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Irradiation Effects upon Ischemic Regenerating Rat Liver Cells

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ABSTRACT

Starch particles injected into the arterial and portal systems of the liver of the rat caused a temporary blockage of the liver circulation and consequent hypoxia in the liver cells. In the regenerating liver this resulted in a 30-40% decrease of thymidine incorporation into DNA, when analysed 1.5 hours after injection. Irradiation-induced cell damage, evaluated by thymidine incorporation 1.5 hours after irradiation with a single dose of X-rays, was not ameliorated by the ischemic condition. It is suggested that this depends on an inhibited nucleotide metabolism and DNA synthesis leading to an additive metabolic hypoxic effect of the starch particles on radiation damage. An equal level of thymidine incorporation, however, was found in an ischemic and a non-ischemic group of animals 16 hours after irradiation. In this case the liver cells in the ischemic group had overcome the additional inhibition of DNA synthesis caused by temporary hypoxia.

INTRODUCTION

Low LET-radiation induces DNA damage by free radicals. An extremely low tissue concentration of molecular oxygen reduces damage. The degree of damage can be studied histologically (5,6) or, at the molecular level, effects on DNA synthesis can be studied by the incorporation of radioactively labelled precursors e.g. thymidine (1,14). Rapidly growing liver cells offer a good opportunity for such studies.

After partial hepatectomy liver cells are fairly well synchronized when entering the S-phase of the cell cycle within 12-30 hours of regeneration. The various stages of the cell cycle have been thoroughly investigated and the well known time course for the growth of the regenerating liver (9,10) allows studies of radiation effects at different stages (1,2,8).

In the present study we analysed the effects of irradiation on DNA-synthesis in regenerating ischemic liver tissue. Ischemia was temporarily induced by means of intravascularly injected, degradable starch microspheres (4). The animals

were whole-body-irradiated during the prereplicative or the replicative phase of liver growth. DNA-synthesis was evaluated by thymidine incorporation into DNA during the replicative phase of growth.

MATERIAL AND METHODS

Animals

Young adult male Sprague-Dawley rats weighing 160-180 g (SPF-quality, Anticimex, Sweden) were used. The animals were kept under constant conditions, and had free access to standard laboratory diet before and after the experimental procedures.

Surgical procedures

The surgical procedures have been thoroughly described earlier (11). The experiments were accomplished in four different series. Series I and III comprised sham-operated and partially hepatectomized animals. Series II and IV comprised partially hepatectomized animals with one catheter in the gastroduodenal artery, one in the ileo-colic vein and one catheter emptying into the peritoneal cavity. The catheters were inserted into the vessels or fixed to the peritoneal wall in connection with partial hepatectomy as described previously (11). Each catheter was flushed, filled with Fyskosal (Pharmacia) + Heparin (Vitrum) 500 IE/100 ml and plugged. The catheters were kept open by intermittent flushing. Injection of starch particles into the vascular catheters was performed 8 or 25 hours after surgery. To avoid diurnal variation all operations were performed between 8 - 11 a.m.

Irradiation

The rats were exposed to total doses of 4 - 16 Gy whole body irradiation. Series I, II and IV were irradiated with 8 MV X-rays from a linear accelerator at a dose rate of 4 Gy/min. Series III was irradiated in an irradiation chamber with γ-rays from a ^{137}Ce source (Scanditronix, B 349, Scanditronix instruments AB, Uppsala, Sweden) at a dose rate of 0.84 Gy/min. Non-irradiated control groups were included in each series.

Starch particles

Digestible starch particles (Spherex 60 mg/ml, batch 78 08 24, Pharmacia) were administered into the portal and arterial catheters 3.5 minutes before irradiation (series II and IV). Mean diameter of the spheres was 30 µ. 200 µl of Spherex suspension was injected into each catheter and the catheters were flushed with 200 µl saline (Fyskosal, Pharmacia).

Radionuclides

Thymidine incorporation into DNA was analysed on the second day after partial hepatectomy and 16 or 1.5 hours after irradiation. 100 µl containing 185 kBq (5 µCi) of (metyl-^{14}C) thymidine (185 GBq, Amersham) or 100 µl containing 3.7 MBq (100 µCi) of (metyl-^{3}H) thymidine (555-1110 GBq, Amersham) was administered i.p. In animals with peritoneal catheters injection was made by way of this catheter. Animals irradiated 1.5 hours before analysis of DNA synthesis were prelabelled with ^{3}H-thymidine one hour before irradiation to give the pretreatment level of DNA synthesis in each animal. In these animals ^{14}C-thymidine was used for analysis of thymidine incorporation into DNA 1.5 hours after irradiation. The double-labelling allowed analysis of effects of radiation in each animal (11).

