. After craniotomy and opening of the dura, the microdialysis probe (membrane length, 10 mm; membrane diameter, 0.5 mm) was introduced into the brain parenchyma distantly from the resection. We used CMA/20 microdialysis probes (molecular size cutoff, 20,000 Da; molecular size of rifampin, 823 Da), a CMA/102 perfusor, and a CMA/142 collector (Microdialysis AB, Stockholm, Sweden). The probe was then perfused with two solutions of different rifampin concentrations (0.25 and 0.5 μg/ml in the first two patients and 0.5 and 1.0 μg/ml in the following six patients) for 1 h each at a rate of 3 μl/min, resulting in 180 μl for each dialysate. As described in our animal study (11), rifampin concentrations in the CES were determined by calculating the loss or gain of rifampin in the two solutions, using the no-net-flux method (also called the equilibrium method, or the Lönnroth method) (6). The resulting rifampin concentrations in the CES represent the average concentration in the CES over a period of 2 h. For the calculation of the rifampin concentrations in the CES, the actual rifampin concentrations in the perfusates were determined, since rifampin solutions were stored in plastic vials before the commencement of the experiments. Due to the well-known adherence of rifampin to plastic surfaces, these determinations are indeed crucial in order to obtain accurate results. We found a 60.5% ± 14% ($n = 8$) reduction with the 0.5-μg/ml perfusates and a 54.8% ± 18% ($n = 6$) reduction with the 1.0-μg/ml perfusates.

Tissue samples. Intraoperatively, tissue samples were taken from unaffected brain tissue which was resected with pole resections, from perifocal tissue sur-

TABLE 1. Patient characteristics

Patient	Gender[a]	Age (yr)	Tumor location	Operation	Histological diagnosis
1	M	57	Temporo-occipital left	Left occipital lobectomy	Glioblastoma multiforme
2	F	62	Occipital left	Left occipital lobectomy	Glioblastoma multiforme
3	M	25	Frontal right	Right frontal craniotomy	Astrocytoma WHO II
4	M	71	Frontal right	Right frontal pole resection	Anaplastic astrocytoma WHO III
5	F	71	Frontal left	Left frontal pole resection	Oligodendroglioma WHO II[b]
6	M	70	Occipital left	Left occipital lobectomy	Glioblastoma multiforme
7	M	40	Temporal left	Left temporal pole resection	Astrocytoma WHO II[c]
8	M	77	Temporal left	Left temporal pole resection	Glioblastoma multiforme

[a] M, male; F, female.
[b] Intraoperative histology was anaplastic astrocytoma WHO III.
[c] Small pockets of astrocytoma WHO III.

rounding the tumor, and from tumor tissue. At the same time, 5 ml of peripheral venous blood was drawn from the patient in order to determine concentrations of rifampin in serum. Brain tissue samples were briefly and gently rinsed with 0.9% saline until they appeared macroscopically free of contaminating blood. The gently rinsed brain tissue was homogenized with a Teflon homogenizer in 0.9% saline (1:1 [wt/vol]) before the determination in the bioassay. Tissue concentrations were calculated by multiplying the concentration in the homogenate by 2.

Bioassay and standard solutions. The rifampin concentrations of all samples were determined by an agar diffusion bioassay using *Sarcina lutea* as the test strain, as previously described (11, 17). In addition, the perfusates' concentrations, which were used for calculation, were determined by the same bioassay. This was realized to be important for accurate results because of a considerable loss of rifampin at plastic surfaces. Standard solutions were prepared from a commercially available intravenous preparation of rifampin by diluting stock solutions with pooled serum, resulting in a final concentration in serum of 50%. Identical standard curves were obtained with standards prepared in 0.9% saline or 50% serum, respectively. Therefore, all specimens (rifampin perfusate, serum, CES dialysates, and tissue homogenates) were assayed on the same plate with the same 50% serum standards. The bioassay has a detection limit of 0.05 μg/ml and does not differentiate between rifampin and its metabolites. The agar diffusion assay has an excellent correlation ($r = 0.988$) with high-pressure liquid chromatography (18). Since this assay does not differentiate between rifampin and its metabolites, the total antimicrobial activity is determined, which is adequate for the question under investigation. The reproducibility of our assay was good, with an intraassay coefficient of variation of 2.3% and an interday coefficient of variation of 4.4%.

