ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1991, p. 1953–1959
0066-4804/91/101953-07$02.00/0

Vol. 35, No. 10

Antibiotic Tissue Penetration and Its Relevance: Impact of Tissue Penetration on Infection Response

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Fluid penetration. Given that distribution in extracellular fluid (ECF) is important, a variety of methods have been applied to the collection and study of data on ECF distribution. Antibiotic concentrations have been measured in the lymphatic drainage of organs and tissues (95, 96), fluids obtained from tissue cage reservoirs (82, 90), chemically or mechanically induced skin blisters (1, 81), surgically implanted cotton threads (28, 81), implanted fibrin clots (3), and directly from inflammatory exudates (72). This diversity of models has confused the interpretation of antibiotic extravascular fluid distribution, particularly in comparisons between members of the same class of compounds. Whenever possible, comparisons of drugs should be made under identical conditions.

Antibiotics leave the vasculature and enter ECF via passive diffusion through the spaces between vascular endothelial cells. Thus, the surface area of vascular tissue in relation to the total volume of the tissue to be sampled is the most important consideration in the modeling of ECF (26). The commonly used tissue penetration models differ in the vascular tissue surface area/volume ratio at the measurement site (28, 77, 81, 93). In addition, the inflammatory response induced by the procedure (81, 104) and the presence of cellular debris in the sample (i.e., paper disks or cotton threads) (81) may influence results. Given these multiple sources of confusion, it is vital to define general principles of antibiotic extravascular fluid penetration and then interpret the outcomes of specific fluid models within this context.

In the tissue cage method, small, inert spheres or tubes are surgically implanted in subcutaneous tissue (12). The surface of the device contains multiple pores, each 1 to 2 mm in diameter. The implantation procedure causes an inflammatory response that may continue for 2 to 3 weeks (31). After 4 weeks, the fluid contained in the reservoir is biochemically similar to ECF (25, 31). One great advantage of this method is that multiple samples can be obtained over a period of days or weeks. One must be careful to maintain sterility when puncturing the device to collect fluid samples, or infection may complicate the data. After several weeks, the growth of vascular or fibrous tissue may limit the proper functioning of the device. The tissue cage device itself is usually spherical, with a relatively small diffusion surface area in relation to a large fluid volume. As a result, the peak antibiotic concentration in the device generally lags behind the peak antibiotic concentration in the blood or in true ECF (30, 93). Steady-state concentrations normally reflect complete equilibration between the fluid in the device and the free drug concentration in serum (30, 92).

Skin blister models are perhaps more useful in estimating antibiotic concentrations in ECF. Noninflammatory blisters are usually induced by applying a block containing 8-mm-diameter holes to the inner surface of the forearm. A vacuum of 4.3 lb/in^2 is applied to each hole for approximately 2 h. This procedure raises blisters, each containing 0.1 to 0.2 ml of fluid. The fluid is transudate with a protein content lower than that of serum. Inflammatory and exudative blisters can be chemically induced with a plaster containing 0.2% cantharidin ointment (102). Since several blisters are formed with either of these procedures, multiple samples can be obtained by emptying the contents of individual blisters with a sterile needle and syringe at appropriate sampling times. Fluid present in the inflammatory blisters contains a higher protein content than does ECF, so the antibiotic concentration appears higher than that present in true ECF for protein-bound drugs (88, 104).

Another technique for measuring antibiotic diffusion into ECF involves implanting cotton threads in subcutaneous tissue (81). The cotton threads are sterile and uniform in length and weight. At various times, the threads are removed and weighed to determine fluid uptake. Antibiotic concentrations are measured by coiling the thread into the wells of a microbiological assay plate. Inaccuracies may result because of drug adherence to the cotton fibers or the presence of tissue proteins that become attached to the cotton fibers.

Skin abrasions may be formed with a high-speed rotating buff. Sterile paper disks of known weight are then applied to the abraded skin site to absorb transudate fluid. After removal, the filter paper disks are reweighed and assayed by a standardized microbiological assay. Weighing and assaying must proceed immediately to avoid errors caused by the evaporation of fluid from the disks.

