3. RESULTS

3.1. Expression of multidrug resistance-associated genes after irradiation

After completion of radiation with 27 Gy, most of the cell lines had undergone growth arrest and about a 1 log cell kill, but the remaining cells were vital as assessed by trypan blue exclusion and with preserved adherent growth pattern. To examine the relationship between radiation and the expression of multidrug resistance-associated factors, the expression levels of three genes, namely lrp, mrp1 and mdr1 were evaluated on the basis of their mRNA/PBGD ratio. The studied breast and colon cancer cell lines had a basal expression of lrp mRNA (ratio marker/PBGD), ranging from 0.01 to 13.38, median being 0.78. The median ratio in the breast cancer cell lines was 0.05, as in the colon cancer cell lines - 1.5. However, after 27 Gy of radiation therapy over three weeks and three days of rest, the lrp mRNA content ranged from 0.03 to 7.42 (Fig. 3.1.1), median being 2.44. There was statistically significant 4.7-fold lrp mRNA increase in the irradiated breast cancer cell lines compared to the non-irradiated parental controls (n = 5). However, no statistically significant difference in lrp mRNA expression between irradiated and non-irradiated colon cancer cell lines was observed (Fig. 3.1.2). Nevertheless, the irradiated SW620 colon cancer cell line had increased its lrp mRNA content about 10 times compared to its non-irradiated control (Fig. 3.1.2). The mrp1 mRNA gene expression varied from 0.0006 to 0.17, median being 0.028 among the non-irradiated cell lines and from 0.01 to 0.7. In the breast cancer cell lines (Fig. 3.1.1), there was a 14.5-fold statistically significant mrp1 mRNA overexpression after irradiation. No significant difference in mrp1 mRNA expression between irradiated and non-irradiated control colon cancer cell lines was detected (Fig. 3.1.2). However, the irradiated Colo320 colon cancer cell line increased notably its mrp1 mRNA content compared to its non-irradiated control (Fig. 3.1.2). The expression of mdr1 mRNA among the non-irradiated cell lines was at fairly low levels, ranging from 0.000001 to 1.01. After radiation therapy, a 15-fold

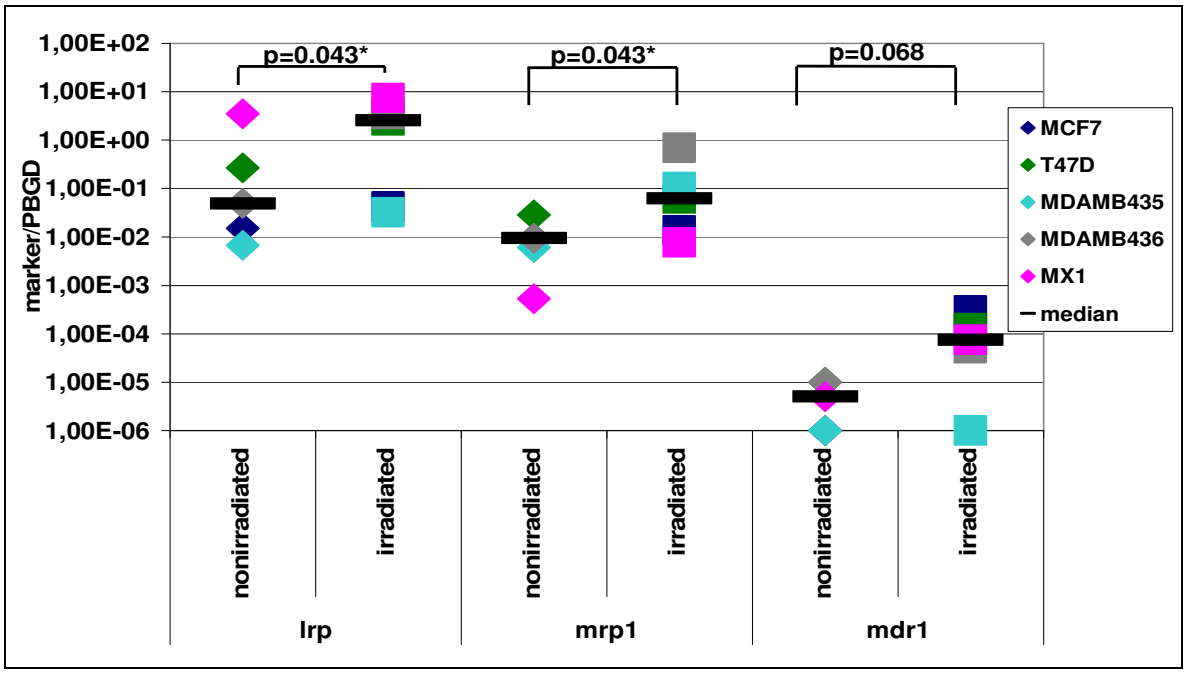

Fig. 3.1.1. Expression of lrp, mrp1 and mdr1 mRNA among five breast cancer cell lines before and 3 days after irradiation with 27 Gy. The mRNA content is given as a ratio between the marker and the PBGD mRNA. The asterisks (*) indicates statistical significance at a level of $p < 0.05$, attained by the Wilcoxon rank test.

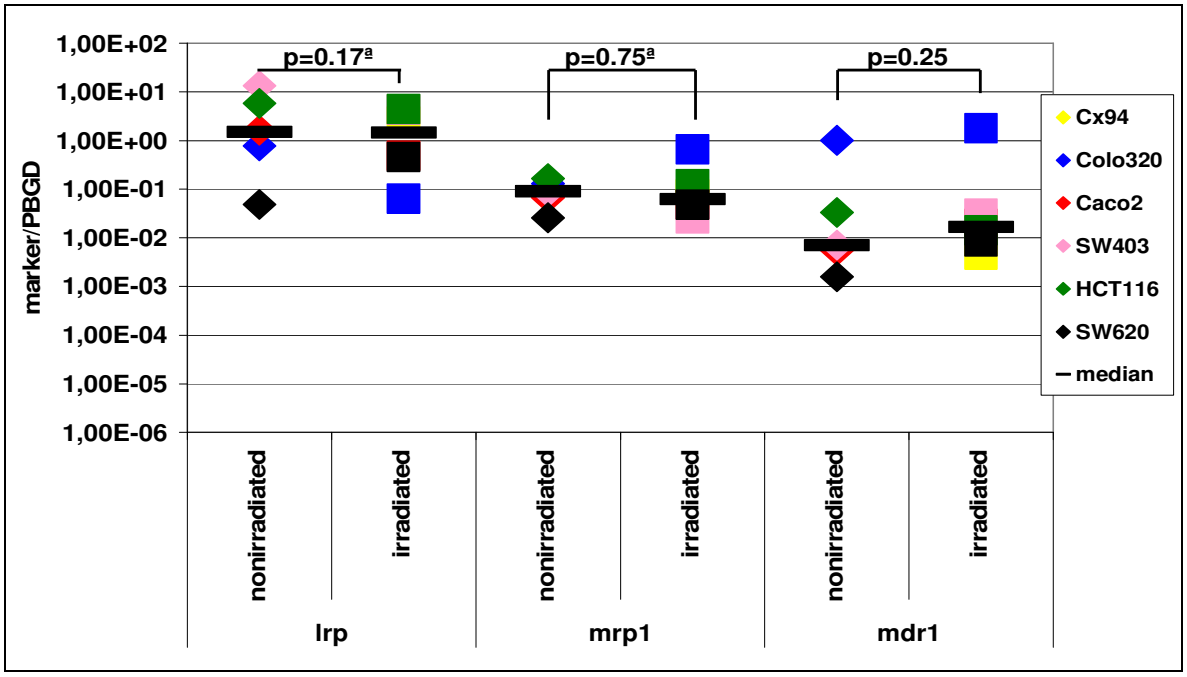

Fig. 3.1.2. Expression of lrp, mrp1 and mdr1 mRNA among six colon cancer cell lines before and 3 days after irradiation with 27 Gy. The p-value is obtained by the Wilcoxon's rank test. [a] – based on the positive ranks.

overexpression close to the statistical significance was observed in the breast cancer group. However, although the irradiated SW403, SW620 and Caco2 colon cancer cell lines increased their mdr1 mRNA content compared to the non-irradiated controls, no statistically significant difference in mdr1 mRNA expression between irradiated and non-irradiated colon cancer cell lines was detected (Fig. 3.1.2).

3.2. Expression of multidrug resistance-associated proteins after irradiation

As mentioned in Materials and Methods the protein expression was evaluated on a flow cytometer using monoclonal and fluorescence labeled antibodies (2.3.9). The fluorescence of each isotype control was subtracted from the mean fluorescence and arbitrary units were achieved (AU). The results for LRP, MRP1 and P-glycoprotein are summarized in Fig. 3.2.1 and Fig. 3.2.2 and presented as medians.

The lung resistance protein (LRP) was detected at various levels in the studied cell lines prior to irradiation. The LRP fluorescence in the non-irradiated cell lines ranged from 0.5 AU to 19.4 AU (Fig. 3.2.1 and 3.2.2). The LRP fluorescence in the irradiated group of cell lines was significantly higher vs. the non-irradiated parallel controls, ranging from 4 AU to 34.7 AU. In the colon cancer cell lines there was a 5.2-fold median fluorescence increase in the irradiated cell cultures compared to the non-irradiated controls. Although in the irradiated T47D, MCF7 and MDAMB435 breast cancer cell lines there was an increased LRP fluorescence, overall the difference compared to the non-irradiated controls was not statistically significant (Fig. 3.2.1). Most of the non-irradiated cell samples stained entirely negative for P-gp and MRP-1. The level of multidrug resistance-associated protein 1 (MRP1) ranged from 0 AU to 9.2 AU and in 7 of the non-irradiated controls was fairly undetectable (n = 11). However, in the irradiated cell lines there was a statistically significant increase of MRP1 vs. the non-irradiated control cultures, ranging from 0 AU to 17.5 AU. This was due predominantly to a 25.9-fold increase of the MRP1 fluorescence in the irradiated colon cancer cell lines compared to the non-irradiated (Fig. 3.2.2). In the irradiated T47D breast cancer cell line an MRP1 fluorescence increase was detected.

