Fyziologie buněčných systémů

Eikosanoidy a cytokinetika:

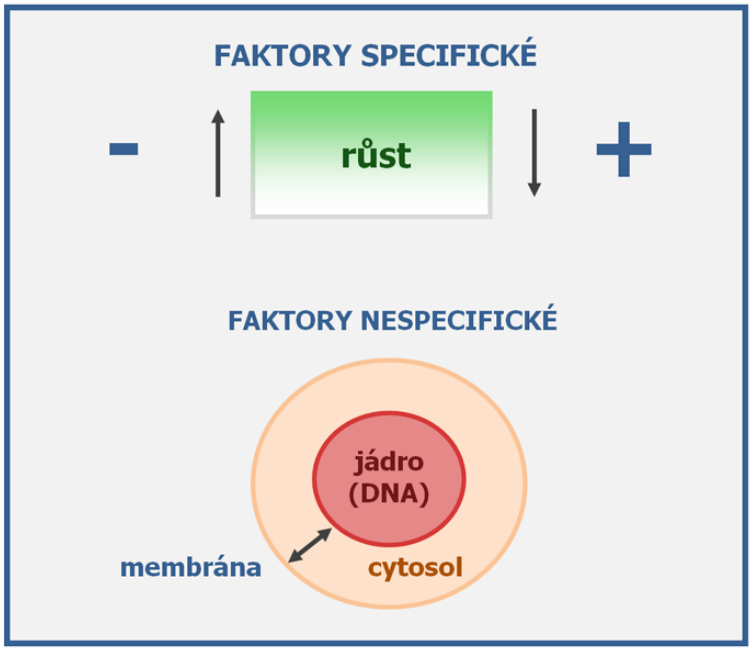
(Možnosti modulace – I)

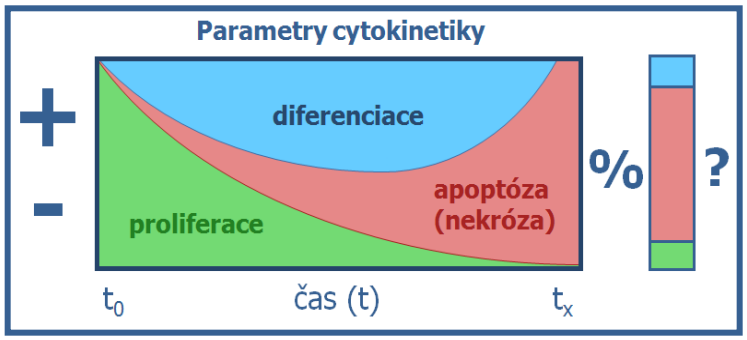
A. Kozubík

Biofyzikální ústav AVČR, v.v.i., *(Oddělení cytokinetiky)*
Ústav experimentální biologie, PřF MU
(*Oddělení fyziologie a imunologie živočichů*)
Brno

Spolupůsobení „regulátorů růstu“ rozdílné chemické povahy na proliferaci a diferenciaci

NĚKTERÉ HLAVNÍ CÍLE SOUČASNÉHO VÝZKUMU



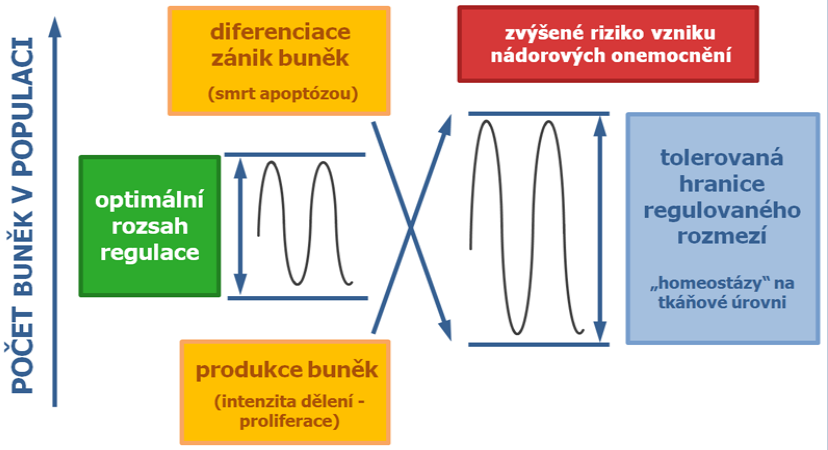


Poznání mechanismů působení látek lipidové povahy, zejména VNMK a jejich derivátů,
***v mezi- a vnitrobuněčných* komunikacích podílejících se**

v regulaci cytokinetiky *(proliferace, diferenciace a apoptózy)*
v kontextu jejich interakcí s

