

CHEMOTHERAPEUTIC STUDIES ON MOUSE HEPATITIS VIRUS (I)

Effect of Anti-viral Agents on the Swelling of Mice Liver *Mitochondria in vitro*

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Introduction

It has been well known that isolated *mitochondria* from rat liver caused gradually the swelling during the incubation *in vitro*. The mechanism is not well known, but it has been stated that the phenomenon may be coupled to the oxidative phosphorylation of *mitochondria*. DNP and other uncoupling agents including some bacterial endotoxins prevent the mitochondrial swelling *in vitro*. Recently JUDAH (1960) reported that antihistamin drug which was effective to mice hepatitis virus *in vivo*, prevented the mitochondrial swelling of rat liver *in vitro*, and GLICK (1965) described the reversal effect of Adenosin 5'-triphosphate to the mitochondrial swelling induced by thyroxin.

Liver mitochondrial swelling has been also observed *in vivo* in some cases of liver damage by the injection of tetrachlorocarbon (BASSI 1966), by the injection of thyroxin (GLICK 1965) or by the infection of mice hepatitis virus (TONES, 1962). Mitochondrial swelling is one of the typical marker of the liver damage.

So that it is interesting to find the relationship of the drug activity on mouse hepatitis virus between *in vivo* and *in vitro*.

Present reports described on the effect of some antiviral agents on the mice liver mitochondrial swelling *in vitro*.

Materials and Methods**1) Preparation of mitochondria**

Mitochondria was prepared from normal and infected mice weighing 9~10 g, according to the method of HOGEBOM (1948), resuspension and recentrifugation of the nuclear fraction being omitted. The washing and centrifugation were repeated and the final mitochondrial suspension was prepared in 10 ml of isotonic sucrose solution from 1 g of fresh liver.

2) Virus

EH-120 strain of mouse hepatitis virus (MHV)

was used. After injection of virus intraperitoneally to normal mice, three or four days later, mice which showed typical symptoms were sacrificed and liver was removed aseptically and ten percent of liver homogenate was prepared using sterilized isotonic sucrose solution.

3) Drugs

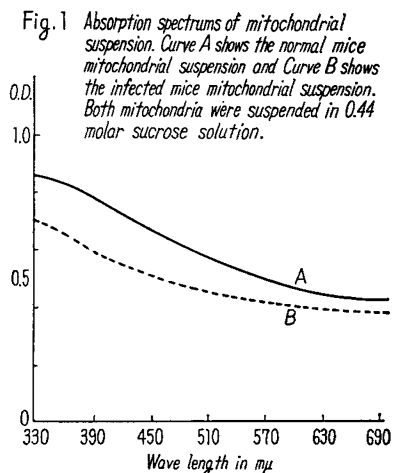
The following drugs were used; Xenaldial (MAGRASSI 1961), Benadryl (Farmitalia Co.). The drugs were resolved in isotonic sucrose solution and stored in the cold.

4) Experiment of mitochondrial swelling

Estimation of mitochondrial swelling was carried out according to the method of TAPLEY (1956) at 23°~25°C with 520 m μ of wave length.

Experiments**I) Absorption spectrums of mitochondrial suspension**

It has been well known that mice liver infected with hepatitis virus became white yellowish according to the increase of adipose tissue. To decide the adsorption spectrum of mitochondrial suspension, normal and infected mice mitochondrial suspension

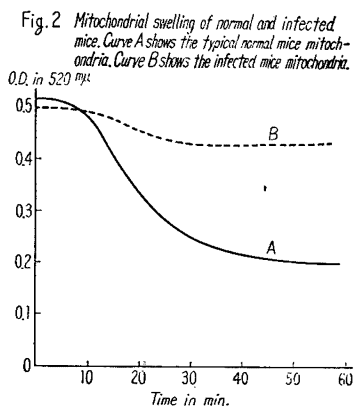


were prepared and absorption spectrums were estimated. The results were shown in Figure 1. As shown in the figure, the spectrum of normal mice *mitochondria* was same as the infected one.

II) Mitochondrial swelling of normal and infected mice

It has been observed that mice liver *mitochondria* caused the swelling with infection of virus *in vivo*, but has never been reported that whether the *mitochondria* prepared from the infected mice liver caused the swelling *in vitro* or not.

To study the infected *mitochondria* swelling *in vitro*, it was prepared from the infected mice liver three or four days after infection and estimated the degree of swelling. The results were shown in Figure 2. As shown in the figure, infected



mitochondria did not cause the swelling, as compared with the normal one. These phenomenon might be thought that infected *mitochondria* has already caused the swelling *in vivo* and lost its biological activities according to the virus infection.