Fluid penetration at passive diffusion sites. At the steady state, the free concentration of drug in serum is equal to the free concentration of drug in ECF, provided that passive diffusion is the only operational mechanism. ECF models which provide a small sampling compartment with a large diffusible surface area/volume ratio lead to rapid equilibration with serum (28, 81). Examples include cotton threads and paper disks on abraded skin. However, if the surface area/volume ratio is small, such as in large fluid-filled capsules, the antibiotic concentrations will not reflect true ECF distribution, except at the steady state on continuous infusion. This is particularly true of long-half-life antibiotics (30). For drugs with low protein binding (aminoglycosides, quinolones, and some β-lactams), concentrations in ECF are virtually identical to concentrations in serum with large surface area/volume ratio models like cotton threads (28, 81). The ECF penetration of cephalosporins which show relatively high protein binding is also affected by the protein concentration in ECF. Models can then be constructed to account for ECF binding (91). These models must consider the concentration of albumin in extravascular spaces (8, 67) and model changes in albumin when applicable. The closer

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TABLE 1. ECF distribution ratios for β-lactams, quinolones, and aminoglycosides at the steady state[a]

Site	Fluid/serum ratio for:		
	Aminoglycosides	β-Lactams	Quinolones
CSF	0.08–0.25	0.1–0.3	0.2–0.4
Pleural fluid	1.00	1.00	1.00
Synovial fluid	0.90	1.00	1.00
Peritoneal fluid	1.00	1.00	1.00
Saliva	0.05	0.05	1.00
Urine	>500:1	>200:1	>500:1

[a] Data are adapted from reference 83.

the ECF protein concentration to serum protein concentration, the closer the agreement in total concentration between serum and ECF. For protein-bound drugs, the area under the concentration-time curve for free drug in ECF is equivalent to that for free drug in serum at the steady state (22, 26, 67). In general, fluids such as pleural fluid, peritoneal fluid, noninflamed wound drainage, or synovial fluid equilibrate with serum by passive diffusion. A comprehensive listing of concentrations at these sites for individual antibiotics has been published (32). We have compressed and summarized the essence of these data in Table 1 (83). The data in this table represent our interpretation considering both known principles of passive distribution and the variety of models used. In addition, Table 1 contains data from sites which do not obey the rules of passive diffusion, such as urine, cerebrospinal fluid (CSF), and saliva.

Sites affected by diffusion barriers or transport processes. There are extravascular sites in which antibiotic concentrations cannot be predicted by use of passive diffusion principles alone. Some of these, such as urine or bile, are sites of active transport. Others, like the aqueous humor and CSF, represent permeability barriers. As in the case of penicillins, active transport mechanisms may also influence concentrations in CSF by transporting penicillins out of CSF. In general, all three major classes of antibacterial agents are concentrated in urine, as all are eliminated by glomerular filtration and/or renal tubular secretion. Biliary secretion appears passive for aminoglycosides, while it is active for a few β-lactams and quinolones. Some β-lactams, such as cefamandole and cefoperazone, are highly transported into bile. Bile is the primary route of excretion for cefoperazone.

Serum protein binding. Comprehensive studies and reviews of the effect of serum protein binding on tissue penetration are abundant in the literature (4, 15, 17, 18, 39, 44, 60, 68, 70, 100–103). Protein binding appears to affect such pharmacokinetic parameters as distribution volume and renal elimination (33, 34, 64) and clearly influences in vitro susceptibility testing (14, 45). The effect on in vivo antibacterial activity is not as clear. The in vivo effects of protein binding are still surrounded by controversy, although there are a few clinical demonstrations of poor activities of highly bound drugs (13, 51, 54). Most of these examples involve staphylococci, both in vitro and in vivo.

Most antibiotics bind preferentially to albumin but may also bind to other proteins. Binding may occur via ionic, hydrogen, and hydrophobic interactions; therefore, minute variations in chemical structure may produce great differences in the extent of protein binding (16). Factors affecting the extent of protein binding include antibiotic concentration, nature and concentration of protein, pH, lipid solubility, competition with other drugs or biological components

