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Permeability of the Dura Mater of the Spinal Cord in Dogs for Low Molecular Weight Substances in Serum

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The permeability of the dura mater (DM) of the dog spinal cord for low molecular weight serum components – urea, creatinine, glucose, lactate, cholesterol, calcium, and inorganic phosphate – was studied in in vitro conditions. DM permeability for a high molecular weight serum component – albumin – was assessed as a reference compound. Most of the study components had permeabilities of 8–15%. The greatest DM permeability was for lactate (33.6%) and the lowest was for cholesterol (1.3%). Values for urea and creatinine were 8.0 and 7.5%, respectively; there was a nonlinear relationship between permeability and the initial substrate concentration in the serum. The DM permeability threshold for urea was 4.83 mM and that for creatinine was 97 μM. The functional characteristics of DM permeability may be determined by its structural features – dense packing of fibrillar connective tissues structures, high content of sulfated and the absence of nonsulfated glycosaminoglycans.

Keywords: dura mater of the spinal cord, permeability, low molecular weight compounds.

The meninges are part of the natural biological barrier system, protecting the parenchyma of the brain and spinal cord from the entry of substances both of natural origin and xenobiotics (particularly high molecular weight compounds) [7]. Analysis of reports published in recent years shows that much attention has been paid to the permeabilities of brain structures forming the blood-brain barrier [6, 18, 22].

Studies of the meninges of the spinal cord have mainly addressed their permeability for the anesthetics and analgesics used in epidural anesthesia [7–11].

At the same time, questions of the permeability of the meninges of the spinal cord for parenterally administered xenobiotics and endogenous substrates from tissue fluid, which is a filtrate of serum/plasma, have revealed virtually no investigation. In part, we believe that there are no baseline data on the abilities of low molecular weight endogenous compounds, which are constantly present in the plasma and tissue fluid, to cross the meninges of the spinal cord. Another aspect of this problem is the ability of natural metabolites to influence the kinetics of the permeability of the meninges of the spinal cord. In fact, some authors take the view that a number of substances present in the blood can alter the permeability of the meninges of the spinal cord in inflammation, as they have significant actions on both the development of the pathological process and on drug bioavailability [12, 21]. In addition, excessive quantities of endogenous catabolic products circulating in the blood and present in the tissues can lead to impairments to metabolism in the spinal cord and neurointoxication [3, 13, 14].

The aim of the present work was to undertake in vitro studies of the histostructural features and permeability of the dura mater of the dog spinal cord for low molecular weight serum substances.

Methods. The mechanisms of the permeability of the meninges of the spinal cord were studied using a method based on a diffusion chamber in which the semipermeable membrane was the dura mater (DM) from the experimental animals [8]. Experiments were performed using spinal cord DM from 12 mongrel dogs aged 1–3 years and weighing 18–25 kg. The experiments were approved by the Ethics

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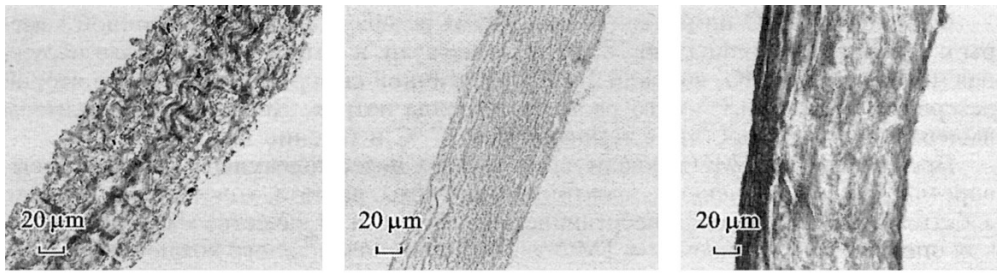

Fig. 1. Histochemical characteristics of the connective tissue matrix of the DM of the dog spinal cord. *a*) Intense PAS-positive staining of collagen-associated neutral glycoproteins; *b*) negative reaction on detection of nonsulfated glycosaminoglycans with Alcian blue at pH 2.5; *c*) intense Alcian-positive straining for sulfated glycosaminoglycans at pH 1.0. Magnification ×630. Longitudinal sections. The outer surface of DM of spinal cord is at left.

Committee of the Russian Ilizarov Restorative Traumatology and Orthopedics Scientific Center, Ministry of Health of the Russian Federation.

Autologous serum was obtained by collecting venous blood into Vacuette® vacuum tubes (Greiner Bio-one, Austria) containing clotting activator, which were left for 30 min at room temperature and centrifuged for 10 min at 3000 rpm.

Immediately after euthanasia by i.v. administration of 5% thiopental sodium solution, the spinal cord was harvested at the level of T_{VI}–L_{IV}. Blood and epidural fatty tissue were removed by washing spinal cord in 0.9% sodium chloride solution, after which spinal cord was separated into segments at the level of the spinal nerve roots. The DM of each segment was incised in the ventral surface and accurately separated from the arachnoid mater, cutting the dentate ligaments.

