Foods 2020

1st International Electronic Conference on Food Science and Functional Foods

CYTOTOXIC EFFECT OF CHOLESTEROL METABOLITES ON HUMAN COLONIC TUMOR (CACO-2) AND NON-TUMOR (CCD-18CO) CELLS AND THEIR POTENTIAL IMPLICATION IN COLORECTAL CARCINOGENESIS

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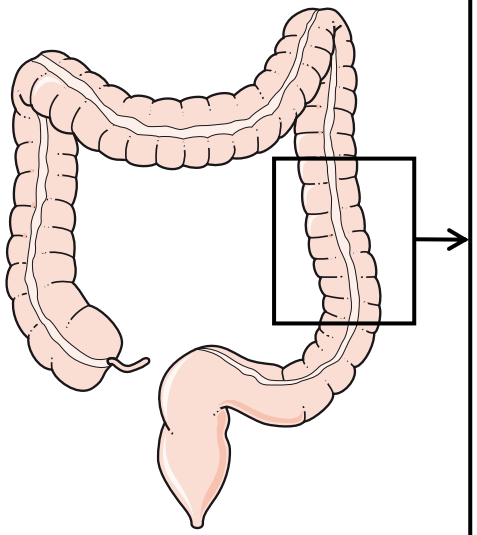
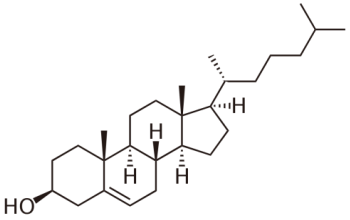
CHOLESTEROL METABOLISM BY COLONIC MICROBIOTA



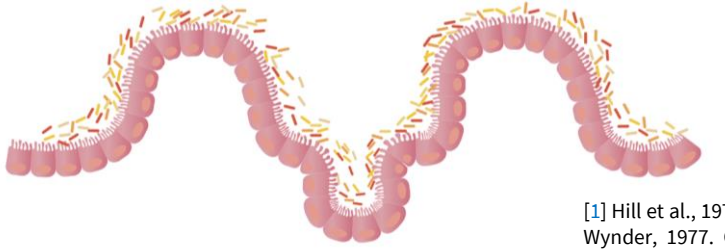


Cholesterol
(1 g/day)

Metabolites

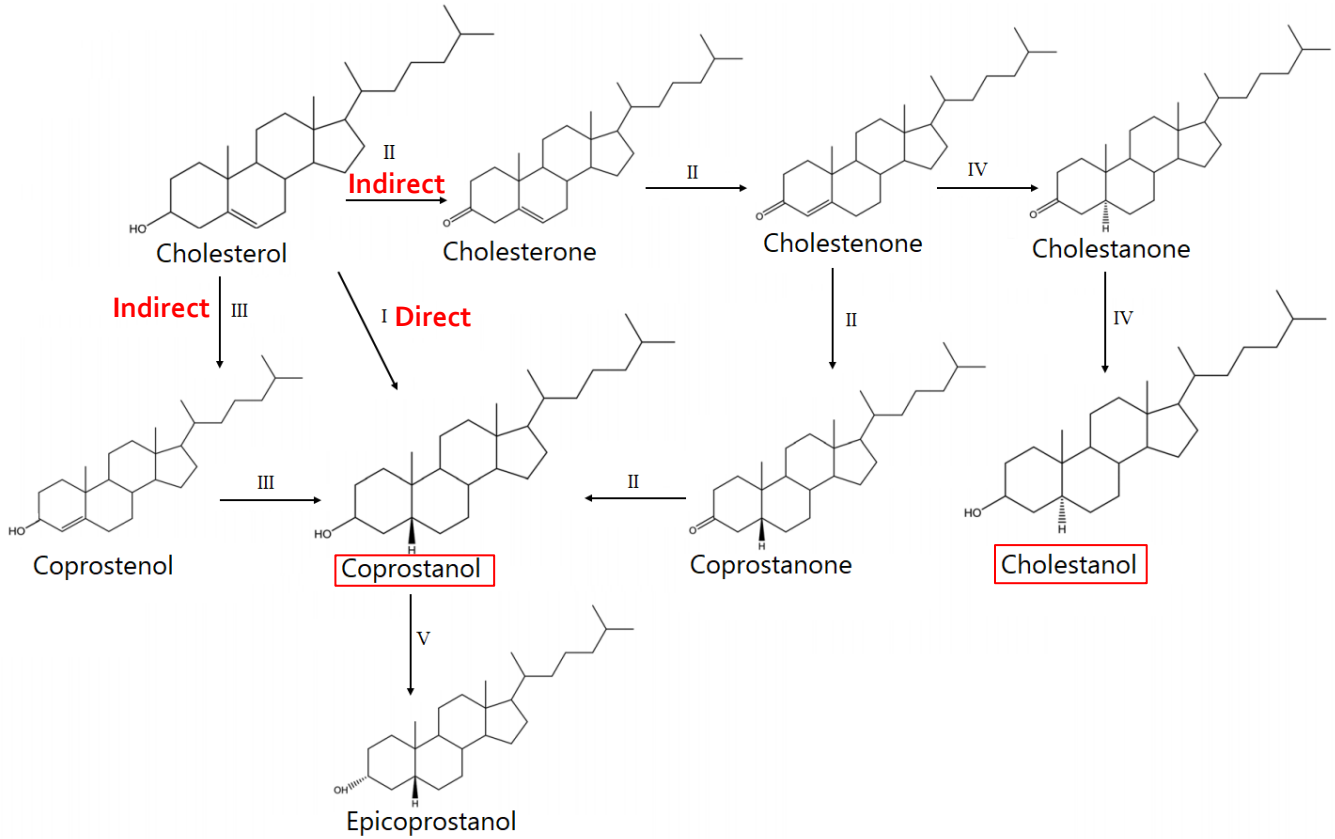
Promoters of colorectal cancer (CRC) [1-3]

Colonic microbiota: *Eubacterium* and *Bacteroides*



[1] Hill et al., 1971. Lancet 1971. 297: 95-100; [2] Reddy & Wynder, 1977. Cancer. 39: 2533-39. [3] Korpela et al., 1988. Scand J Gastroenterol. 23: 277-83.

CHOLESTEROL METABOLISM BY COLONIC MICROBIOTA



Adapted from Cuevas-Tena et al., 2018. Eur J Lipid Sci Technol. 120: 1800054.

CHOLESTEROL METABOLITES AND COLORECTAL CANCER

Metabolite	Conditions	Fecal *vs*. control (mg/g dry or wet* feces)	Referencia
Coprostanol	CRC	**14.2-26.4** *vs*. 6.8-14.7	[1-4]
	Ulcerative colitis	**19.9-26.6** *vs*. 6.7-12.9	[5, 6]
	Adenomatous polyps	**19.7** *vs*. 12.4	[3]
	Western diet *vs.* rich vegetables	**3.7-6.6** *vs*. 1.2-1.4*	[7, 8]
Coprostanone	CRC	**3.1-3.6** *vs*. 4.2-2.1	[3, 4]
	Ulcerative colitis	**3.4** *vs*. 2	[6]
	Adenomatous polyps	**4.2** *vs*. 2.1	[6]
	Western diet *vs.* rich vegetables	**2.1-3.4** *vs*. 0.2-2 / **0.65** *vs*. 0.12*	[3, 9]/[8]
Cholestanol	CRC	**0.6** *vs*. 0.4	[10]

[1] Reddy et al., 1975. Cancer Res. 35: 3403-06; [2] Reddy et al., 1977. Cancer. 39: 1815-19; [3] Reddy & Wynder, 1977. Cancer. 39: 2533-39; [4] Peuchant et al., 1984. Clin Chim Acia. 141:151-68; [5] Reddy et al., 1977. Cancer Res. 37: 1697-701; [6] Reddy & Wynder, 1973. J Natl Cancer Inst. 50: 1437-42; [7] van Faassen et al., 1987. Am J Clin Nutr. 45: 962-7; [8] Hill et al., 1971. Lancet. 297: 95-10; [9] Reddy et al., 1978. Cancer Lett. 4: 217-22; [10] Kanazawa et al., 1996. Cancer. 77: 1701-06.

CHOLESTEROL METABOLITES AND COLORECTAL CANCER

Animal studies

- ↓ **[LDL-C],** ↑ **[metabolites]** in feces and No. of **chemoinduced large intestine** tumors in rats with a diet rich in unsaturated fat *vs*. saturated fat [1]
- **Cholestanone** and **cholestenone** ⟶ nuclear aberrations in mice colonic epithelium [2]
- **Cholestenone** ⟶ sister chromatid exchange in mice colonic epithelium [3]
- ↑ Incidence of **chemoinduced large intestine** tumors in rats with a diet rich in unsaturated fat + neomycin + cholesterol (*vs*. basal diet) [4]

  neomycin → Inhibitor of intestinal cholesterol absorption

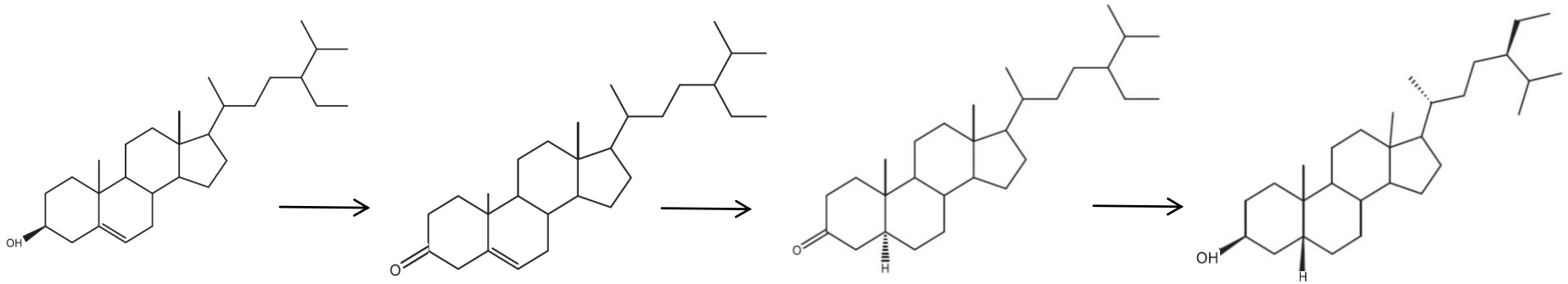
[1] Broitman et al., 1977. Cancer. 40:2455-63; [2] Suzuki et al., 1986. Cancer Lett. 33: 307-16; [3] Kaul et al., 1987. Mutagenesis. 2: 441-4; [4] Panda et al., 1999. Br J Cancer. 80: 1132-36.