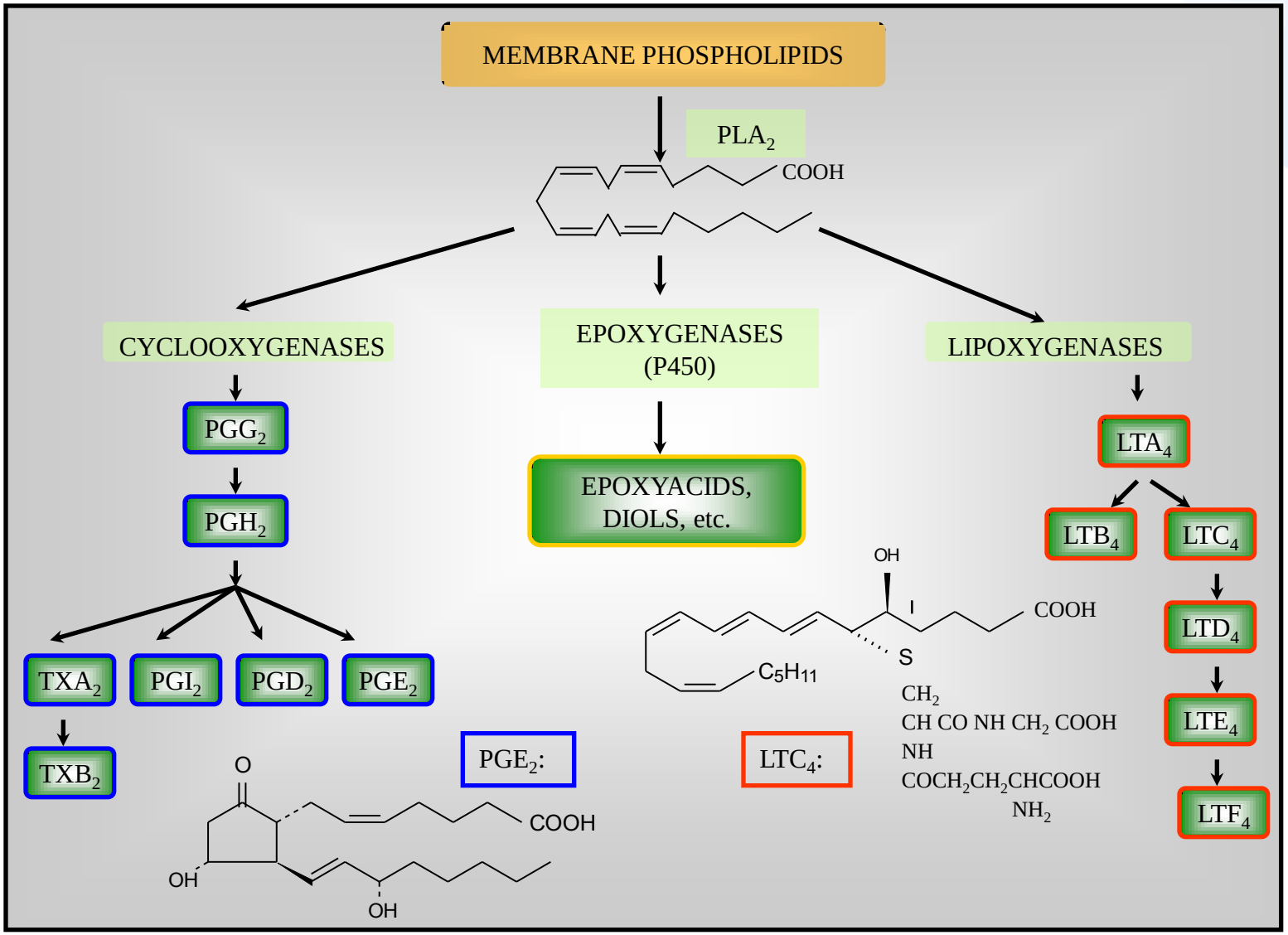
- **fyziologickými regulátory růstu,**
- **environmentálními polutanty**
- **vybranými farmaky.**

Jedním z praktických cílů je využít tyto znalosti v rámci přípravy **lipidových nutričních preparátů,** případně **cytostatik.**