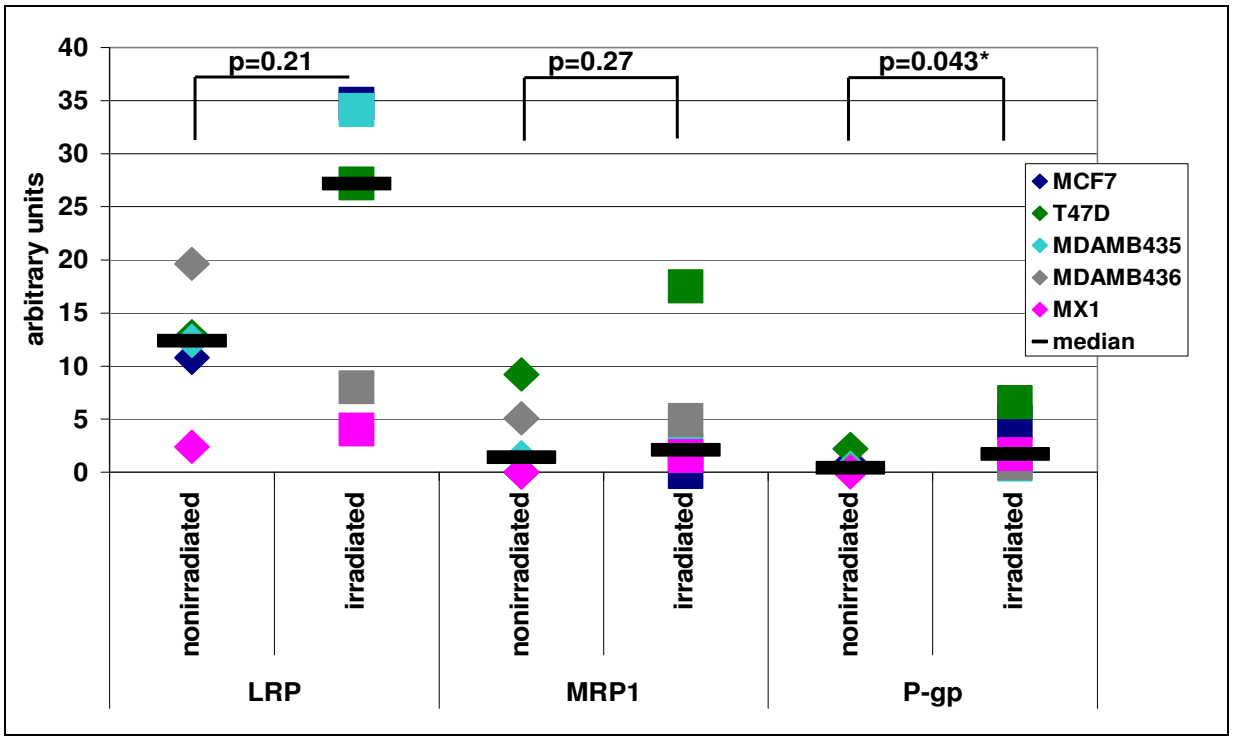

Fig. 3.2.1. Expression of LRP, MRP1 and P-glycoprotein among five breast cancer cell lines before and 3 days after irradiation with 27 Gy.

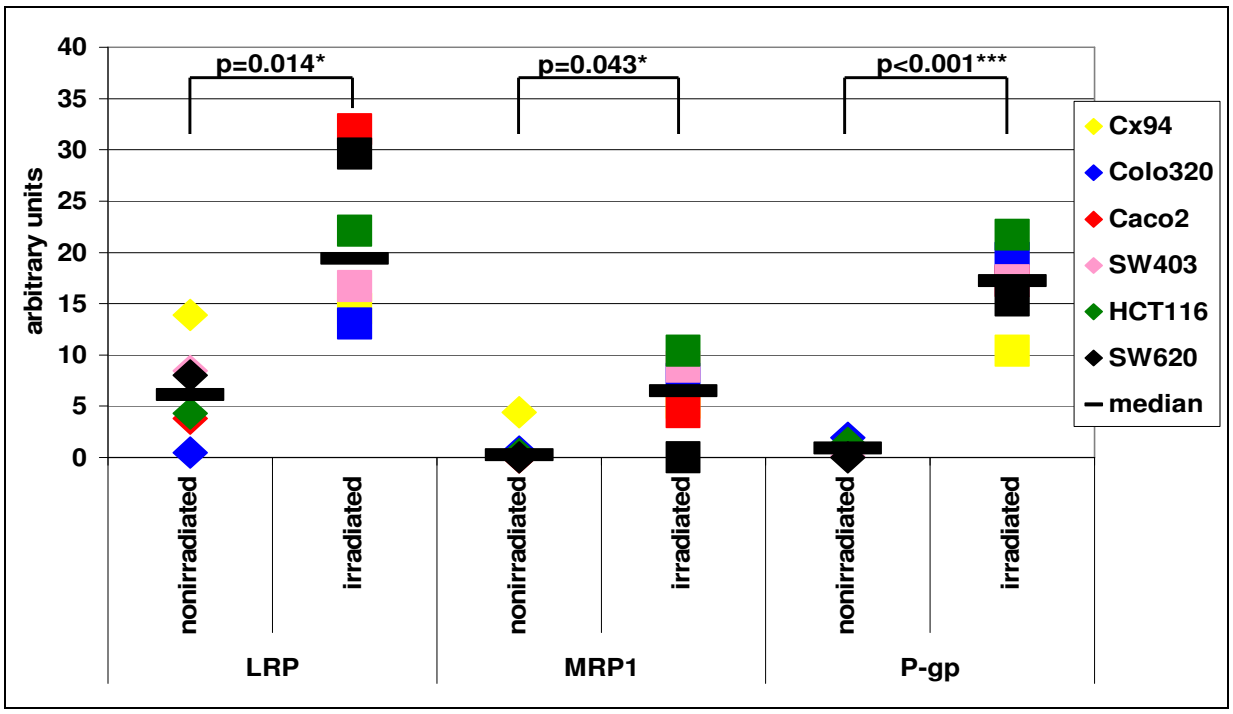

Fig. 3.2.2. Expression of LRP, MRP1 and P-glycoprotein among six colon cancer cell lines before and 3 days after irradiation with 27 Gy. The three asterisks (***) indicate significance at level $p < 0.001$

However, no statistically significant overexpression was found in the irradiated breast cancer cell lines compared to the non-irradiated controls (Fig. 3.2.1). All investigated cell lines exhibited a low P-glycoprotein basal level with median 0.6 AU for both groups of cell lines, ranging from undetectable level to 2.2 AU. In agreement to previous studies (Berger et al., 2005, Hill et al., 2000+2001), we observed a P-glycoprotein content increase after irradiation. In both subgroups, statistically significant overexpression of P-glycoprotein was observed. In the irradiated colon and the breast cancer cell lines there was a 17.3-fold, respectively 3.5-fold P-glycoprotein content increase vs. the non-irradiated parallel control cell cultures (Fig. 3.2.1 and 3.2.2).

3.3. Correlation between the expressions of multidrug resistance-associated genes and their protein products

As a next step of analysis, the expression levels of lrp, mrp1 and mdr1 mRNA levels (as marker/PBGD ratio) were compared to their protein products LRP, MRP1 and P-glycoprotein, respectively. The Pearson correlation coefficient demonstrated a trend between mdr1 mRNA and P-glycoprotein in the irradiated breast cancer cell lines, but no significance was reached. In the irradiated breast cancer cell lines a negative correlation was observed between lrp mRNA and the LRP expression (Table 3.3.1).

	All (n = 11)	Breast (n = 5)	Colon (n = 6)
LRP/lrp mRNA (r)	-0.7*	-0.914*	-0.31
P	0.013	0.03	0.55
MRP1/mrp1 mRNA (r)	0.1	0.015	0.3
P	0.78	0.98	0.6
P-gp/mdr1 mRNA (r)	0.4	0.7	0.33
P	0.24	0.2	0.52

Table 3.3.1. Correlation between the expression of multidrug resistance genes mRNA (obtained as marker/PBGD ratio) and the level of multidrug resistance-associated proteins in irradiated cancer cell lines, 3 days after the last irradiation.

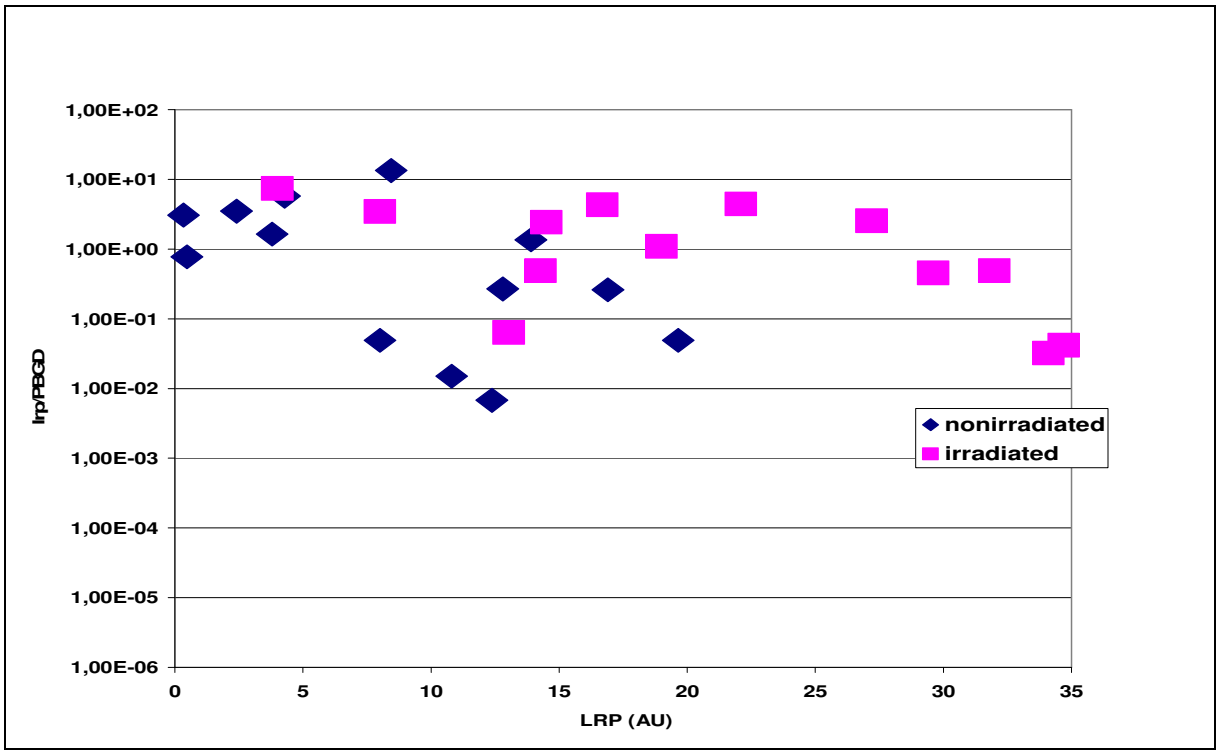

Fig. 3.3.1.Correlation between the lrp gene expression and LRP level before and 3 days after the last irradiation of 27 Gy, in 11 cell lines- r = - 0.7 (irradiated group).

