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Effect of severe protein-calorie malnutrition on the penetration kinetics of trimethoprim and sulfamethoxazole to the deep tissues of Wistar rats

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Abstract

This study shows the effect that severe malnourishment has on the kinetics of antibiotic penetration in tissues. A total of 104 male Wistar rats, 21 days old, were randomly divided into eight groups. Five groups of experimental rats were severely malnourished (SM) and three further groups were considered well-nourished control groups (WN). A single dose of trimethoprim–sulfamethoxazole (TMP-SMX) was administered intraperitoneally. Blood samples were taken by heart puncture and five organs were extracted 0–24 h after the administration of the drug. HPLC was used to assess the amount of trimethoprim and sulfamethoxazole in fluids. The elimination half-life for trimethoprim from plasma was longer in SM rats with a median of 3.15 h; in WN rats, it was 0.390 h. Clearance was slower in SM rats: 646.72 mL μg^{-1} h^{-1} vs 3036.38 mL μg^{-1} h^{-1} in WN rats ($P < 0.05$). Tissue penetration was much higher for trimethoprim, with penetration indexes of 0.80–5.66 in WN rats, compared with 0.35–2.14 in SM rats. In the case of sulfamethoxazole, penetration indexes were 0.029–1.13 for WN and 0.075–0.657 for SM rats. Similarly, the penetration ratio to muscle and heart tissue was lower in SM rats. However, penetration to kidney, lung, liver and spleen was greater in SM rats. It is evident that severe SM decreases the capacity of trimethoprim more importantly than sulfamethoxazole biotransformation.

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Introduction

When antibiotics are administered, they usually penetrate in adequate concentrations to sites of biological action where microorganisms commonly reside. Therapeutic success will depend on the balance between the dynamic interaction of the antibiotic and the host's response mechanism, as well as other factors such as the sensitivity to the antibiotic (Mattie et al 1987).

In studies on the therapeutic assessment of antibiotics, tissue penetration plays an important role, since many infections are located in deep tissues, or more specifically in the extracellular tissue space (Bruun et al 1981). Due to methodological reasons, many of the experimental trials on the penetration of antibiotics to tissues are frequently based on artificial compartments, similar to tissue cages or swollen skin. However, it is worth noting that the relationship between time and the concentration in these compartments does not exactly correspond to real tissue concentrations (Chisholm 1978).

Studies on antibiotic distribution have shown that blood (serum) levels do not necessarily reflect the concentration in deep tissues, which is frequently 50% less (Chisholm et al 1973). Studies by Waterman & Kastan (1972) emphasize that there is a direct relationship between the drug dose (cefalotin) and the level that can be achieved in the interstitial fluid; antibiotic concentrations in the serum and interstitial fluids showed the same time–concentration response regardless of the dose. The immediate high serum concentrations decreased to less than those of the interstitial fluid in two hours. After fifteen minutes, interstitial fluid concentrations were not significantly different from maximum levels recorded later. Other authors

(Chisholm et al 1976; Agerso & Friis 1998) state that, in spite of high serum concentrations, it may be that adequate drug concentrations comparable to those reached in blood are never attained in tissue, because they are often modified by various factors.

Antibiotic penetration to superficial and deep tissues determines the clinical efficacy and the toxic potency of antibiotics; therefore, knowledge of tissue distribution principles is essential for the rational management of antimicrobial agents (Nix et al 1991a).

Certain pharmacokinetic characteristics of trimethoprim and sulfamethoxazole are similar. Both are rapidly absorbed, but differ in tissue distribution. Sulfamethoxazole binds to plasma proteins ($62 \pm 5\%$), is eliminated unaltered through urine (60%), and it has been shown that it is the active chemotherapeutic fraction of a sulfonamide that is not metabolized and not linked to proteins. The active chemotherapeutic fraction of trimethoprim comprises metabolites I, III and IV in the same proportion as the unaltered compound; it binds to proteins ($37 \pm 5\%$) and is eliminated unaltered through urine (80%) (Sigel et al 1973). These observations also hold true for other antibacterial compounds, as well as trimethoprim (Acar et al 1973; Mandell & Petri 1996). This concurs with the fact that drug concentration in tissue interstitial fluid is the same as in plasma. In fact, the changes in plasma are reflected at tissue level, reaching different concentrations depending on drug distribution and penetration index. This information is very significant to assess the amount of drug available in any of an organism's tissues (Nix et al 1991b).

This study shows the effect of severe malnourishment on the kinetics of antibiotic penetration in tissues, and uses mathematical models to describe the distribution of these antimicrobial compounds in different tissues. This is based on the fact that severe malnourishment causes pathogenic changes that may modify the penetration of antibiotics, often characterized by biochemical, functional and anatomical variations as described by Jolliffe and Barac-Nieto (Jolliffe et al 1950; Barac-Nieto et al 1978). Consequently, changes produced in tissues may require a modification in organ size (anatomical changes) as a consequence of tissue depletion, secondary to low-protein food content (Ramos & Cravioto 1958; Tirapegui & De Angelis 1985). In addition, there are changes in the bodily composition of extra- and intracellular spaces (biochemical changes), characterized by an increase in total water content and in tissue spaces, as well as functional and pharmacokinetic changes that cause malnourishment (Lares-Asseff et al 1992, 1997).