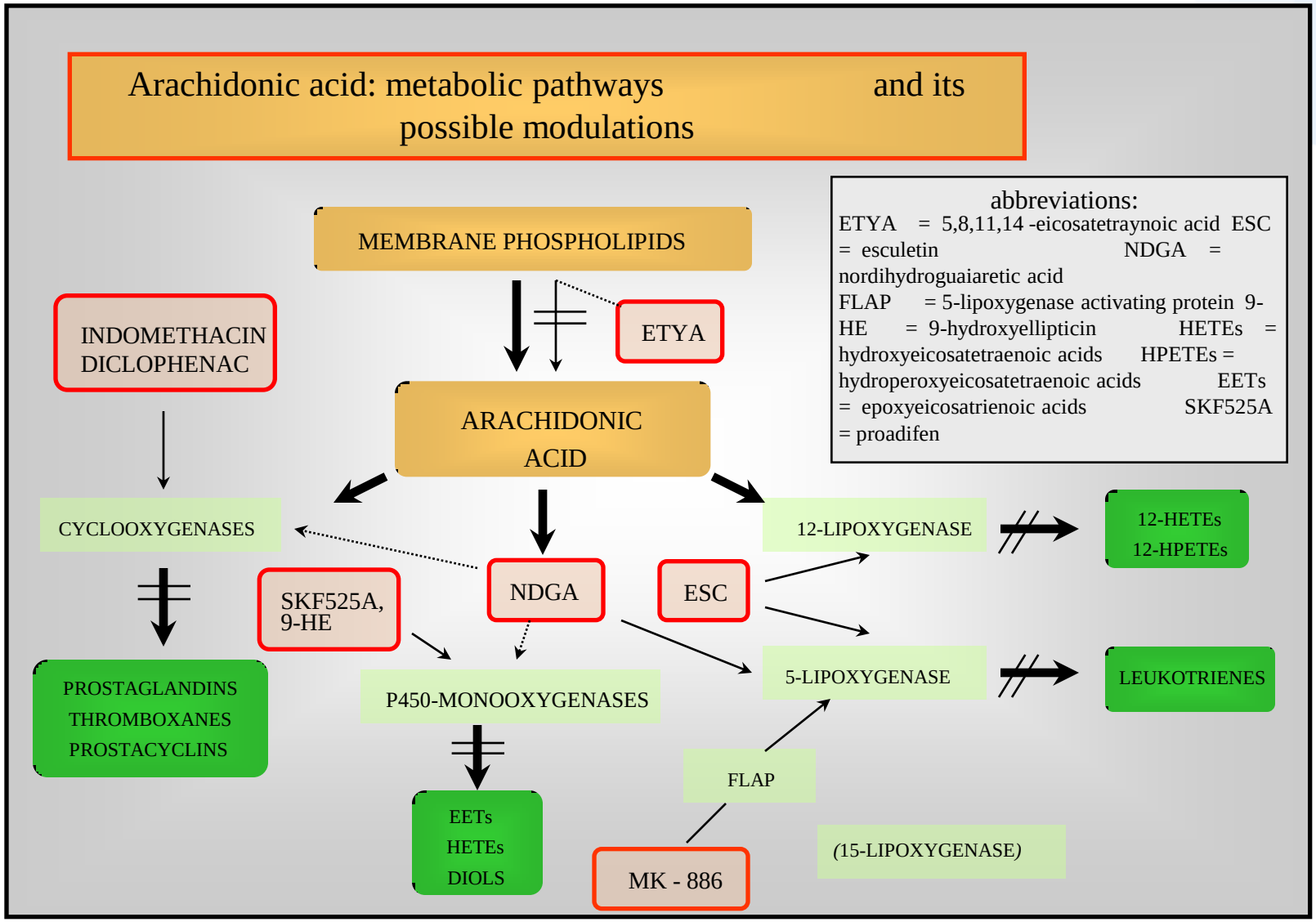


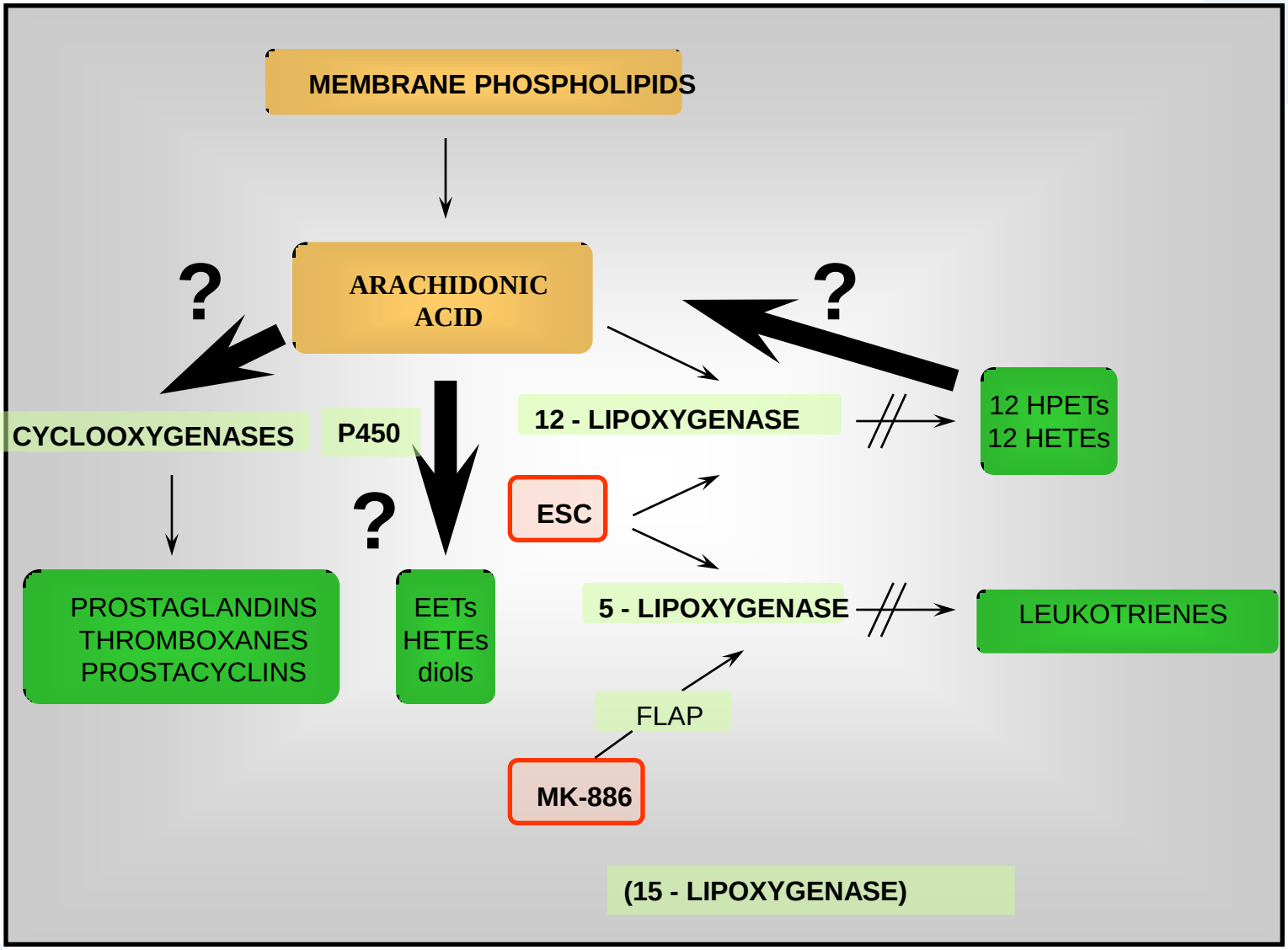
Efekty inhibitorů metabolismu AA

význam rovnováhy
v přísunu prekursorových PUFAs
a
v produkci jednotlivých jejich metabolitů

