Effects of 5-fluorouracil on cell cycle arrest and toxicity induced by X-irradiation in normal mammalian cells

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Abstract. From clinical studies in cancer patients and experimental *in vitro* studies, there is evidence of an increased cytotoxic effect, and even synergy, when irradiation is combined with 5-fluorouracil (5-FU). The mechanism for this is unclear.

Materials and Methods: Mouse fetuses (C₃H) have been exposed *in vivo* to X-irradiation and 5-fluorouracil (5-FU) as single agents or in combination. Cell proliferation, cell cycle progression, fetal survival and incidence of fetal malformations have been studied.

Purpose: The aim of this study was to determine possible synergistic cytotoxic effects when 5-FU and ionizing radiation were combined, particularly concerning the regulation of cell cycle progression in proliferating, non malignant mammalian cells *in vivo*.

Results: The combination of low-toxic doses of X-irradiation and 5-FU had a synergistic toxic effect in nonmalignant mouse fetuses *in vivo*. The cell cycle regulation was perturbed and the radiation-induced G₂-arrest was eradicated by 5-FU during the initial hours.

Conclusions: The time for repair of radiation induced DNA-damage is probably reduced, which may explain the increased toxicity of this combination.

INTRODUCTION

In a variety of clinical tumour treatments, the concomitant use of cytostatic drugs and radiation has been introduced aimed at improving the antitumoural effect and patient survival. Documented improvement of the antitumoural effect has been recorded in various settings, for example the combination of radiation and 5-fluorouracil (5-FU) in treating squamous cell carcinoma of the head and neck region (Browman *et al.* 1994) and gastrointestinal adenocarcinomas (Pu *et al.* 1995).

The mechanism behind the potentiated or synergistic effect is unclear. However, redistribution of cells to a more radiosensitive cell cycle phase (Vietti *et al.* 1971; Fu & Phillips 1991; Lawrence & Maybaum 1993), deranged pyrimidine pools with reduced capacity for repair of DNA damage (Lawrence & Maybaum 1993) and activation of apoptosis mechanisms (Lawrence

& Maybaum 1993; Yoneda *et al.* 1998) have been found to contribute to the increased effect, but the importance of each of the different mechanisms has not yet been elucidated.

Ionizing radiation of cells induces DNA-damage as a direct effect and also indirectly via free radicals. The cell cycle is reversibly blocked in all its phases, particularly in G₂, with inhibition of cell proliferation (Skog & Tribukait 1985). Cells exposed to methyl-xanthines, such as caffeine, show increased sensitivity to radiation due to the release of cells from the radiation-induced G₂-arrest (Rowley 1992). The cytotoxic effect of DNA-damaging cytostatic drugs, such as cisplatin, have similarly been shown to increase when cells are released by caffeine from a drug-induced G₂-block (Rowley 1992; Sivak 1994). Also the lack of a G₂ checkpoint delay after irradiation of p53 deficient cells (Hendry *et al.* 1997) and to AT-cells (Rudolph & Latt 1989) is shown to be associated with increased toxicity. The G₂-block seems therefore to be important for repair of damaged DNA prior to mitosis.

The cytostatic agent 5-fluorouracil (5-FU) is an antimetabolite which exerts a cytotoxic effect primarily through inhibition of thymidylate synthase (TS) (Heidelberg *et al.* 1983; Pinedo 1988). Other mechanisms also contribute to the cytostatic activity. Hence, 5-FU is incorporated in DNA and RNA (Pinedo 1988; Skog *et al.* 1994) and the maturation process and metabolism of RNA is influenced (Dolnick & Wu 1993; Ghoshal & Jacob 1994).

The mouse fetus model has made it possible to study the consequences of the cytotoxic treatment in highly proliferative, nonmalignant, mammalian cells *in vivo*. The aim of this study was to determine possible synergistic effects when 5-FU and ionizing radiation were combined, particularly the cytotoxicity and the regulation of cell cycle progression. To elucidate the cytotoxic effect, cell proliferation, cell cycle progression, fetal survival and incidence of fetal malformations were studied. Possible mechanisms for the observed effects are discussed in this paper.

MATERIALS AND METHODS

Experimental animals

C₃H strain primiparous mice, 3–5-month-old (25–30 g), were kept on a 12-h light and 12 h dark cycle at a constant temperature of 23 °C and relative humidity 50–55%. Water and standard food were given *ad libitum*.

