

Silymarin Studies

Li LH, Wu LJ, Tashiro SI, Onodera S, Uchiumi F, Ikejima T.

Activation of the SIRT1 pathway and modulation of the cell cycle were involved in silymarin's protection against UV-induced A375-S2 cell apoptosis.

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Abstract

Silymarin, derived from the milk thistle plant, *Silybum marianum*, has been traditionally used in the treatment of liver disease. Our previous study demonstrated that silymarin has an anti-apoptotic effect against UV irradiation.

In this study, SIRT1, a human deacetylase that was reported to promote cell survival, was activated by silymarin (5×10^{-4} mol/L) in UV-irradiated human malignant melanoma, A375-S2 cells, followed by down-regulated expression of Bax and decreased release of cytochrome c. Cleavage of procaspase-3 and digestion of its substrates, the inhibitor of caspase-activated DNase (ICAD) and poly(ADP-ribose) polymerase (PARP), were also reduced. Consistent with its protective effect on UV-induced apoptosis, silymarin (5×10^{-4} mol/L) also increased G2/M phase arrest, possibly providing a prolonged time for efficient DNA repair.

Consequently, that silymarin protected A375-S2 cell against UV-induced apoptosis was partially through SIRT1 pathway and modulation of the cell cycle distribution.

Keywords: Silymarin; A375-S2 cell; UV irradiation; Anti-apoptosis; SIRT1; Cell cycle arrest

... Here, we found that silymarin's inhibitory mechanism on UV-induced A375-S2 cell apoptosis has a relationship with SIRT1, a member of the conserved sirtuin family of nicotinamide adenine dinucleotide (NAD⁺)-dependent deacetylases, which is a key regulator of cell defences and survival in response to a variety of stresses 4-6 ...

2. Results and discussion

2.1 Silymarin protected A375-S2 cells against UV-induced cell death

Table 1 shows the viability ratio of A375-S2 cells, which were treated with various concentrations of silymarin for 1 h and then further incubated for 12 h after UV irradiation. It was found that silymarin protected

UV-irradiated A375-S2 cells from death in a dose-dependent manner (cell viability ratio was increased to 92.5% at the concentration of 5×10^{-4} mol/L) and that it had no cytotoxic effect on the cells.

Table 1. Protective effect of silymarin on cell viability in UV-irradiated A375-S2 cells (%).

Silymarin (mol/L)---Cell viability (%)		
---	UV irradiation	Without UV irradiation
0	18.2±3.4	100.0±2.8
1×10^{-5}	17.8±2.6	100.3±2.4
5×10^{-5}	18.9±4.3	98.5±3.7
1×10^{-4}	28.3±2.5	103.1±1.6
2×10^{-4}	34.7±4.2	105.7±4.9
3×10^{-4}	45.6±1.6	105.4±4.1
4×10^{-4}	69.7±3.8	107.3±6.2
5×10^{-4}	92.5±5.6**	103.5±3.1

Mean±SD, n = 3.

**P < 0.01 vs 0 mol/L.

2.2 Silymarin reversed UV irradiation-induced morphologic changes in A375-S2 cells

In response to cellular insults, cells attempt to repair and defend themselves, but if unsuccessful, they often undergo programmed cell death, or apoptosis. Therefore, in order to determine whether silymarin protected A375-S2 cells against UV-induced cell death through anti-apoptotic pathway, the morphologic changes were observed. When A375-S2 cells were cultured for 12 h after UV irradiation, marked morphologic changes were observed as compared with the untreated control (figure 2a,c). The majority of cells became round, and some of these cells showed membrane blebbing (figure 2c), which were hallmarks of apoptosis, while silymarin pre-treatment (5×10^{-4} mol/L) reversed these morphologic changes (figure 2d).

2.3 The expression of SIRT1 was up-regulated in UV-irradiated A375-S2 cells after silymarin pre-treatment

SIRT1 play an important role in cell defences and survival in response to stress 4-6. To investigate whether SIRT1 might be responsible for the ability of silymarin to protect A375-S2 cells from UV-induced apoptosis, the expression of SIRT1 was examined by Western blot analysis, which was found to be markedly up-regulated by silymarin (5×10^{-4} mol/L) in UV-irradiated A375-S2 cells as compared to that of silymarin-untreated cells (figure 3), suggesting that silymarin's protection against UV irradiation might be through SIRT1 pathway.

2.4 The protein expressions involved in SIRT1 pathway

Since up-regulated SIRT1 activity [6] could deacetylate the DNA repair factor Ku70, causing it to sequester the proapoptotic factor Bax away from the outer mitochondrial membrane to the cytoplasm, forming a complex with Ku70, the subsequent release of cytochrome c was inhibited as the result of Bax protein relocalisation. Downstream events including caspase activation and cleavage of ICAD and PARP were attenuated, thereby inhibiting stress-induced apoptotic cell death. In our study, it was found that the expression of Bax and release of cytochrome c from mitochondria were attenuated in UV-irradiated A375-S2 cells after silymarin pre-treatment (figure 3). Cleavage of procaspase-3 to caspase-3 (figure 4) and digestion of its substrates, ICAD and PARP, were also inhibited subsequently (figure 5).

2.5 The effect of silymarin on UV-induced cell cycle modulation

Cell cycle progression is important for maintaining homeostasis, especially when there is an insult to DNA [12,13]. Physiological stress or an insult to DNA could cause arrest in different stages of the cell cycle. Since UV irradiation is known to damage DNA directly, the effect of UV irradiation and silymarin pre-treatment on cell cycle progression was assessed. It was found that UV exposure caused a S arrest (29.62 versus 16.00% in control) at the expense of a decrease in G2/M phase cells (0 versus 7.86% in control) (figure 6a,c, table 2). Pre-treatment with silymarin (5×10^{-4} mol/L), however, reversed the UV-induced S arrest, resulting in an increase in G2/M phase cells (7.45% in silymarin+UV versus 0 in UV alone) (figure 6c,d, table 2). In general, an arrest in G2/M phase of the cell cycle allows cells more time to repair damaged DNA before mitosis (M phase), until the damage of the genome is repaired. Since silymarin treatment resulted in an accumulation of UV-irradiated cells at G2/M phase, part of the protective effect of silymarin against UV-induced apoptosis might be due to its effect on cell cycle distribution. However, detailed studies remain to be conducted to delineate the molecular mechanism involved in this action of silymarin. In addition, apoptotic sub-G0/G1 phase peak, caused by UV irradiation, was reduced obviously by silymarin pre-treatment (figure 6c,d).

Table 2. Effects of silymarin on cell cycle distribution (%).

Group	G0/G1 (%)	S (%)	G2/M (%)
Medium control	76.14	16.00	7.86
Silymarin (5×10^{-4} mol/L)	75.60	16.45	7.95
UV irradiation (52.1 J/m ²)	70.38	29.62	0
Silymarin (5×10^{-4} mol/L)+UV irradiation (52.1 J/m ²)	76.39	16.16	7.45

In conclusion, silymarin promoted UV-irradiated A375-S2 cell survival partly

through SIRT1 pathway. It also modulated the distribution of the cell cycle to allow more time for the damaged cells to repair. Present results may broaden silymarin's potential therapy use for many diseases in the future.

[B]