CHOLESTEROL METABOLITES AND COLORECTAL CANCER

Plant sterols lower the risk of CRC

↓

By reducing the biotransformation of cholesterol by the colonic microbiota



β-Sitosterol → Sitostenone → Ethylcoprostanone → Ethylcoprostanol

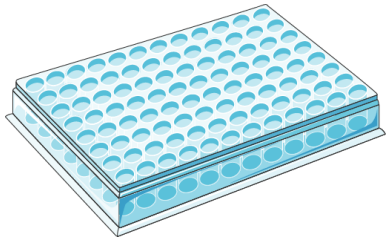
Adapted from Cuevas-Tena et al., 2018. Eur J Lipid Sci Technol. 120: 1800054.

OBJECTIVE

To evaluate the cytotoxic activity of the main cholesterol-derived metabolites (coprostanol, cholestanol, coprostanone, and cholestenone) at physiologically relevant concentrations on tumoral (Caco-2) and non-tumoral (CCD-18Co) human colon cells.

- **CELL CULTURE AND TREATMENT**

Seeded



24 h

Caco-2 and CCD-18Co cells cultured in 75 cm^2 Falcon flasks

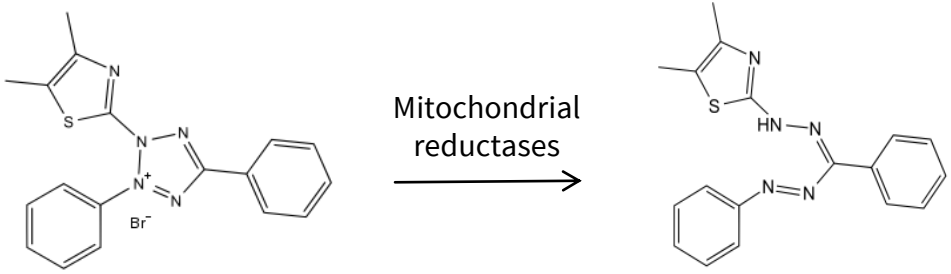
96-well plates at 25,000 cells/ well

TREATMENT

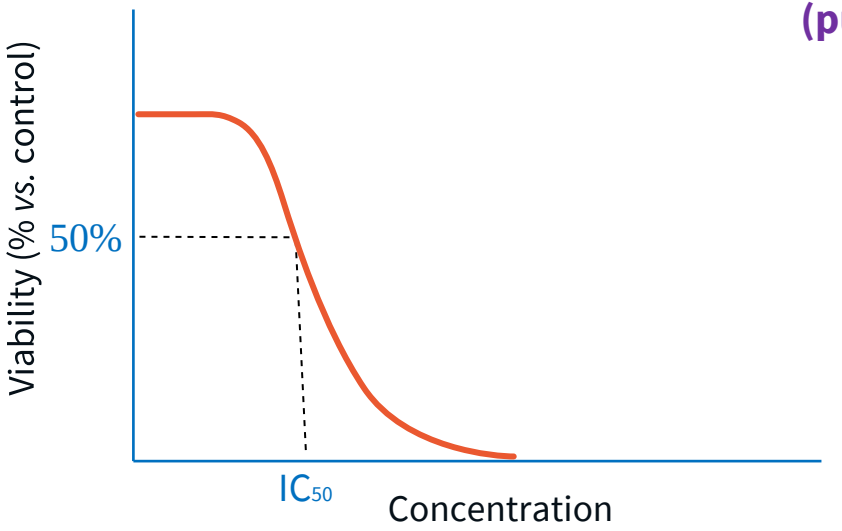
- Cholesterol metabolites (commercially available)
- 9.4-300 µM [1, 2]
- 24-72 h
- D-MEM with ethanol 1.4% (v/v) (control)
- 5-Fluorouracil (25 µM): chemotherapeutic drug for CRC [3]

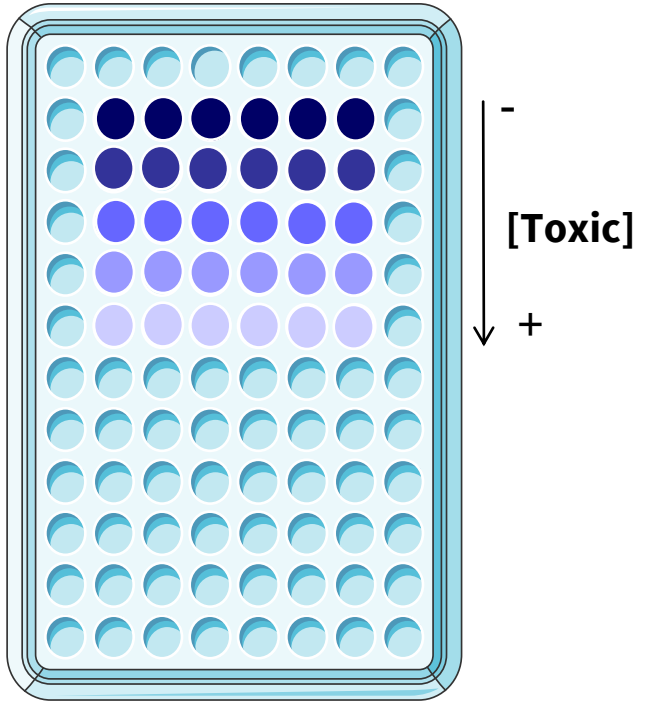
[1] Cuevas-Tena et al., 2018. Eur J Lipid Sci Technol. 120: 1800054; [2] Pem et al., 2018, Water Research, 132, 222-240; [3] Álvarez-Sala et al., 2018. J Funct Foods. 49: 52-60.