Female C₃H mice were mated overnight and examined for the presence of a vaginal plug the following morning, which was also day zero of the pregnancy. On day 9 of the pregnancy, the females were exposed to 5-FU, irradiation or a combination of these agents (Table 1). At the time of analysis the mice were sacrificed by cervical dislocation.

Irradiation procedure

On day 9 of the pregnancy the mice were exposed to whole body X-ray irradiation by a single dose of 0.12, 0.5, 0.75, 1.00 or 1.75 Gy (Table 1). The animals were kept in a plastic cage encompassed by the radiation field and were allowed to move freely in the cage during irradiation. Siemens Stabilopan radiotherapy equipment at 250 kV and 15 mA with 0.5 mm Cu filter was used. The dose rate was 1.33 Gy/min as measured by lithium-fluoride thermoluminescence dosimetry and checked by a simplex universal dosimeter.

5-fluorouracil treatment

5-FU (5-fluorouracil, Roche, 0.6 mg/animal corresponding 24 mg/kg body weight) was diluted in normal saline to a total volume of 0.2 ml/mouse. The drug was injected intraperitoneally on

Table 1. Results of teratological investigation of mouse fetuses. Irradiation and 5-FU (5-FU 1 h before irradiation) was administered at day 9 after gestation and dissection of the fetuses was performed at day 18 as described in 'Materials and Methods'. In 'surviving fetuses' and 'resorptions' the numbers in brackets indicate ratio to total implants. In 'malformations' the numbers in brackets indicate ratio to surviving fetuses

Experimental conditions	Litters	Total implants	Surviving fetuses	Resorptions	Malformations
Control	36	156	140 (0.90)	14 (0.090)	6 (0.043)
0.12 Gy	16	80	76 (0.95)	4 (0.050)	0
0.50 Gy	15	82	77 (0.94)	5 (0.061)	0
0.75 Gy	26	143	128 (0.90)	15 (0.10)	3 (0.023)
1.0 Gy	20	121	107 (0.88)	14 (0.12)	6 (0.056)
1.75 Gy	36	175	62 (0.35)	113 (0.65)	37 (0.60)
5-FU 0.6 mg	26	138	110 (0.80)	28 (0.20)	26 (0.24)
5-FU + 0.12 Gy	15	73	47 (0.64)	26 (0.36)	15 (0.32)
5-FU + 0.50 Gy	17	79	24 (0.30)	55 (0.70)	10 (0.42)
5-FU + 0.75 Gy	15	72	16 (0.22)	56 (0.78)	15 (0.94)
5-FU + 1.0 Gy	17	75	14 (0.19)	61 (0.81)	8 (0.57)
5-FU + 1.75 Gy	6	33	0	33 (1.00)	—
5-FU + 0.75 Gy*	4	26	8 (0.31)	18 (0.69)	7 (0.88)
0.5 Gy + 5-FU**	15	60	34 (0.57)	26 (0.43)	14 (0.41)

*5-FU 4 h before irradiation, **5-FU 2 h after irradiation.

day 9 of the pregnancy 1 or 4 h before or 2 h after irradiation (Table 1). The control mice were injected with the same volume of normal saline at corresponding times.

Cell cycle composition

For measurement of the cell cycle composition in the 2–3 mm large mouse fetuses, a suspension of single nuclei was prepared as previously described (Tribukait *et al.* 1975). The cells were then fixed in ice-cold ethanol and the DNA was stained with ethidium bromide.

The distribution of cells in the cell cycle was determined by flow-cytofluorometry described previously (Tribukait *et al.* 1975). The relative numbers of cells in G_1 , S and $G_2 + M$ phases were calculated using a computer program (Ahrens, Germany).

Determination of cell number

The number of cells per fetus was determined by converting the measured DNA content using a standard curve depicting relation between DNA-content and cell number in spleen cells. Since the size of the fetuses at day 9–10 was small (2–3 mm) and the number of cells limited, the DNA content was determined by a fluorescence technique. For conversion, a standard curve made from adult C_3H mice spleen cells was used. More than 90% of the spleen cells were in G_1 and therefore regarded as a homogenous G_1 cell population.