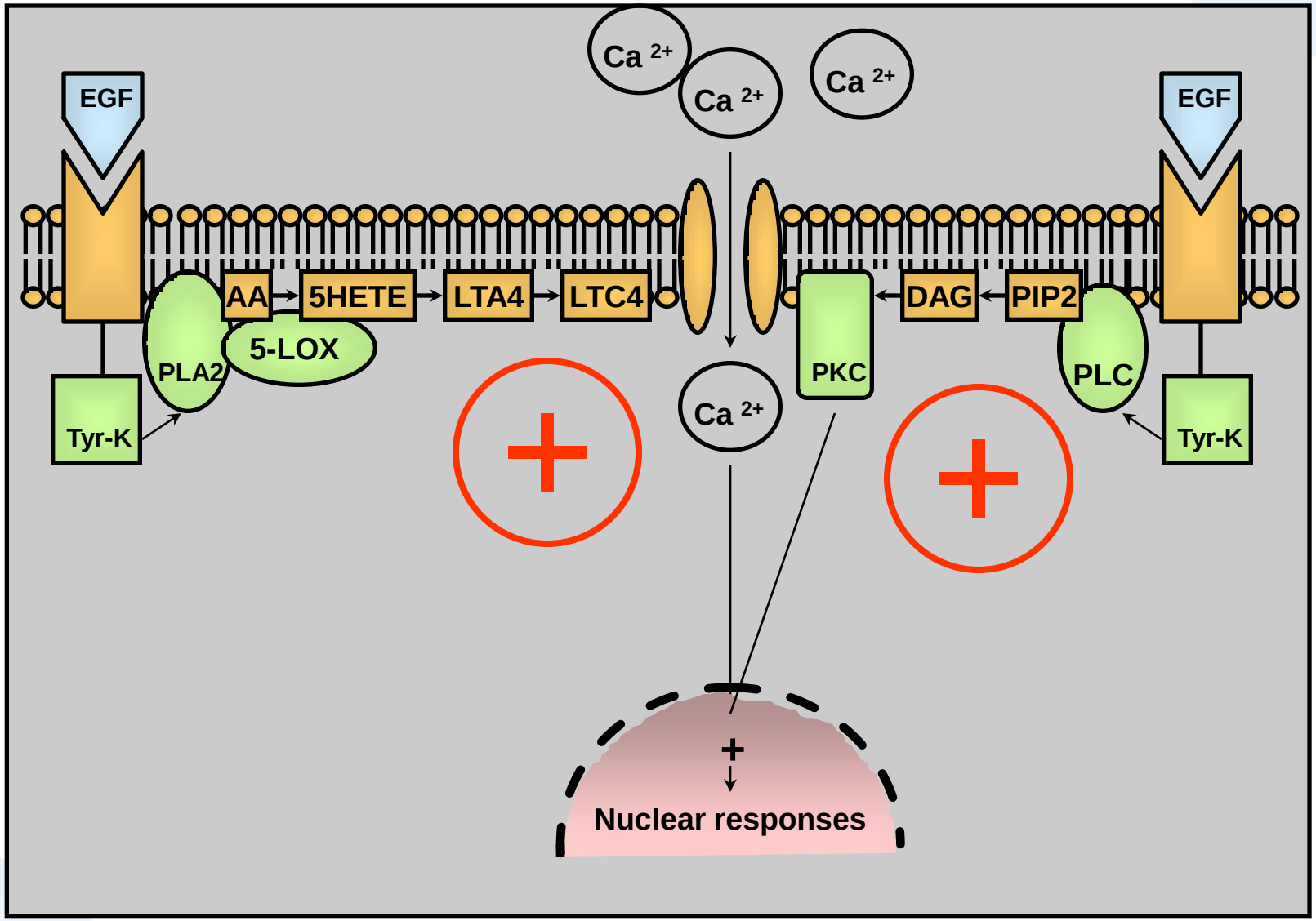

MEMBRANE PHOSPHOLIPIDS
PLA2
COOH
CYCLOOXYGENASES
EPOXYGENASES (P450)
LIPOXYGENASES
PGG2
PGH2
EPOXYACIDS, DIOLS, etc.
LTA4
LTB4
LTC4
LTD4
LTE4
LTF4
TXA2
PGI2
PGD2
PGE2
TXB2
OH
COOH
S
C5H11
CH2
CH CO NH CH2 COOH
NH
COCH2CH2CHCOOH
NH2
PGE2:
LTC4:
O
OH
OH
COOH