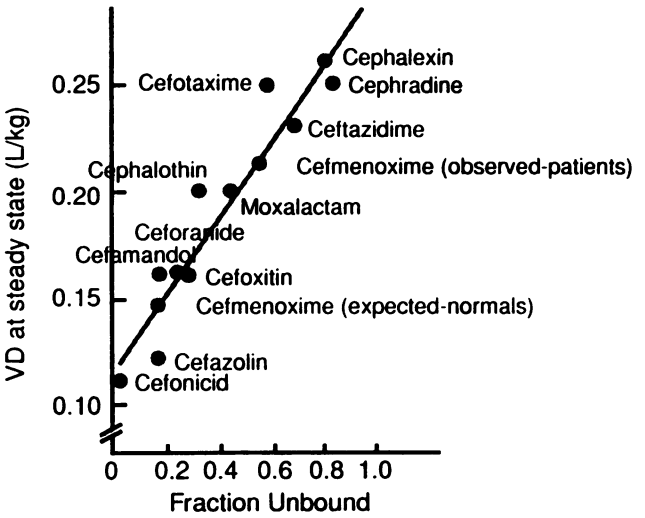

FIG. 1. Relationship between fraction of unbound drug (F_c) in serum and total volume of distribution at steady state (V_{ss}) for 11 cephalosporins (1-g intravenous dose). The cefmenoxime coordinate observed in 20 pneumonia patients is illustrated, as is the expected coordinate for normal sera on the basis of the observed protein binding in normal individuals. The regression equation is as follows: total $V_{ss} = 0.0167F_c + 0.115$ ($r = 0.948$). This figure is adapted from Fig. 1 of reference 75.

(e.g., bilirubin or fatty acids), and disease states, such as renal failure (53, 60, 65, 74, 89). Critical illness and a host of associated conditions presumably shift protein binding in patients, as shown in Fig. 1, in which cefmenoxime protein binding was shown to be different in critical-care patients and normal volunteers (75). Disease-associated alteration of protein is a novel observation and clearly needs much more study. Virtually all human protein binding data are collected in healthy subjects.

Two methods are commonly used to determine the free fraction of an antibiotic: equilibrium dialysis and ultrafiltration. Ultracentrifugation has also been reliably used to determine the extent of protein binding; however, its use is limited (69). All three methods have been discussed extensively in the literature (5, 43, 46). Because each method is complicated by methodological problems, Jusko and Gretch (41) suggested measuring protein binding by at least two different methods. A comparison of data from several methods can yield similarities or differences which require further exploration. Antibiotic serum protein binding has been tabulated with the method of determination specified (17). In general, the aminoglycosides (37) and quinolones (62) have low plasma protein binding. The β-lactams, on the other hand, have low to very high plasma protein binding (101).

Fluid distribution but exclusion from cells of β-lactam antibiotics. It is apparent that extravascular β-lactam concentrations depend on the method of measurement. Conventional assays of homogenized tissue assume a uniform distribution of the drug throughout the tissue (79). This assumption is particularly incorrect for β-lactam antibiotics, because they do not penetrate well into eucaryotic cells (71) and because they are almost exclusively confined to the much smaller space approximating the sum of plasma water and interstitial fluid (79). Measurement of β-lactam concentrations in homogenized tissue artificially lowers the estimate of the concentrations in tissue by dilution and thereby gives the false impression that these drugs diffuse poorly into

infection sites. Concentrations of ampicillin, cefuroxime, and ceftazidime in homogenized tissue sections are approximately 1/5 to 1/10 concentrations obtained simultaneously in ECF and serum (78, 79). The lack of intracellular penetration of these β-lactams explains these results. Calculations such as those in Table 1 in the accompanying paper (60a) show how drugs which do not penetrate cells but which are evenly distributed throughout blood and ECF can produce the false impression of low biophase penetration. In contrast, steady-state or postdistributive concentrations of these antibiotics in ECF obtained with cotton threads or paper disks are similar to the corresponding concentrations in serum (78–80). On the basis of ECF models, one would conclude that β-lactam antibiotics penetrate extracellular infection sites very well. This conclusion is underscored by the successful clinical use of β-lactam antibacterial agents in the prophylaxis and treatment of extracellular infections.