The fetuses were stored in 0.1 ml of TRIS-buffer at -20°C . At the time of DNA measurement the fetuses were completely dissolved in 0.2 ml to 1.0 ml of 0.3 M KOH over night. The solution (0.2 – 1.0 ml) was mixed with 3.0 – 15.0 ml DAPI 100 $\mu\text{g/ml}$ (4'-diamidino-2-phenylindol HCl in TRIS-buffer [0.01 M TRIS; 0.1 M NaCl; 0.01 M EDTA, pH = 7.0]) and measured in a spectrofluorometer (Brunk *et al.* 1979). The excitation and emission wave-lengths were 360 nm and 450 nm, respectively. The DNA content was then converted into a cell number using the standard curve. Since not all fetal cells were in G_1 phase, the cell number obtained from the standard curve had to be used for further calculations to determine the total relative

DNA content of G_1 , S and $G_2 + M$ cells. A multiplication of the relative number of these cells in each cell cycle phase (e.g. G_1 0.40, S 0.40 and $G_2 + M$ 0.20) with the corresponding relative DNA content (G_1 1.0, S 1.5 and $G_2 + M$ 2.0) was made. The total relative DNA content was then multiplied with the total number of cells obtained from the spleen standard curve. The number of cells in G_1 , S and $G_2 + M$ and thus the total cell number of each fetus was obtained.

An analysis of cell cycle composition in different fetal organs was performed. Different fetuses from the same treatment group were used for cell cycle composition analysis, determination of cell number and teratological examination.

Cell cycle progression

The cell flow through the cell cycle was analysed from sequential analyses of the total number of cells at various time intervals after treatment and the evaluation of the proportion of cells in the various parts of the cell cycle. The minimal values for the flow of cells from one compartment to the next could then be calculated (Skog & Tribukait 1985). The cell outflow from each cell cycle phase was expressed as percent per hour (%/h). The cell cycle duration time could be obtained by inversion of the cell flow values (Skog *et al.* 1985).

Teratological examination

On day 18 of gestation the animals were killed by cervical dislocation and dissected. Each pregnant uterus was examined for the total number of implantations. Implanted fetuses dead before day 16 of gestation (denoted resorptions) and dead fetuses were recorded. The living fetuses were examined for gross malformations, individually weighed and fixed in ethanol for 3–5 days. Under a dissection microscope they were then further examined for external malformations (exencephaly, umbilical hernia, club foot, syndactyly, ectodactyly or adactyly). The fetuses were then cleared in 1% potassium-hydroxide solution and stained with alizarin-red-S for examination of skeletal and eye malformations (Wilson & Warkany 1965). Results were summarized per litter, prior to calculation of results for the different treatment groups and variance analysis. The presence of synergy was investigated, as described in Fig. 3 (Valeriotte & Lin 1975).

RESULTS

Teratology

In untreated mice 90% of the total implants survived to gestation day 18 and out of those approximately 4% show malformations (Table 1, Fig. 1). The teratologic effect of X-irradiation and 5-FU, given *in vivo* as single agents or in combination at day 9 after gestation, was determined at day 18. Irradiation with doses up to 1.0 Gy slightly increased the fetal death rate (resorption and late fetal death) and caused a minor increase in the frequency of malformations. When the radiation dose was further increased, the frequency of fetal death increased to 65% and the malformations rose to 60% (Table 1, Fig. 1).

Doses of 0.3–0.6 mg 5-FU/mouse gave a fetal death rate of approximately 20% and elevated the frequency of malformations to 24%. When the 5-FU-dose was 0.8 or 1.2 mg/mouse the fetal death rose to approximately 80% (Fig. 2). For the experiments with 5-FU in combination with irradiation the dose of 0.6 mg/mouse was used.

Addition of 5-FU (0.6 mg/mouse) 1 h before irradiation (0.12–1.75 Gy) had a marked effect on both survival and the frequency of fetal malformations as compared to a single agent treatment (Table 1, Fig. 1). Combined treatment with a radiation dose of 0.12 Gy increased the fetal death

Figure 1. Fetal death and malformations after treatment. Irradiation and 5-FU (0.6 mg 5-FU/mouse) were administered at day 9 after gestation with 5-FU preceding irradiation by 1 h. The figures show the results at day 18 after gestation (= 9 days after treatment). Striped bars indicate irradiation alone, black bars indicate treatment with 5-FU + irradiation. At '0 Gy' the striped bar represents untreated controls and the black bar represents single agent 5-FU. SE-values are shown. (a) Fetal death after treatment. The y-axis shows ratio of resorptions and fetal death to all implants. (b) Fetal malformations after treatment. The y-axis shows ratio of malformations to all living fetuses. At 0.12 Gy and 0.5 Gy no malformations were recorded. At 1.75 Gy the combination of irradiation and 5-FU caused resorption of all implants and thus no investigation of malformations could be done.