Material and Methods

Population

A total of 104 male Wistar rats, 21 days old (at weaning), were randomly divided in eight groups (13 rats in each group). Five groups of experimental rats were severely malnourished (SM) and three further groups were considered as being well-nourished control groups (WN). From that moment on, the groups were fed as follows: all groups had free access to food and water; the control group was fed rat chow with an average of 4.20 g of protein per day; malnourished rats received food altered in protein, carbohydrate and fat content, so that they consumed 2.4 g of protein per day. Both groups had a daily energy consumption of 88.00 Kcal per day, the normal daily requirements of a rat (Newberne et al 1978). All rats were weighed daily and the pharmacokinetic study was performed when any of the SM rats lost 40% or more body weight with respect to their control (criteria used for assessing severe malnourishment) (Gomez 1946).

The study protocol was approved by the Institutional Care and Use of Laboratory Animals Committee (CULAC), in accordance with the Mexican Official Norm (NOM-062-ZOO-1999, Daily Gazette, 22 August 2001) of the National Institute of Pediatrics-SSA.

Pharmacokinetic study

For the purpose of this study, a single intraperitoneal dose of trimethoprim–sulfamethoxazole (TMP-SMX) (Bactrim, Roche) ($8\,mg\,kg^{-1}$ of trimethoprim and $40\,mg\,kg^{-1}$ of sulfamethoxazole) was administered. Blood samples were taken by heart puncture and rats were bled to death. Organs (spleen, kidney, liver, heart, lungs and muscle) were extracted at time 0 (first sample, untreated rats), 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10 and 24 h after the administration of the drug, using one rat per sampling time. The organs were weighed and later homogenized in 10 mL saline solution and frozen at $-70\,^{\circ}C$ until analysed.

Analytical procedure

The Vree T. B. method for high performance liquid chromatography (HPLC) was used to assess trimethoprim and sulfamethoxazole amounts in biological fluids (Vree et al 1978). This method was validated in our laboratory, obtaining the following results: sulfamethazine was used as internal standard, with a linearity of $0.3–5\,\mu g\,mL^{-1}$ for trimethoprim and $5–100\,\mu g\,mL^{-1}$ for sulfamethoxazole ($r = 0.996$ for trimethoprim; $r = 0.998$ for sulfamethoxazole); 97.7% accuracy was found for trimethoprim in plasma, 96% in muscle, 96% in lungs, 97% in spleen, 97.1% in heart, 96.9% in kidneys and 93.7% in liver; 96% for sulfamethoxazole in plasma, 91.7% in muscle, 94.8% in lungs, 102.0% in spleen, 99.1% in heart, 91.6% in kidneys and 93.4% in liver. The variation coefficient for trimethoprim was 11% for plasma, 4.4% for muscle, 3.3% for lungs, 6.9% for spleen, 2.9% for heart, 4% for kidneys and 6.37% for liver, and for sulfamethoxazole it was 10.01% for plasma, 4.2% for muscle, 4.3% for lungs, 2.2% for spleen, 4.0% for heart, 2.6% for kidneys and 3.9% liver. Recovery for trimethoprim was $98.5 \pm 3.5\%$ in plasma and 101.3 ± 9.8 for muscle, 94.3 ± 11.7 for lungs, 93.6 ± 8.1 for spleen, 104.7 ± 9.4 for heart, 100.2 ± 3.5 for kidneys and 96.0 ± 6.7 for liver; and for sulfamethoxazole it was $96.97 \pm 4.5\%$ in plasma and 95.7 ± 5.1 for muscle, 100.1 ± 5.8 for lungs, 99.3 ± 6.5 for spleen, 94.3 ± 4.9 for heart, 99.2 ± 7.1 for kidneys and 101.7 ± 4.3 for liver.

Pharmacokinetic analysis

The plasma and organ pharmacokinetic profiles from the concentration versus time data were adjusted using the non-linear regression method and the minimum weighted squared procedure (1/C), employing the Winnonlin program version 1.1. Adjustment of the experimental data to the pharmacokinetic model was assessed using the following criteria: sum of the square of the residuals, F test, Akaike's information criteria (Yamoaka et al 1978) and lack of systemic deviations with respect to the adjusted curves. All kinetics were adjusted to a one-compartment open model (MAUC) (Gibaldi & Perrier 1982a). The relative exposure of trimethoprim and sulfamethoxazole (tissue penetration) to the different tissues was assessed using the area under the curve (AUC) of the trimethoprim and sulfamethoxazole concentrations versus time in each compartment in comparison to the plasma compartment. The AUC was calculated using the trapezoid method (Gibaldi & Perrier 1982b), obtaining percent of penetration for both drugs in each of the studied organs.

Statistical analysis

The pharmacokinetic parameters between the groups of well-nourished and malnourished rats were compared using $x \pm$ s.d. values with Student's *t*-test whenever data complied with the criteria to apply parametric tests (interval scale, normal distribution and small data dispersion) and with the Mann-Whitney U test using median and range values. When data did not comply with criteria to apply parametric tests, the option was to apply non-parametric tests (nominal or ordinal scales, asymmetric distribution and wide data dispersion) (Castilla & Cravioto 1991); $P < 0.05$ was considered significant.

Results

No statistically significant differences were found in average weight at weaning (21 days old) and before proceeding to misfeed the malnourished group of rats: the body weight of well-nourished rats was 56.7 ± 5.1 g and that of malnourished rats was 59.8 ± 6.5 g ($P > 0.05$). However, the body weight of the malnourished rats at 43 days of age (71.9 ± 7.3 g) showed statistically significant differences compared with the weight of the well-nourished rats (144.1 ± 12.8 g; $P < 0.05$).

