Induction of Fas mRNA and brain apoptosis following Rat and Sheep Fetus transient ischemia.

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Introduction
Periventricular leukomalacia (PVL) is a perinatal hypoxic ischemic encephalopathy that is largely responsible for various neurological sequelae in premature infants. In this study we examined histochemically for the presence of post-ischemic brain apoptosis using sheep fetus chronic preparation system. The pathophysiological roles of apoptosis in ischemic tissue damage have been recently reported in several organs including neural tissues. Appearance of morphologically detectable apoptosis or oligonucleosomal fragmentation of adult animal brain DNA is reported in transient global cerebral ischemia as well as focal ischemia. This is the first report of fetal brain apoptosis induced by perinatal generalized ischemia.

Materials and methods
Three pregnant ewes (127 +/- 7 days of gestation, term 145 days) were obtained from Nippon Ikadohbutsu (Tokyo, Japan) ands kept under a 16 hour light, 8 hour dark regimen, with food and water available ad libidum. Fetal lambs were delivered from hysterotomy incision. By means of the technique previously reported by Clapp et al., an inflatable umbilical cord occluder was placed around the umbilical cord, close to the incision site on the fetal abdomen. Umbilical cord occlusion was carried out by 15 seconds infusion/aspiration of warm sterile saline solutions followed by a 30 seconds of occlusion. This procedure was repeated 40 times over 2 hours.

We fixed fetal lamb brains 24 hours after ischemic insults using a 10% formaldehyde perfusion. Whole brain paraffin sections were made and stained for apoptosis using TUNEL stain as described elsewhere.

We then examined for the expression of apoptosis related genes Fas and Fas ligand in post ischemic fetal rat brains by RT-PCR. Unilateral uterine cornu (including 3-4 fetuses) of 4 pregnant rats were subjected to transient ischemia (15 min.) by clipping of ipsilateral uterine arteries and reperfusion (2 hours). Fetal rat brains were prepared for histological and molecular examinations.

Results
Sheep model By transient ischemic insults, we observed appearance of apoptotic cells in both neurons and glial cells. Apoptotic cells are abundant in the peri-ventricular areas and basal ganglia.

Rat model; By occlusion of the uterine artery, we also observed increased histochemically detectable apoptosis in fetal brains but their precise localization was difficult to identify due to the relative small size of fetal rat brains. Expression of Fas mRNA was induced by ischemic insults while expression of Fas L was not changed.
Discussions

Apoptosis is regarded as physiological or programmed cell death induced by mild chemical or thermal stress, cytotoxic agents, hormones or their deprivation, cytokines and attacks by specific CTL or activated NK cells, in contrast, necrosis is associated with severe cell and tissue injury. In this study, we have demonstrated fetal brain apoptosis induced by transient occlusion and reperfusion by histochemical methods and the detection of DNA fragmentation. We believe apoptosis possibly contributes to the development of infarctive brain damage and PVL after transient fetal ischemia. Onset of PVL is rather latent after delivery and its direct relationships between prenatal ischemic insults are still controversial. But we feel it is possibly induced by perinatal ischemia because Li et al. reported prolonged presence of rat apoptotic brain cells from 0.5 hour to 28 days after 2 hr. MCA occlusion. If apoptosis continues for several hours or days after ischemia, then it is reasonable to expect for postnatal administration of substances that may prevent this process. Several mechanisms have been proposed to explain the pathophysiology of fetal ischemic cerebrovascular disorders, including excitotoxicity, intracellular calcium overload, inhibition of protein synthesis and altered expression of autotoxic suicide genes. In this study we observed induction of Fas mRNA in ischemic fetal rat brains. Expression of Fas was detected in various cell types including T and B lymphocytes, thymus, ovary, liver, intestine and heart but not in the brain, spleen and bone marrow. Interestingly, even though expression of Fas in adult murine brain is absent in non treated animals but is strongly induced by ischemia and reperfusion. 6) They reported Fas induction peaked at 6 hours after ischemic insults and discussed its roles in vulnerable hippocampal neurons. Fas antigen is a membrane associated receptor protein which can mediate apoptosis if bound to anti Fas monoclonal antibody and Fas ligand. Recently, Fas ligand, which induces apoptosis in Fas antigen expressing target cells, has been identified and cloned in mice, rats and humans. Fas ligand is constitutively expressed in immunologically privileged sites including prostate, cornea and brain and possibly contributes to eliminate deleterious cytotoxic lymphocytes infiltration. 7) Further studies including the localization of Fas antigen and its time course of expression are required to evaluate the role of Fas antigen in postischemic fetal brain injury and its relationship to the development of PVL.

7) Saito, S. personal communication
8) Masaoka N. submitted
9) Hayakawa S. submitted