Figure 2. Mouse fetal death in response to increasing doses of 5-FU. 'Fetal death' indicates total number of early resorptions and late fetal death. Mean values from 20 litters (76–123 implants) per dose level. For further investigations in combination with irradiation, the dose of 5-FU 0.6 mg per mouse was selected.

to 36% and the malformations to 32%. At 0.5 Gy the effects were even more pronounced. When 0.75 Gy was combined with 5-FU, 78% of the fetuses were resorbed or dead and 94% of the surviving fetuses showed malformations. When 1.75 Gy was combined with 5-FU all fetuses died (Table 1, Fig. 1).

When 5-FU was given 2 h after a radiation dose of 0.5 Gy, the frequency of fetal death was 43% compared to 70% when 5-FU was given 1 h prior to the irradiation. However, the frequency of malformations in the living fetuses were unchanged (41%) (Table 1).

An analysis of variance showed that the rates of fetal death and malformations were significantly related to both radiation dose and the addition of 5-FU (Fig. 1). An interaction between 5-FU and radiation dose was also observed (Fig. 1). Furthermore, when the observed result and the expected outcome in fetal survival were compared, there was clear evidence of synergy when 5-FU was added to irradiation (Fig. 3). Synergy was present also in regard to the malformations.

Cell proliferation

The fetuses could be regarded as a homogenous cell population, as the cell cycle distribution was similar in the different fetal organs and tissues analysed, with the exception of the liver, where the fraction of S-phase cells was high until day 17 of pregnancy. The fetal heart could be

Figure 3. Synergy in induction of fetal death after treatment. 'Observed' shows the proportion of surviving fetuses after combined treatment with 5-FU and irradiation (also in Figure 1). Expected survival is calculated as the survival ratio after irradiation alone $\times$ the survival ratio after 5-FU alone (Valeriote & Lin 1975). If the combination treatment has an additive effect the ratio will be equal to one. If the ratio of observed/expected < 1.0 a synergy is indicated (Valeriote & Lin 1975). For single agent 5-FU (0.6 mg/mouse) the fetal survival ratio (treated/untreated) was 0.80, which is shown at radiation dose 0 Gy in the graph. SE-values are shown.

Table 2. Cell cycle distribution of untreated fetuses. Untreated whole mouse fetuses and different organs were analysed at gestational day 13–18, as soon as each individual organ was detected during the fetal development. The heart, however, could be identified from day 10 (fetus 2–3 mm in size). Each value is a mean of 8–16 samples

Fetal organ	% G1	% S	% G2 + M
Whole fetus	69.0	22.7	8.3
Lung	67.0	23.3	9.7
Thymus	66.1	26.5	7.4
Spleen	79.4	14.9	5.7
Brain	83.7	10.4	6.2
Intestine	74.3	16.5	9.2
Testis	80.6	12.8	6.6
Muscle	75.5	15.6	9.0
Pancreas	80.0	13.1	6.9
Kidney	70.1	20.8	9.2
Heart	80.3	12.5	7.2
Liver	48.0	37.6	14.3

identified from gestation day 10, the liver from day 12, but other organs could not be separated until day 13–14 after gestation (Table 2).

The total number of cells in the untreated fetuses increased exponentially with a doubling time of 8 h from approximately 0.2×10^6 on day 9 to approximately 1×10^7 on day 11. After

Figure 4. Fetal cell proliferation. Cell number in whole fetuses, that were untreated, irradiated (1.75 Gy, 2.50 Gy) or treated with 5-FU (0.6 mg/mouse). Mean and SE-values are shown in a and b. For each observation 8–13 fetuses were analysed. 0, 2, 4, 6 and 10 h after treatment (b) 0, 24, 48, 72, 96, 120, 144 and 168 h after treatment. (Due to small SE-values error bars hardly visible.)

that point in time the increase in rate declined gradually, reaching cell numbers of 7×10^8 on day 18. The influence on cell proliferation by single agent or combined treatment at gestation day 9 was studied up to 168 h after treatment. For the effect of radiation, there was a dose-response relationship (Fig. 4).

Figure 5. Cell number per fetus 48 h after treatment. Influence of irradiation and 5-FU (0.6 mg/mouse) on the total number of cells per mouse fetus is shown. The mice were treated at day 9 after gestation and the cell number in the fetuses was analysed 48 h after treatment. '1 h' and '4 h' indicate the interval between injection of 5-FU and irradiation. Bars indicate SE-values. The results are based on analysis of at least 5 fetuses per treatment group.