The pharmacokinetic parameters obtained with both drugs and for both groups in all the studied organs are shown in Table 1 for trimethoprim and Table 2 for sulfamethoxazole. The elimination half-life (K10HL) for trimethoprim from plasma was longer in malnourished rats with a median of 3.15 h (range 1.11–3.31 h), while in well-nourished rats it was 0.390 h (0.375–0.626 h, $P = 0.01$). Clearance (CL_t) was slower in malnourished rats: 646.72 mL μg^{-1} h^{-1} (range 497.1–2820.1) vs 3036.38 mL μg^{-1} h^{-1} (range 2322.1–3050.0) in well-nourished rats ($P < 0.05$). It should be noted that the plasma area under the curve (AUC) was practically four times larger in malnourished rats, with a median equal to 12.33 and range of 2.63–16.08 μg mL^{-1}·h vs 2.62 (2.60–3.44 $\mu g\,mL^{-1}$·h) in well-nourished rats ($P < 0.05$). The same behaviour was seen in some of the organs studied (liver and spleen) where clearance was slower (longer half-life or lesser clearance, with a greater AUC in malnourished rats). However, the AUC for muscle tissue was twice as large in well-nourished rats. It should be noted that the distribution volume in organs was significantly lower in the liver and heart of malnourished rats.

Table 2 shows the differences observed in the pharmacokinetic parameters of plasma sulfamethoxazole between well-nourished and malnourished rats, none considered to be statistically significant with a 0.05 confidence level. As seen for trimethoprim pharmacokinetics, there is an evident increase in the lung and spleen AUC, as well as an increase in the maximum concentrations in malnourished rats with respect to the values observed in well-nourished rats in the lung, spleen, kidney, liver and heart. However, the AUC and maximum concentration (C_{max}) were lower in malnourished rat muscle. All other pharmacokinetic parameters were statistically significant.

Figure 1 shows sulfamethoxazole concentration profile in plasma and the various organs. There are evident differences between the concentration-vs-time profiles for well-nourished and malnourished rats with higher concentrations and larger AUCs in malnourished rats (except for muscle tissue).

Figure 2 describes the trimethoprim concentration profiles in plasma and the different organs vs time, clearly showing that the AUCs are larger in malnourished rats, and that in both groups the AUC for the spleen and kidney are larger than for plasma.

Figure 3 and Table 3 show the penetration indexes (organAUC/plasmaAUC) for sulfamethoxazole and trimethoprim in different tissues. In the case of sulfamethoxazole, statistically significant differences were only found in the spleen when penetration indexes were compared, although the tendency in most organs was for penetration indexes to be larger in malnourished than in well-nourished rats (with the exception of muscle, which showed the opposite tendency ($P = 0.03$)). For trimethoprim, the penetration indexes were significantly higher in the kidney, lung and muscle tissue ($P = 0.01$) in well-nourished rats. Despite the penetration indexes being higher in the liver, spleen and heart tissue of well-nourished rats, not all differences were statistically significant for trimethoprim (Table 3).

Discussion

Craig & Kunin (1973) studied the tissue distribution of trimethoprim and sulfamethoxazole in Rheusus monkey tissues after establishing a balance of serum levels comparable to those for humans, showing that non-acetylated sulfamethoxazole concentrations are higher in serum than in kidney, lung and heart tissue. In contrast, the trimethoprim tissue concentrations were much higher than in serum, except in the brain, skin and fatty tissue. This was confirmed by studies conducted by Schwartz &

Table 1 Pharmacokinetic parameters of trimethoprim in well-nourished and malnourished rats.

Parameter	Well-nourished rats	Malnourished rats	*P*	Significance
Plasma				
K10 (h^{-1})	1.77 (1.10–1.83)	0.219 (0.209–0.618)	0.01	s**
AUC ($\mu g\ mL^{-1}{\cdot}h$)	2.62 (2.60–3.44)	12.33 (2.63–16.08)	0.03	s**
K01HL (h)	0.050 (0.021–0.094)	0.118 (0.026–0.165)	0.03	s**
K10HL (h)	0.390 (0.375–0.626)	3.15 (1.11–3.31)	0.01	s**
CL_t (mL $\mu g^{-1}\ h^{-1}$)	3036.38 (2322.11–3050.04)	646.7 (497.1–2820.1)	0.03	s**
Lungs				
K10HL (h)	2.15 (1.89–2.31)	2.50 (2.24–2.84)	0.03	s**
Spleen				
AUC ($\mu g\ mL^{-1}{\cdot}h$)	8.97 (6.50–18.54)	26.36 (10.10–36.40)	0.03	s**
C_{max} ($\mu g\ g^{-1}$)	2.67 (1.28–4.03)	5.35 (2.70–12.35)	0.03	s**
Cl_t (mL $\mu g^{-1}\ h^{-1}$)	889.39 (431.03–1225.86)	302.72 (219.42–790.06)	0.03	s**
Kidneys				
VD/F (mL μg^{-1})	1208.24 ± 207.81	1931.23 ± 580.50	0.04	s*
K10 (h^{-1})	2.43 ± 0.662	9.63 ± 2.43	0.001	s*
t_{max} (h)	0.843 ± 0.136	0.335 ± 0.071	0.001	s*
K01HL (h)	0.272 (0.228–0.401)	0.079 (0.051–0.100)	0.01	s**
Liver				
VD/F (mL μg^{-1})	14023.55 ± 2847.68	6336.74 ± 1456.54	0.005	s*
AUC ($\mu g\ mL^{-1}{\cdot}h$)	2.42 ± 0.709	4.56 ± 0.962	0.01	s*
C_{max} ($\mu g/g$)	0.528 ± 0.158	1.16 ± 0.238	0.004	s*
CL_t ($ml/\mu g/h$)	3459.27 ± 887.08	1799.79 ± 299.14	0.01	s*
Heart				
VD/F (mL μg^{-1})	3747.76 ± 355.50	1644.80 ± 427.1	0.0006	s*
K01HL (h)	0.090 ± 0.027	0.433 ± 0.037	0.00008	s*
t_{max} (h)	0.440 ± 0.072	1.182 ± 0.156	0.0003	s*
C_{max} ($\mu g\ g^{-1}$)	1.89 ± 0.342	3.31 ± 0.919	0.02	s*
K01 (h^{-1})	6.78 (6.36–11.66)	1.62 (1.52–1.82)	0.01	s**
Muscle				
AUC ($\mu g\ mL^{-1}{\cdot}h$)	14.54 ± 2.27	7.36 ± 2.57	0.006	s*
C_{max} ($\mu g\ g^{-1}$)	2.42 (2.40–2.43)	1.33 (0.424–2.06)	0.01	s**

s, significant statistical difference; *Student's *t*-test; **U Mann-Whitney test.

