Preliminary report on the effects of Praziquantel and Oxamniquine on autophagy in liver sinusoidal cells of schistosomotic mice treated with vitamin-A

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Abstract
The *in vivo* effects of Praziquantel and Oxamniquine combined withVitamin-A on autophagy of sinusoidal cells in mice infected with *S. mansoni* are reported.

Keywords: Schistosomiasis. Cellular autophagy. Liver sinusoidal cells.

The present study reports some *in vivo* effects of Praziquantel (PZQ) and Oxamniquine (OXQ) combined with Vitamin-A (VIA) on autophagy of sinusoidal cells in mice infected with *S. mansoni*. To our knowledge no ultra structural changes have been described to date in these cells under such experimental conditions. Eight-week-old out bred male Swiss mice were infected with 30 cercariae. Six months post infection, the experimental group (EG) composed of 35 animals received subcutaneously a total of 210,000 IU of retinyl palmitate (VIA, Arovit, Roche); followed one week later by OXQ (100 mg/kg). In the control group (CG) 35 infected mice were similarly treated with VIA, but not with OXQ and PZQ. No other group of mice was used in this study. Five animals of the EG and none of the CG groups died during the treatment. Five animals of both groups were killed weekly post the treatment. Liver tissue was examined using standard techniques by light (LM) and transmission electron microscopy (TEM).

In the EG group the periovular granulomas appeared in various stages. Fusiform cells were seen together with macrophages, lymphocytes, eosinophils and collagen fibrils. Many granulomas presented variable fibrosis at the periphery. Vacuolated cells were seen by LM in the perisinusoidal area outside and within periovular granulomas. Under the TEM the fibroblast like cells showed fat droplets and were surrounded by collagen fibrils at the granuloma periphery. Fibrous septa, formed by collagen rich matrix containing prominent liver stellate fat storing cells (FSC) and myofibroblast like cells,

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were often seen near the granulomas radiating into the parenchyma. Outside the granulomas, numerous autophagic vacuoles (AV) defined as areas of still recognizable cytoplasm, limited by a membrane from the remaining cytoplasm, were found in FSC and endothelial cells (EC), especially three and four weeks after the treatment with OXQ and PZQ (Fig.1a e Fig.1b). The AV were variable in size. The segregated cytoplasmic components in the FSC consisted chiefly of fat droplets. In the EC mixed AV of ground substance and profiles of endoplasmatic reticulum were seen. No quantitative evaluation was done.

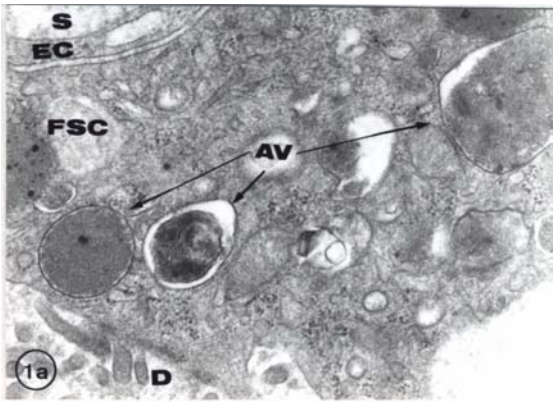

FIGURE 1a - Example of autophagic vacuoles (AV) in liver stellate fat storing cells (FSC).
The AV are in different stages of segregation and destruction, S=sinusoid; D=Disse's space; C=collagen fibrils; P=parenchymal cell. TEM a) x 50,000; b) x 54,000

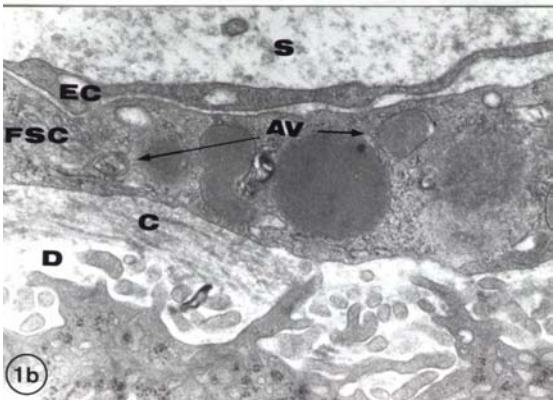

FIGURE 1b - Example of autophagic vacuoles (AV) in liver stellate fat storing cells (FSC)
The AV are in different stages of segregation and destruction, S=sinusoid; D=Disse's space; C=collagen fibrils; P=parenchymal cell. TEM a) x 50,000; b) x 54,000

In CG group by LM and TEM no basic difference was noted in the granulomas compared with those of EG. In the CG group no AV were identified in the liver sinusoidal cells.

The importance and role of the liver sinusoidal cells, especially FSC and myofibroblast like cells in liver fibrosis and granuloma formation, experimentally induced by schistosomiasis is well known.[1]

Macroautophagy or autophagy, first described more than 50 years ago and represented morphologically by the AV or autophagosomes, is an intracellular degradation system for the majority of proteins and some organelles.[2] It is a non-selective bulk process whereby cellular macromolecules fated for degradation gain access to the lysosomes.[3] Upon induction of autophagy, cytoplasm is sequestered into vesicles and delivered to a degradative organelle, the vacuole or the lysosome in mammalian cells. This complex cellular process is unique in that it converts material that is topologically intracellular into topologically extracellular[4] and represents an important mechanism of intracellular turnover[5, 6] involving dynamic membrane rearrangements under a range of physiological conditions. It is also a highly regulated process that plays a role in cellular maintenance and development[4]; being crucial for survival during starvation and cell differentiation.[7] The increase of cellular autophagy is known to play a role in cellular atrophy.[8]

The presence of many AV in the sinusoidal cells observed in this study could suggest a connection between this experimental treatment, i.e. drug-induced autophagy and atrophy of sinusoidal cells. However, the significant increase in volume fraction and numerical density of AV could be only detected confidently by large sample morphometry. Further studies will have to show if the observed increase in the cellular autophagy of liver sinusoidal cells could influence the pathogenesis of schistosomiasis.

Relato preliminar dos efeitos do Praziquantel e Oxamniquine na autofagocitose de células sinusoidais hepáticas de camundongos esquistossomóticos tratados com vitamina A

Resumo

São descritos os efeitos in vivo *do Praziquantel e Oxamniquine combinados com a Vitamina-A na autofagocitose de células sinusoidais hepáticas em camundongos infectados com* S. mansoni.

Palavras-chave*: Esquistossomose. Autofagocitose celular. Células sinusoidais hepáticas.*

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