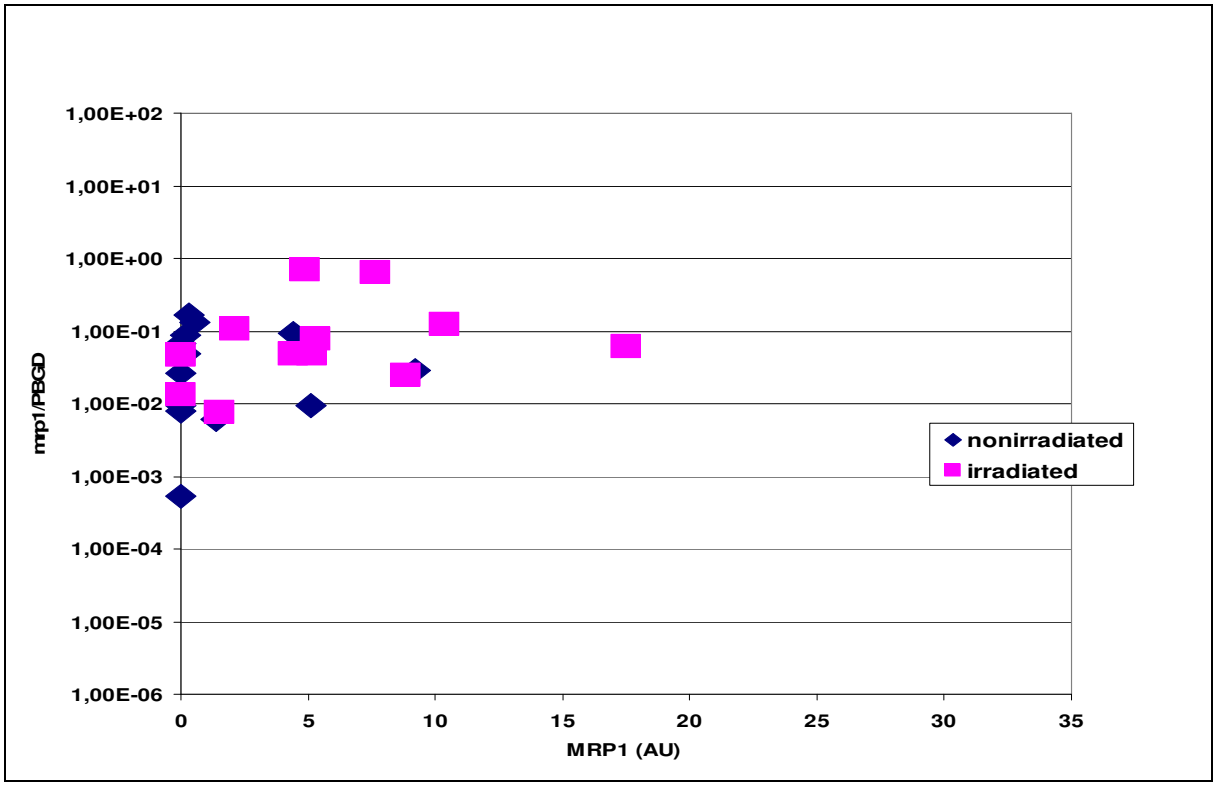

Fig. 3.3.2.Correlation between the mrp1 gene expression and MRP1 level before and 3 days after the last irradiation of 27 Gy, in 11 cell lines - r = 0.1 (irradiated group).

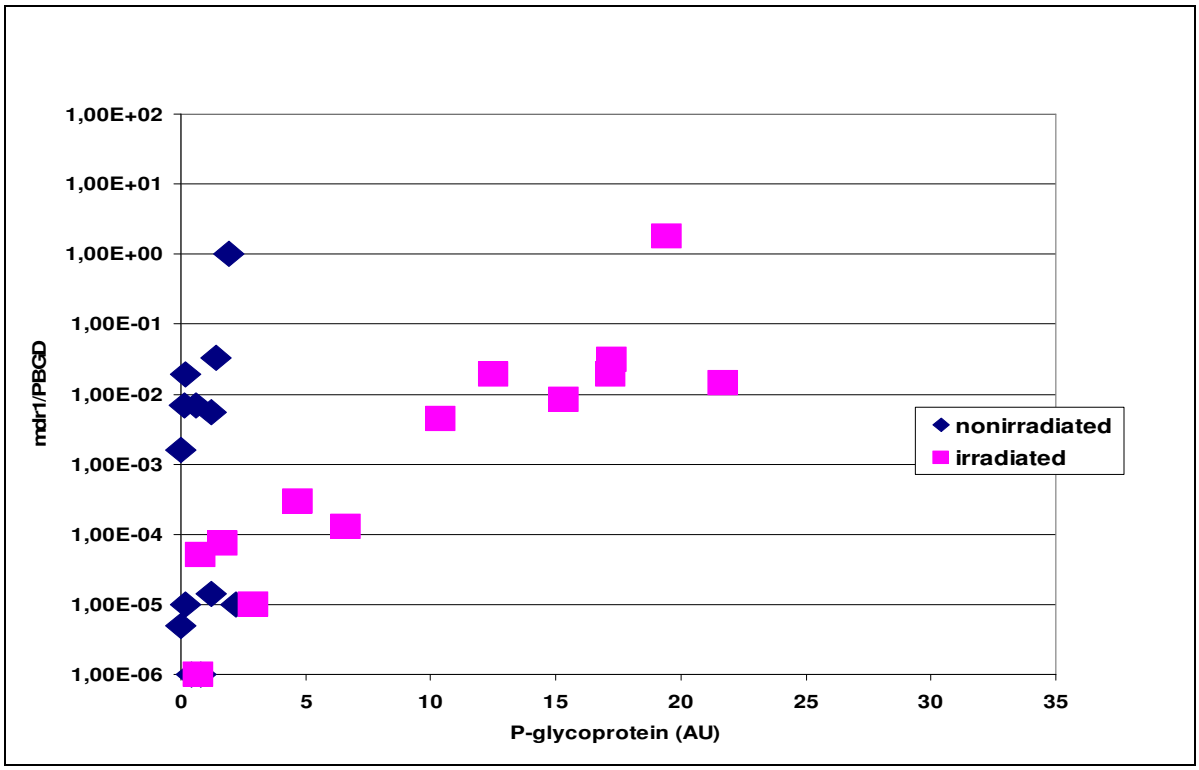

Fig. 3.3.3.Correlation between the mdr1 gene expression and P-glycoprotein level before and 3 days after the last irradiation of 27 Gy, in 11 cell lines – r = 0.4 (irradiated group).

A subsequent study using the non-parametric Spearman method proved a strong correlation between mdr1 mRNA and P-glycoprotein - $r = 0.91$ ($p < 0.001$).
However, no relationship between the lrp and mrp1 mRNA level and the expression of LRP and MRP1, respectively, was observed in the cell lines treated with radiation (Table 3.3.1).

3.4. Co-expression of multidrug resistance associated genes and proteins

From previous studies we know that after chemotherapy a variable co-expression of mdr1 and mrp1 genes and their protein products – P-glycoprotein and MRP1 occurs. Nonetheless, no such co-expression has been reported after radiation treatment. In this study we observed a correlation between mdr1 mRNA and mrp1 mRNA expression in

the irradiated cell lines (Table 3.4.1). Nevertheless, no statistically significant co-expression was observed in the multidrug resistance-associated proteins after irradiation (Table 3.4.2).

	All (*n* = 11)	Breast (*n* = 5)	Colon (*n* = 6)
lrp/mdr1 (r)	-0.3	0.4	-0.48
P	0.35	0.5	0.3
lrp/mrp1 (r)	-0.12	-0.45	-0.43
P	0.7	0.4	0.4
mdr1/mrp1 (r)	0.63*	-0.37	0.98***
P	0.039	0.53	0.0001

Table 3.4.1. Correlation (r) in the expression of multidrug resistance-associated genes in cancer cell lines, 3 days after the last irradiation. The gene expression was obtained as a marker/PBGD mRNA ratio.

	All (*n* = 11)	Breast (*n* = 5)	Colon (*n* = 6)
LRP/P-glycoprotein (r)	0.08	0.43	0.1
P	0.81	0.47	0.85
LRP/MRP1 (r)	-0.1	0.1	-0.58
P	0.78	0.9	0.23
P-gp/MRP1 (r)	0.3	0.65	0.56
P	0.38	0.24	0.2

Table 3.4.2. Correlation (r) in the expression of multidrug resistance-associated proteins in cancer cell lines, 3 days after the last irradiation. The protein expression is obtained as arbitrary units.

3.5. Cell sensitivity to cytotoxic drugs, following radiation treatment

Two breast cancer cell lines - MCF7 and Mx1 and six colon cancer cell lines - Caco2, Colo320, Cx94, HCT116, SW403 and SW620, which in the preliminary studies had shown to be sensitive to physiologically relevant concentrations of bendamustine,

cisplatin and doxorubicin (2.3.10), were introduced in the drug sensitivity assays after fractionated radiation therapy. Three weeks of radiation treatment caused growth arrest and cell death. However, the HCT116 colon cancer cell line exhibited a decreased sensitivity to irradiation and did not change its growth speed. For six colon and two breast cancer cell lines, drug cytotoxity curves were obtained. The values of resistance levels of each cell line were obtained and the medians for each drug concentration were compared. The cell viability was assessed after 48 hours of incubation with bendamustine, cisplatin and doxorubicin. In the cell lines studied, a comparatively low in vitro non-drug induced cytotoxity was detected – 9.8 % (median) in the irradiated and 14.1 % (median) in the non-irradiated lines, which was not significant ($p = 0.33$; z).

