An Improved Janus Green Technique for Detection of Mitochondrial Structure with Electron Microscope

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Abstract

Previous studies have shown that Janus Green have a role in mitochondrial localization in light microscopy. In this study, Janus green have been used in electron microscopic technique to demonstrate fine mitochondrial structure. Tissue specimens from mice liver was preceded for electron microscope examination according to Hayat method, 1986. Certain modification was performed to use Janus green with uranyl acetate. The result has shown that Janus green gives a good contrasts to the membraneous system not only to the mitochondria but also to other cytoplasmic organelles (rough endoplasmic reticulum and nuclear envelop). Using Janus Green in electron microscopic preparation reflected the interaction between Janus Green and uranyl acetate and gives a good contrast to many cell components.

Introduction

Numerous procedure have been used to demonstrate mitochondria localization and their fine structure , potentiometric dyes was used to demonstrate the cytochrome P-450 SA in the mitochondria of kidney tubule (1), while Snyder and Small (2) found that using LDS-751 achieved a good procedure to demonstrate the mitochondria.

Furthermore rhodamine 123 has been restricted to the evaluation of mitochondria respiratory function (3). Since Janus Green was used to demonstrate mitochondria in light microscopic preparation (4, 5), the present study was designed as a modifying procedure in which the mitochondrial structure will be studied within the electron microscope by using Janus Green.

Materials and Methods

Tissue specimens from mice liver were used in this study. Tissue specimens were divided into two groups, each group was processed for electron microscope examination according to Hayat, (6). One of the two groups was prepared by using Janus Green as in the followings:

1. Tissue specimens were primary fixed with 2.5% glutaraldehyde diluted in phosphate buffer pH (7.4).
2. The specimens were rinsed in 1% Janus Green dissolved in the same buffer solution for several times and left overnight in Janus Green-buffered solution.
3. The specimen were post fixed with 1% osmium tetraoxide for one hour then, washed for ten minutes in distilled water.
4. Specimens were dehydrated through a series of ethanol with concentration (30%, 50%, 70%, 80%, 90% and 100%).
5. Specimens were cleared in propylene oxide for 15 minutes (2 times).
6. Specimens were placed in a mixture of propylene oxide and embedding material (araldite) for 1hour and then left in araldite for 12hours at lab temperature.
7. Each specimen was cleaned from adherent araldite by a filter paper, then placed in plastic capsule and filled in araldite then left in the oven for 48hours at 60 C.

All specimens were sectioned and stained with uranyl acetate & lead citrate, then examined with CM10 electron microscope.

Results

Janus Green gave good contrast to the membrane of all cytoplasmic organelle (Mitochondria, Rough endoplasmic reticulum (RER) & Nuclear envelope). The results are summarized in table (1) and Fig (1, 2, and 3).

Table (1): Showing the Cyto- architecture of liver cells proceeded with Janus Green.

Cytoplasmic Organelle	Specimen treated with Janus Green	Specimen without Janus Green
RER	More contrast	Normal appearance
Mitochondria	+	+
Nuclear envelope	+	+
Ribosomes	+	+

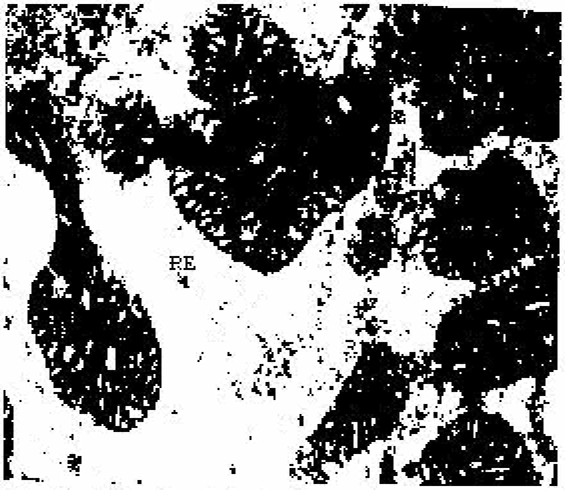

Fig (1): Mitochondria in liver cell from specimen stained with uranyl acetate & lead citrate only (31000X). M: Mitochondria, RE: Rough Endoplasmic Reticulum, R: Ribosome

Fig (2): Mitochondria in Liver cell from specimen proceeded with Janus Green and stained with uranyl acetate & lead citrate (31000X). Note the increase in the contrast of their membrane.

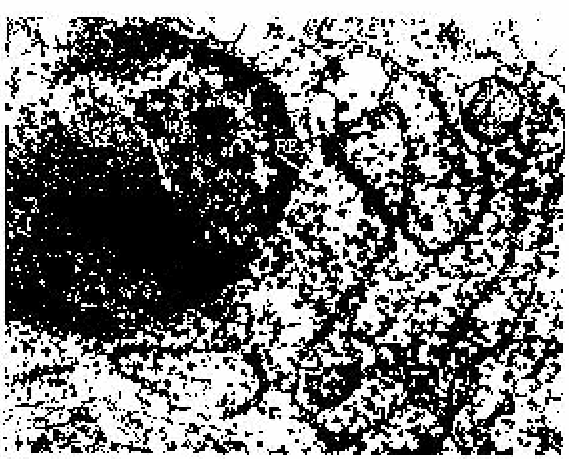

Fig (3): Liver cell from specimen proceeded with Janus Green and stained with uranyl acetate and lead citrate (31000X). RE: Rough Endoplasmic Reticulum, R: Ribosome, N: Nuclear envelope

Discussion

Mitochondria are cytoplasmic organelles found in variable numbers in all planets and animal cells .They also vary greatly in size ;only large ones are very visible by light microscope and can be demonstrated by the following types of techniques(6):

1. Electron microscopy
2. Enzyme histochemistry
3. Histological methods

Since electron microscopy is the most satisfactory method used to demonstrate mitochondria even in cells where they are small and very sparse, in this technique Janus Green was used to demonstrate the mitochondria in E.M. The results have shown that Janus Green gives more contrast to the mitochondrial structure and other cytoplasmic organelles, these results might reflect an interaction between Janus Green and uranyl acetate, since uranyl acetate is considered as a good stain to demonstrate various proteins including: Histone, Ribonucleoprotein and Phosphoproteins (6). which are the main constituents of endomembranous system in all eukaryotic cells.

References

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الخلاصة

أشارت الدراسات السابقة الى دور اليانوس الاخضر في بيان موقع المتقدرات باستخدام المجهر الضوئي . في هذه الدراسة استخدم اليانوس الاخضر لايضاح التركيب الدقيق للمتقدرات في المجهر الالكتروني . حضرت عينات مأخوذة من كبد الفئران للفحص بالمجهر الالكتروني وحضرت وفقا لما جاء بالطرق المعتمدة من قبل (Hayat,1986) مع اجراء بعض التحويرات باستخدام اليانوس الاخضر . اظهرت النتائج ان اليانوس الاخضر يعطي تمايزا جيدا لاغشية العضيات الخلوية (المتقدرات والشبكة الاندوبلازمية والغلاف النووي) يمكن الاستنتاج ان استخدام اليانوس الاخضر عند التحضير للمجهر الالكتروني يعكس التفاعل الجيد بين اليانوس الاخضر وخلات اليورانيل والتي تؤدي الى اعطاء تمايز جيد لمكونات الخلية.