Analysis of DNA

DNA was extracted according to Schmidt-Tannhäuser as modified by Munro & Fleck (12) and quantified according to Burton (3) or isolated and quantified according to Kasche Amneus (7). Incorporation of labelled thymidine was analysed by liquid scintillation (Mark II Scintillation Counter) and the specific activity of DNA was calculated as the amount of radioactivity per mg of DNA per injected amount of radioactivity. The effect of radiation dose was calculated as the ratio between the difference in DNA specific activity in irradiated and non-irradiated animals and the radiation dose.

RESULTS

Table 1. Thymidine incorporation into liver DNA. Series I. Effect of partial hepatectomy and radiation dose. Irradiation performed 8 hours after partial hepatectomy. Mean ± standard deviation, relative values.

Liver status	Radiation		^{3}H-thymidine hours after part.hep.	Killing hours after part.hep.	Number of animals	^{3}H-spec. activity in DNA M ± SD	Effect of radiation dose x 10^{3}		
	dose Gy	hours after part.hep.							
Intact	-	-	24	25	2	0.24 ± 0.08	-		
Regen.	-	-	24	25	4	2.30 ± 1.12			
Regen.	4	8	24	25	3	0.86 ± 0.16	0.4		
Regen.	8	8	24	25	4	0.62 ± 0.19		0.2	
Regen.	16	8	24	25	4	0.54 ± 0.12			0.1

Thymidine incorporation increased tenfold in liver tissue 24 hours after partial hepatectomy (Table 1). The variation of thymidine incorporation between different animals was high. Irradiation 8 hours after surgery decreased thymidine incorporation 24 hours postoperatively. The effect of the radiation

dose was highest at 4 Gy. Increased radiation dose (Table 1) reduced thymidine incorporation.

An increased thymidine incorporation into liver tissue made ischemic during irradiation was not found (Table 2). The catheters in the arterial and portal systems, necessary for administration of the starch particles, depressed thymidine incorporation (Table 2) as has been noted before (11).

Table 2. Thymidine incorporation in liver DNA on the second day after partial hepatectomy. Series II. Effect of digestible starch particles during irradiation 8 hours after partial hepatectomy. ^{3}H-thymidine was adminis tered i.p. 24 hours after partial hepatectomy, and the rats were killed 25 hours after partial hepatectomy.

Catheters in a. gastrod. v.ileo-colica	Starch particles	Radiation		Number of animals	^{3}H-spec. activity in DNA M ± SD
		dose Gy	hours after part.hep.		
-	-	-		4	2.43 ± 0.61
+	-	-		4	1.67 ± 1.13
+	-	7	8	7	0.62 ± 0.26[1)]
+	+	7	8	7	0.85 ± 0.36[1)]

1) Difference not significant. Student's t-test.

The fact that no ischemia-induced protective action on thymidine incorporation was found motivated us to analyse the immediate effect of radiation and temporary blood exclusion on thymidine incorporation. Since thymidine incorporation in the regenerating liver is low 8 hours after operation the animals were irradiated and injected with thymidine 24-27 hours after partial hepatectomy.

Table 3. Thymidine incorporation into liver DNA on the second day after partial hepatectomy. Series III. Immediate effect of radiation 25 hours after partial hepatectomy. The animals were prelabelled with ^{3}H-thymidine 24 hours after partial hepatectomy. Eight rats in each group.

Radiation		^{14}C-thymidine hours after part.hep.	killed hours after part.hep.	^{14}C-spec. activity in DNA M ± SD	Effect of radiation dose x 10^{3}		^{14}C/^{3}H in liver DNA M ± SD
dose Gy	hours after part.hep.						
-	-	26.5	27.5	1.23±0.42[1,2)]			0.56±0.17[3,4)]
3	25	26.5	27.5	0.74±0.27[1)]	0.16		0.39±0.09[3)]
7	25	26.5	27.5	0.59±0.21[2)]		0.09	0.34±0.10[4)]

1) Significant difference, 0.02 > p > 0.01. Student's t-test.
2) " " , 0.002 > p > 0.001.
3) " " , 0.01 > p > 0.002.