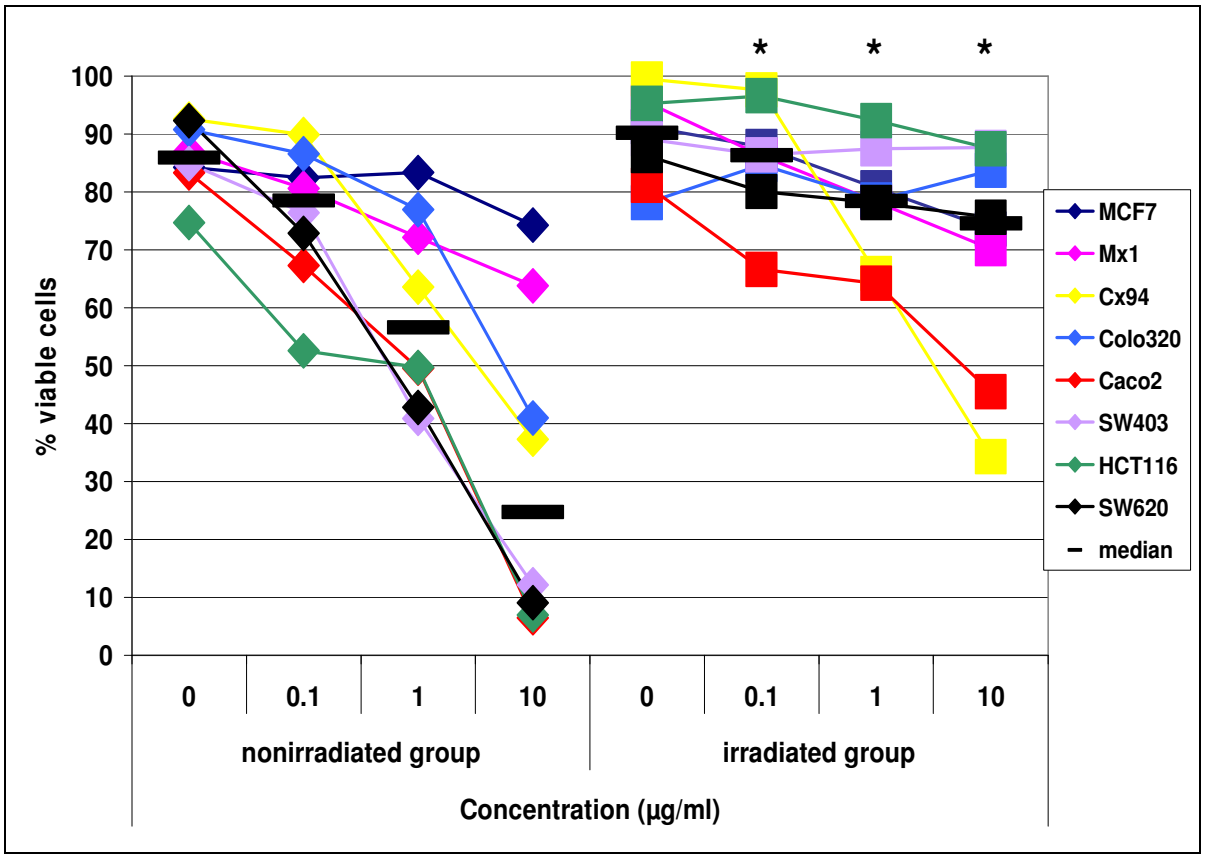

Fig. 3.5.1. Sensitivity of eight breast and colon cancer cell lines to doxorubicin, before and 3 days after the last irradiation of 27 Gy.

After irradiation, the cell samples were statistically significant less susceptible to undergo apoptosis after incubation with doxorubicin than the non-irradiated parental controls (Fig. 3.5.1). The irradiated cell lines had 1.9-fold increase in cell drug resistance (median) to 0.1 mg/ml doxorubicin than the non-irradiated ($p = 0.036$; z). The level of resistance to physiological (1 µg/ml) and supra-physiological concentrations (10 µg/ml) of doxorubicin increased to 2.5-fold ($p = 0.036$; z) and 3.8-fold ($p = 0.036$; z) respectively. Moreover, two irradiated colon cancer cell lines, namely SW403 and Colo320, had become highly resistant to 10 µg/ml doxorubicin compared to their parallel non-irradiated controls (Fig. 5.3.1). However, in the Cx94 colon cancer cell line and the breast cancer MCF7 cell line, no change of cell sensitivity to doxorubicin was observed following irradiation (Fig. 3.5.1).

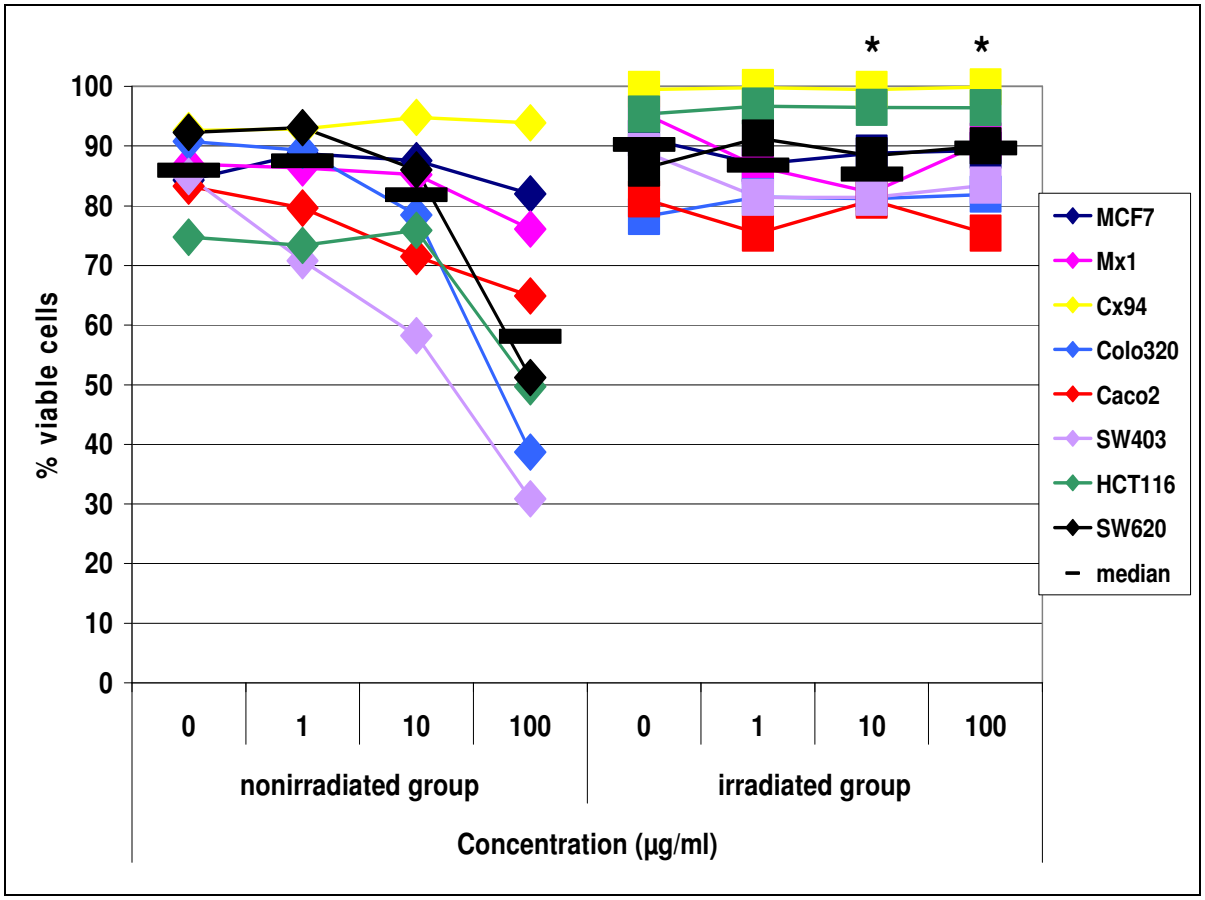

Fig. 3.5.2. Sensitivity of eight breast and colon cancer cell lines to bendamustine, before and 3 days after the last irradiation of 27 Gy.

It was obvious that 1 µg/ml bendamustine could not trigger apoptosis among most of the irradiated cell lines and also their non-irradiated control (p = 0.4; t) (Fig.3.5.2). However, the cell lines treated with radiation had a statistically significant 1.9-fold increase in drug resistance to 10 µg/ml bendamustine (physiological concentration) than the non-irradiated controls (p = 0.05; t). At 100 µg/ml bendamustine, this effect was even more obvious - 11-fold (p= 0.005; t). Nonetheless, both non-irradiated and irradiated MCF7 and Mx1 breast cancer cell lines remained resistant to bendamustine.

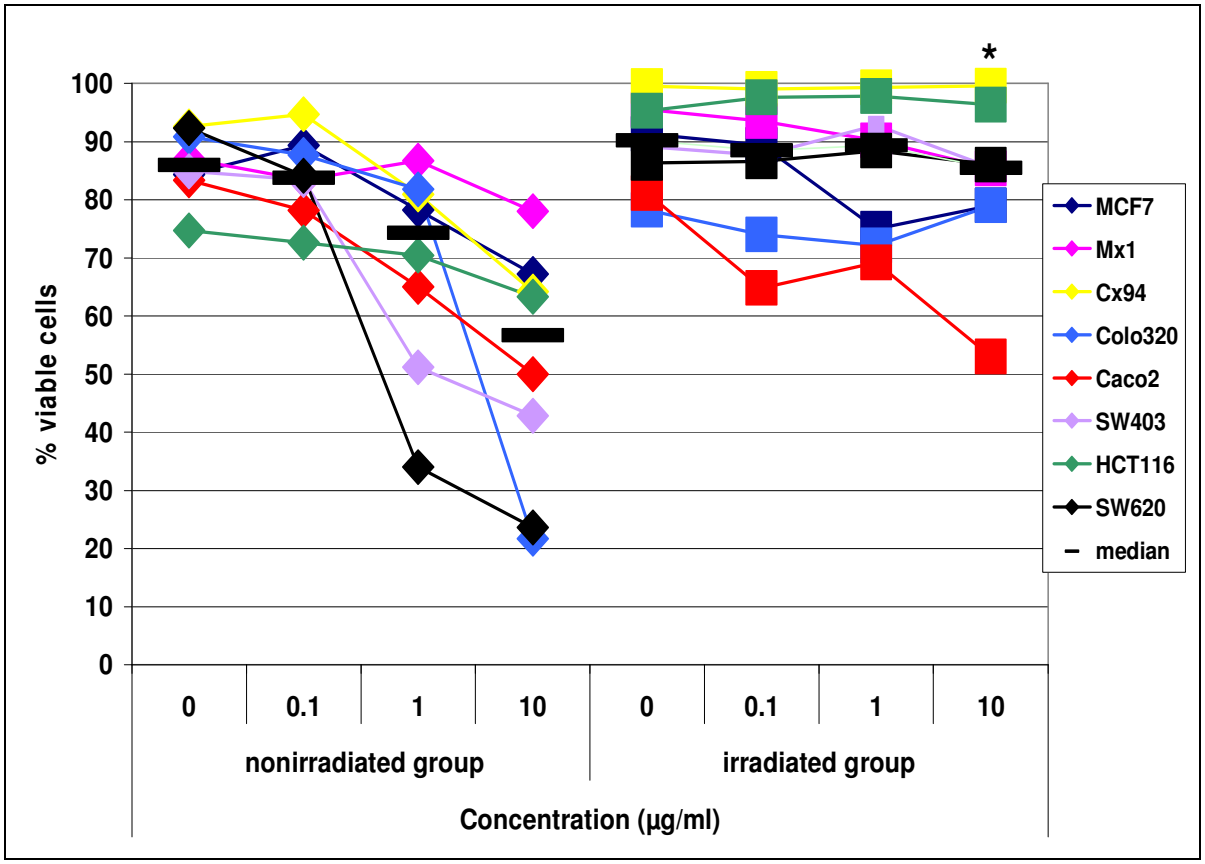

Fig. 3.5.3. Sensitivity of eight breast and colon cancer cell lines to cisplatin, before and 3 days after the last irradiation of 27 Gy.