Rieder (1970), using rats as the experimental model and finding that sulfamethoxazole plasma concentrations were higher, while trimethoprim plasma concentrations were lower than in tissues.

In a study carried out by Tartaglione et al (1991), antimicrobial tissue penetration was studied in a rat model with epididymitis due to *E. coli*. They found that the AUC ratio of infected vs non-infected rats was 1.05 for trimethoprim and 1.58 for sulfamethoxazole, except for trimethoprim where tissue concentrations were significantly higher in infected rats than in epididymal levels of non-infected rats ($P < 0.05$). The antibiotic concentrations in infected epididymi compared with serum concentrations revealed a penetration of 66% for sulfamethoxazole and 256% for trimethoprim.

In another study, Reeves & Wilkinson (1979) showed that trimethoprim and sulfamethoxazole concentrations in diverse body fluids and in different tissues differ considerably with respect to blood concentrations, given the different distribution patterns of trimethoprim and sulfonamides in the body. As a rule, the ratios of trimethoprim-to-sulfonamide concentrations are higher in most of the fluids and tissue secretions than in blood. These observations were confirmed by our results despite severe malnourishment. Figure 3A shows that tissue penetration is much higher for trimethoprim, independently of the accompanying nutritional state, than for sulfamethoxazole, with penetration indexes that vary from 0.80 to 5.66 in well-nourished rats for trimethoprim, compared with penetration indexes of 0.35–2.14 in malnourished rats. For sulfamethoxazole, penetration indexes are 0.029–1.13 in well-nourished and 0.075–0.657 in malnourished rats. Similarly, the penetration ratio to tissue is lower in malnourished rats. However, penetration to the spleen is greater in malnourished rats.

Figure 3B shows that, in practically all organs, the penetration ratio of trimethoprim is much lower in malnourished rats ($P < 0.05$ in all cases).

Several other studies have confirmed lower penetration of trimethoprim in tissues. This was observed for trimethoprim in lungs by Morel et al (1982) and by Seppänen (Seppänen 1980), where trimethoprim tissue penetration is

Table 2 Pharmacokinetic parameters of sulfamethoxazole in well-nourished and malnourished rats.

Parameter	Well-nourished rats	Malnourished rats	*P*	Significance
Plasma				
VD/F (mL μg^{-1})	334.18 ± 93.90	262.85 ± 29.94	0.2	n.s*
K10 (h^{-1})	0.122 ± 0.020	0.127 ± 0.034	0.20	n.s*
K10HL (h)	5.77 ± 0.873	5.60 ± 1.13	0.18	n.s*
C_{max} ($\mu g\ g^{-1}$)	121.24 ± 37.01	142.76 ± 15.3	0.037	n.s*
CL_t (mL $\mu g^{-1}\ h^{-1}$)	40.67 ± 12.89	32.55 ± 4.32	0.33	n.s*
K01 (h^{-1})	10.25 (7.80–28.75)	8.64 (4.41–9.88)	0.12	n.s**
AUC ($\mu g\ mL^{-1}$·h)	911.57 (773.69–1497.13)	1262.4 (999.38–1328.87)	0.28	n.s**
K01HL (h)	0.068 (0.024–0.088)	0.080 (0.070–0.150)	0.12	n.s**
t_{max} (h)	0.450 (0.185–0.547)	0.510 (0.457–0.840)	0.12	n.s**
Lungs				
C_{max} ($\mu g\ g^{-1}$)	8.14 ± 1.48	12.22 ± 2.19	0.02	s*
AUC ($\mu g\ mL^{-1}$·h)	44.48 (38.51–57.74)	100.04 (55.12–158.22)	0.03	s**
CL_t (mL $\mu g^{-1}\ h^{-1}$)	889.29 (690.47–1034.19)	394.22 (220.86–717.23)	0.03	s**
Spleen				
AUC ($\mu g\ mL^{-1}$·h)	234.24 ± 17.24	842.42 ± 169.87	0.04	s**
CL_t (mL $\mu g^{-1}\ h^{-1}$)	158.12 ± 17.96	47.17 ± 11.20	0.0002	s*
VD/F (mL μg^{-1})	2700.1 (2308.80–4345.36)	972.9 (654.05–1813.69)	0.01	s**
K01 (h^{-1})	6.45 (5.64–32.14)	3.94 (0.370–45.11)	0.01	s**
C_{max} ($\mu g\ g^{-1}$)	14.09 (9.12–16.49)	33.74 (20.61–59.74)	0.01	s**
Kidneys				
VD/F (mL μg^{-1})	2919.37 ± 722.18	1605.06 ± 291.23	0.02	s*
t_{max} (h)	0.220 ± 0.069	1.20 ± 0.321	0.0009	s*
C_{max} ($\mu g\ g^{-1}$)	14.08 ± 3.11	24.01 ± 4.27	0.009	s*
K01 (h^{-1})	33.48 (24.38–34.61)	4.61 (2.14–5.06)	0.01	s**
K01HL (h)	0.020 (0.020–0.020)	0.160 (0.136–0.322)	0.01	s**
Liver				
VD/F (mL μg^{-1})	6554.9 (6454.71–11677.0)	3486.8 (1537.4–4667.2)	0.01	s**
K10 (h^{-1})	17.33 (1.16–32.94)	36.85 (26.54–48.25)	0.03	s**
K01HL (h)	0.039 (0.020–0.590)	0.018 (0.010–0.020)	0.01	s**
t_{max} (h)	0.251 (0.180–2.32)	0.160 (0.110–0.210)	0.03	s**
C_{max} ($\mu g\ g^{-1}$)	5.75 (2.72–6.12)	11.30 (8.40–25.68)	0.001	s**
Heart				
t_{max} (h)	0.747 ± 0.163	1.88 ± 0.150	0.0002	s*
VD/F (mL μg^{-1})	5654.31 (2691.41–7089.8)	2031.0 (1454.0–2774.09)	0.03	s**
K01 (h^{-1})	6.38 (5.15–12.40)	1.90 (1.61–2.41)	0.01	s**
K01HL (h)	0.100 (0.50–0.130)	0.360 (0.286–0.420)	0.01	s**
C_{max} ($\mu g\ g^{-1}$)	7.03 (5.48–14.08)	7.48 (13.28–23.12)	0.03	s**
Muscle				
C_{max} ($\mu g\ g^{-1}$)	58.61 ± 15.57	24.93 ± 6.94	0.01	s*
VD/F (mL μg^{-1})	665.00 (413.16–858.97)	1616.10 (132.50–1896.4)	0.01	s**
AUC ($\mu g\ mL^{-1}$·h)	1031.87 (1003.75–1464.3)	438.98 (207.48–987.75)	0.01	s**

