Possible Role of the Blood-Testicular Barrier in Dominant Lethal Testing

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During dominant-lethal testing for mutagenic effects, chemicals are usually administered intraperitoneally or orally to male animals as a single dose. These treated animals are subsequently housed for mating with virgin females which are replaced at weekly intervals. For screening purposes, a reduction in total living implants is monitored as an indication of the induction of chromosomal anomalies which result in fetal deaths. This serial mating approach further allows the investigator to identify the spermatogenic cell type affected by the test chemical (*1*).

It is generally assumed that the test chemical gains access readily to the cells in the seminiferous tubules, tubuli recti, rete teste, efferent ducts, epididymis, and vas deferens. However, such an assumption ignores many pharmacokinetic concepts regarding the distribution of chemicals in the body and the role of biologic barriers (*2*). An especially important barrier in this regard could be the so-called blood–testicular barrier which has been adequately described only in the last few years (*3–6*). These reports and recent studies in our own laboratory (*7, 8*) as well as other physiologic and anatomical aspects of the testes suggest the possibility of many "false negative" results of the dominant-lethal test, i.e., instances where the test results could be negative for a true mutagenic agent.

To produce any toxic effect, an environmental chemical must achieve adequate concentrations at its sites of action. This is obviously a function of exposure. However, the concentrations attained also depend upon the extent and rate of the chemical's absorption, distribution, binding, or localization in tissues, inactivation, and excretion. These factors are diagrammed in Figure 1 and are discussed further by Rall (*9*).

After a chemical is absorbed into the blood stream, it must enter or pass through the various body fluid compartments—plasma, extracellular (interstitial) fluid, transcellular fluids, and intracellular fluids. Some chemicals cannot pass cell membranes easily and therefore are restricted in their distribution and in their potential effect, whereas others readily pass through cell membranes and thereby distribute throughout all fluid compartments. Thus, cellular and intracellular membranes present a formidable barrier to the penetration of many environmental agents to their intracellular sites of action. Even if the membranes serve only to slow somewhat the rate of penetration of a particular chemical, they still afford a degree of protection for the target tissue. This is especially true in cases of acute exposure, in which they restrict the exposure to the agents to sites of accumulation, metabolism, and excretion.

Despite anatomical differences, the diffu-

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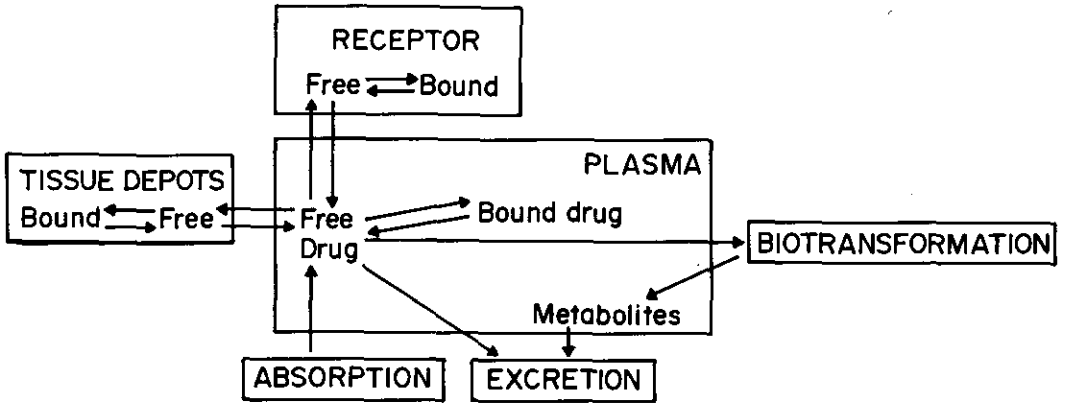

FIGURE 1. Pharmacokinetic factors which interact to determine the penetration of a chemical to its site of action or toxicity.

sion and transport of chemicals across these various biological boundaries are remarkably similar. The ways in which substances move across biologic membranes can be grouped under two general headings: passive transfer and specialized transport processes. When a chemical penetrates a living membrane by simple passive diffusion, its rate of transfer is directly proportional to the concentration gradient across the membrane. A number of lipid-insoluble substances of relatively small molecular size diffuse across the membrane as if it were interspersed with small, water-filled channels or pores. Other substances move across the membrane as if it were a layer of lipoid material, the speed of passage being determined by the lipid-to-water partition coefficient of the substances. Most drugs and other environmental agents are weak acids or bases and exist in solutions as a mixture of the ionized and nonionized forms. The nonionized form is usually lipid-soluble, and the ionized moiety lipid insoluble. Only the nonionized substance will diffuse rapidly across a lipoid membrane. The proportion of drug in the nonionized form depends on the dissociation constant (pK) of the compound and on the pH of the solution in which it is dissolved (*2*).