DM fragments were placed between two reservoirs in the diffusion chamber with openings of area 28 mm^2. Autologous serum (2 ml) was placed in the reservoir in contact with the outer surface of the DM, while 2 ml of 0.9% sodium chloride solution was placed in the second reservoir. Diffusion chambers were placed in an incubator at 37°C for 120 min.

The permeability of the DM for the main low molecular weight substances present in serum – urea, creatinine, glucose, lactate, and cholesterol – and the electrolytes calcium and inorganic phosphate was studied. For controls, the permeability of the DM for a high molecular weight serum component – albumin – was also studied.

The concentrations of study substances in the initial serum and the dialysate passing through the DM were determined using a Hitachi/BM 902 automated biochemical analyzer (F. Hoffman-La Roche Ltd./Roche Diagnostics GmbH, Switzerland/Germany) using a regent kit from Vital Diagnostics (Russia). Filtration activity was assessed as the percentage concentration of the substance in dialysate to its initial concentration in serum ($C_{DIAL}/C_{INIT} \times 100$).

Significant differences in permeability to low molecular weight substances were identified using the nonparametric Kruskal–Wallis test followed by multiple comparisons using Dunn's test. Permeability for low molecular weight compounds relative to albumin was assessed by pairwise comparisons using the nonparametric Wilcoxon W test for independent sets.

For histological investigations, samples of dog spinal cord DM (n = 12) were fixed in cold mix of 2% glutaraldehyde and paraformaldehyde solutions in phosphate buffer pH 7.4 supplemented with 0.1% picric acid. Some of the specimens (n = 3) were embedded in paraffin by standard methods. Longitudinal and transverse sections were stained with Alcian blue at pH 1.0 and 2.5 [4]. Semithin sections were prepared from some specimens (n = 3) by postfixation in 1% osmium tetroxide solution containing 1.5% potassium ferricyanide and embedding in Araldite. Longitudinal and transverse semithin sections were stained with methylene blue/PAS. After fixation and dehydration in ascending alcohols, some specimens (n = 3) were stained by the Weigert method and total film preparations were made. Light microscopic studies of histological properties were performed using an Axioscope A1 stereomicroscope and an AxioCam ICc 5 digital camera, along with the Zen Blue program (Carl Zeiss MicroImaging GmbH, Germany). Some of the specimens (n = 3) were prepared for electron microscopy using camphene [5]. After creating an electrically conducting coating in an ion spray (JEOL, IB-6, Japan), specimens were examined in a secondary electron regime with an acceleration voltage of 20 kV in a JSM-840 scanning electron microscope (JEOL, Japan).

Results. Dog spinal cord DM consists of a dense connective tissue film of thickness 0.1–0.2 mm. Electron microscopy data show that the fibrillar basis of the meninges is formed from spirally curved collagen fibrils grouped into dense parallel bundles. Individual fibroblast-like cells are located between the bundles.

Histochemical studies of spinal cord DM preparations stained with Schiff reagent identified intense staining of neutral glycoproteins associated with collagen fibrils (Fig. 1, *a*).

TABLE 1. Permeability of the Dura Mater of the Dog Spinal Cord for Serum Components

Component	Permeability, % (median, min–max)
Low molecular weight compounds	
Lactate	33.6 (24.4–39.7)*#
Inorganic phosphate	15.4 (8.8–18.4)#
Total calcium	15.2 (9.7–18.0)#
Glucose	9.6 (6.2–15.5)#
Urea	8.0 (3.0–14.3)#
Creatinine	7.5 (3.2–12.0)#
Cholesterol	1.3 (0.9–3.8)
High molecular weight compounds	
Albumin	0.3 (0.0–1.3)

*Permeability values significantly different from other low molecular weight substrates, $p < 0.05$; #permeability values significantly different from that of albumin, $p < 0.05$.

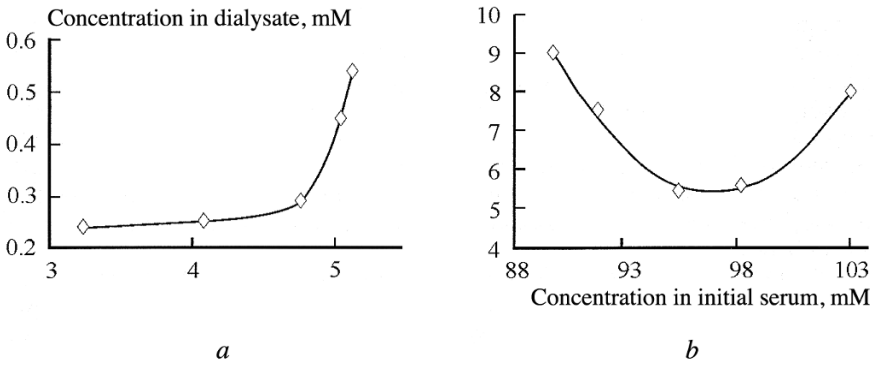

Fig. 2. Relationships between concentration of urea (*a*) and creatinine (*b*) in dialysates and their initial serum concentrations.