- **CYTOTOXICITY ASSAY (MTT)**

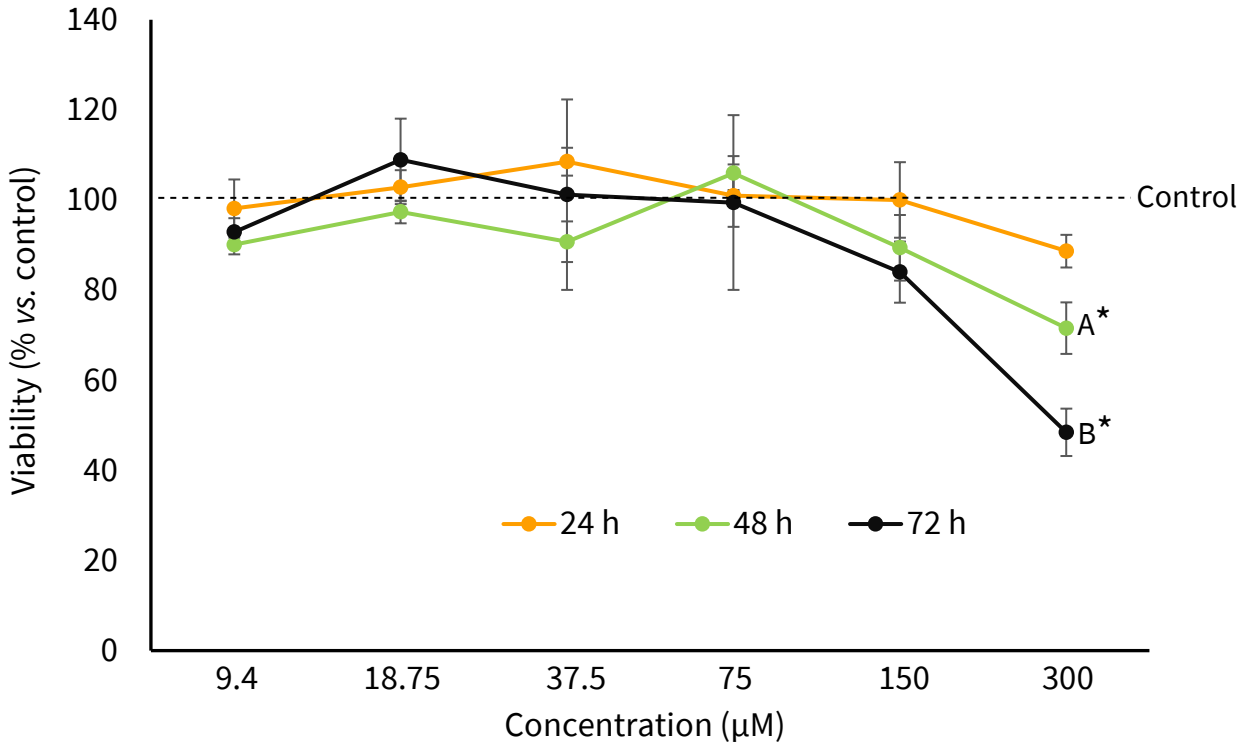


MTT → **Formazan (purple)**



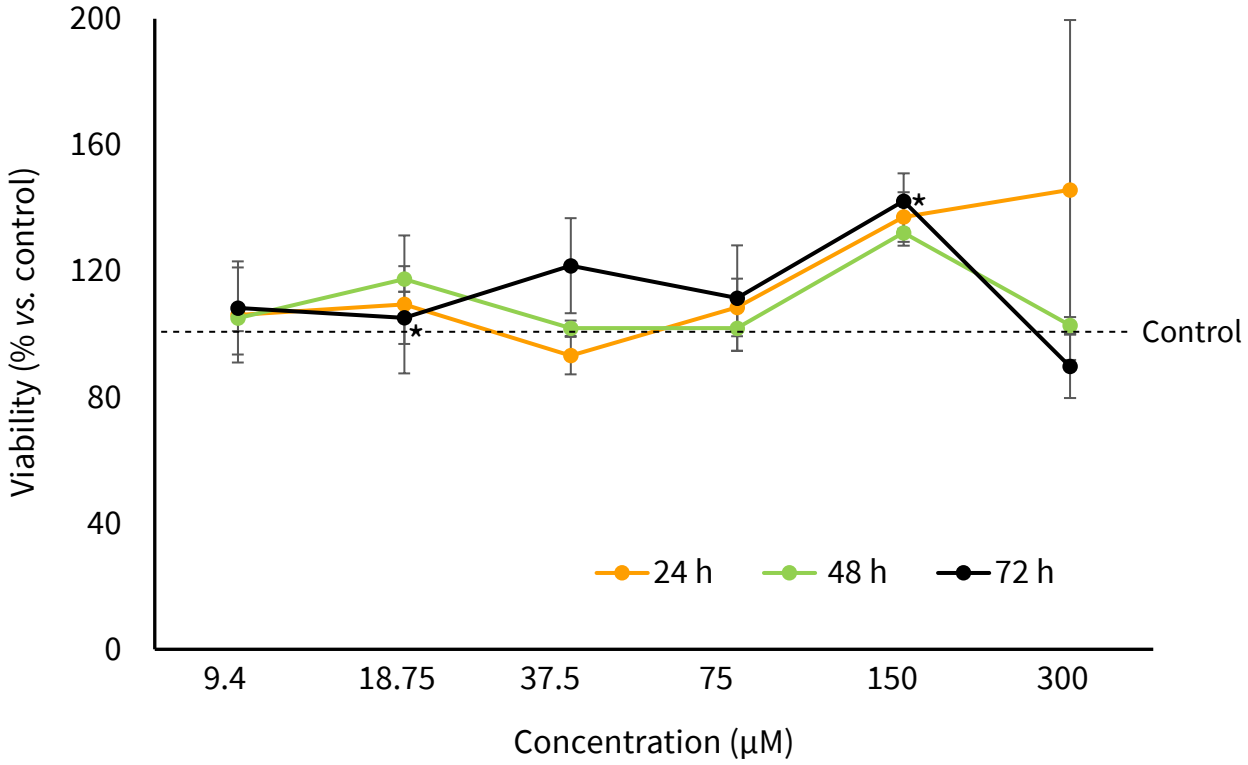


- **COPROSTANOL IN CCD-18CO CELLS (NON-TUMORAL)**



The * indicates statistically significant differences ($p < 0.05$) between the treatments and the control. Different uppercase letters (A-B) indicate statistically significant differences ($p < 0.05$) at different times at the same concentration

- **COPROSTANOL IN CACO-2 CELLS (TUMORAL)**



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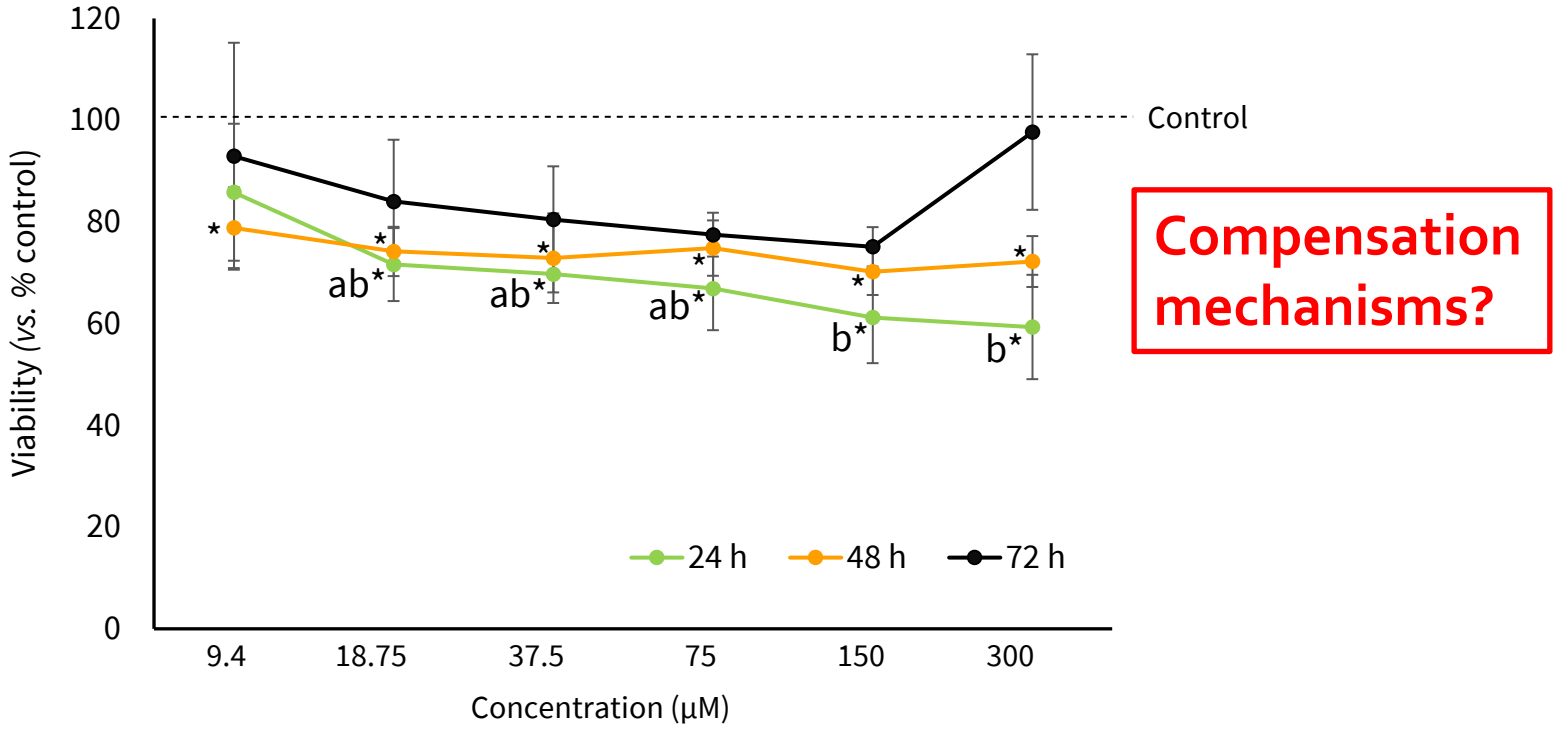
- **CHOLESTANOL IN CCD-18CO CELLS (NON-TUMORAL)**



Compensation mechanisms?

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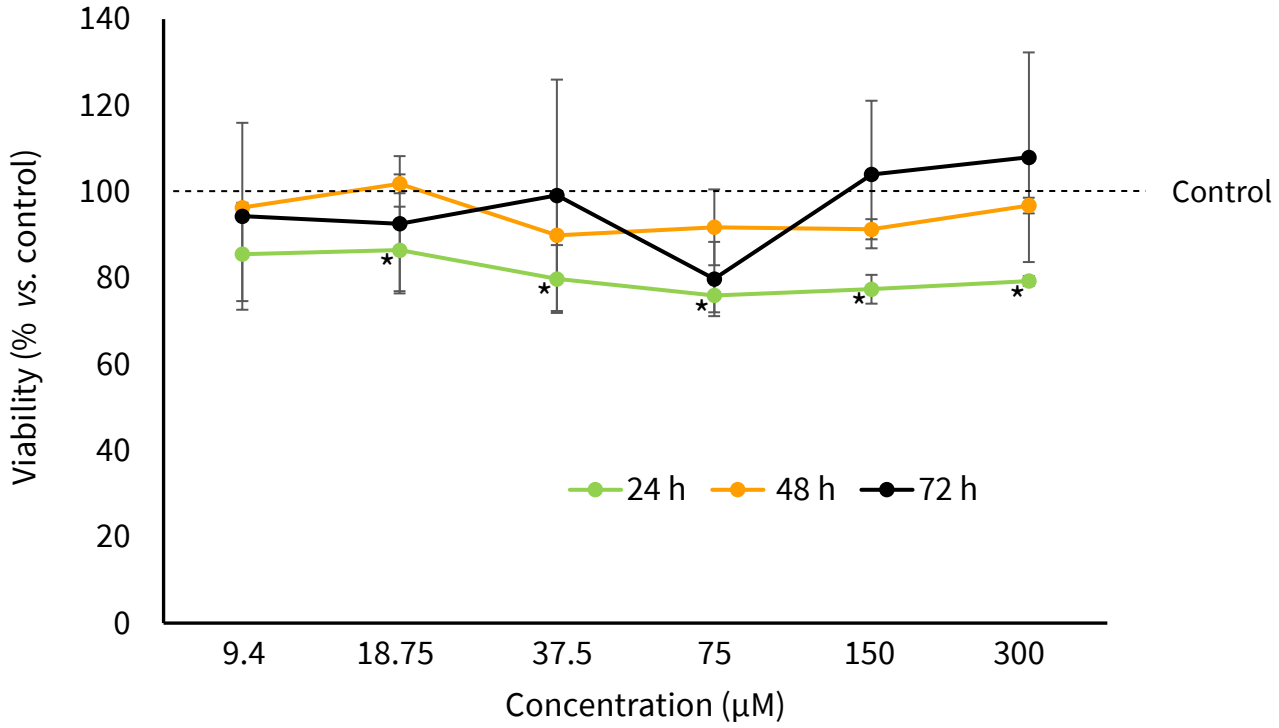
- **CHOLESTANOL IN CACO-2 CELLS (TUMORAL)**