Similar effects of the radiation treatment on the drug sensitivity were observed also in the treated with cisplatin group of cell samples (Fig. 3.5.3). At 1 µg/ml cisplatin (physiologic concentration), the irradiated cell lines had 5.75-fold increase in cisplatin resistance than the non-irradiated parallel controls and that was close to the level of statistical

significance (p = 0.07; t). However, at 10 µg/ml cisplatin, the irradiated cell lines had 5.5-fold increase in cell drug resistance vs. the non-irradiated lines (p= 0.005; t). The irradiated Cx94 and HCT116 colon cancer cell lines remained highly resistant to any concentration of cisplatin.

3.6. Correlation between multidrug resistance-associated proteins expression and cell viability following incubation with cytotoxic drugs

We evaluated the relationship between multidrug resistance-associated proteins overexpression and the relative drug resistance occurring after radiation therapy. The latter was defined as: % viable cells in the non-irradiated cell line - % viable cells in the irradiated cell line at maximal concentrations of bendamustine, cisplatin and doxorubicin (Table 3.6.1).

	bendamustine 100 µg/ml	cisplatin 10 µg/ml	doxorubicin 10 mg/ml
LRP (r)	0.03	-0.14	0.48
P	0.94	0.73	0.22
MRP1 (r)	0.67	0.4	0.71*
P	0.068	0.34	0.048
P-glycoprotein (r)	0.65	0.58	0.87**
P	0.08	0.13	0.005

Table 3.6.1. Correlation (r) between the overexpression of multidrug resistance proteins and the appearance of multidrug resistant phenotype in a group of eight breast and colon cell lines. The two asterisks (**) indicate statistical significance at a level of p < 0.01.

A significant correlation was detected between the P-glycoprotein overexpression and the relative cell viability following a 10 µg/ml doxorubicin incubation (p = 0.005). A significant level of correlation was also found between MRP1 overexpression and the relative cell viability after doxorubicin incubation (p = 0.048). Similar results were obtained in the bendamustine cluster, where P-glycoprotein and MRP1 overexpression correlated with the relative cell viability following bendamustine incubation at a level

near the statistical significance (Table 3.6.1). A tendency of correlation was found also between P-glycoprotein and the level of cell resistance to cisplatin (p= 0.13). However, no relationship was observed between LRP overexpression and the occurrence of multidrug resistance after irradiation.

3.7. Modulation of P-glycoprotein and MRP1 activity using verapamil

Calcium channel blockers and especially verapamil were reported to block the activity of P-glycoprotein and MRP1 in a dose-dependent fashion and to restore the tumor cell sensitivity to cytotoxic drugs (Salerno et al., 2004; Takara et al., 2002). The breast cancer cell lines were excluded from this study as they poorly reacted to the cytotoxic drugs used in this study. The chemosensitizing effects of verapamil on doxorubicin, cisplatin and bendamustine sensitivity were first assessed on SW620NaBr cell line, a positive control for P-glycoprotein, MRP1, and LRP expression (2.3.13) and a model for multidrug resistance (Fig. 3.7.1). Verapamil enhanced the sensitivity to doxorubicin in a dose-dependent manner, but poorly to cisplatin and bendamustine, as only doxorubicin is a proved P-glycoprotein substrate (Fig. 3.7.1).

Verapamil was tested for possible cytotoxity in absence of cytotoxic drugs in the cell lines used in the cell cytotoxity assays. Cell samples of the six colon cancer cell lines, which participated previously in the drug cytotoxity assays, were incubated in medium containing verapamil, diluted to 25 mM and 75 mM, in the absence of cytotoxic drugs. The chemosensitizer alone did not show significant cytotoxity at doses 25 mM after 24 and 48 hours of incubation. However, 75 mM verapamil induced significant cell apoptosis after 48 hours of incubation in Cx94 colon cancer cell line and this line was excluded from the successive study (data not shown). In subsequent experiments, we analyzed the impact of 25 mM and 75 mM verapamil as a multidrug resistance sensitizer on five pre-irradiated colon cancer cell lines, treated with increasing concentrations of doxorubicin, bendamustine and cisplatin for 48 hours (Fig. 3.7.2).

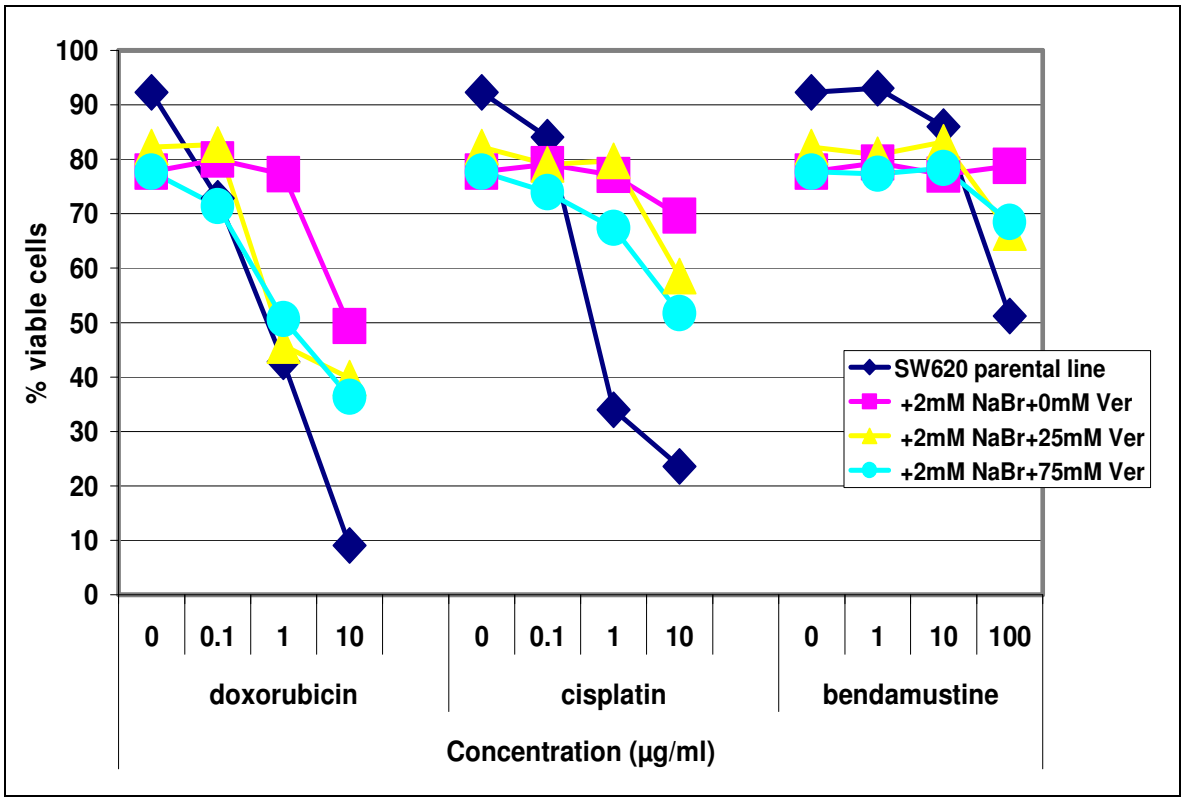

Fig. 3.7.1. Chemosensitizing effects of verapamil to cytotoxic drugs in SW620 colon cancer cell line incubated with 2 mM Natrium butyrate. NaBr – Natrium butyrate, Ver – verapamil.

The survival curves and presented in Fig. 3.7.2 show that 25 mM and 75 mM verapamil slightly increased the cytotoxity of doxorubicin. However, no statistical significance was observed, while comparing the cell groups incubated with 0 mM, 25 mM and 75mM verapamil and increasing concentrations of bendamustine, cisplatin and doxorubicin (Fig. 3.7.2).

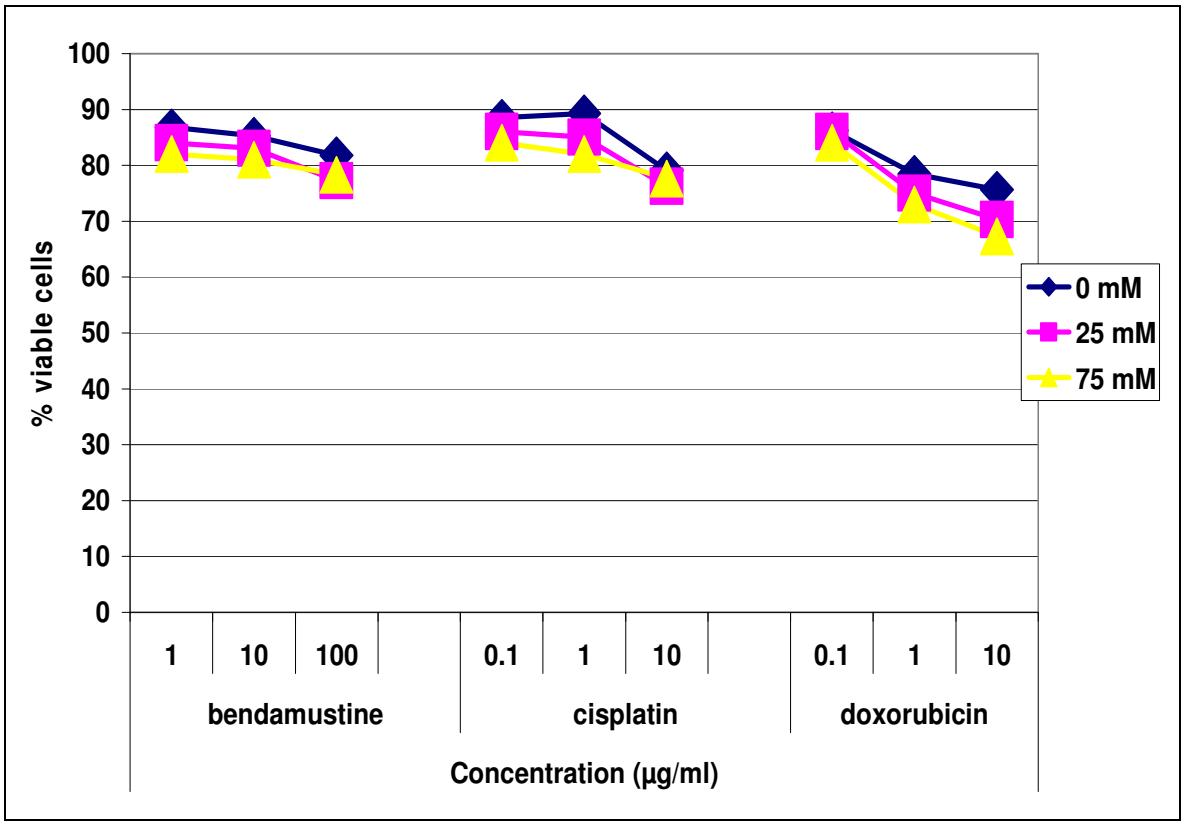

Fig. 3.7.2. Median sensitivity of five colon cancer cell lines to bendamustine, cisplatin and doxorubicin supplemented with 25 mM or 75 mM verapamil, 3 days after completion of the irradiation treatment. No statistical difference was observed in both subgroups.