ns, non-significant differences; s, significant statistical difference; *Student's *t*-test; **U Mann-Whitney test.

evident (prostate gland, epididymis, testicles in man) and 2–4 times lower compared with sulfonamide penetration.

Based on the results obtained with respect to the elimination half-life of trimethoprim, which was longer in malnourished rats with slower clearance, it is evident that severe malnourishment decreases the capacity of trimethoprim elimination more markedly than that of sulfamethoxazole, which although somewhat modified, is not statistically significantly different. In addition, it should be noted that for trimethoprim, the lower clearance observed in SM rats is probably explained by decreased renal clearance, since this drug is eliminated 80% unaltered compared with sulfamethoxazole, which is eliminated 70% unaltered. Malnourishment is known to decrease renal clearance as well as metabolic clearance (Lares-Asseff et al 1992, 1997, 1999). Sulfamethoxazole undergoes extensive hepatic metabolism (hydroxylation via CYP2C9 is the major pathway, with acetylation and glucuronidation also taking place). Only 30% of sulfamethoxazole is recovered unchanged in the urine, with total urinary recovery of parent drug and metabolites being in the range 84–100%. We think that the changes in

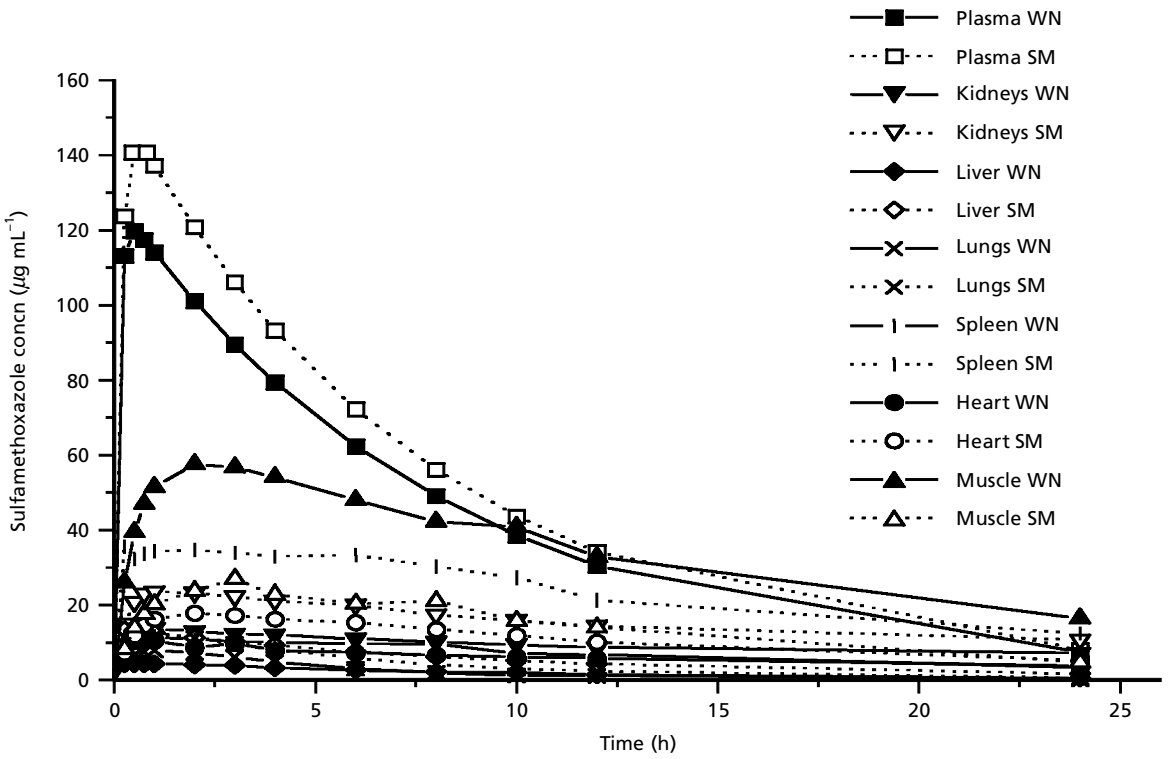

Figure 1 Plasma and tissue concentrations of sulfamethoxazole in the studied organs.