Tissue binding. Although not as important for the β-lactams, the effect of antibiotic binding to cellular components cannot be neglected because the serum antibiotic concentration is the net result of binding in both serum and tissues. In addition to extravascular albumin, antibiotics may bind to such tissue components as cell membranes, intracellular organelles, macromolecules, and products of inflammation (11, 27). β-Lactams are primarily bound to extracellular serum proteins, and quinolones are primarily bound inside cells (24, 27, 38, 40). The aminoglycosides undergo extensive and nearly irreversible intracellular binding to organelles and lysosomes (47). Aminoglycoside intracellular binding is a slow process because of poor membrane permeability, and its antimicrobial impact is negligible. However, these antibiotics serve as the prime example of the role of tissue binding in toxicity. The impact of aminoglycoside tissue binding can be appreciated by comparing the single-dose tissue/serum ratio with the value at the steady state (see Table 2 in the accompanying paper [60a]). This table demonstrates the impact of slow tissue accumulation on the aminoglycoside tissue/serum ratio in a nonexcretory organ, such as muscle or liver (85). Tissue/serum ratios for aminoglycosides are 200-fold higher in the kidneys than in nonexcretory organs (85). Although not all investigators agree, the weight of evidence favors the conclusion that these high intracellular concentrations interact with phospholipids, leading to the development of nephrotoxicity in both animal models (6, 9, 49) and patients (36, 84). Although aminoglycosides are negligibly bound to serum proteins, binding may be interrupted by high concentrations of divalent cations (37, 73). These cations also antagonize their antibacterial activity (35, 56).

The quinolones have very large volumes of distribution and high tissue/serum ratios, apparent even after a single dose (see Table 3 in the accompanying paper [60a]). Currently approved quinolones are less than 40% bound by serum proteins and are widely distributed throughout the body (62). Their large volumes of distribution primarily reflect the large fraction of the total body load (at least 60%) which is inside cells (see Table 3 in the accompanying paper [60a]). The intracellular binding of quinolones is of lower affinity than that of aminoglycosides. Consequently, the terminal half-lives of the former are measured in hours rather than in days.

In summary, serum protein binding appears to be most important for the β-lactam antibiotics and far less important for the available members of the other two classes. Tissue binding in lysosomes and mitochondria plays a major role in toxicity (but not efficacy) for aminoglycosides (36, 47). Quinolones inside cells are thus far not linked to any serious toxicity, but the large intracellular fraction clearly alters pharmacokinetic parameters, such as apparent volume of distribution and serum half-life, and it may enhance efficacy against intracellular pathogens.

ECF distribution with essentially irreversible intracellular binding of aminoglycosides. As is apparent from Table 2 in the accompanying paper (60a), aminoglycoside ECF distribution profiles are somewhat similar to those of β-lactams, while their intracellular distribution profiles are more similar to those of quinolones. Their tissue homogenate profiles are depicted in Fig. 4 in the accompanying paper (60a). Like quinolones, these drugs are not significantly bound to serum proteins and demonstrate a rapid distributive phase after intravenous injection. For the first dose, the distribution volume approximates the ECF volume, and as a result, aminoglycoside concentrations in ECF are similar to concentrations in serum at virtually all permeable, nonexcretory sites (83, 85). After single doses, tissue/serum ratios can be approximated by estimating the volume of ECF in each tissue. There is virtually no penetration of the drugs into cells after single doses. Table 2 in the accompanying paper (60a) shows how these drugs behave after single doses and why the relevant antibacterial concentrations of aminoglycosides are most likely the concentrations in serum, as suggested by studies correlating concentrations in serum to clinical cure (57, 63).

When multiple dosing is considered, aminoglycosides slowly accumulate inside cells. Intracellular aminoglycoside transport is essentially a one-way process because of the high-affinity bond which forms inside cells at the level of lysosomes and mitochondria (36, 47). In sharp contrast, the binding of quinolones is more avid and of a higher capacity but is readily reversible (as revealed by the 10-fold-shorter half-life of these drugs). Aminoglycosides translocated into cells are tightly bound and microbiologically inactive. These drugs offer little potential benefit for treating intracellular pathogens (20, 42) and, in fact, are now being explored as liposomal formulations in an attempt to increase their intracellular activity (42). Aminoglycoside tissue/serum ratios approach 2:1 at the steady state in most organs (see Table 2 in the accompanying paper [60a]), except in the kidneys, in which this ratio approaches 200:1 (83, 85). The processes of intracellular uptake are similar between the kidneys and other organs, but the rate of renal tissue uptake is much more rapid. As a consequence, nephrotoxicity is the major dose-limiting toxicity. As the steady state is approached, serum and ECF aminoglycoside concentrations rise and the steady-state volume of distribution exceeds 2 liters/kg (85). The central compartment volume of distribution, a marker of ECF space, remains unchanged at 0.25 to 0.3 liter/kg (85). the microbiologically active portion of the aminoglycoside body load is only that in serum and ECF. This fraction of the body load is accessible to extracellular bacteria. Thus, serum aminoglycoside concentrations are predicted to be the most useful index of the antimicrobial actions of these drugs (83), as has been recently demonstrated in two studies (57, 63).