Arachidonic acid: metabolic pathways and its possible modulations

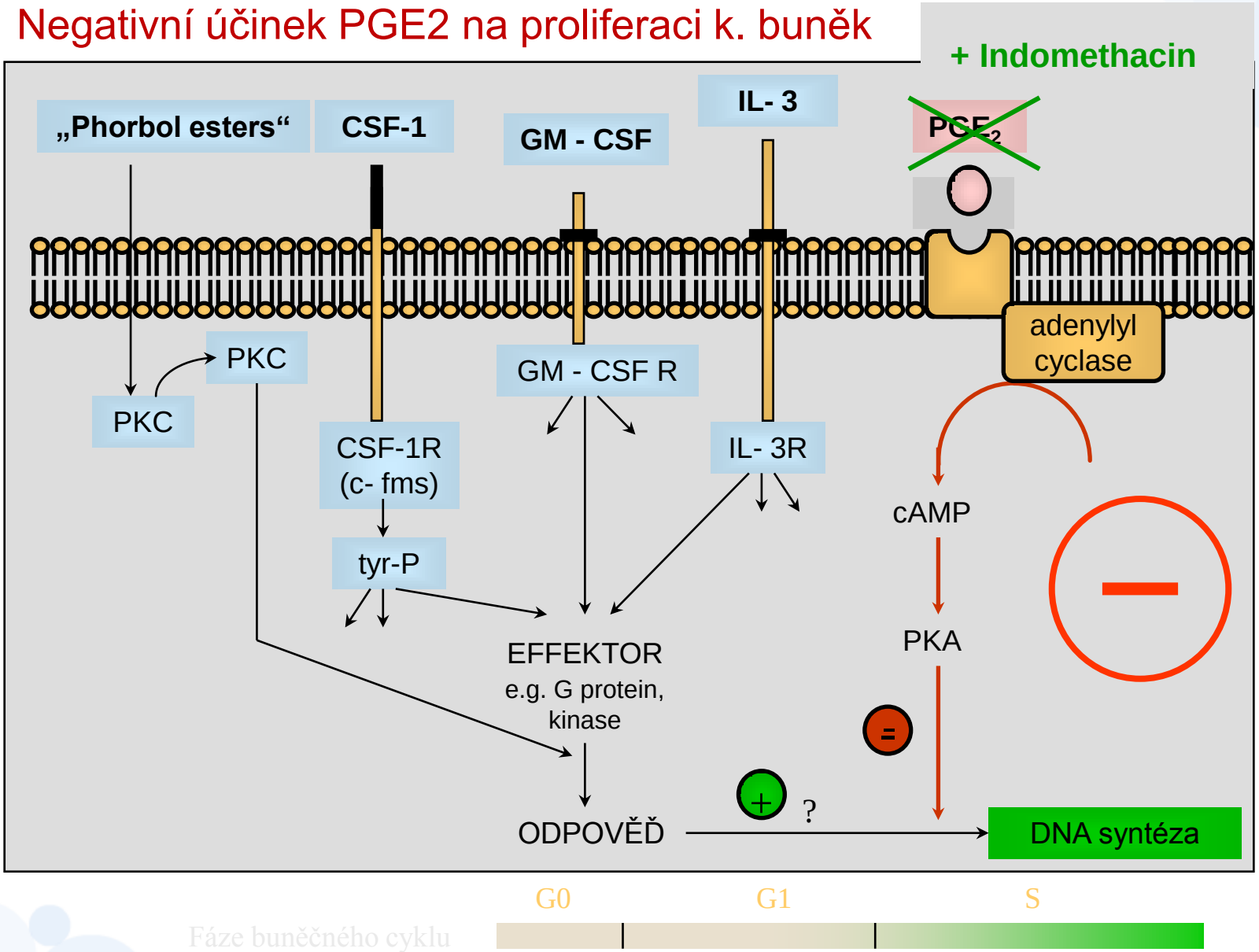


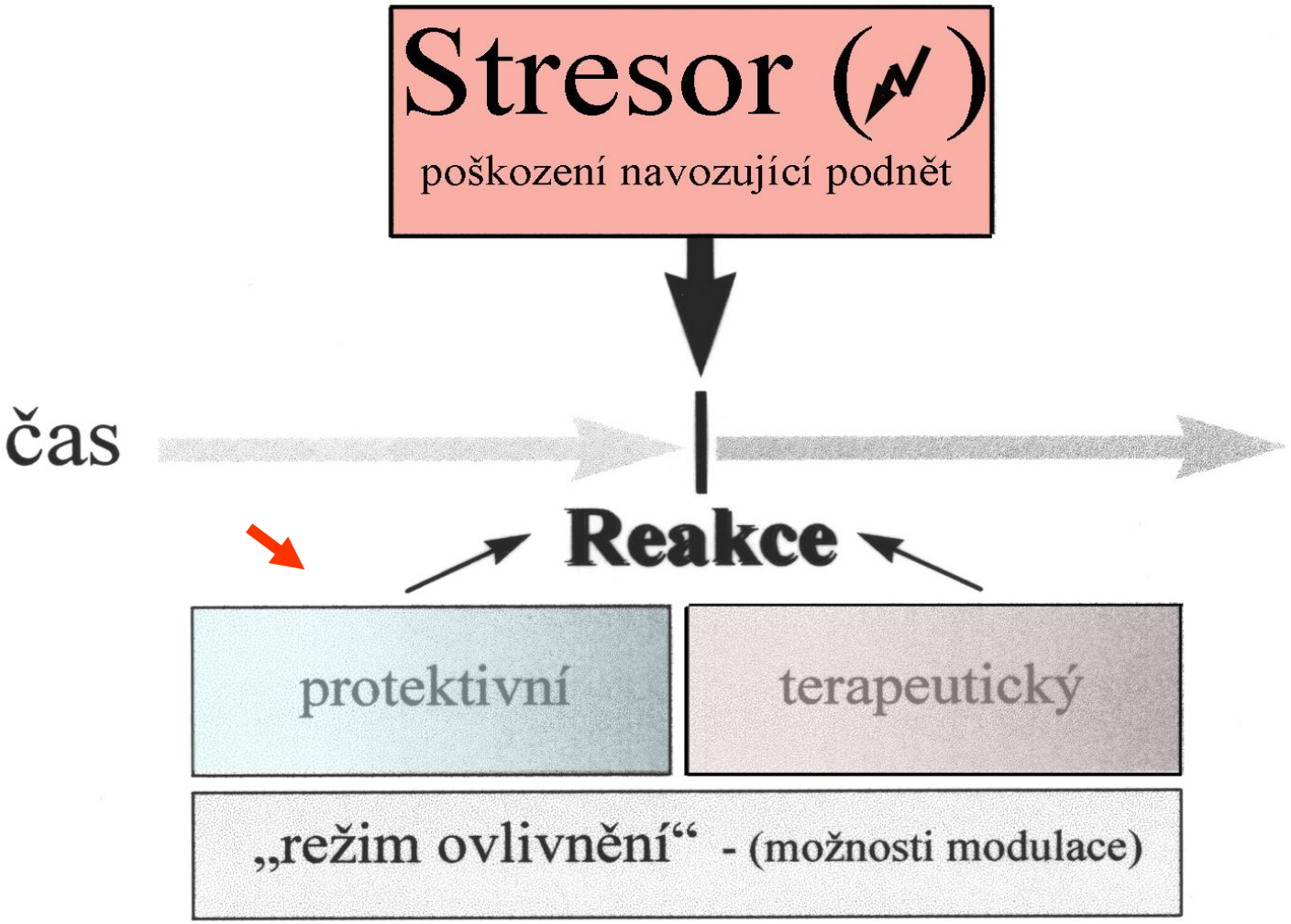

MEMBRANE PHOSPHOLIPIDS
ARACHIDONIC ACID
?
?
CYCLOOXYGENASES
P450
12 - LIPOXYGENASE
12 HPETs
12 HETEs
?
ESC
PROSTAGLANDINS
THROMBOXANES
PROSTACYCLINS
EETs
HETEs
diols
5 - LIPOXYGENASE
LEUKOTRIENES
FLAP
MK-886
(15 - LIPOXYGENASE)

Příklad pozitivního efektu na proliferaci



Negativní účinek PGE2 na proliferaci k. buněk

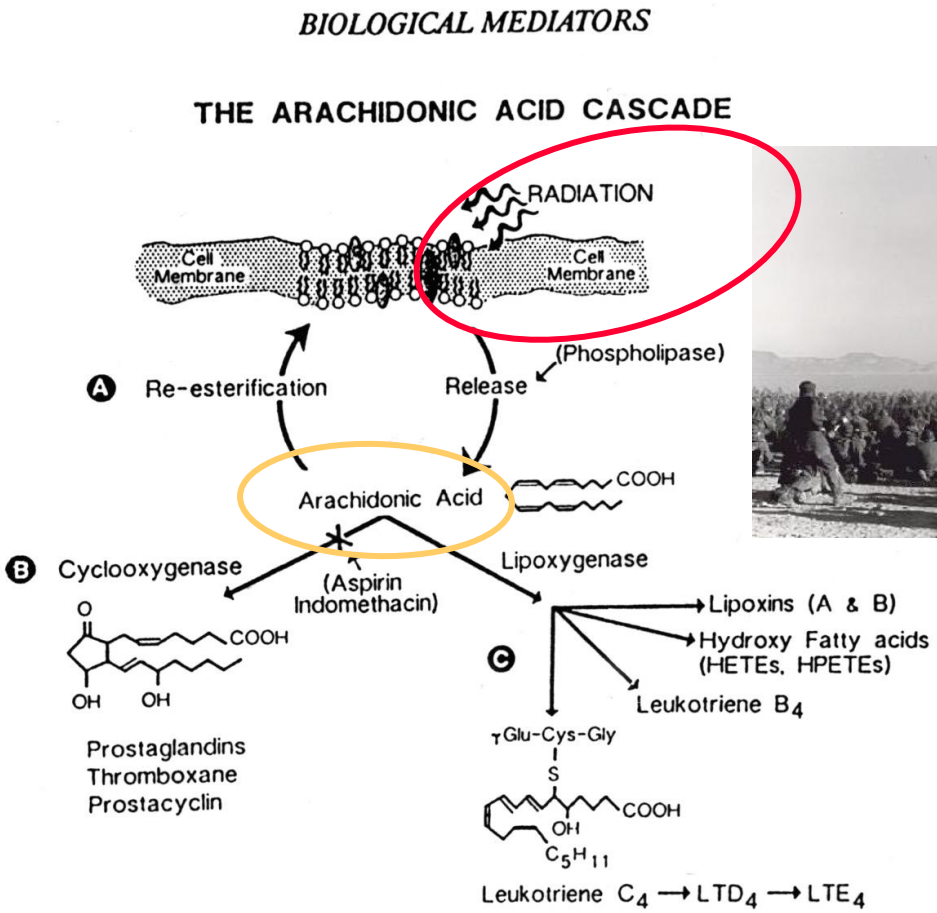




Laboratoř cytokinetiky

Biofyzikální ústav AVČR, BRNO

Fosfolipidový metabolismus a působení ioniz. radiace (škodlivých faktorů životního prostředí)