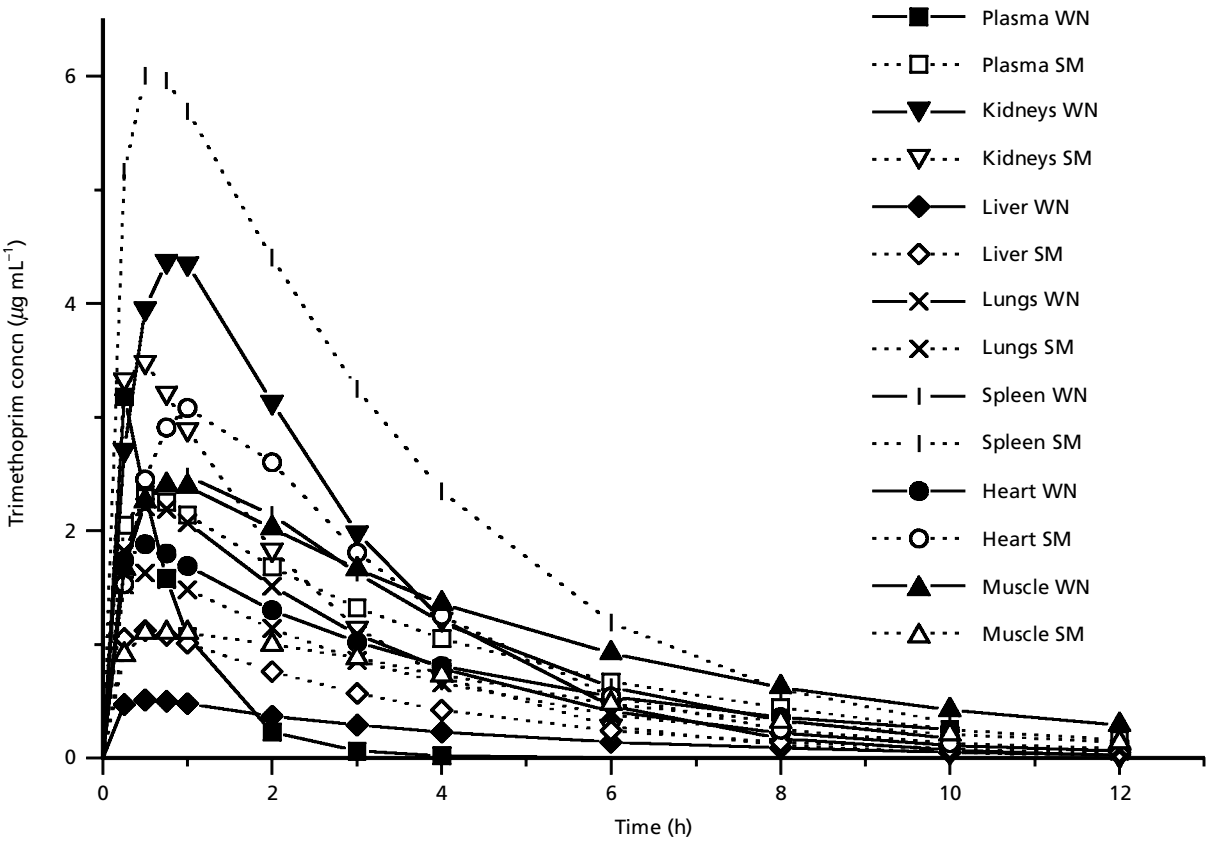

Figure 2 Plasma and tissue concentrations of trimethoprim in the studied organs.