In addition to the blood–testicular barrier, which will be considered in more detail, there are other examples of biologic compartments which display characteristics of restricted entry for various foreign chemicals. A classic example is the entry of chemicals into the central nervous system. The blood–brain barrier is well studied and can serve as a model system with which to compare the blood–testicular barrier. The so-called blood–brain barrier is not located at the surface of brain cells. Transfer of chemicals across neuronal cell membranes is like that across any other cell membrane. The barrier exists between the plasma and extracellular space of the brain at the capillary walls or the surrounding layers of glial cells. Lipid-insoluble chemicals and inorganic and organic ions enter the brain much more slowly than do lipid-soluble sub-

stances. The rate of entrance is also proportional to the size of the molecule. Carrier-mediated transport is generally restricted to endogenous substances. Nonionized chemicals of high fa+ solubility readily enter the central nervous system. Penetration is least with quaternary ammonium ions, various conjugated metabolites, and other chemicals possessing a high degree of dissociation and/or low lipid solubility or high molecular weight. After a substance has entered the central nervous system, it returns to the blood stream in two or three different ways: (1) by passive diffusion, (2) by filtration as the cerebrospinal fluid (CSF) drains into the blood through the large channels of the arachnoid villi which is analogous to the systemic lymphatics, and/or (3) by active transport from CSF to blood across the epithelium of the choroid plexus. In addition, the continual production of cerebrospinal fluid results in its circulation from the ventricles over the surfaces of the brain and down the spinal cord. CSF production is an active process which can act against the hydrostatic pressure of the venous circulation to insure a continuous turnover of the fluid which bathes the nervous tissues (*10*).

The blood–testicular barrier is similar to the blood–brain barrier in many ways. Physiologic studies by Setchell and co-workers (*3, 4*) have described the existence of a permeability barrier surrounding the seminiferous tubules of the mammalian testis. By cannulating the rete testis of rams, these investigators were able to collect samples of testicular fluid for analysis. It was found that proteins, while abundant in blood plasma and testicular lymph, were present in very low concentrations in the rete testis fluid. Significant differences in concentration were also found for amino acids and certain ions. It was concluded, therefore, that there is a blood–testis barrier located around the seminiferous tubules which is capable of excluding from the lumen of these tubules many substances normally present in testicular blood and lymph to create and maintain a unique luminal milieu.

Other studies have revealed additional aspects of the blood-testicular barrier. It develops postnatally in most eutherian mammals and is a prerequisite for both secretion of a characteristic tubular fluid and for the production of spermatozoa. Evidence also suggests that the barrier functions immunologically to protect the autoantigenic spermatozoa.

The rate of penetration of various test substances from blood plasma into the testicular (rete testis) fluid and testicular lymph has been examined, and these fit generally into three groups (*4*).

1. The first group comprises those chemicals that pass readily into both testicular lymph and testicular fluid and includes tritiated water, urea, ethanol, and bicarbonate. These same substances equilibrate readily between plasma and the central nervous system.

2. The second group consists of those substances that pass readily into testicular lymph but only slowly into testicular fluid so that there are differences in concentration between testicular fluid and testicular lymph when compared to blood plasma. This group includes creatinine, various drugs such as actinomycin D, L-dopa, and serotonin, and various ions such as sodium, potassium, chloride, and iodide. The fluid-to-plasma ratios and transfer constants are similar to those for aqueous humor and cerebrospinal fluid.

3. In the third group are those substances which, like those of the other two groups, pass readily into testicular lymph but which do not appear at all in the testicular fluid. This group includes albumin, inulin, and chromium EDTA. The latter two compounds are filtered by the kidney. A chemical that is actively secreted by the renal proximal tubules, *p*-aminohippuric acid (PAH), is also excluded from the testicular fluid. These same chemicals penetrate the central nervous system either very slowly or not at all. The fact that an active transport system exists for PAH in the kidney and choroid plexus of the brain suggests the possibility

of a similar mechanism in the seminiferous tubules.

Foreign compounds may be present in any of three fluids of the testis. The first such fluid is obviously blood. The anatomy of the blood supply of the testis of animals with scrotal testes is more complicated than those with inguinal or abdominal testes. Arteries and veins are so arranged that there is precooling of the arterial blood before it reaches the testes and blood flow becomes slower and almost nonpulsatile.