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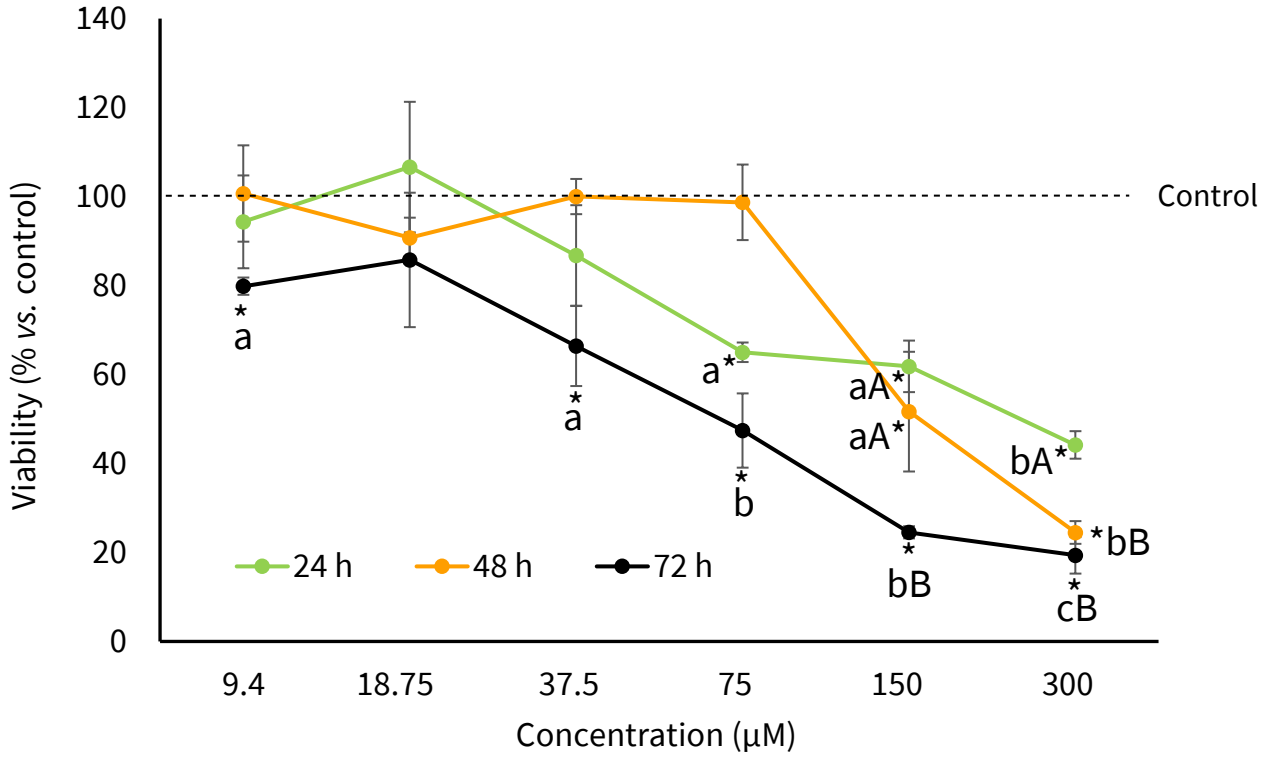
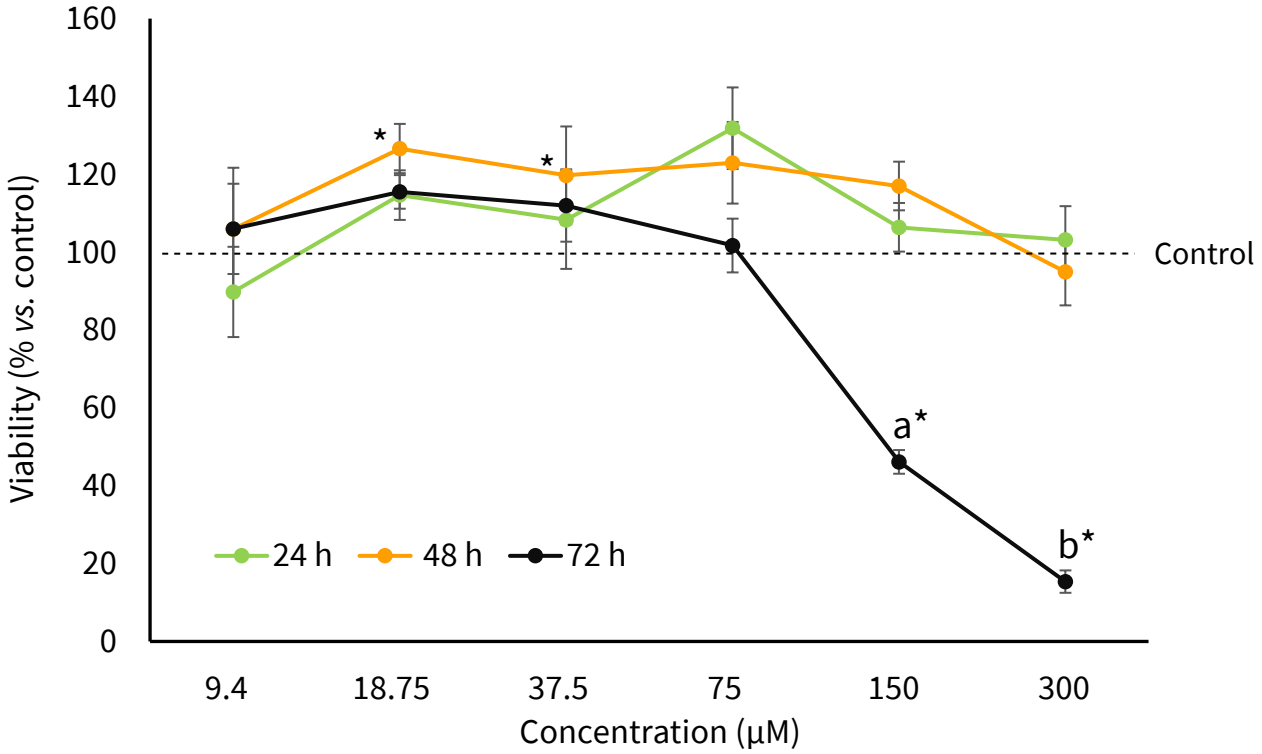
- **COPROSTANONE IN CCD-18CO CELLS (NON-TUMORAL)**



The * indicates statistically significant differences ($p < 0.05$) between the treatments and the control. Different lowercase letters (a-b) indicate statistically significant differences ($p < 0.05$) at different concentration at same time. Different uppercase letters (A-B) indicate statistically significant differences ($p < 0.05$) at different times at the same concentration