3.8. Expression of multidrug resistance-associated genes and their proteins as a late response to radiation

In a next step, we studied the dynamics of lrp, mrp1 and mdr1 gene expression and LRP, MRP1 and P-glycoprotein level among five colon cancer cell lines, namely - Caco2, Colo320, HCT116, SW403 and SW620 at a second endpoint – day 18 after the last irradiation. At the second end point almost all cell lines had achieved logarithmic growth; however, the irradiated Colo320 colon cancer cell line had grown substantially slower than its nonirradiated control. We observed that the mdr1 mRNA had a 2.6-fold increase in the irradiated cell lines vs. the non-irradiated controls (p= 0.08; z). On mrp1 mRNA

level no substantial changes were detected after irradiation compared to the non-irradiated parallel cultured cell lines (p = 0.12; t). Among three out of five cell lines, the median mrp1 mRNA content did not change throughout the experiment on day 3 as well as on day 18. The lrp mRNA level on day 18 was comparable to that of the non-irradiated controls (p = 0.69; z). However, SW403 kept a trend to downregulate the lrp gene, as on day 18 it reached 1 log lower level than the non-irradiated parental line. The SW620 colon cancer cell line upregulated the lrp gene over 1 log vs. the non-irradiated cell line (Fig. 3.8.1).

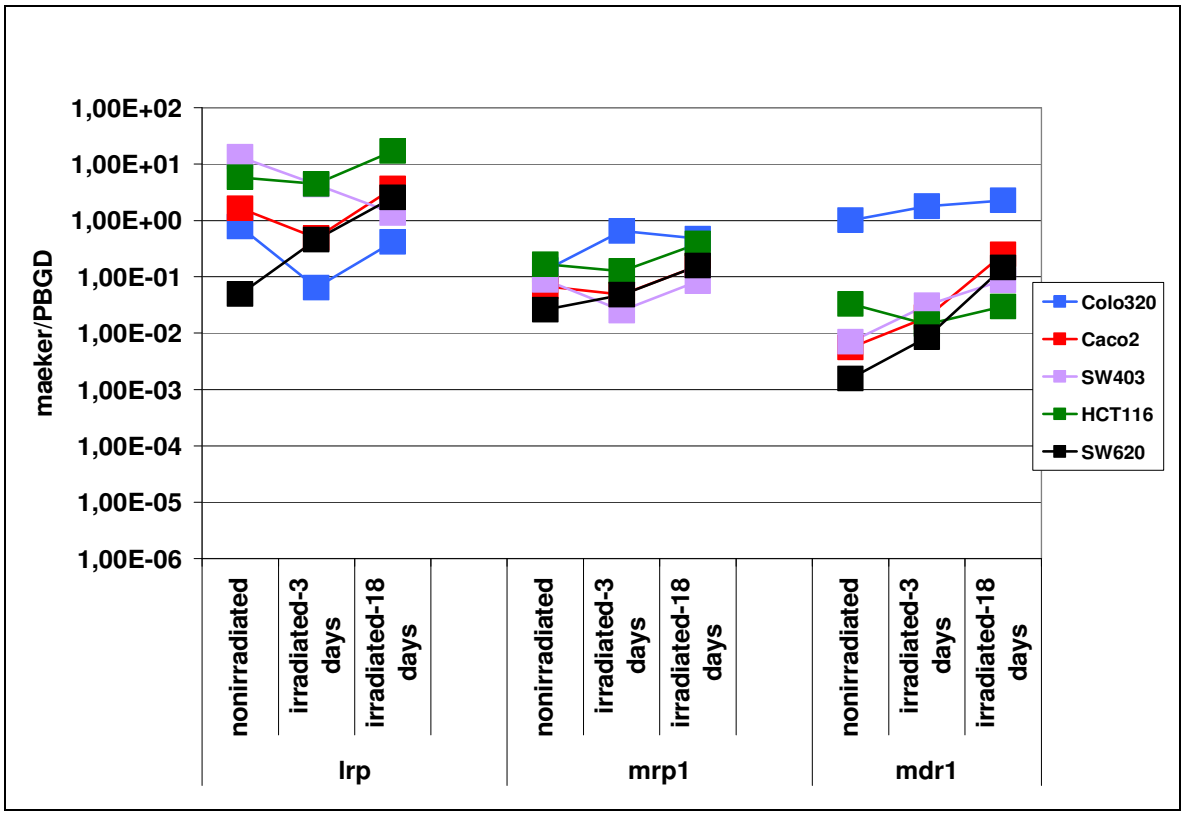

Fig. 3.8.1. Modulation of the lrp, mrp1 and mdr1 gene expression 3 and 18 days after the last irradiation with 27Gy in five colon cancer cell lines. The gene expression is obtained as marker/PBGD ratio.

Although all five cell lines overexpressed significantly LRP on day 3 after the last irradiation (3.2), only HCT116 retained a notably higher level of LRP expression on day 18 vs. the parallel non-irradiated line (Fig. 3.8.2). Overall, there was no significant

increase in the level of LRP expression after irradiation compared to the non-irradiated control cell lines (p = 0.75; t). Similar effects were observed on MRP1 level - three out of five cell lines simultaneously downregulated the MRP1 on day 18 and no difference compared to the non-irradiated controls was observed (p = 0.68; t). However, SW403 and HCT116 retained their high MRP1 level on day 18 (Fig. 3.8.2). In the studied irradiated cell lines, the P-glycoprotein underwent a statistically significant 2-fold increase compared to the non-irradiated parental lines (p = 0.043) on day 18 after the last irradiation.

We detected a significant correlation between the LRP and MRP1 expression on day 18 after the last irradiation (Table 3.8.1. A). However, no other significant correlation between gene expression and protein level (data not shown), as well as gene and protein co-expression was monitored on day 18 (Table 3.8.1. A and B).

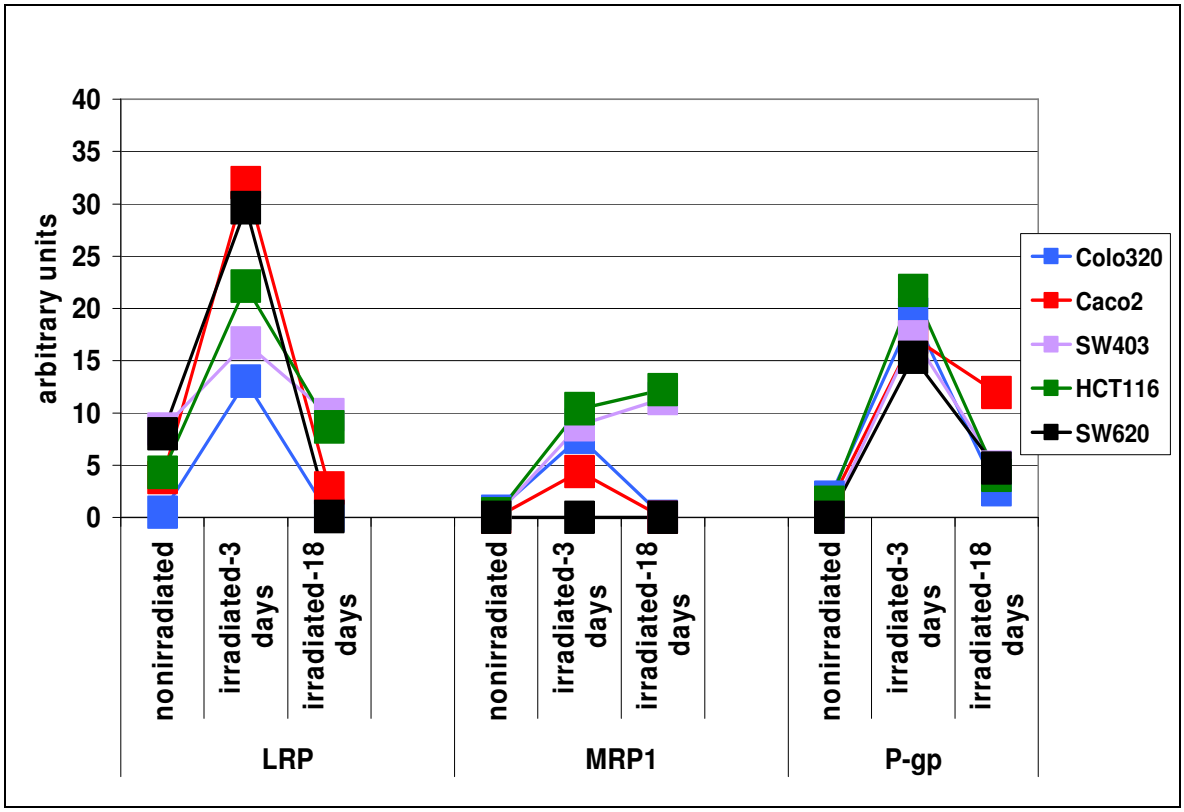

Fig. 3.8.2. Modulation of LRP, MRP1 and P-glycoprotein level 3 and 18 days after the last irradiation of 27 Gy in five colon cancer cell lines. The protein level was obtained in arbitrary units (AU).

	colon cancer cell lines (*n* = 5)
lrp/mdr1 (r)	-0.42
P	0.48
lrp/mrp1 (r)	0.34
P	0.57
mdr1/mrp1 (r)	0.7
P	0.19

A.

	colon cancer cell lines (*n* = 5)
LRP/P-gp(r)	-0.07
P	0.9
LRP/MRP1 (r)	0.96**
P	0.009
P-gp/MRP1(r)	-0.3
P	0.61

B.