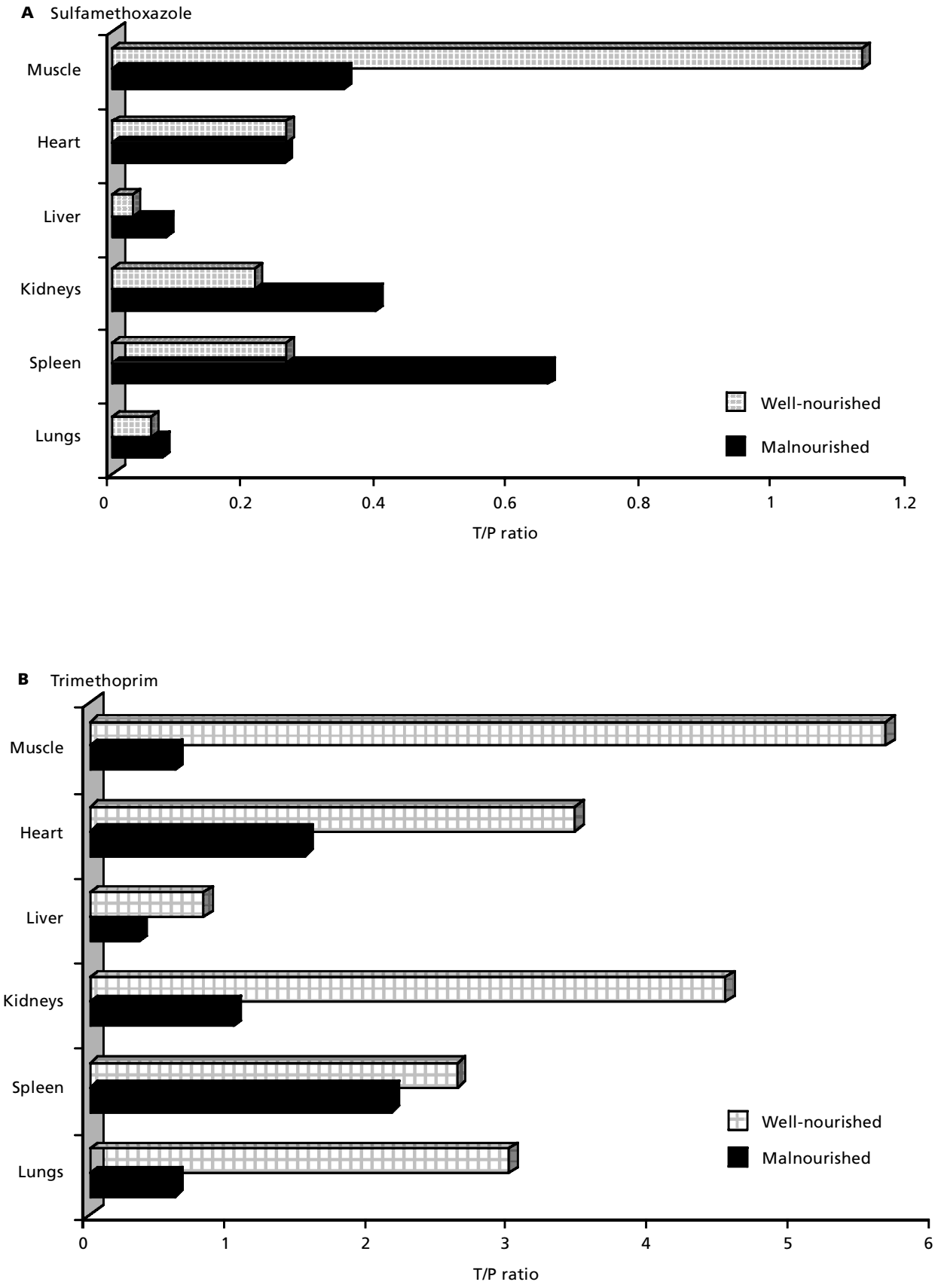

Figure 3 Penetration indexes (T/P ratio) of (A) sulfamethoxazole and (B) trimethoprim in organs of well-nourished and malnourished rats.

Table 3 Penetration indexes of sulfamethoxazole and trimethoprim to the different tissues (T/P ratio) in well-nourished and malnourished rats.

Tissue	Sulfamethoxazole T/P ratio		U value	Trimethoprim T/P ratio		U value
	WN rats	SM rats		WN rats	SM rats	
Kidney	0.214	0.397	6	4.52	1.02	0*
Liver	0.029	0.081	3.5	0.80	0.35	3
Lungs	0.057	0.075	4	2.98	0.606	0*
Spleen	0.263	0.657	6.9*	2.61	2.14	3
Heart	0.262	0.259	7	3.45	1.53	4
Muscle	1.13	0.348	1*	5.66	0.605	0*

*Significant statistical differences.

trimethoprim and sulfamethoxazole elimination half-life and clearance, as well as the differences in volume of distribution, may influence the changes in drug availability.

This is evident in Figure 1 wherein plasma concentration profiles vs time are compared in well-nourished and malnourished rats. Higher concentration profiles are found in malnourished rats with larger AUC.

With respect to these results, it is evident that the nutritional state of the rat has certain influence on trimethoprim and sulfamethoxazole pharmacokinetics. For both drugs, the kinetics vary in malnourished and well-nourished rats, as well as the functional changes and body-composition alterations caused by severe malnourishment (Gomez 1946; Ramos & Cravioto 1958; Tirapegui & De Angelis 1985; Whitehead & Alleyne 1972). This is also the case when total body-water content increases, and when the extracellular-space water content rises in subjects with severe malnourishment. This, in turn, exerts an important influence on antibiotic tissue penetration.

Even if the recorded data were obtained from experimental models that cannot be directly extrapolated to man, special care should be taken when a treatment scheme is to be recommended with these antibiotics in infected patients with severe malnourishment.

References

Acar, J. F., Goldstein, F., Chabbert, Y. A. (1973) Synergistic activity of trimethoprim-sulfamethoxazole on Gram-negative bacilli: observations in vitro and in vivo. *J. Infect. Dis.* **128** (Suppl.): 470–477

Agerso, H., Friis, C. (1998) Penetration of amoxycillin into the respiratory tract tissues and secretions in pigs. *Res. Vet. Sci.* **64**: 245–250

Barac-Nieto M., Spurr G. B., Lotero H., Maksud M. G. (1978) Body composition in chronic undernutrition. *Am. J. Clin. Nutr.* **31**: 23–40

Bruun, J. N., Ostby, N., Bredesen, J. E., Kierulf, P., Lunde, P. K. (1981) Sulfonamide and trimethoprim concentrations in human serum and skin blister fluid. *Antimicrob. Agents Chemother.* **19**: 82–85

Castilla, L., Cravioto, J. (1991) *Estadística simplificada para la investigación en ciencias de la salud.* Trillas, México, pp 174–181

Chisholm, G. D. (1978) The tissue cage model in the distribution of antibacterial agents. *Scand. J. Infect. Dis. Suppl.* **14**: 118–124

Chisholm, G. D., Waterworth, P. M., Calnan, J. S., Garrod, L. P. (1973) Concentration of antibacterial agents, in interstitial tissue fluid. *Br. Med. J.* **1**: 569–573

Chisholm, G. D., Smith, C. B., Waterworth, P. M., Calnan, J. S. (1976) Factors influencing the distribution of antibacterial agents in interstitial tissue fluid: molecular size and protein binding. *Infection* **4** (Suppl. 2): 123