ECF distribution with reversible intracellular binding of quinolones. The fluoroquinolone antibiotics have homogenate tissue/serum ratios markedly different from those of the β-lactams and single-dose aminoglycosides. Quinolone distribution in a tissue homogenate is shown in Fig. 3 in the accompanying paper (60a). The quinolone distribution ratios are in excess of 1:1 at most tissue sites (59, 83). Tissue/serum ratios of this magnitude create excitement among investiga-

tors who believe that tissue homogenate ratios reflect infection site concentrations higher than those in serum. It is tempting to conclude that blood quinolone concentrations would not need to exceed the MIC for the pathogen for a drug to be effective. However, the intracellular penetration of the quinolones may not confer automatic advantages in the treatment of extracellular pathogens, since one consequence of high tissue partitioning is low extracellular concentration. On the basis of leukocyte partitioning and measured tissue/serum ratios, at least 60% of the body load of quinolones is bound inside cells (24, 83). The large apparent volume of distribution for these agents, exceeding 2.0 liters/kg, is consistent with extensive intracellular penetration (62). The calculations in Table 3 in the accompanying paper (60a) show that intracellular quinolone concentrations must exceed those in serum by as much as fivefold to produce tissue/serum ratios of 2:1 (62). This distribution profile is advantageous when the infecting bacterial are intracellular. For intracellular pathogens, quinolones may be the antibiotics of choice, since aminoglycosides and β-lactams are virtually excluded from intracellular infection sites.

Blister fluid penetration studies show good penetration of these agents into extravascular fluids (1, 19, 48), but the ECF concentrations are virtually identical to those in serum (Table 1). Unlike tissue homogenate ratios, ECF concentrations are not twofold greater. Furthermore, recent studies in our pneumonia patients suggest that the response of extracellular pathogens is best when peak concentrations in serum exceed four to eight times the MIC and trough concentrations in serum remain higher than the MIC (61, 66). Dose regimens must be designed to achieve concentrations in serum or ECF above the bacterial MIC for the treatment of extracellular infections. This goal is relatively easy to achieve, given the fact that ECF models show concentrations similar to those shown by serum models (19, 48). From a pharmacokinetic perspective, the difference between quinolones and β-lactams is reflected in a quinolone distribution volume in excess of 2.0 liters/kg and a resulting lower clearance from serum. Quinolones have a longer serum half-life, even in the absence of high serum protein binding. Thus, the major impact of quinolone tissue binding is on the pharmacokinetic parameters steady-state distribution volume and half-life.

Tissue distribution of other antibiotic classes. The tissue distribution principles discussed above also apply to other classes of antibiotics. On the basis of their large volumes of distribution and limited partitioning studies, it can be postulated that vancomycin and teicoplanin are partitioned into most cells (99) and are concentrated in the kidneys (105). Thus, their normal tissue/serum ratios are approximately 1:1 (94). Both of these agents, particularly teicoplanin, show some tissue binding and accumulation on multiple dosing (94). In this aspect, they resemble the aminoglycosides. Both of these agents are large molecules, being eliminated only by glomerular filtration. Their serum half-lives exceed those of most β-lactams, and in the case of teicoplanin, the half-life approaches the terminal half-lives of the aminoglycosides. Chloramphenicol, rifampin, and macrolides such as erythromycin and the azalide azithromycin behave in many ways like the quinolones. These drugs reversibly penetrate cells and are highly partitioned into many body cells. Particularly in the case of azithromycin, the long serum half-life is partially a result of this intracellular partitioning. In contrast to quinolones, however, many of these agents are extensively metabolized. Finally, colistin, polymyxin, and amphotericin B display kinetic, tissue binding, and toxicology profiles analogous to those of the aminoglycosides. They enter cells slowly, but their high-affinity intracellular binding produces large steady-state volumes of distribution and very long terminal half-lives.