Film preparations of spinal cord DM showed the development of a network of uniformly distributed branching elastic fibers. Alcian-negative stained sections at pH 2.5 demonstrated the almost complete absence of nonsulfated glycosaminoglycans (hyaluronic acid) in spinal cord DM specimens (Fig. 1, *b*). Positive staining with Alcian blue at pH 1.0 revealed a high content of sulfated glycosaminoglycans in the superficial outer layer and smaller quantities in the deeper layers of the spinal cord DM (Fig. 1, *c*).

The study metabolites were ranged in decreasing order of permeability through the DM (see Table 1). Most of the study compounds had permeabilities in the range 8–15%. There was no clearly marked relationship between the permeability of low molecular weight substrates and their molecular weights or charges. The permeability of albumin was close to 0%. Among low molecular weight compounds, cholesterol had the lowest permeability, approaching that of albumin: mean DM spinal cord permeability for cholesterol was not statistically significantly different from that of albumin, while the permeabilities of all other metabolites were significantly different from that of albumin. The greatest permeability was seen for lactate, at 33.6%. Multiple comparisons showed that these differences were statistically significant ($p < 0.05$) relative to other serum components.

Two compounds – urea and creatinine – has permeabilities of 8.0% and 7.5%, respectively. There was a nonlinear relationship between permeability and the initial substrate concentrations in serum within the normal ranges. The permeability of spinal cord DM for urea displayed a hyperbolic relationship with a turning point (permeability threshold) of 4.83 mM. Above this concentration, there was a sharp increase in ability of this substrate to cross the DM (Fig. 2, *a*). In the case of creatinine, permeability showed a parabolic relationship with a minimum at 97 μM (Fig. 2, *b*).

Discussion. Histochemical analysis demonstrated that the connective tissue of the DM of the dog spinal cord did not contain hyaluronic acid, which correlated with the dense distribution of collagen fibrils. The high level of sulfation of the extracellular matrix of the DM of the spinal cord suggested that its main structural proteoglycans are sulfated glycosaminoglycans (GAG). These latter are involved in forming the fibrillar structures of the DM of the spinal cord due to formation of disulfide bonds between collagen fibrils [15]. This structure for DM protects the spinal cord from the penetration of large molecules – potential carriers of antigenic information (foreign and intrinsic proteins). The high negative charge of sulfated proteoglycans is important in this, as it prevents the passage of other negatively charged molecules (various polysaccharides and proteins) through the basal membrane, as well as negatively charged erythrocytes.

Overall, these properties of the extracellular matrix of the DM of the spinal cord determine the functional characteristics of this sheath, namely the filtration of large and charged molecules and cells. A similar function is provided by the basal membrane of renal glomeruli, which also contains a highly sulfated GAG, i.e., heparan sulfate [1].

In addition, the significant hydrophilicity of sulfated GAG promotes the penetration of low molecular weight substances with high solubility in water, which we believe explains the permeability of the DM of the spinal cord for lactate. Such a mechanism for lactate transport across the DM is a reserve mechanism in case of energy deficiency for spinal cord cells, as this compound can be metabolized by aerobic degradation or gluconeogenesis. It can also be suggested that the permeability of lactate, urea, and creatinine across the DM of the spinal cord operates by passive diffusion.

The permeability thresholds established for urea and creatinine many be analogs of the renal permeability thresholds for various substances, such as glucose. In states accompanied by hyperuricemia and hypercreatininemia (renal disease, severe trauma), increases in spinal cord DM permeability for these compounds can be seen, which can result in neurointoxication.

The histological structure of the organization of the human DM has similarity to that in dogs – it consists of dense fibrillar connective tissue, with a small number of cellular elements (fibrocytes) among collagen bundles, elastic fibers, and amorphous material, in which glycoproteins and glycosaminoglycans are identified [2, 23]. Data from the meta-analysis reported by Richman et al. [19] show that the DM of the lumbar segment of the human spinal cord contains a network of chaotically oriented collagen structures. However, studies reported by several authors contain convincing evidence of the mainly longitudinal organization of bundles of collagen fibers in this part of the DM [17, 20], which is consistent with the results of our study of the DM of the dog spinal cord. The thickness of the DM in the lumbar segment of the spinal cord in adult men varies over the range 0.1–0.25 μm [23], which is also consistent with the thickness of the DM of the dog spinal cord. Thus, despite possible differences in the organization of the fibrous base of the the DM of the human and dog spinal cord [16], these data may be useful for interpreting the results of clinical studies.

Conclusions. Thus, this in vitro study showed that the DM of the dog spinal cord has moderate permeability for low molecular weight serum substances. For most (urea, creatinine, glucose, total calcium, and in organic phosphate), permeability values were 8–15%. Cholesterol had the lowest permeability, close to that of the control marker albumin. The greatest permeability was seen for lactate, which is a highly hydrophilic organic compound. Catabolites (urea, creatinine) showed nonlinear relationships between the permeability of the DM of the spinal cord and the serum contents of these substances and demonstrated that they have threshold concentrations. These data were interpreted in relation to the morphohistochemical characteristics of DM structure.

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