Statistical methods. Data are reported as means ± standard deviation. For multiple comparisons we performed the analysis-of-variance test.

RESULTS

From February 1996 until July 1997, eight patients fulfilled the study criteria and were evaluated. Patient characteristics are listed in Table 1. None of the eight patients had any side effects of the study drug, and no intraoperative or postoperative complications occurred. Rifampin concentrations in the various compartments are listed in Table 2. Serum samples and whole tissue samples were taken beginning 77 ± 43 min after the infusion of rifampin was completed. Sampling of sera and tissues was performed within a period of 23 ± 15 min. Microdialysis was started 67 ± 28 min after the infusion of rifampin was completed in seven patients and 44 min before the completion of the infusion in one patient (patient 6). Microdialysis samples were obtained within a 125-min time period for all patients. Rifampin concentrations in tissue samples from brain tumors were higher than in tissue samples from normal brain tissue or the CES. A typical example of the determination of rifampin concentrations in the CES according to the no-net-flux method is shown in Fig. 1. For the first two patients, the CES values could not be calculated because the actual rifampin concentrations in the perfusates were not determined. For these two patients (patients 1 and 2), we determined the rifampin concentrations in the serum and tissue samples only (Table 2). For patient 3, the rifampin concentration in normal brain tissue could not be determined because no pole resection was performed (Table 1). The differences in rifampin concentrations in the various CNS compartments did not gain statistical significance due to the high variability of the levels of rifampin in the tissue samples.

DISCUSSION

This study is unique because it is the first in which actual drug concentrations were measured in the CES in humans. Microdialysis allows for direct in vivo measurement of drug concentrations in the CES. Interstitial drug concentrations are

TABLE 2. Rifampin concentrations in the various compartments

Patient	Amt (μg/ml [%]) of rifampin in:				
	Serum[a]	Tumor tissue	Perifocal tissue	Normal brain tissue	CES
1	13.34	2.74 (20.5)	0.86 (6.5)	0.42 (3.2)	ND[b]
2	9.85	3.92 (39.8)	2.08 (21.1)	0.30 (3.0)	ND
3	12.20	0.36 (2.9)	0.22 (1.8)	ND	0.39 (3.2)
4	7.48	1.60 (21.4)	0.90 (12.0)	0.20 (2.7)	0.49 (6.6)
5	14.40	1.20 (8.3)	0.48 (3.3)	0.56 (3.9)	0.23 (1.6)
6	9.86	0.33 (3.3)	0.15 (1.5)	0.18 (1.8)	0.24 (2.4)
7	11.76	0.20 (1.7)	0.03 (0.3)	0.18 (1.5)	0.34 (2.9)
8	8.32	0.58 (7.0)	0.24 (2.9)	0.16 (1.9)	0.20 (2.4)
Mean ± SD	10.90 ± 2.43	1.37 ± 1.34 (12.6)	0.62 ± 0.67 (5.7)	0.29 ± 0.15 (2.7)	0.32 ± 0.11 (2.9)

[a] The concentration of rifampin in serum is taken as 100%.
[b] ND, not done.