Target concentrations and active sites for efficacy. At nonexcretory sites permeable to these drugs, aminoglycosides, β-lactams, and quinolones show predictable distribution volumes and predictable tissue/serum ratios. However, the tissue homogenization method is a seriously misleading index of bacterial exposure to antibiotics. This fact is particularly true for drugs, such as the β-lactams and aminoglycosides, not homogeneously distributed. Although some bacteria are trapped inside macrophages or leukocytes (50), bacteria in tissue infections reside primarily in the extracellular space of that tissue. β-Lactams and aminoglycosides are excluded from these intact cells (10, 50) and are confined to blood and ECF. Thus, in extracellular infections, concentrations in serum reflect infection site drug concentrations. The concentration in serum is thus the most likely correlate of antibacterial efficacy. Quinolones are located primarily intracellularly. Therefore, much of the quinolone body load is sequestered away from the extracellular infection site. However, the sequestered quinolone serves as a reservoir to prolong exposure of bacteria in the extracellular compartments and to extend the half-life in serum. Quinolone dosages against intracellular pathogens should also be selected to achieve target concentrations in serum in relation to the bacterial MIC or MBC.

Thus, all three classes of antibiotics are likely to have antibacterial activities which can be closely related to their concentrations in serum. Evidence is rapidly accumulating in both animal models (29, 55, 76, 97, 98) and humans (22, 57, 61, 63, 86) that concentrations in serum in relation to the MIC are the best predictors of response, even for tissue site infections.

Examining these relationships for free drug is the most conservative way to evaluate new and existing agents (22). Both the low homogenate tissue/serum ratios of aminoglycosides and β-lactams and the high homogenate tissue/serum ratios of quinolones are potentially misleading when they are used to describe the responses of extracellular gram-negative bacteria. Concentrations in serum may predict antibacterial responses better than concentrations in tissue, provided that bacteria are extracellular. This hypothesis would hold true at all sites which do not have permeability barriers. It may not extend to central nervous system infections or abscess cavities, which potentially have barriers to passive diffusion. At such sites, concentrations must be measured to determine their relationship to those in serum. Further studies must then be done to measure site-specific antagonism of their concentration-versus-effect relationships.

For antibiotics in general, the inadequate tissue penetration hypothesis has been strongly challenged by studies demonstrating correlations between concentrations in serum and efficacy against tissue infections. Studies with aminoglycosides (21, 22, 57, 63), β-lactams (22, 23, 29, 86), and quinolones (55, 61, 66) have demonstrated correlations between concentrations in serum and responses of infections at extravascular sites. Some of the same studies have demonstrated good relationships independent of the degree of serum protein binding (86). The primary effects of protein binding are slowing of excretion (30) and reduction of volumes of distribution (60). While further study of the bound fraction versus antibiotic efficacy is clearly needed, clinicians appear generally satisfied with the efficacy of antibiotics showing high and low protein or tissue binding

(58). Decreased efficacy has only been demonstrated with staphylococci exposed to antibiotics showing very high protein binding, exceeding 90% (13). Perhaps sufficient free drug is always present to inhibit most bacteria, given that antibiotics showing high protein or tissue binding show the resulting increases in half-lives and contact times.

Therefore, dosages and dosing intervals for the common extracellular gram-negative and gram-positive pathogens should be designed with full consideration of the relationships between serum antibiotic concentrations and MICs (2, 87). Tissue homogenate ratios distract investigators from this important consideration. For β-lactams (86) and tentatively for quinolones (55, 61, 66), trough concentrations in serum should be maintained above the MIC and/or MBC. Studies of the phenomenon of breakthrough bacteremia strongly amplify the concept of maintaining antibiotic concentrations above the inhibitory levels for the entire dosing interval (2). For aminoglycosides, trough concentrations may fall below the MIC for a period of time, allowing the advantageous use of the postantibiotic effect (52). The length of time that aminoglycoside trough concentrations may safely be below the MIC without the loss of efficacy remains an unanswered question, actively being studied at the present time.

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