When analysed 48 h after 2.5 Gy the total number of cells per fetus was less than half (4.5×10^6 cells) of that in the untreated control group (10.2×10^6 cells). 5-FU had a marked inhibitory effect on cell proliferation with a clear dose-response relationship. When the low toxic dose of 0.6 mg 5-FU was added 1 or 4 h prior to the irradiation, the inhibitory effect of irradiation on proliferation was potentiated. The effect of 5-FU + 0.5 Gy was the same as 1.75–2.5 Gy given as single agent. Prolongation of the interval between 5-FU and irradiation from 1 to 4 h further increased the synergy (Fig. 5).

Cell cycle progression

On gestation day 9 in the untreated mouse fetuses, 30% of the cells were in cell cycle phase G_1 , 50% in S and 20% in $G_2 + M$, corresponding to a phase duration of approximately 2, 5 and 1 h, respectively (Figs 6, 7). The phase durations were obtained from the cell cycle flow calculations described in 'Material and Methods' and shown in Fig. 7.

Irradiation resulted in typical changes in the cell cycle distribution with an accumulation of cells in G_2 and a corresponding decrease in G_1 (Figs 6, 7). Calculation of cell cycle flow after, for example, the administration of 1.75 Gy, showed a marked rise in outflow from G_1 within 2 h followed by an arrest at 4 h, while the S-phase cells were practically unaffected, except at 4–6 h. There was a continuous decrease up to 4 h in the outflow from G_2 followed by a slow normalization (Fig. 7a).

Figure 6. DNA-histograms of untreated and treated fetal cells. Example of DNA-histograms showing the cell cycle distribution in mouse fetal cells up to 24 h after exposure to irradiation and 5-FU at day 9 after gestation. The peak to the left represents G₁ cells, the peak to the right G₂ cells and the area in between the S-phase cells.

5-FU (0.6 mg/mouse) as a single agent caused a marked change in the distribution of cells in the different cell cycle phases (Fig. 6). The maximum changes were obtained at 6 h after administration of 5-FU, where the proportion of cells in S-phase increased to more than 75%. There were corresponding decreases of the proportion of cells in G₁–15% and of cells in G₂ + M to below 10%. Thus, the outflow from G₁ and G₂ was almost doubled during the first 4 h, while the outflow from the S-phase was unchanged. After 6–10 h there was almost a complete arrest in all cell cycle phases, which was slowly released after 10 h (Fig. 7b).

The addition of 5-FU 1 hour before irradiation with 1.75 Gy changed the continuous decrease in the G₂-outflow, seen when 1.75 Gy was used as single agent, to a rise in the G₂-outflow-rate during the initial 2 h. At 4–8 h there was a complete arrest in the outflow from all cell cycle phases. When the cells started to recover from the arrest, there was a higher cell

Figure 7. Cell cycle progression after treatment. The cell flow through the cell cycle was analysed as described in Material and Methods. Cell outflow from G₁, S and G₂-M are shown 0–96 h after exposure of mouse fetuses to 1.75 Gy (a), 0.6 mg 5-FU/mouse (b) and 0.6 mg 5-FU/mouse + 1.75 Gy (c). In (d) the outflow from G₂ is shown for the initial 10 h after treatment. The results are based on analysis of at least 5 fetuses per treatment group.

outflow rate from G_1 and G_2 after the combination treatment in the 10–24 h time interval compared to irradiation alone (Fig. 7c).

DISCUSSION

This study revealed that, in an *in vivo* mouse fetus model, the combination of low-toxic doses of 5-FU and irradiation has a marked effect on cell proliferation. The fetal cell growth was inhibited and there was a synergistic increase of cytotoxicity and fetal malformations. The radiation-induced G_2 -arrest was irradiated by 5-FU during the initial hours when DNA damages are repaired. The molecular mechanism behind the observed action of 5-FU is not known.

Mechanisms behind the effects of cytotoxic drugs and radiation should be generally applicable in malignant and nonmalignant proliferating cells. The mouse fetus model here employed gave us the opportunity to expose large amounts of highly proliferative normal cells *in vivo* to homogeneous cytotoxic influence. Toxicity was observed as a decreased rate of cell proliferation as well as elevated rates of fetal death and malformations. The system used, responded to both single agent treatment and to the combination.

