Protective Effects of Human Osteoblast-like Cells to Apoptotic Threat of PK 11195, a Synthetic Translocator Protein 18 kDa (TSPO) Ligand...

Introduction
The 18 kDa mitochondrial Translocator Protein (TSPO) is an important factor in the regulation of cellular apoptosis and steroid synthesis. The role of the TSPO in metabolism of human osteoblasts is unknown. Previously we found that TSPO is abundant in human osteoblasts (1). We hypothesized that human osteoblast metabolism may be modulated by the TSPO. Therefore we evaluated the effect of its synthetic ligand PK 11195 on these cells. This ligand is known to have a pro-apoptotic effect in malignant cell lines (2, 3).

Methods
We assessed the impact of the exposure of explant cultures of human osteoblast-like cells to PK11195 (10^-5 M) on protein levels (Western blotting), gene expression (RT PCR) of TSPO, voltage dependent anion channel (VDAC), and hexokinase 2. These proteins are functionally interconnected in the regulation of the mitochondrial membrane potential (ΔΨm). Additionally we investigated cell maturation (cellular alkaline phosphatase activity), cell viability (PI incorporation), cell death (LDH activity in culture media), apoptosis (TUNEL assay), mitochondrial mass (Mito Tracker Green, MTG, staining for cytometry), ΔΨm (by JC1 staining and cytometry), cellular ATP content (bioluminescence method) and glycolytic activity ([18F]-FDG incorporation into cells).

Results
PK 11195 did not affect significantly cell proliferation, cell death, cellular viability, maturation and glycolysis. But PK 11195 exerted a suppressive effect on VDAC protein levels (mean 36749 +/- 2521 OD in controls vs. 42531 +/- 3761 OD in controls, n=6, p < 0.001) and increased TSPO protein levels (mean 228026 +/- 41564 OD in treated cells vs. 146071 +/- 22728 OD in controls, n=6, p < 0.001) (Figure 1), without affecting hexokinase 2 gene expression or protein levels. The elevated TSPO protein levels were accompanied with a parallel increase in the expression of the TSPO gene, as determined with RT PCR (mean RQ = 1.7, range 1.522-1.963). Additionally, PK11195 induced increase in mitochondrial mass (cytometric analysis stained by MTG; mean 257 +/- 2 counts in treated cells vs. 225 +/- 3 counts in controls, n=6, p < 0.05, Figure 2) and mitochondrial ATP content (mean 55.6 +/- 5.6 bioluminescence/10^6 cells in treated cells vs. mean 24.9 +/- 2.3 bioluminescence/10^6 cells in controls, n = 6 , p < 0.001) and a reduction in ΔΨm collapse (mean 55.6 +/- 5.6 bioluminescence/10^6 cells in treated cells vs. mean 24.9 +/- 2.3 bioluminescence/10^6 cells in controls, n = 6 , p < 0.001).

Discussion:
Thus, it appears that PK11195 (10^-5 M) stimulates mitochondrial activity in human osteoblast-like cells without affecting glycolytic activity or cell death and without mitotic effect. These findings might indicate that TSPO has a unique role in human osteoblast that involves the determination of the energetic level of these cells and the existence of cell preserving compensatory pathways that involve TSPO. It also appears that these pathways were involved in the response of the studied normal human osteoblasts to the apoptotic “threat” of PK 11195.

Significance:
This dual effect of the TSPO ligand, previously known as pro-apoptotic in malignant cells and currently found to be overall inert in normal mature human osteoblasts, may indicate on the possibility of the future development of a selective anti-malignant agent based on TSPO ligand analog.

Figure 1: Western blot analyses of TSPO and VDAC following exposure of osteoblast-like cells to PK 11195 (10^-5 M). Representative examples of SDS-PAGE of assayed A: TSPO, B: VDAC are given, showing decrease in VDAC and increase in TSPO following treatment with PK 11195.

Figure 2: A representable example of flow cytometry overlay histograms of cells stained by MTG. The histogram of the mitochondrial mass in osteoblast-like cells following treatment with PK 11195 (10^-5 M) is shifted, showing an increase in the mitochondrial mass relatively to the control.

References: