IN VITRO NEUROTOXICITY OF ANTIBIOTICS

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Prediction, measurement, and monitoring of neurotoxicity of antibacterial agents are extremely difficult. We studied the effects of antibiotics on growth and differentiation in dissociated rat brain cell cultures. Drugs were added from 7 to 14 days in cultures (DIC) in order to attain concentrations representing 10 and 2 times the levels reached by intraventricular antibiotic administration. At DIC 14, 21 and 24 the following evaluations were performed and compared to controls: content of protein and DNA reflecting cell growth, two enzymes of brain cell differentiation and staining for oligodendrocytes and astrocytes. No effects showed penicillin G, amoxicillin, sulbactam, aztreonam and chloramphenicol. Only marginal and always reversible influences produced ceftriaxone, vancomycin and ciprofloxacin. Clear inhibition of both cell growth and cell differentiation with only partial or absent regeneration was induced by cefepime, ceftazidime, cefotaxime, cefuroxime, imipenem, temafloxacin and amikacin. These results are in accordance with other in vitro experiments (bone marrow cells, fibroblasts) and also with clinical findings. We conclude that the sensible use of this model on the cellular level can predict potential neurotoxicity of antimicrobial agents.

GENETICS

EXPRESSION OF DIRECTLY TRANSFERRED GENES IN SKELETAL AND CARDIAC MUSCLE OF NORMAL RODENTS AND DYSTROPHIC (MDX) MICE IN VIVO

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Skeletal and csrdiac muscles of rodents are able to take up and express directly injected plasmids containing reporter genes such as luciferase (pRSVL), E. coli B-galactosidase (pRSVLac-z). After injection of pRSVLac-z DNA into heart or skeletal muscle muscle, gene expression was localized histochemically to cardiocytes or myocytes, respectively. Seven days after injection of pRSVL DNA, luciferase activity (mean±SEx10^{3} light units per ug DNA injected) was 1325±287 in normal mouse skeletal muscle, 1543 ±407 in mdx skeletal muscle,3015±1295 in rat cardiac muscle. After injection of pRSVL, gene expression was present in cardiac and mdx skeletal muscle for only 1 month while it persisted in normal skeletal muscle for one year. Gene expression was stable in cardiac muscle of athymic and ciclosporin treated rats for at least 2 months that suggest the role of immune response in heart. Rapid turnover of myofibers in mdx muscle can explain the instability of gene expression. Direct transfer of genes into muscle has applications for gene therapy.
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EVIDENCE FOR A LOW INTERLEUKIN 1ß RESPONSE IN SEPTIC NEWBORNS.

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The role of cytokines in neonatal sepsis is not yet fully understood. We studied 10 septic newborns and 22 controls, aged 1-10 days. Tumor necrosis factor (TNFα), IL-1ß and Interleukin 6(IL-6) concentrations were measured in EDTA-plasma (Easia,Medgenix). In the septic group we sampled every 8 to 12 houres during 2-3 days. Maximum values are summarized in the table. IL-6 correlated inversely with the degree of hypotension (R=-0.562,p=0.045). With the improvement of the clinical picture both TNFα and IL-6 concentration decreased.

CYTOKINE	CONTROLS	SEPSIS	p value
TNFα	36±4	476±189	<0.001
IL-1ß	8±1	44±19	<0.005
IL-6	55±28	79690±37578	<0.005

values expressed in pg/ml(mean±SEM).

In conclusion, in septic newborns the highly pyrogenic mediator IL-1ß is only slightly elevated or normal. This may explane the lack of a febrile response in septic newborns.

MOLECULAR GENETIC ANALYSIS IN HUNTER DISEASE

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Clinical and biochemical studies have revealed a great phenotypic variability in mucopolysaccharidosis type II (Hunter disease), probably due to different mutations in the IDS gene that has been localized in Xq28. Using a cDNA probe containing almost the entire coding region of the human IDS gene, we performed a molecular analysis on 7 patients with Hunter disease. In one patient, a complete deletion of the IDS coding sequences was found. Another patient had structural alterations of the IDS gene including a partial deletion. In 5 patients, however, after restriction digestion of the DNA by PstI and TaqI and Southern hybridization with the IDS cDNA, the audiographic patterns obtained were similar to those found in controls. These patients may probably have small deletions or point mutations in the IDS gene. Genotype analysis offers now the possibility to detect carrier among females at risk and to perform prenatal diagnosis in this X-linked disorder.

INTRACISTERNALLY (ic) GIVEN E.COLI O 111 B 4 ENDOTOXIN (LPS) OPENS THE BLOOD-BRAIN BARRIER (BBB) FOR NA-FLUORESCEIN (NaF) IN NEWBORN PIGLETS

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Despite decades of investigating the BBB, there remains a distinct paucity of data regarding its response to infections. We have studied the pial-arachnoid microvasculature in vivo in newborn piglets through an open cranial window using fluorescence photomacroscope giving 20 µg (n=6) -Group 1- and 200 µg (n=6) -Group 2- LPS ic. Control animals (n=6) -Group 3- were given mock cerebrospinal fluid (CSF). CSF LPS concentration was determined using chromogenic limulus amebocyte lysate assay. NaF (MW=376) given intravenously was used as a BBB permeability marker and brain NaF uptake (BNU) was measured by a spectrofluorometer. In Group 3 the BBB remained tight through the experimental period (4 hours), there were neither fluorescein extravasation nor significant BNU. In Group 1 the BBB was opened (extensive fluorescein leakage) 70.5±10.5 min after the ic LPS injection, and CSF LPS levels was significantly elevated (0.70±0.10 EU/ml) at that time compared to the value measured at the start of the experiment (0.14± 0.04 EU/ml; p<0.05). There was an elevation in BNU (3.0±0.7 µg NaFxmg^{-1} protein/µg NaFxµl^{-1} serum) at the time of sacrifice (4 hours). In Group 2 the BBB was opened significantly earlier (55.2±4.1 min), and CSF LPS content (1.18±0.10 EU/ml) and BNU (5.9±0.9 µg NaFxmg^{-1} protein/µg NaFxµl^{-1} serum) were also highly elevated compared to the Group 1 (p<0.05 in each parameter). It is concluded that ic given LPS, as a model of neonatal purulent meningitis, results in a dose dependent opening of the BBB for NaF, and in marked changes in the extracellular fluid space of the brain. (All values are mean±SD.)

FFU COMPLEX: AN ANALYSIS OF 491 CASES.

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The original description of the femur-fibula-ulna (FFU) complex included cases which femur, fibula and ulna show defects and which tend to be associated. These cases are usually sporadic.
We present an analysis of 491 cases, registered in the Institute of Human Genetics in Münster, from many different populations and from different years.
Our result, showing nearly equal proportions of the most common malformations in four analysed groups (with one, two, three and four limbs affected) supports the hypothesis that even if one arm or one leg is affected, the cases still may belong to the FFU-complex.
In our study upper limbs are affected slightly more often than lower. All malformations tend to be unilateral than bilateral. The right-side and male-sex are preferentially affected. The limb malformations present in the FFU complex are usually different from those seen in most other types of limb defects. There is virtually no overlap between FFU complex and other limb anomalies, however differential diagnosis will be briefly discussed.