It can be questioned whether the mouse fetus really behaves as a homogenous tissue in response to treatment. However, judging from the DNA-histograms, the cell cycle regulation and proliferation in different tissues and organs in the mouse fetus was very similar. Furthermore, the extensive effect of only 5-FU makes it plausible that the availability of 5-FU in different fetal tissues is quite homogeneous. We find it justified to regard the whole fetus as a homogeneously responding tissue. Furthermore, mouse fetus systems have earlier been shown to be suitable for studies of cytotoxic effects by 5-FU (Dagg 1960).

Various models for administration of 5-FU in combination with X-rays, such as continuous long-term infusions or bolus injections, have been employed both clinically and *in vitro*. Synergistic responses have been observed when 5-FU has been given within 14–24 h before irradiation or within 8 h afterwards. *In vitro*, there were differences in response between different cell lines, but the effect was seen irrespective of whether a bolus – or a continuous exposure – model was used (Smalley *et al.* 1991). The dependence on the duration of the time interval between radiation and 5-FU was not studied, but the present data demonstrate a synergy in the toxic effect of the combination, where the injection of 5-FU precedes the radiation dose. Our data indicate a less toxic, however, still synergistic, effect when the irradiation precedes an injection of 5-FU. Visible effects caused by addition of 5-FU to irradiation, irrespective of the sequencing, is in line with earlier published results.

The influence of 5-FU at the cellular level is still unclear, but interesting parallels to the 5-FU-mediated release from the G_2 -block after radiation is the action of cordycepin, which inhibits synthesis of messenger RNA and methyl-xanthines, such as caffeine, which inhibit protein kinases. These agents release the radiation induced G_2 -block (Rowley 1992) and increase teratogenic toxicity when combined with ionizing radiation in mice (Sivak 1994). In earlier studies on mouse sarcoma cells *in vivo*, there was evidence of a synergistic cytotoxic effect when cisplatin and 5-FU were combined. Cisplatin causes a G_2 -arrest, which is irradiated by the addition of 5-FU (Lewin *et al.* 1990) without any change in the rate of induction or elimination of cisplatin-induced DNA crosslinks (Lewin *et al.* 1995) or evidence of increase in misincorporations of pyrimidines in DNA (Nylén *et al.* 1996). Thus, there is strong evidence, that the combination of a DNA-damaging agent with an agent shortening or abolishing the G_2 arrest involves increased toxicity.

From the study results, it was obvious that 5-FU influences the G₂-M checkpoint regulation. However, clear indications are still missing as to which level is influenced. Besides the well documented antiproliferative effect by inhibition of TS, 5-FU is incorporated in DNA and RNA. The consequences of this are less well known. There is however, evidence, that the DNA-directed, TS-mediated effects of 5-FU are important for the radiosensitizing effect of the drug (Lawrence *et al.* 1994), rather than the RNA-directed effects. In the context of the results presented, 5-FU could exert an effect on the G₂-M regulation via altered expression of regulatory proteins on the gene level.

The radiation-induced, reversible cell cycle block in G₂ is believed to be dependent on modification by DNA-damage related factors, which regulate the cell cycle. DNA-dependent protein kinase (DNA-PK) may act as a link from the damaged DNA to the G₂ check-point (Morozov *et al.* 1994). The protein kinase wee-1 phosphorylates p34^{cdc2}. Dephosphorylation of the p34^{cdc2}-enzyme is mandatory for progression from G₂ to M and persistent phosphorylation of p34^{cdc2} prevents the progression into mitosis (Coleman & Dunphy 1994). The wee-1 has been shown to be necessary for the radiation-induced mitotic delay (Rowley *et al.* 1992). It has also been shown, that irradiation may decrease the normal synthesis of cyclin B mRNA in late S + G₂ (Muschel *et al.* 1992), which would inhibit the normal production of cyclin B-p34^{cdc2}-complexes needed for the normal progression into mitosis. 5-FU has been shown to modify and perturbate the processing of mRNA and it has been proposed, that primarily short-lived mRNA, such as mRNA for growth factors and other factors important for mastering the cell proliferation, are influenced (Dolnick & Wu 1993). Action by 5-FU, as a modifier of the cellular response to DNA damage by mRNA-production, might be in line with the observations, that 5-FU has to be present at the moment of irradiation or within hours afterwards. This would be during the time period when the cellular reaction on the radiation induced damage takes place. An explanation for our present findings of a 5-FU-related release of radiation-induced G₂-arrest and the ensuing increase in mouse fetal toxicity, could be that 5-FU affects the regulation or production of cell cycle regulating factors at the mRNA-level. This issue is being addressed in ongoing experiments.

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