Experimental Hematology 21:138–142 (1993)

Experimental Hematology

The effect of nordihydroguaiaretic acid, an inhibitor of prostaglandin and leukotriene biosynthesis, on hematopoiesis of gamma-irradiated mice

Alois Kozubík, Jiřina Hofmanová, Jiřina Holá, Jaromíra Netíková

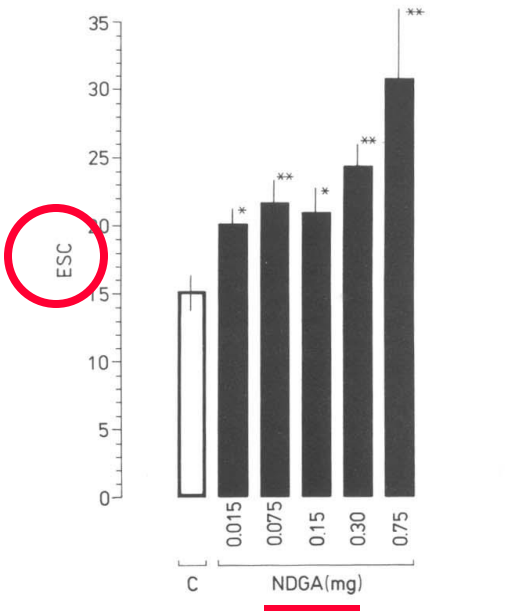


Fig. 1. Numbers of endogenous macroscopic spleen colonies (ESC) in mice irradiated with a dose of 5 Gy 1 hour after i.p. injection of various doses (0.015 to 0.75 mg) of NDGA. The respective groups comprised 20 to 30 animals.
* p<0.05, ** p<0.01 as compared with control (C) mice