A second fluid is the testicular lymph, which is derived from the interstitial tissue and the tunica albuginea. Apparently the endothelium of testicular capillaries acts as a nonselective molecular sieve that allows extracellular components to pass readily to the lymph circulation as a function of hydrostatic pressure. Its composition is similar to that of lymph from other lymphatic vessels except for a high concentration of testosterone. The fact that lymphatics continuously drain the interstitial tissues of the testis could further limit the penetration of chemicals to the seminiferous tubules by not allowing an accumulation of chemicals in this extracellular space.

A third fluid arises within the seminiferous tubules and carries the spermatozoa through the rete testis and efferent ducts into the epididymis, where almost all of the fluid is reabsorbed. The rate of flow of this fluid from the rete testis is lower than the flow of either blood or lymph and it is not closely related to sperm production. Rete testis fluid has a unique composition and is actively secreted, probably by the Sertoli cells. When the efferent ducts are ligated the seminiferous tubules dilate and an internal fluid pressure in excess of normal hydrostatic pressure is developed. In addition, ion concentrations cannot be accounted for by filtration, and isolated perfused testis secrete fluid independent of perfusion pressures. The continued formation of testicular fluid in mice made devoid of spermatogenic elements except Sertoli cells suggests indirectly a role for these cells in fluid secretion. Histologic studies also support a secretory role for the Sertoli cells.

The morphologic basis of the blood–testis barrier has been examined by electron microscopy by Fawcett and Dym and others (*5*, *6*). Various electron-opaque particles were injected interstitially, and their localization at different time intervals was determined by examination of electron micrographs. It was found that the larger particles, carbon and thorium dioxide, were excluded from the seminiferous tubules, apparently by the surrounding layer of contractile cells. Smaller tracers such as ferritin and perioxidase, though generally excluded, did penetrate into the epithelium in certain areas of the tubules. Where they did gain access to the epithelium, they entered an intercellular cleft surrounding the spermatogonia but did not penetrate more deeply toward the tubular lumen. It was suggested by these studies that the primary barrier to penetration of the seminiferous tubules is the surrounding layer of contractile cells, but where this is breached, specialized cell-to-cell junctions within the epithelium constitute a secondary barrier to passage of materials into the testicular fluid. Further studies have demonstrated that the myoid layer does constitute a significant permeability barrier but that some of its cell-to-cell junctions are not closed and therefore permit penetration of tracers into the germinal epithelium at certain sites along the length of the tubules. The specialized junctions between the Sertoli cells, on the other hand, present multiple sites of focal contact of the apposed membranes. These provide a second, and apparently a more effective barrier to the passage of large molecules through the epithelium.

A number of questions arise concerning the role of the blood–testicular barrier and testicular fluid which require further experimentation. Those that apply directly to the area of mutagenesis include the following. Do dominant lethal effects usually appear to be associated with postmeiotic spermatogenic cells? Does meiosis somehow limit the functional expression of dominant

lethality? What is the relative ease of chemical penetration to cells in the seminiferous tubules versus the epididymis and/or vas deferens? Do environmental chemicals alter the secretion of testicular fluid; if so, what is the physiologic consequence? Are there cellular differences in the rate at which a chemical can penetrate the various spermatogenic cells? What is the role of testicular fluid secretion and the blood-testicular barrier in the reliability of the dominant-lethal test for mutagens?

In summary, it has been shown that the blood–testicular barrier retards the penetration of many chemicals to the spermatogenic cells in the seminiferous tubules. In some cases, for instance *p*-aminohippurate, chemicals never gain significant entry into the seminiferous tubules possibly due to processes of active secretion similar to that demonstrated in the kidney proximal tubules and the choroid plexus. The lymphatic circulation and the architecture of the cells in the seminiferous tubules further limit the penetration of the test compound. When these physiologic and anatomical considerations are coupled with the fact that the test compound is usually administered only once as a single injection, it seems reasonable to ask whether the test chemical even penetrated to the target cells during this brief exposure. It must be stressed that the body is not a single-compartment system, but rather a much more complicated multicompartmental arrangement that limits the distribution of chemicals, especially after acute exposure. Even during periods of chronic exposure, fluid turnover such as exists in the brain and testis, specialized secretory processes, and characteristics of accumulation and elimination prevent some chemicals from ever achieving concentrations in these restricted areas which are comparable to their plasma concentration. Thus, the possibility of obtaining "false negative" results from the dominant lethal test, due in part to the blood–testicular barrier and other pharmacokinetic factors, must be recognized and considered further.

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