Effect of Irradiation on Amino Acid in vitro Incorporation in the Course of Liver Regeneration

Bistra Tasheva, George Markov, and Roumen Tsanev

Biochemical Research Laboratory, Bulgarian Academy of Sciences, Sofia, Bulgaria

Protein biosynthesis, irradiation, liver regeneration

The in vitro incorporation of 14C-leucine by rat liver microsomes is studied under four different physiological states: 1. Normal liver of adult rats (NL), 2. irradiated (4000 rads) liver (IL), 3. liver after partial hepatectomy (RL) and 4. regenerating liver in preirradiated rats (IRL)

2, 5, 12, and 24 hours after treatment.

Both irradiation and partial hepatectomy, when applied separately, stimulate the endogenous activity of microsomes, whereas preirradiation abolishes the stimulatory effect in the early hours after partial hepatectomy.

The results are in agreement with the concept that irradiation stimulates the operating synthetic programme of the cell but inhibits cellular reprogramming.

It has been suggested that irradiation does not suppress directly the operating synthetic programme of the cell but prevents the process of cellular reprogramming. Studies on the radiosensitivity of RNA biosynthesis in regenerating liver have supported this view by showing that irradiation enhances the synthesis of ribosomal RNA (rRNA) and DNA-like RNA (dRNA) in normal liver but inhibits the synthesis of dRNA in regenerating liver. The purpose of the present study was to check this hypothesis on the level of protein biosynthesis using rat liver in four different physiological states: 1. Normal liver of adult rats (NL), 2. liver of adult irradiated rats (IL), 3. liver after partial hepatectomy (RL), and 4. regenerating liver in preirradiated rats (IRL).

The results presented in this paper show that both irradiation and partial hepatectomy, when applied separately, stimulate the endogenous activity of microsomes, whereas preirradiation abolishes the stimulatory effect in the early hours after partial hepatectomy.

Methods and Materials

Treatment of experimental animals

The experiments were performed on 200 male albino rats (160–180 g body wt.), fasted 18 hours before killing. The animals were irradiated with 4000 rads of γ-rays by a Co60 unit under conditions ensuring homogeneity of the field controlled by a ferro-sulphate dosemeter. The dose rate was 159 rads/min. Liver regeneration (compensatory hyper trophy) was induced by removing two thirds of the liver by the method of Higgins and Anderson, 30 min after irradiation.

Preparation of purified supernatant (S-100) and microsomes

The animals were killed by decapitation under light ether anaesthesia. The liver was rinsed and chilled in ice-cold medium A (0.05 M Tris/HCl, pH 7.6, 0.025 M KCl, 0.005 M MgCl2, 0.25 M Sucrose), minced with scissors, then homogenized in 2.5 volumes of medium A by means of a Potter-Elvehjem glass-Teflon homogenizer (two up-and-down strokes, 1800 rpm, clearance - 0.2 mm). The homogenate was centrifuged 15 min at 10,000 x g to remove cell debris, nuclei and mitochondria.

The upper two thirds of the supernatant were centrifuged 60 min at 105,000 x g to sediment microsomes. The clear part of the postmicrosomal supernatant was dialysed overnight against 50 volumes of medium A with 0.005 M /β-mercaptoethanol, frozen in small portions and stored at -20°C (S-100). For isolation of microsomes the livers were homogenized with 4 volumes of medium A and the preparation was performed as described above. The microsomal pellets were suspended in medium A by gentle manual homogenization and used immediately.

Estimation of RNA content of microsomes

RNA was determined spectrophotometrically with a correction for ferritin.

Abbreviations:

Pyruvate kinase — E. C. 2.7.1.40.

Requests for reprints should be sent to Bistra Tasheva, Biochemical Research Laboratory, Bulgarian Academy of Sciences, Sofia 13, Bulgaria.
Method of incubation

The endogenous activity of microsomes was determined by cell-free incorporation of \(^{14}\)C-leucine. The incubation mixture contained in one ml the following components in micromoles: MgCl\(_2\) - 10, Tris/HCl, pH 7.6 - 30, \(\beta\)-mercaptoethanol - 2, ATP - 1, GTP - 0.4, PEP - 10, PEP kinase (E. C. 2.7.1.40) - 30 \(\mu\)g, \(^{14}\)C-leucine (85mCi/mM - 1 \(\mu\)Ci, mixture of all amino acids without leucine) - 0.01 \(\mu\)mole of each, sRNA and soluble enzymes added as S-100 from normal liver (about 2 mg of protein), microsomes - about 15 OD\(_{280}\) units. Incubation was at 37 °C for the time specified.