Craig, W. A., Kunin, C. M. (1973) Distribution of trimethoprim-sulfamethoxazole in tissues of Rhesus monkeys. *J. Infect. Dis.* **128**: S575–S579

Gilbaldi, M., Perrier, D. (1982a) *Pharmacokinetics.* Marcell Dekker, New York, pp 1–43

Gilbaldi, M., Perrier, D. (1982b) *Pharmacokinetics.* Marcell Dekker, New York, pp 293–296

Gomez, F. (1946) Desnutrición. *Bol. Med. Hosp. Infant. Mex.* **3**: 543

Jolliffe, N., Tisdall, F. F., Cannon, P. R. (1950) *Clinical nutrition.* Paul B Heeber Inc., New York

Lares-Asseff, I., Cravioto, J., Santiago P., Pérez-Ortíz, B. (1992) Pharmacokinetics of metronidazole in severely malnourished and nutritionally rehabilitated children. *Clin. Pharmacol. Ther.* **51**: 42–50

Lares-Asseff, I., Zaltzman, S., Pérez, G. G., Camacho, G. A., Murguía, T, López, M. C., Toledo, A. R., Zaltzman-Rudy, A. B., Cravioto, J. (1997) Pharmacokinetics of cyclosporine as a function of energy-protein deficiency in children with chronic renal failure. *J. Clin. Pharmacol.* **37**: 179–185

Lares-Asseff, I., Flores, P. J., Juárez, O. H., Ramírez, L. M., Loredo, A. A., Carbajal, R. L. (1999) Influence of nutritional status on the pharmacokinetics of acetylsalicylic acid and its metabolites in children with autoimmune disease. *Am. J. Clin. Nutr.* **69**: 318–324

Mandell, L. G., Petri, A. W. (1996) Fármacos antimicrobianos (continuación) sulfonamidas, trimetoprim-sulfametoxazol, quinolonas, y fármacos contra infecciones de vías urinarias. In: Goodman, G. A., Hardman, G. J., Limbird, L. E., Molinoff, P. B., Ruddon, R. W. (eds) *Las bases farmacologicas de la terapéutica.* 9th edn, vol II, McGraw-Hill Interamericana Editores, S. A. de C.V. México, pp 1123–1140

Mattie, H., Hoogeterp, J., Hermans, J. (1987) The relation between plasma and tissue concentrations of antibiotics. Description of a method. *J. Pharmacokinet. Biopharm.* **15**: 191–202

Morel, C., Langeard, M., Vergnaud, M., Monrocq, N. (1982) Lung tissue diffusion of intravenous trimethoprim-sulfamethoxazole combination. *Pathol. Biol. (Paris)* **30**: 380–384

Newberne, P. M., Bieri, J. G., Briggs, G. M., Nesheim, M. C. (1978) Control of diets in laboratory animal experimentation. *ILAR News* **XXI**: A2–A12

Nix, D. E., Goodwin, S. D., Peloquin, C. H. A., Rotella, D. L. (1991a) Antibioitic tissue penetration and its relevance: models of tissue penetration and their meaning. *Antimicrob. Agents Chemother*. **35**: 1947–1952

Nix, D. E., Goodwin, S. D., Peloquin, C. H. A., Rotella, D. L., Schentag, J. J. (1991b) Antibiotic tissue penetration and its relevance: Impact of tissue penetration on infection response. *Antimicrob. Agents Chemother*. **35**: 1953–1959

Ramos, G. R., Cravioto, J. (1958) Desnutrición, concepto y ensayo de sistematización. *Bol. Med. Hosp. Infant. Mex*. **15**: 763

Reeves, D. S., Wilkinson, P. J. (1979) The pharmacokinetics of trimethoprim and trimethoprim/sulphonamide combinations, including penetration into body tissues. *Infection* **7**: S330–S341

Schwartz, D. E., Rieder, J. (1970) Pharmacokinetics of sulfamethoxazole + trimethoprim in man and their distribution in the rat. *Chemotherapy* **15**: 337–365

Seppänen, J. (1980) The penetration of sulfadiazine, sulfamethoxazole and trimethoprim into the prostate gland, epididymis and testis in man. *Ann. Clin. Res.* **25**: 47–51

Sigel, C. W., Grace, M. E., Nichol, C. A. (1973) Metabolism of trimethoprim in man and measurement of a new metabolite: a new fluorescence assay. *J. Infect. Dis.* **128** (Suppl.): 580–583

Tartaglione, T. A., Taylor, T. O., Opheim, K. E., See, W. A., Berger, R. E. (1991) Antimicrobial tissue penetration in a rat model of E. coli epididymitis. *J. Urol.* **146**: 1413–1417

Tirapegui, J. O., De Angelis, R. C. (1985) Marginal protein deficiency in pregnant rats. Changes in offspring body composition. *Arq. Gastroenterol*. **22**: 83–87

Vree, T. V., Hekster, Y. A., Baar A. M., Damsma J. E., Van der Kleijin, E. (1978) Determination of trimethoprim and sulfamethoxazole (co-trimoxazole) in body fluids of man by means of high-performance liquid chromatography. *J. Chromatogr*. **146**: 103–112

Waterman, N. G., Kastan, L. B. (1972) Interstitial fluid and serum antibiotic concentrations. *Arch. Surg*. **105**: 192–196

Whitehead, R. G., Alleyne, G. A. (1972) Pathophysiological factors of importance in protein-calorie malnutrition. *Br. Med. Bull.* **28**: 72

Yamoaka, K., Nakagawa, T., Uno, T. (1978) Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetics equations. *J. Pharmacokinet. Biopharm*. **6**: 165–175