- **COPROSTANONE IN CACO-2 CELLS (TUMORAL)**



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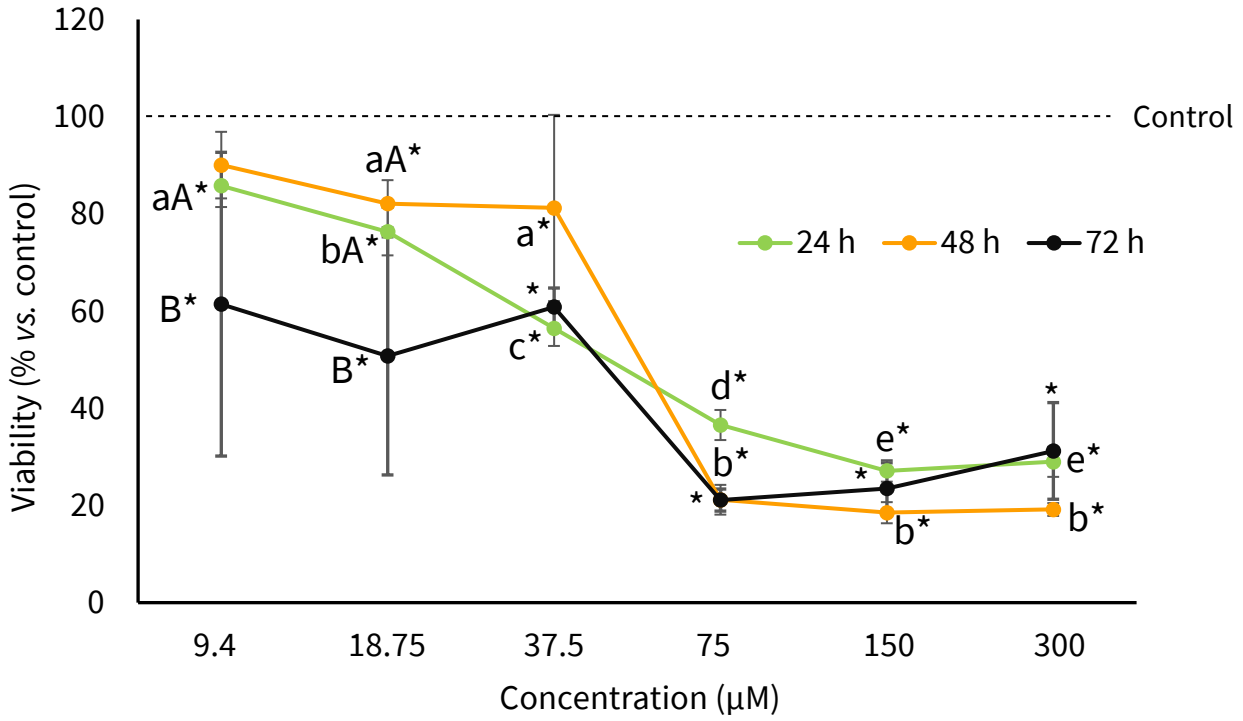
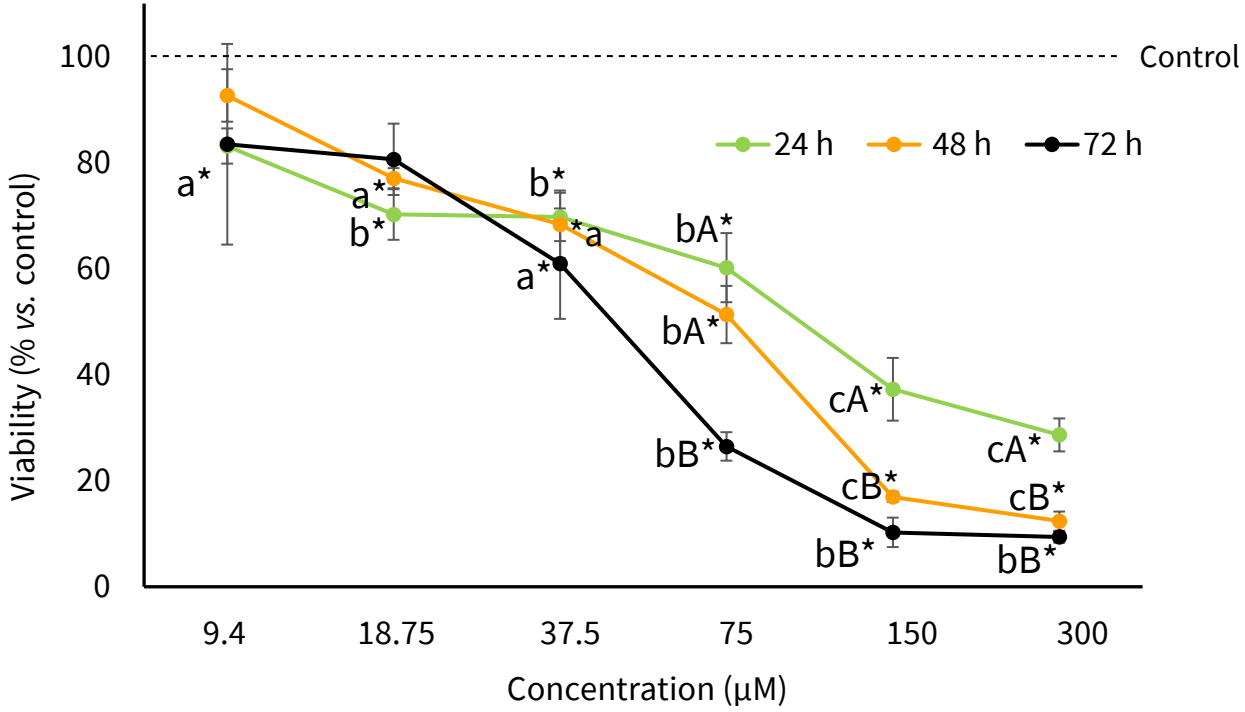
- **CHOLESTENONE IN CCD-18CO CELLS (NON-TUMORAL)**



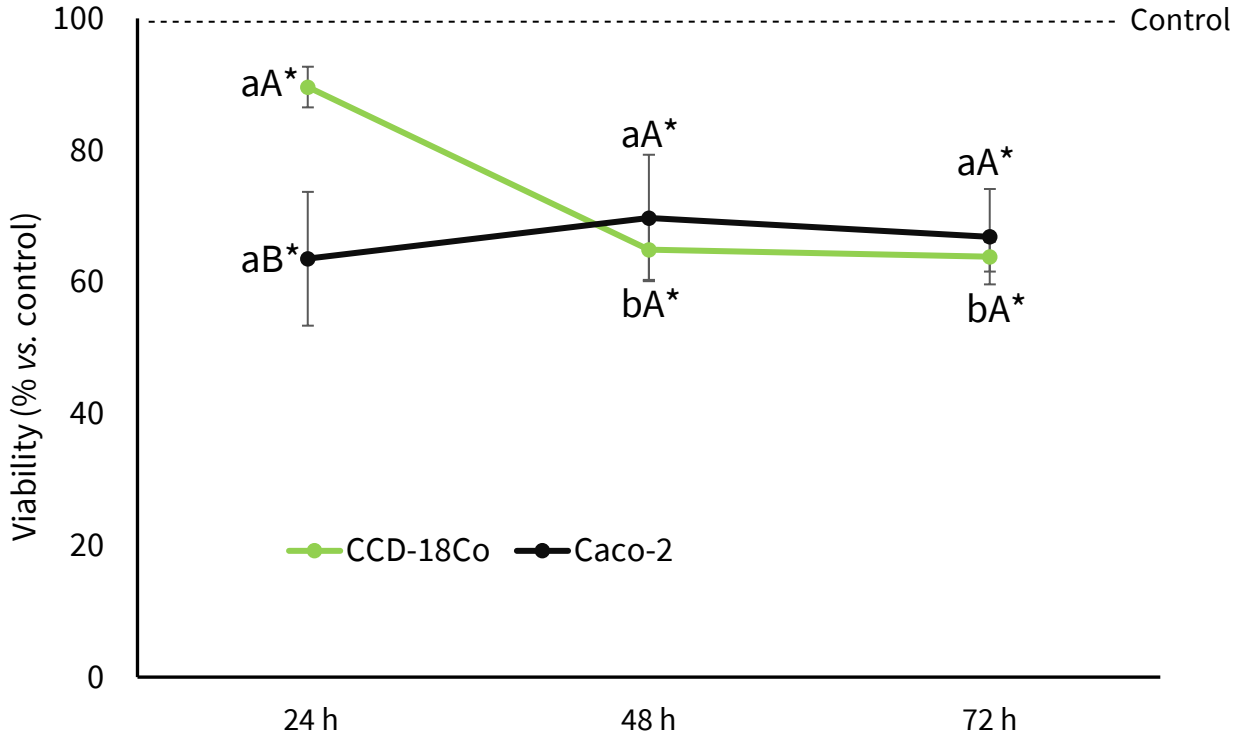
The * indicates statistically significant differences ($p < 0.05$) between the treatments and the control. Different lowercase letters (a-e) indicate statistically significant differences ($p < 0.05$) at different concentrations at the same time. Different uppercase letters (A-B) indicate statistically significant differences ($p < 0.05$) at different times at the same concentration

- **CHOLESTENONE IN CACO-2 CELLS (TUMORAL)**



The * indicates statistically significant differences ($p < 0.05$) between the treatments and the control. Different lowercase letters (a-c) indicate statistically significant differences ($p < 0.05$) at different concentrations at the same time. Different uppercase letters (A-B) indicate statistically significant differences ($p < 0.05$) at different times at the same concentration

- **5-FU (25 µM)**



The asterisk indicates statistically significant differences ($p < 0.05$) between the treatments and the control. Different lowercase letters (a-b) indicate statistically significant differences ($p < 0.05$) between the different times for a cell line. Different uppercase letters (A-B) indicate statistically significant differences ($p < 0.05$) between the two cell lines for the same time

- **CYTOTOXICITY OF CHOLESTEROL METABOLITES**

		IC_{50} (µM)	
		CCD-18Co	**Caco-2**
Coprostanol	24 h	>300^{aA}	>300^{aA}
	48 h	>300^{aA}	>300^{aA}
	72 h	156 ± 17^{*cA}	>300^{aA}
Cholestanol	24 h	>300^{aA}	>300^{aA}
	48 h	>300^{aA}	>300^{aA}
	72 h	>300^{aB}	>300^{aA}
Coprostanone	24 h	59 ± 7^{*aB}	>300^{aA}
	48 h	136 ± 8^{*bB}	>300^{aA}
	72 h	41 ± 4^{*cC}	121 ± 7^{bB}
Cholestenone	24 h	27 ± 1^{*aC}	37 ± 4^{aB}
	48 h	46 ± 3^{bC}	50 ± 3^{bB}
	72 h	13 ± 3^{*cD}	38 ± 3^{aC}

The * indicates statistically significant difference ($p < 0.05$) vs. Caco-2 in the same time. Different lowercase letters (a-c) indicate statistically significant differences ($p < 0.05$) at different times for the same metabolite and cell line. Different uppercase letters (A-D) indicate statistically significant differences ($p < 0.05$) in different metabolites for the same time and cell line

- **CYTOTOXICITY OF CHOLESTEROL METABOLITES**

		IC_{50} (µM)	
		CCD-18Co	**Caco-2**
Coprostanol	24 h	>300^{aA}	>300^{aA}
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The most cytotoxic

The * indicates statistically significant difference ($p < 0.05$) vs. Caco-2 in the same time. Different lowercase letters (a-c) indicate statistically significant differences ($p < 0.05$) at different times for the same metabolite and cell line. Different uppercase letters (A-D) indicate statistically significant differences ($p < 0.05$) in different metabolites for the same time and cell line