After incubation the protein was precipitated and washed as described by Campbell et al.\(^5\). The dried precipitate was dissolved in 85 % formic acid, transferred to aluminum planchets, dried at room temperature and counted in a gas-flow counter (VA-Z-530, Vakutronik).

Results and Discussion

In order to set up appropriate conditions for a precise comparative study the kinetics of the incorporation were examined (Fig. 1 A, B). As seen the incorporation is linear only for time intervals shorter than 10 min and a saturation level for the controls is reached in about 20 min, whereas microsomes from the three experimental groups continue to incorporate \(^{14}\)C-leucine. This effect of increased longevity of incorporation has been described for regenerating liver only by some authors\(^6,7\) but it has not been found by others\(^8\). The effect is more expressed in the later hours following treatment (Fig. 1B). On the basis of these results a 20 min incubation time giving well pronounced differences between microsomes of normal and treated animals was used in all experiments.

The \(^{14}\)C-leucine incorporation in vitro was studied 2, 5, 12, and 24 hours after treatment. As shown in Fig. 2 no stimulatory effect is observed at the 2nd hour. In fact, in all cases there is a slight decrease, which is consistent with the predominence of catabolic processes during this period after partial hepatectomy\(^9\). However, this decrease is not significant and needs further proof. Beginning from the 5th hour, both irradiation and partial hepatectomy, when applied separately, lead to a significant rise in the incorporating activity of microsomes, which is most pronounced at the 24th hour.

Different results are obtained with the combination of the two treatments. In this case the stimulatory effect at the 5th hour is strongly suppressed. At the 12th and the 24th hour an increased incorporation is observed again, although there is no additivity in the effects of the two treatments.

Our results concerning irradiated and partially hepatectomized animals are in good agreement with literature data showing that both partial hepatectomy\(^6,8,10\) and irradiation\(^11,12\) greatly stimulate the incorporating ability of microsomes. The absence of such a stimulatory effect in the early hours after partial hepatectomy in preirradiated animals has not
been observed earlier and is of special interest. This finding suggests that different mechanisms are involved in these two cases. Most probably irradiation increases the endogenous activity of microsomes of intact liver by stimulating the operating synthetic program of the hepatocytes. This view is favoured also by the fact that there is an enhancement of the synthesis of RNA after irradiation. Some data indicate that this effect is not mediated through the adrenal system.

On the other hand, in the case of regenerating liver the increased endogenous activity of microsomes is obviously connected with the process of cellular reprogramming, leading to the synthesis of new RNA species and new proteins.

The lack of stimulation of ¹⁴C-leucine incorporation at the early stages of regeneration in preirradiated rats most probably implies that irradiation affects the process of cellular reprogramming inhibiting in this way the normally observed increased incorporation after partial hepatectomy.

That irradiation does not suppress the operating synthetic programme of the cell but rather the cellular reprogramming is further supported by the results obtained on the level of RNA biosynthesis. It has been found that irradiation enhances the synthesis of rRNA and dRNA in normal liver but inhibits the synthesis of dRNA in regenerating liver, without affecting the increased synthesis of rRNA in the latter case.

It is worthy to mention that at later hours of liver regeneration in preirradiated rats the endogenous activity of microsomes is strongly stimulated again (see Fig. 2). At present it is difficult to explain the mechanisms of this late increased incorporation. It should be pointed out, however, that it is well correlated with the increased synthesis of ribosomal RNA, the increased amount of RNA per liver cell (our unpublished data) and the increased weight of regenerating liver in preirradiated rats.

In conclusion, the results presented are in favour of the concept that irradiation stimulates the operating synthetic programme of the cell but inhibits cellular reprogramming.

This work was partly supported by the International Atomic Energy Agency (IAEA), Research Contract No. 475/RB.