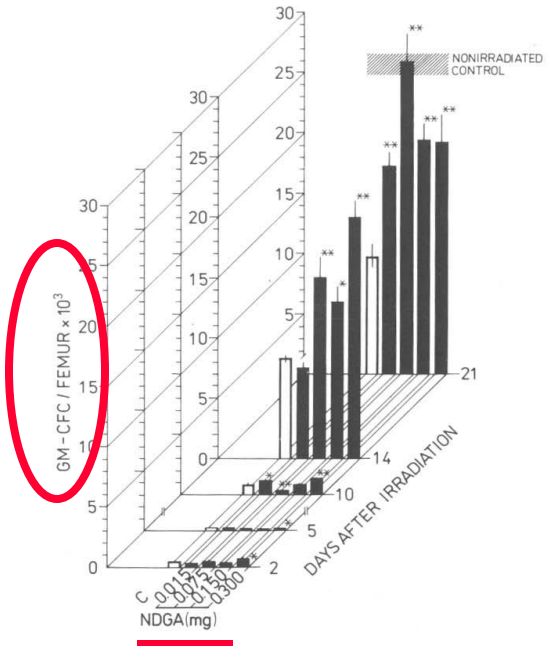


Fig. 2. Dynamics of the postirradiation recovery of GM-CFC in mice irradiated with a dose of 5 Gy 1 hour after i.p. injection of various doses (0.015 to 0.3 mg) of NDGA.
* p<0.05, ** p<0.01 as compared with control (C) mice

Aplikace NDGA „protektivním režimu“

a její dávkově závislé účinky na regeneraci ESC a GM-CFC

Indices	Non-irrad. control	Exp. group	Days after irradiation			
			5	10	14	21
Granulocytes	595.8±52.9	C	406.4±51.8	971.5±130.8	601.1±82.2	1300.4±131.2
		NDGA	717.7*±64.0	1597.8±176.0*	575.7±37.5	1486.4±187.9
Lymphocytes	3184.2±239.4	C	787.5±81.8	830.8±140.0	1575.2±141.6	1831.6±244.9
		NDGA	935.9±83.5	1025.9±113.0	1617.6±105.5	2121.3±268.1
Nucleated cells per femur × 10^7	3.0141±0.1476	C	0.8240±0.0942	1.5486±0.0657	1.8255±0.0778	2.4760±0.0721
		NDGA	0.7577±0.0417	1.6477±0.440	2.2960±0.1500*	3.4919±0.2023**

Numbers of granulocytes and lymphocytes, and bone marrow cellularity measured at selected postirradiational intervals in control (C) and experimental mice treated with NDGA at a dose of 0.3 mg per mouse 1 hour before 5 Gy gamma-irradiation. At least 10 animals per group were used. *p<0.05; **p<0.01 as compared with control (C) mice

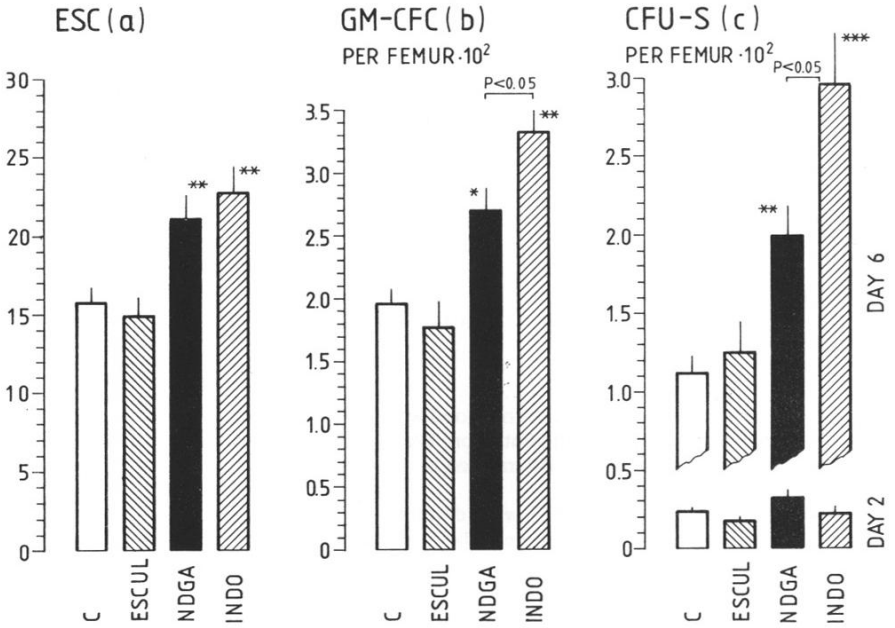


Fig. 3. Endogenous spleen colony numbers (ESC) detected on day 10 **(A)**, GM-CFC numbers in femoral marrow on day 2 **(B)** and exogenous spleen colony numbers (CFU-S) in femoral marrow on days 2 and 6 **(C)** after 5 Gy of gamma-irradiation and experimental treatment (0.3 mg of NDGA, 0.25 mg INDO or 0.51 mg ESCUL, i.e., isomolar doses administered 1 hour before irradiation). Ten mice per group were used for ESC and CFU-S determination; each value for GM-CFC represents the average of 3 independent experiments.
* p<0.05; ** p<0.01 *** p<0.001 as compared with control (C) mice

Periferní krev

Efekty dalších inhibitorů

Kostní dřeň

INT. J. RADIAT. BIOL., 1994, VOL. 65, NO. 3, 369–377

Effects of drugs inhibiting prostaglandin or leukotriene biosynthesis on postirradiation haematopoiesis in mouse