Table 3.8.1. Correlation (r) in the expression of multidrug resistance-associated genes (A) and proteins (B) among five colon cancer cell lines, 18 days after the last irradiation. The protein expression was obtained as arbitrary units.

3.9. Cell drug resistance as a late response to irradiation

We observed in our study that irradiation significantly suppressed the ability of cells to replicate. It is known that cell sensitivity to some cytotoxic drugs is dependent on their cell cycle, i.e., in state of rest or mitosis. It was expected that when the treated with irradiation cell lines regained logarithmic growth and the stress stimulus - irradiation was absent, the cell lines would recover their sensitivity to cytotoxic drugs. A summary of the sensitivity of five colon cancer cell lines before and 18 days after irradiation is shown in Fig. 3.9.1.

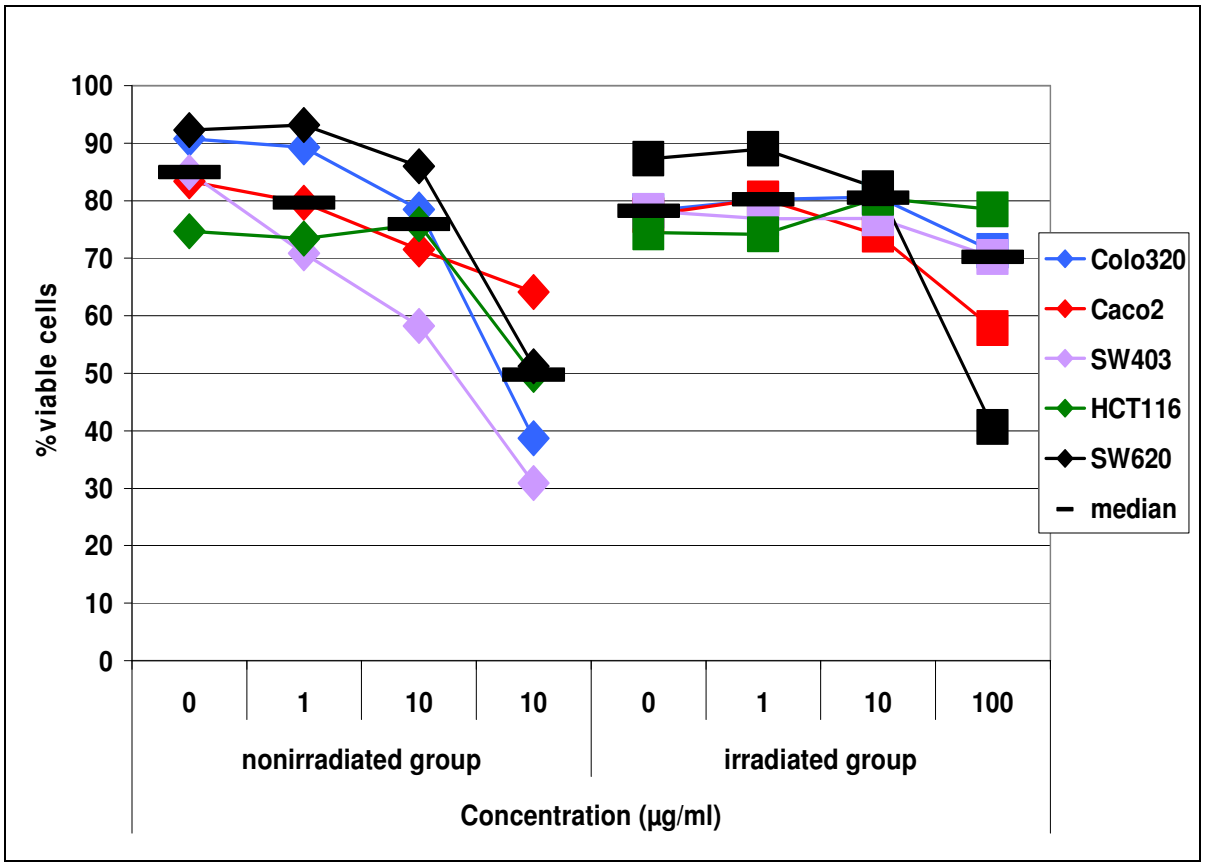

Fig. 3.9.1. Sensitivity of five colon cancer cell lines to bendamustine, before and 18 days after the last irradiation of 27 Gy. No significant difference was detected between irradiated and non-irradiated control cell lines.

From the studied cell lines Caco2 and SW620 restored their sensitivity to bendamustine to the full extent at all concentrations to the levels of their non-irradiated parental lines (Fig. 3.9.1). SW403, HCT116 and Colo320 remained less sensitive to bendamustine. The irradiated cell lines were still more resistant to 10 µg/ml ($p = 0.27$; t) and 100 µg/ml bendamustine ($p = 0.35$; t) than the non-irradiated lines, but this did not reach statistical significance. No significant difference in the cell sensitivity to 1 µg/ml bendamustine was detected ($p = 0.67$; t).

We observed resistance to 1 µg/ml cisplatin in the irradiated cell lines vs. the non-irradiated ($p = 0.038$; t). Only Caco2 restored its sensitivity to cisplatin to the extent of its non-irradiated parental line. Among the rest of the cell lines cisplatin induced lower levels of apoptosis among the treated with irradiation cell lines. However, the resistance

to 0.1 µg/ml (p = 0.59; t) and to 10µg/ml cisplatin was not with a statistically significant difference compared to the non-irradiated (p = 0.33; t).

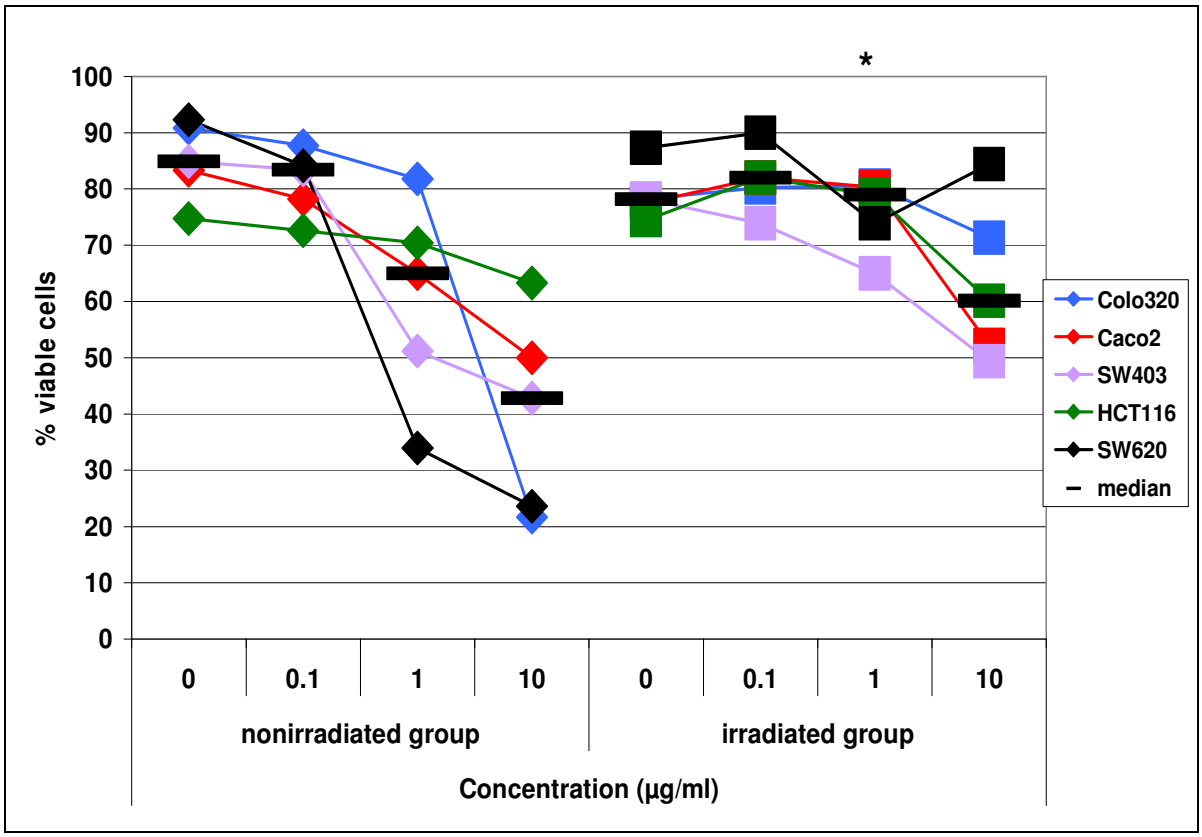

Fig. 3.9.2. Sensitivity of eight breast and colon cancer cell lines to cisplatin, before and 18 days after the last irradiation of 27 Gy.

There was a partial restoration of the doxorubicin sensitivity compared to that on day 3. However, we noticed a 3.5-fold resistance to all three concentrations of doxorubicin (p = 0.043; z). The colon cancer cell lines Caco2, Colo320 and SW620 recovered their sensitivity to 1 µg/ml and 10 µg/ml doxorubicin, compared to the non-irradiated cell lines. Among the SW403 and HCT116 cell lines a considerable level of resistance to doxorubicin was detected on day 18 (Fig. 3.9.3) and coincided with MRP1 overexpression in these two cell lines (Fig. 3.8.2).