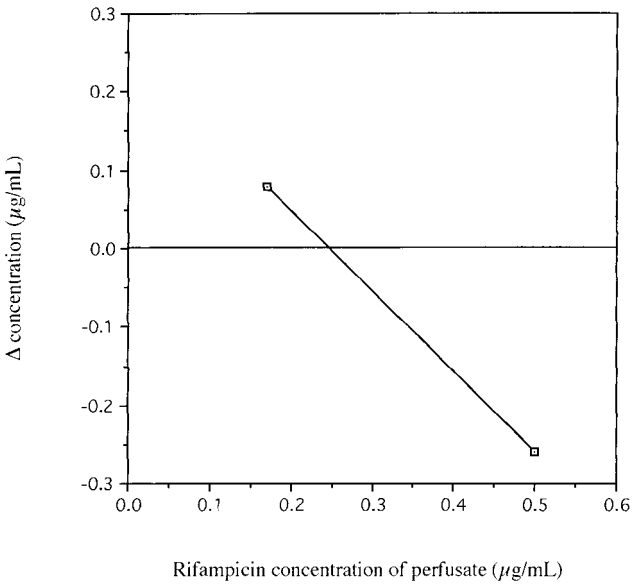

FIG. 1. Rifampin in the CES of patient 6. The determination of the concentration of rifampin in the CES in one patient illustrates the principle of the no-net-flux method. The probe was first perfused with a 0.5-μg/ml solution of rifampin at a constant flow rate of 3 μl/min, resulting in 180 μl of dialysate after 1 h. It was then perfused with a 1.0-μg/ml solution of rifampin under the same conditions. The concentrations of rifampin in the perfusates and the dialysates were determined together with those in the other samples and were used for calculation. The concentration of rifampin in the CES was calculated from the perfusates' loss or gain in rifampin. The intercept with the zero line indicates equilibrium, i.e., equal concentrations inside and outside the microdialysis probe. At this intercept, the concentration of rifampin in the CES (0.24 μg/ml) was determined from the *x* axis. This represents the average concentration of rifampin in the CES over a period of 2 h.

relevant in antibacterial treatment, since bacteria mainly spread through the extracellular space. Until now, skin blisters were the most common model for measurement of drug concentrations in interstitial fluids (19). Recently, microdialysis and microvoltametry have been used to determine concentrations of antibiotics in the CES (3, 8–11). As a method, microvoltametry has the disadvantage that it cannot distinguish between bound and unbound drug. It has the advantage of high temporal resolution. Microdialysis measures unbound drug only, but it has the disadvantage of a worse temporal resolution. In our model, one concentration point of rifampin in the CES represents the average drug concentration during a 2-h period.

Our data show a clear trend in the penetration of rifampin into the various compartments of the human brain. Apart from values in serum, the highest rifampin concentrations were found in the tissue of primary brain tumors, followed by perifocal tissue, CES, and normal brain tissue. Interestingly, concentrations of rifampin in the CES were in the same range as those in samples of normal brain tissue. Both compartments had almost fivefold-lower rifampin concentrations than tumors. Furthermore, the variability of levels of rifampin in tumor and perifocal tissues is high, probably due to the highly variable impairment of the blood-brain barrier. This indicates that the standard method for determining levels of drugs in brain tissues by measuring their concentrations in tissue samples from brain tumors yields nonreproducible results and, in the case of rifampin, overestimates its penetration into normal brain tissue or the CES by a factor of 4 to 5. This overestimation may be especially important in the late treatment course, when the blood-brain barrier is restored.

Anatomically and functionally, the blood-brain barrier differs from the blood-CSF barrier (14). In the case of rifampin, this functional difference becomes obvious, since rifampin penetrates into the CSF at a rate about 10-fold that for the CES (13). Similar discrepancies between the blood-brain barrier and the blood-CSF barrier have been observed for fusidic acid (12) and clindamycin (15).

Our data lend theoretical support to the clinically observed effectiveness of rifampin in the treatment of cerebral staphylococcal and streptococcal infections. Rifampin penetrates into the CES following a standard dose of 600 mg given intravenously, reaching concentrations exceeding those necessary for the treatment of staphylococcal and streptococcal infections. Yet CES concentrations may be below MICs for some mycobacterial strains, indicating why the treatment of brain tuberculoma with a standard dosage of antimycobacterial drugs may fail (5).

This new method for determining the levels of drugs in the CES provides information on their penetration into the interstitial space of the CNS or any other organ in vivo. In the case of rifampin, microdialysis provides data which are significantly less scattered, and therefore more reproducible, than data derived from whole-tissue samples from a variety of CNS compartments. This method should be suitable for measurements of other drugs in the CES, such as other antibiotics, anticonvulsants, or psychotropic drugs.

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