- **CYTOTOXICITY OF CHOLESTEROL METABOLITES**

The most vulnerable ←

		IC_{50} (µM)	
		CCD-18Co	**Caco-2**
Coprostanol	24 h	>300^{aA}	>300^{aA}
	48 h	>300^{aA}	>300^{aA}
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The most cytotoxic ←

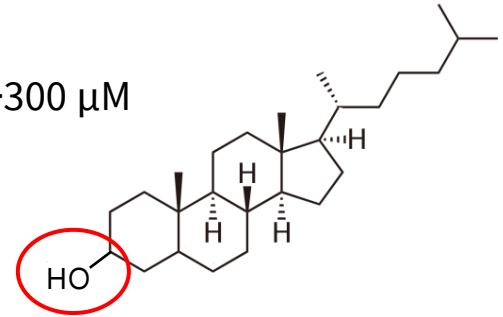
The * indicates statistically significant difference ($p < 0.05$) vs. Caco-2 in the same time. Different lowercase letters (a-c) indicate statistically significant differences ($p < 0.05$) at different times for the same metabolite and cell line. Different uppercase letters (A-D) indicate statistically significant differences ($p < 0.05$) in different metabolites for the same time and cell line

- **CYTOTOXICITY OF CHOLESTEROL METABOLITES**

Coprostanol and cholestanol: IC_{50} >300 µM

↓

Low uptake by the colonocyte →



Equatorial Position

Cholestenone and coprostanone → Relationship between hydrophobicity and cytotoxicity

↓

The most cytotoxic: IC_{50} (CCD-18Co - 72 h): 5 ± 1 µg / mL ←

Accepted cytotoxic values
IC_{50} (48-72 h) < 4 µg / mL [1]

Nuclear aberrations [2] and sister chromatid exchange [3] in the colon of mice

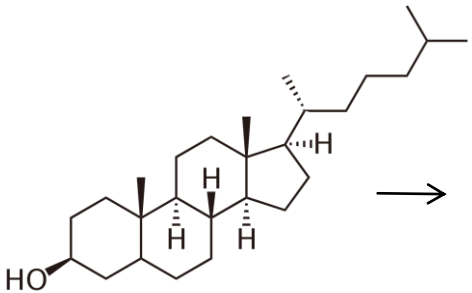
↓

The metabolite with the greatest capacity to promote CRC

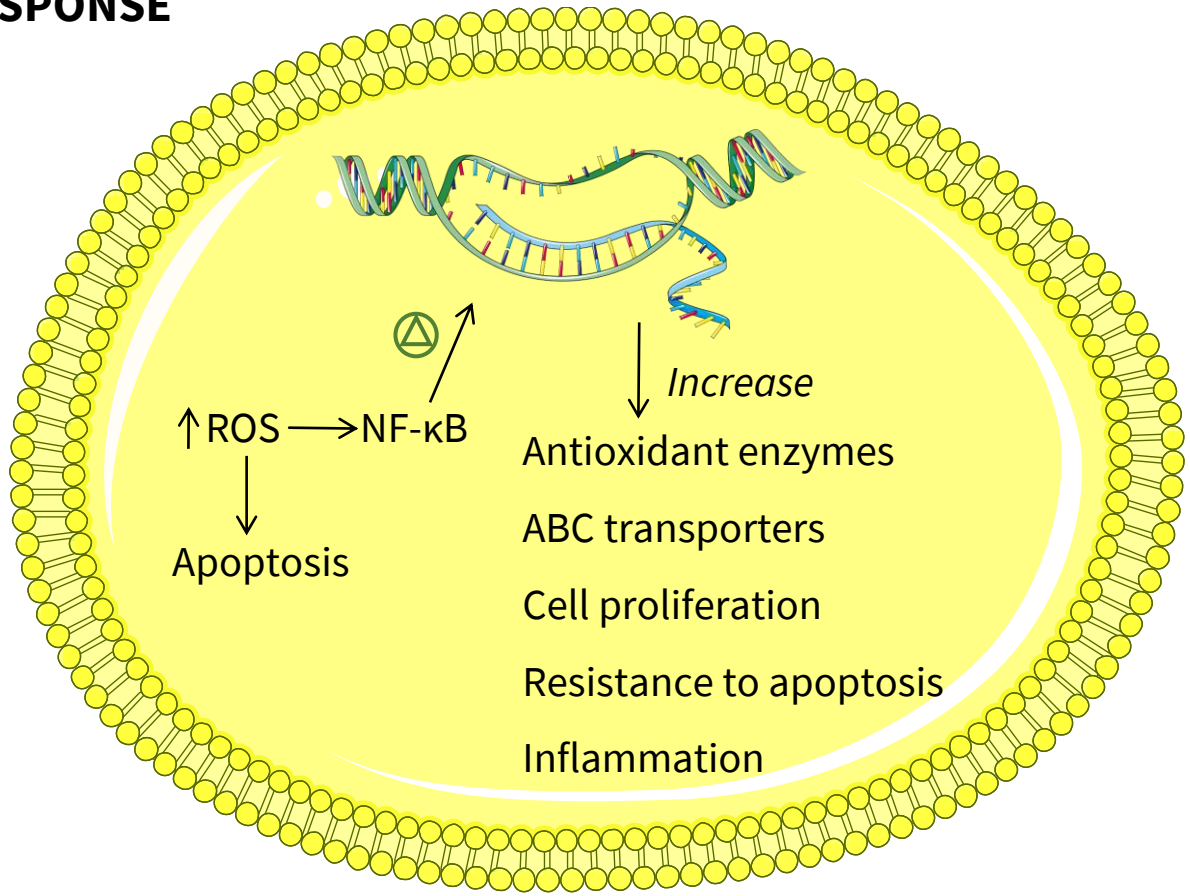
[1] López-García et al., 2019. Nutraceuticals and Natural Product Derivatives: Disease Prevention & Drug Discovery. 1st ed. 145-165.; [2] Suzuki et al., 1986. Cancer Lett. 33: 307-16; [3] Kaul et al., 1987. Mutagenesis. 2: 441-4.

- **COMPENSATORY RESPONSE**

Secondary bile acid mimetic action?



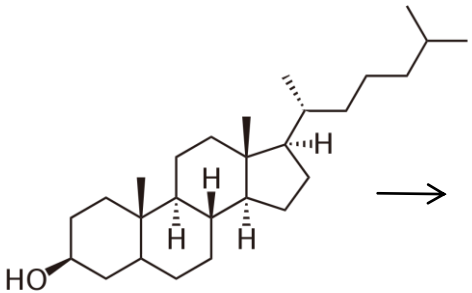
Cholestanol



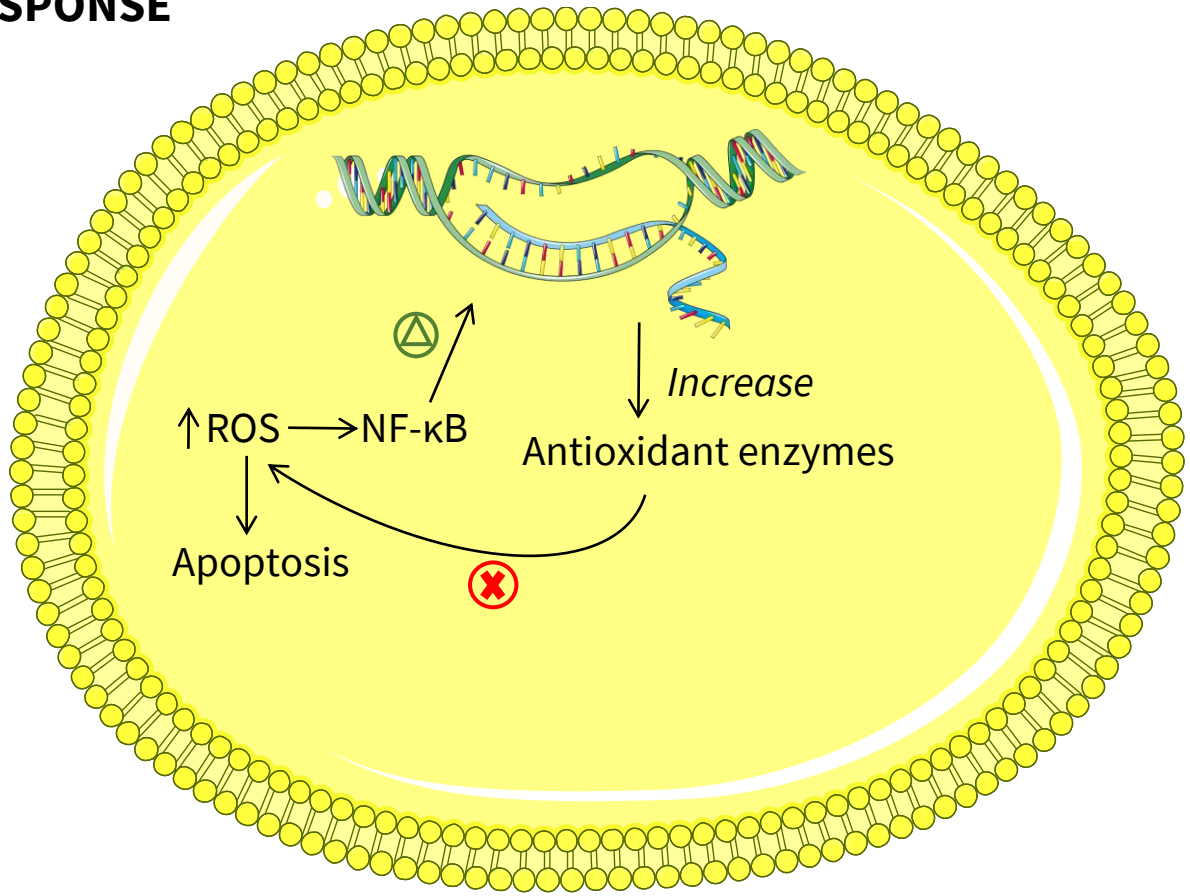
Ajouz et al., 2014. World J Surg. 12, 164; Payne et al., 2007. Carcinogenesis. 28: 215-222; Jarosz et al., 2017. Inflammopharmacology.25, 11-24; Lin et al., 2010. Expert Opin Ther Targets. 14: 45-55.