The depressive effect of radiation on thymidine incorporation was less pronounced on the second day after partial hepatectomy and one hour after irradia-

tion (Table 3 and 4) than on the second day after partial hepatectomy and 16 hours after irradiation (Table 1 and 2). However, the difference between thymidine incorporation in irradiated and nonirradiated animals in the one hour group was significant both at 3 and 7 Gy (Table 3). The significance was slightly improved when the ratio between ^{3}H-thymidine incorporation before irradiation and the ^{14}C-thymidine incorporation after irradiation was calculated for each animal (Table 3).

The temporary exclusion of blood by starch particles decreased thymidine incorporation into DNA as seen from analyses 1.5 hours after injection (Table 4). The ^{14}C-specific activity of DNA decreased from 1.00 to 0.70 or 30% and the ratio $^{14}C/^{3}H$ in each animal from 0.94 to 0.58 or 39%.

Table 4. Thymidine incorporation in liver DNA on the second day after partial hepatectomy. Series IV. Immediate effect of radiation and/or digestible starch particles. All animals had catheters in the gastroduodenal artery and ileo colic vein and were prelabelled with ^{3}H-thymidine 24 hours after partial hepatectomy.

Starch particles	Radiation		^{14}C-thymidine hours after part.hep.	Killed hrs after part.hep.	Number of animals	^{14}C-spec. activity in DNA		$^{14}C/^{3}H$ in DNA	
	dose Gy	hrs after part.hep.				M ± SD	%	M ± SD	%
-	-		26.5	27.5	6	1.00±0.36	100	0.94±0.27	100
+	-		26.5	27.5	8	0.70±0.31	70	0.58±0.18	61
-	3	25	26.5	27.5	6	0.80±0.82	80	0.47±0.35	50
+	3	25	26.5	27.5	9	0.40±0.21	40	0.36±0.16	38
-	7	25	26.5	27.5	8	0.89±0.53	89	0.45±0.14	48
+	7	25	26.5	27.5	9	0.31±0.22	31	0.34±0.18	36

The decreasing effect on thymidine incorporation as a result of temporary ischemia was obvious also in irradiated animals. The mean ^{14}C-specific activity declined from 0.80 to 0.40, i.e. an extra 40% at 3 Gy and from 0.89 to 0.31 or an extra 58% at 7 Gy respectively when starch particles were present during irradiation. The corresponding $^{14}C/^{3}H$ ratios declined from 0.47 to 0.36 and from 0.45 to 0.34 at 3 and 7 Gy respectively. Thus, immediately after irradiation the temporary ischemia acts synergistically with the irradiation in depressing thymidine incorporation. The mean effect of the temporary ischemia caused by the starch particles in non-irradiated and irradiated rats was a 43% decrease of thymidine incorporation (Table 4).

DISCUSSION

The results show no protective action in terms of thymidine incorporation

from temporary ischemia during the first hours after irradiation. On the contrary, the exclusion of blood seemed to act synergistically with the radiation in disturbing cellular DNA synthesis. The disturbances should, however, be of opposite character. Radiation leads to increased amounts of DNA damaging oxygen free radicals. Blood exclusion should lead to a low oxygen tension and decreased amounts of free radicals. Both effects seem to push the liver cells out of or delay their entrance into the S-phase. It has been shown, however, that the protective action of blood exclusion and low oxygen tension during irradiation is quite evident at later stages of recovery (5,6). Thus, cell metabolism is not permanently damaged and the cells should return to the cell cycle and synthesize DNA.

In the present experiment the cells of the 24 hour regenerating livers were probably returning to the cell cycle and the S-phase after being irradiated during ischemia 16 hours earlier. The similar values of DNA specific activity in protected and non-protected animals indicated that the cells in protected livers had overcome the additional decrease caused by the temporarily low oxygen tension.

Prelabelling of DNA by ^{3}H-thymidine and comparisons of $^{3}H/^{14}C$-ratios in DNA was previously shown to improve the significance of results (11). The value of double labelling was not so clear in this study. The period between injection of ^{3}H-thymidine and ^{14}C-thymidine was rather long, i.e. 2.5 hours and the irradiation in the meantime makes handling of the animals necessary. Both may influence thymidine incorporation into DNA.

The observed decrease of thymidine incorporation into DNA during a temporary total block of liver blood supply has implications in the use of starch particles for the local accumulation of drugs (13). Drugs that are energy dependent and have to be metabolized to be effective require uninterrupted blood flow. This would not be a problem when starch particles are used to accumulate drugs with affinity to cell membranes and delayed cellular uptake.

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