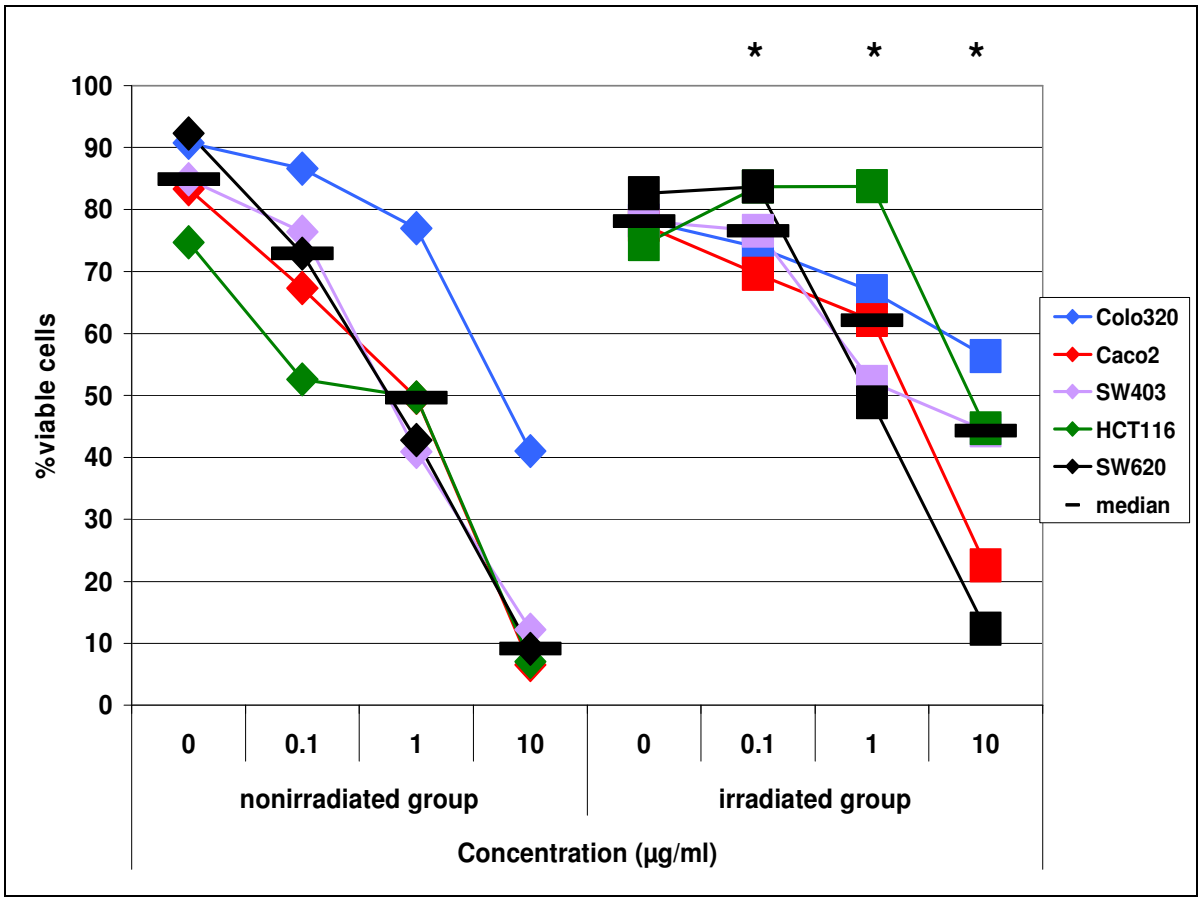

Fig. 3.9.3. Sensitivity of eight breast and colon cancer cell lines to doxorubicin, before and 18 days after the last irradiation of 27 Gy.

3.10. Correlation between the drug sensitivity and LRP, MRP1 and P-glycoprotein expression

As previously described, we compared the relative increase of cell resistance to cytotoxic drugs with the protein overexpression after radiation therapy on day 18. Positive correlation was noticed between the cell viability following incubation with 10 mg/ml doxorubicin and P-glycoprotein overexpression ($p = 0.11$), as well as the LRP expression ($p = 0.039$) after irradiation (3.10.1). However, in case-to-case comparison it was obvious that the MRP1 levels of SW403 and HCT116 was accompanied with cell resistance to bendamustine and doxorubicin. The reduced sensitivity to cisplatin and bendamustine could be attributed to the considerable overexpression of P-glycoprotein in Caco2.

However, the Colo320 colon cancer cell line remained highly resistant to bendamustine, cisplatin and doxorubicin and this could be attributed to the overexpression of LRP, MRP1 and P-glycoprotein.

	bendamustine 100 µg/ml	cisplatin 10 µg/ml	doxorubicin 10 µg/ml
LRP (r)	0.34	-0.36	0.9*
P	0.57	0.5	0.039
MRP1 (r)	0.64	-0.4	0.8
P	0.2	0.5	0.1
P-glycoprotein (r)	-0.62	0.02	0.8
P	0.2	0.97	0.11

Table 3.10.1. Correlation (r) between the overexpression of multidrug resistance proteins and the appearance of multidrug resistant phenotype in a group of five colon cell lines.

3.11. Modulation of P-glycoprotein and MRP1 activity using verapamil

As described above (3.7), verapamil failed to modulate the activity of P-glycoprotein and MRP1 on day 3 after the completion of the irradiation procedure. It was expected that when the cell lines regained logarithmic growth, the effect of verapamil on P-glycoprotein and MRP1 activity could be more distinctive. The survival curves presented in Fig. 3.11.1 show that neither 25 mM, nor 75 mM verapamil increased the cytotoxicity, 18 days after completion of the irradiation treatment. However, verapamil was found to restore partially the sensitivity to doxorubicin in irradiated Caco2, a cell line with a high post-irradiation P-glycoprotein overexpression (Fig. 3.11.2.). However, 75 mM verapamil did not achieve a greater restoration effect of doxorubicin sensitivity than 25 mM verapamil. In SW403 and HCT116, colon cancer lines with high MRP1 overexpression, was not detected an increase of cell sensitivity to any concentration of bendamustine, cisplatin and doxorubicin following incubation with verapamil (data not shown).

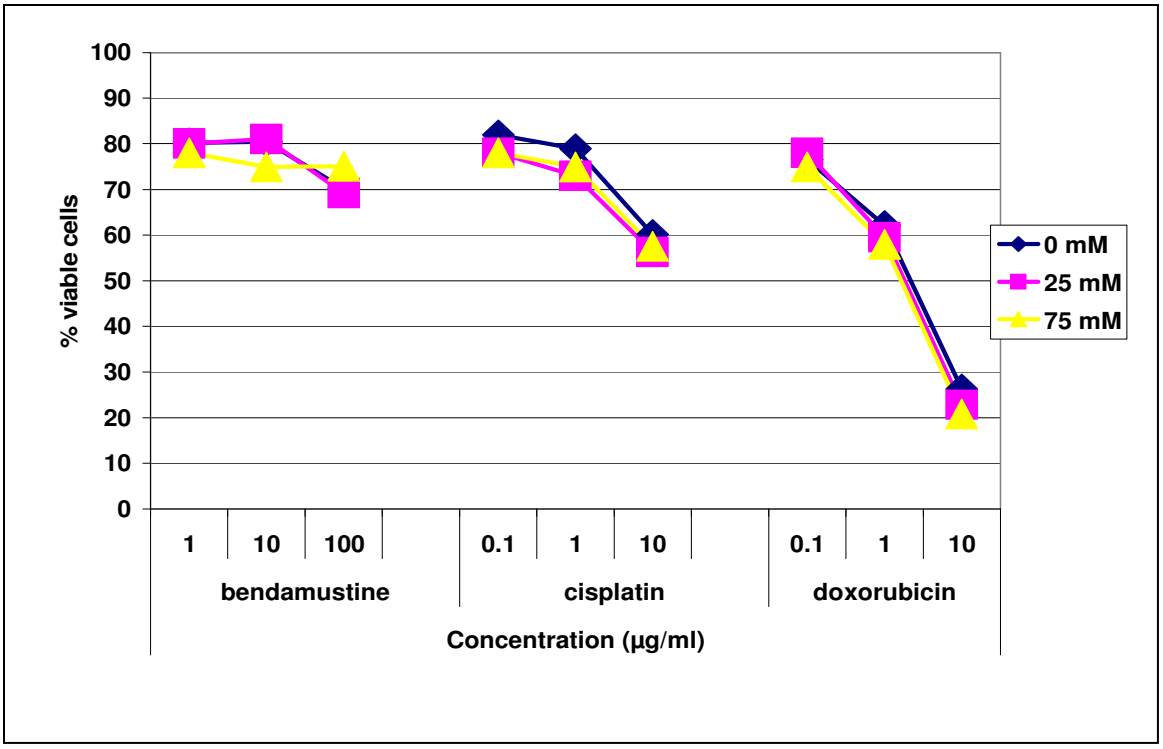

Fig. 3.11.1. Median sensitivity of five colon cancer cell lines to bendamustine, cisplatin and doxorubicin supplemented with 25 mM or 75 mM verapamil, 18 days after completion of the irradiation treatment.

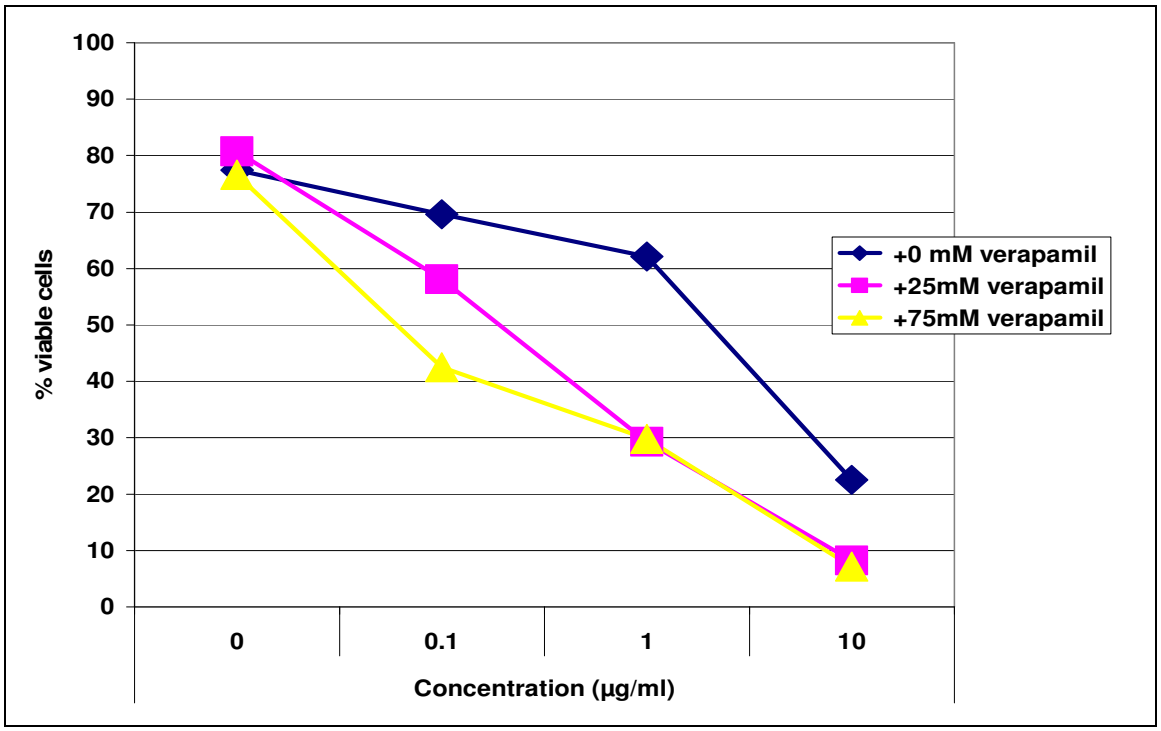

Fig. 3.11.2. Sensitivity of Caco2 colon cancer cell line to doxorubicin, supplemented with 25 mM or 75 mM verapamil, 18 days after completion of the irradiation treatment.