- **COMPENSATORY RESPONSE**

Secondary bile acid mimetic action?



Cholestanol



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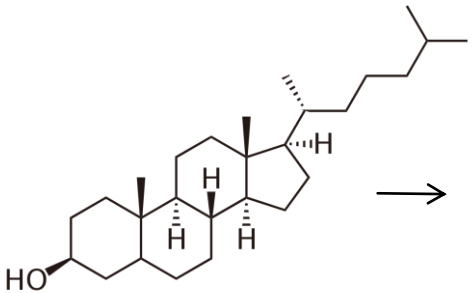
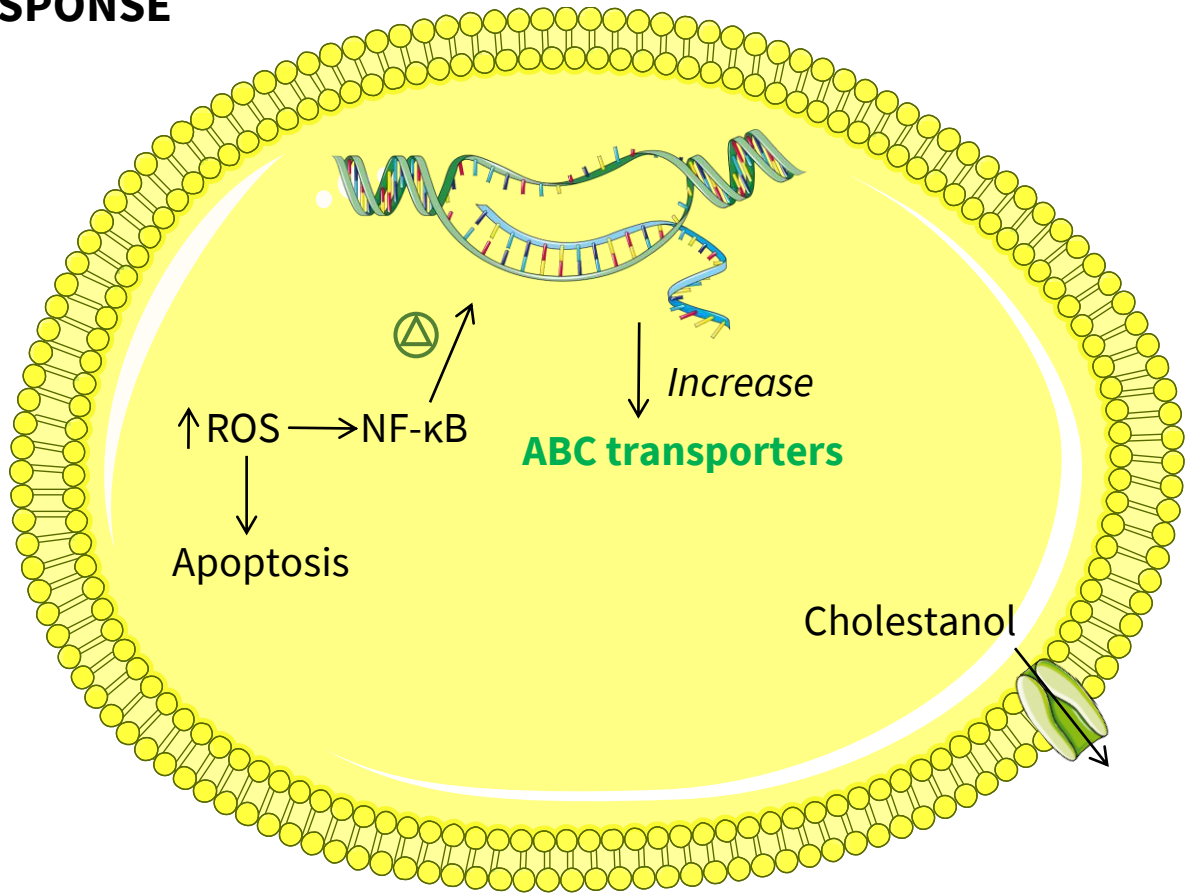
- **COMPENSATORY RESPONSE**

Secondary bile acid mimetic action?



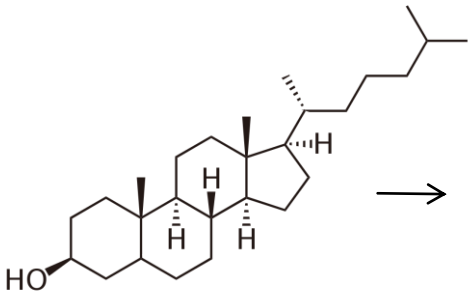
Cholestanol



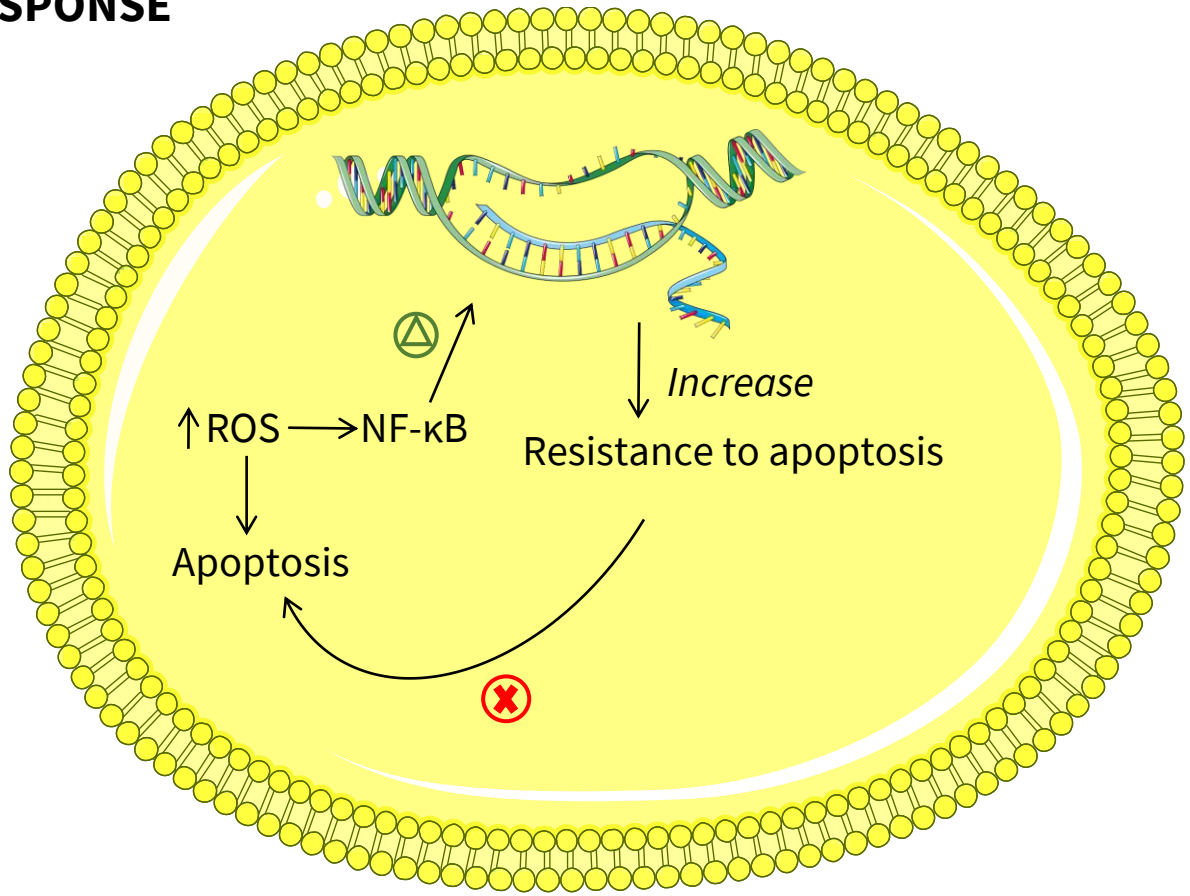
Ajouz et al., 2014. World J Surg. 12, 164; Payne et al., 2007. Carcinogenesis. 28: 215-222; Jarosz et al., 2017. Inflammopharmacology.25, 11-24; Lin et al., 2010. Expert Opin Ther Targets. 14: 45-55.

- **COMPENSATORY RESPONSE**

Secondary bile acid mimetic action?