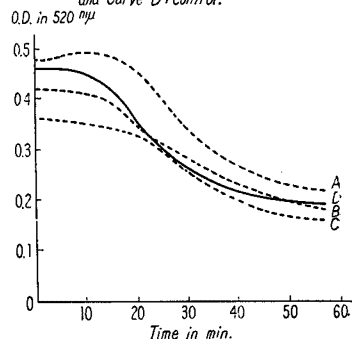
III) Effect of Xenaldial on mitochondrial swelling

There have been reported many drugs which showed the effect on the virus infection *in vivo*, but nothing has been appreciated at the present time to be effective in clinical use.

Xenaldial is one of the antiviral agent reported by MAGRASSI *et al.* (1961). Xenaldial and its relative substances were effective to many viruses including MHV.

It is interesting to study that whether Xenaldial inhibits the mitochondrial swelling *in vitro* or not. From 1.2×10^{-8} mol to 1.2×10^{-6} mol of Xenaldial were added to the normal mitochondrial solution and estimated the degree of inhibition by usual method.

Fig. 3 Effect of Xenaldial on mitochondrial swelling. Curve A: Xenaldial 1.2×10^{-8} mol, Curve B: Xenaldial 1.2×10^{-4} mol, Curve C: Xenaldial 1.2×10^{-6} mol, and Curve D: control.



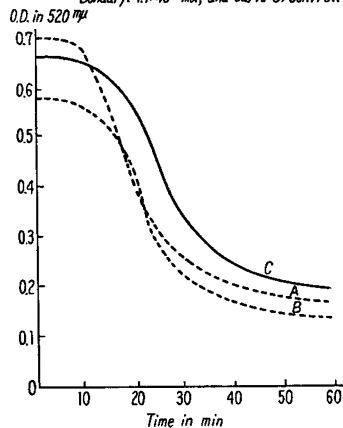
The results were shown in Figure 3. As shown in the figure at each concentration, Xenaldial did not prevent the mitochondrial swelling and it seemed nothing to be influenced by the addition of Xenaldial.

IX) Effect of Benadryl on mitochondrial swelling

JUDAH (1960) reported the protective effect of Benadryl on experimental infection of MHV and later it prevented the swelling of *mitochondria* from normal rat liver. But the effect should be decided to normal mice liver *mitochondria in vitro*.

So that from 1.1×10^{-4} mol and 1.1×10^{-6} mol of Benadryl were added to the normal mitochondrial suspension and estimated the degree of inhibition by usual method. The results were shown in Figure 4.

Fig. 4 Effect of Benadryl on mitochondrial swelling. Curve A: Benadryl 1.1×10^{-4} mol, Curve B: Benadryl 1.1×10^{-6} mol, and Curve C: control.



As shown in the figure at each concentration, Benadryl did not prevent the mitochondrial swelling but seemed to promote the swelling.

Discussion

Mitochondrial swelling has been recognized as one

of the interesting phenomenons in the field of energy transferring system. It is coupled to the oxidative phospholylation of *mitochondria*, and liver damage by the various agents caused the mitochondrial swelling *in vivo*.

Many drugs have been reported to prevent the liver damage caused by carbon tetrachloride and some of them influenced the electron transfer mechanism of NADP and NADPH₂ (SLATER 1966).

Present report showed that mice liver *mitochondria* infected with MHV did not cause the swelling *in vitro*. This showed that after infection, liver cells were damaged with the multiplication of MHV *in vivo*. These interaction of cell damage and virus multiplication of MHV *in vivo* have been clearly demonstrated enzymologically by ALLISON & MALLUCCI (1965) and MAULLUCCI & ALLISON (1965). So that the *mitochondria* from damaged liver completely lost its biological activity, and this is the reason of the present results.

On the contrary, antiviral agents Xenaldial and Benadryl reported that they have been showed potent therapeutic effect *in vivo*. The protective effect of these agents might be thought to be influenced to the mitochondrial swelling *in vitro*. But as shown in Figure 3 and Figure 4 non of them affected the swelling. Benadryl has been reported to be effective on the rat liver *mitochondria* swelling, but it has no effect on the swelling of mice liver *mitochondria*. This discrepancy might be due to the specificity of *mitochondria*.

Summary

1) The mice liver *mitochondria* infected with mice hepatitis virus (MHV) did not cause the

swelling *in vitro*.

2) Xenaldial and Benadryl which protect from the infection of mice hapatitis virus *in vitro* did not prevent the swelling of *mitochondria* prepared from normal mice liver *in vitro*.

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