Cholestanol



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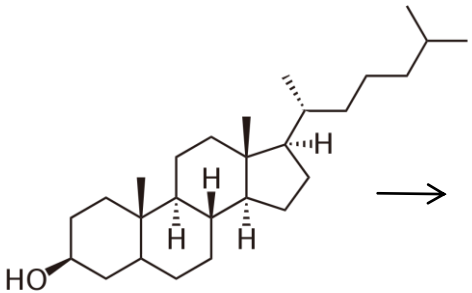
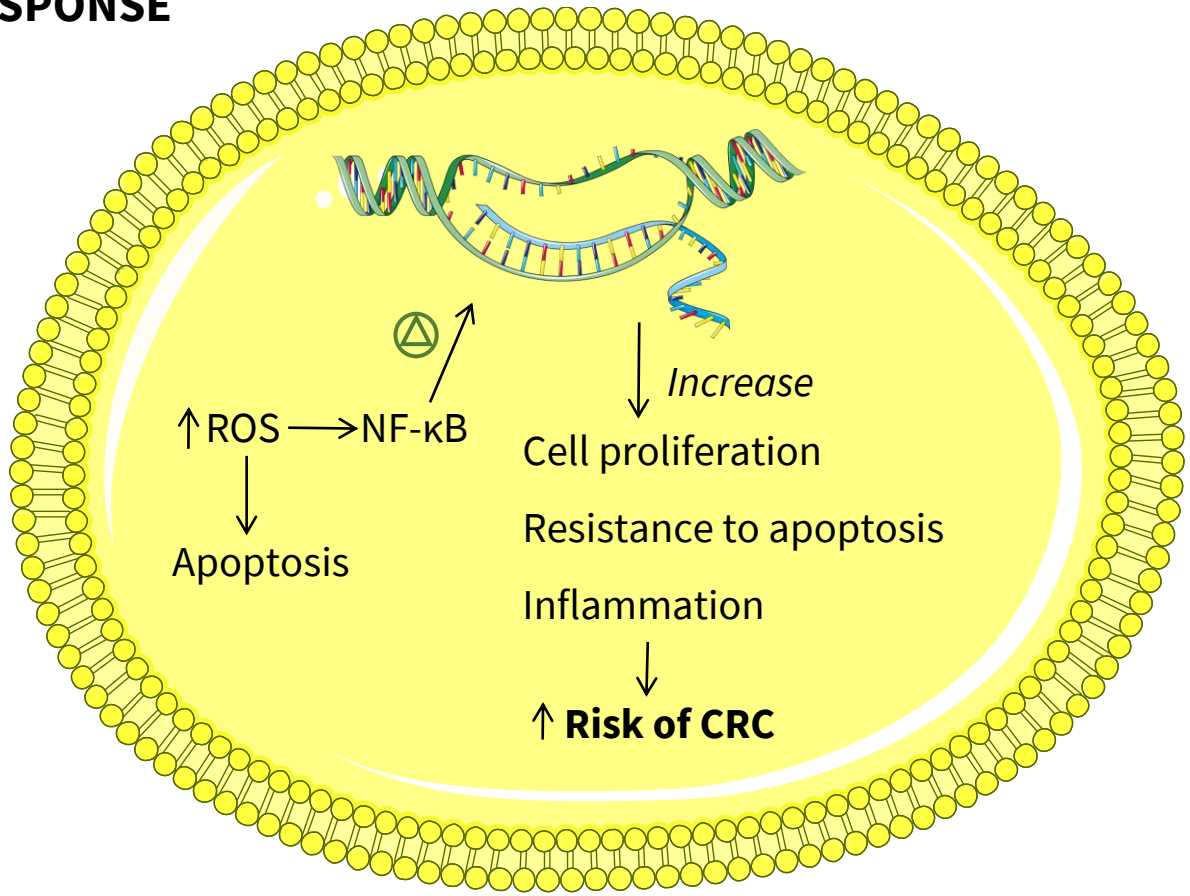


Cholestanol



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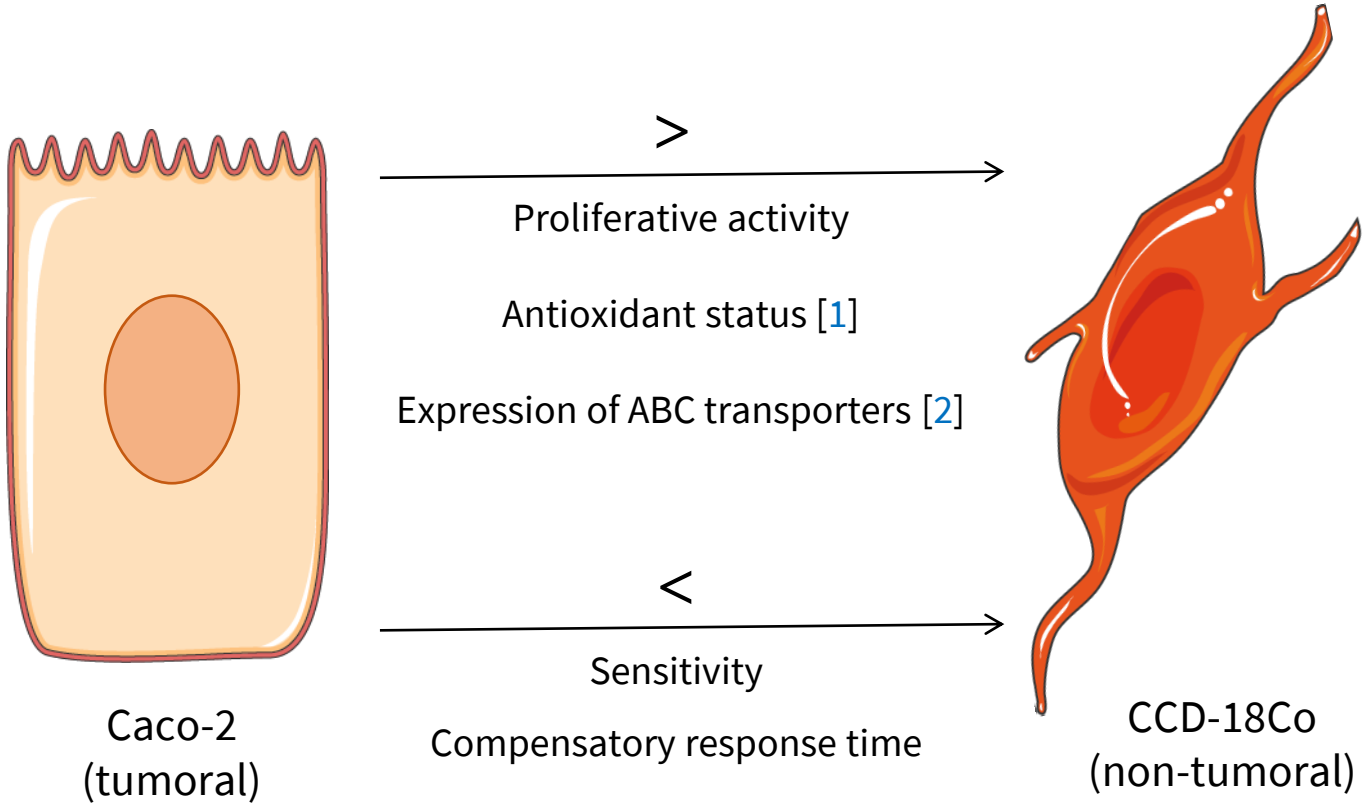
- **CELL LINE RESPONSE**



Proliferative activity

Antioxidant status [1]

Expression of ABC transporters [2]

Sensitivity

Compensatory response time

Caco-2
(tumoral)

CCD-18Co
(non-tumoral)

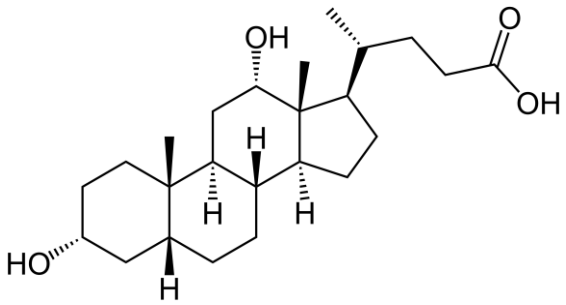
[1] Cermak et al., 1993. Cancer Res. 53, 5308-5313; [2] Lin et al., 2010. Expert Opin Ther Targets. 14, 45-55.

• LIMITATIONS OF STUDY

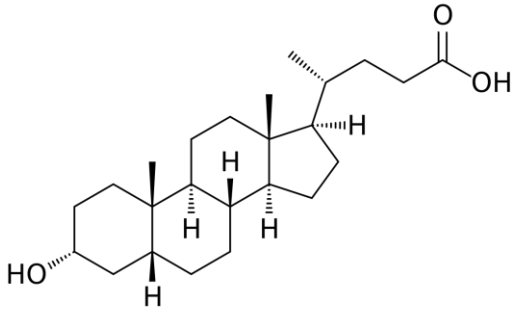
- Preliminary study ⟶ A greater number of analytical repetitions is needed
- Molecular mechanisms have not been studied ⟶ Cell death and cell cycle progression
- **Hypothesis on compensatory response** and cellular sensitivity without experimental support

↓ Based

Structural similarity to secondary bile acids (same activity?)



Deoxycholic acid



Lithocholic acid

1. Metabolites produced by intestinal bacteria from cholesterol, mainly those of a hydrophobic nature (cholestenone and coprostanone), could be involved in colorectal carcinogenesis through their cytotoxic activity.
2. Further studies are needed to determine the molecular mechanisms that mediate the cytotoxicity of cholesterol metabolites, and to define the selectivity of this effect.
3. The study of pathways involved in the compensatory response is needed, since this could contribute to the development of CRC.

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CYTOTOXIC EFFECT OF CHOLESTEROL METABOLITES ON HUMAN COLONIC TUMOR (CACO-2) AND NON-TUMOR (CCD-18CO) CELLS AND THEIR POTENTIAL IMPLICATION IN COLORECTAL CARCINOGENESIS

THANKS FOR YOUR ATTENTION

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