A. KOZUBÍK*, J. HOFMANOVÁ, M. POSPÍŠIL, J. NETÍKOVÁ, J. HOLÁ and A. LOJEK

(Received 21 May 1993; revised 31 August 1993; accepted 15 October 1993)

Příklad aplikace inhibitorů metabolismu AA
v „terapeutickém režimu“

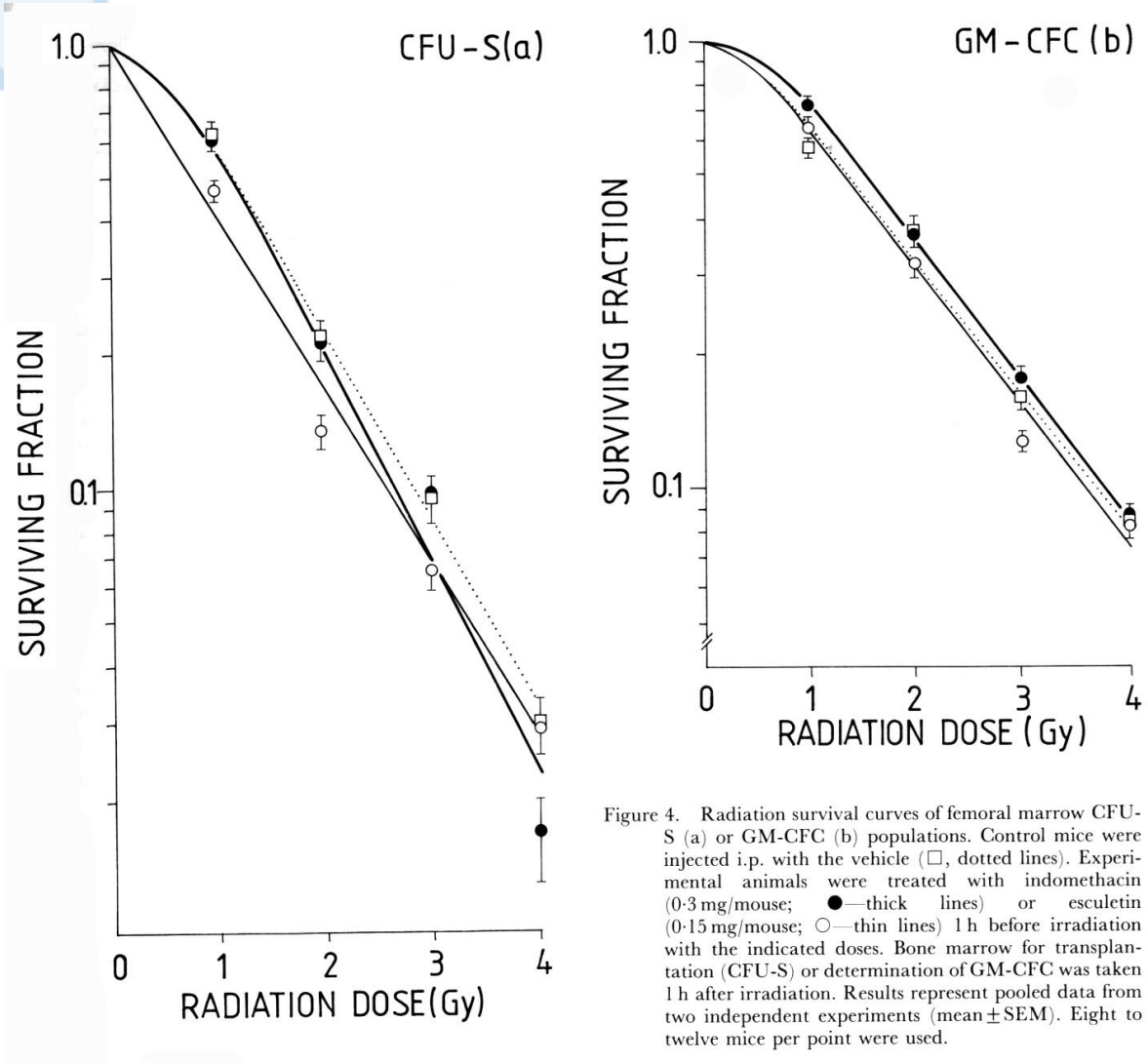


Figure 4. Radiation survival curves of femoral marrow CFU-S (a) or GM-CFC (b) populations. Control mice were injected i.p. with the vehicle (□, dotted lines). Experimental animals were treated with indomethacin (0·3 mg/mouse; ●—thick lines) or esculetin (0·15 mg/mouse; ○—thin lines) 1 h before irradiation with the indicated doses. Bone marrow for transplantation (CFU-S) or determination of GM-CFC was taken 1 h after irradiation. Results represent pooled data from two independent experiments (mean ± SEM). Eight to twelve mice per point were used.

INT. J. RADIAT. BIOL., 1994, VOL. 65, NO. 3, 369–377

Effects of drugs inhibiting prostaglandin or leukotriene biosynthesis on postirradiation haematopoiesis in mouse

A. KOZUBÍK*, J. HOFMANOVÁ, M. POSPÍŠIL, J. NETÍKOVÁ, J. HOLÁ and A. LOJEK

(Received 21 May 1993; revised 31 August 1993; accepted 15 October 1993)

Dílčí shrnutí (MOŽNÉ MECHANISMY)

Radiorezistence kmenových a prekurzorových buněk není oblivněna !!!

Efekty inhibitorů mohou být tedy způsobeny zásahy do biosyntézy eikosanoidů a jejich regulačními účinky na krvetvorbu

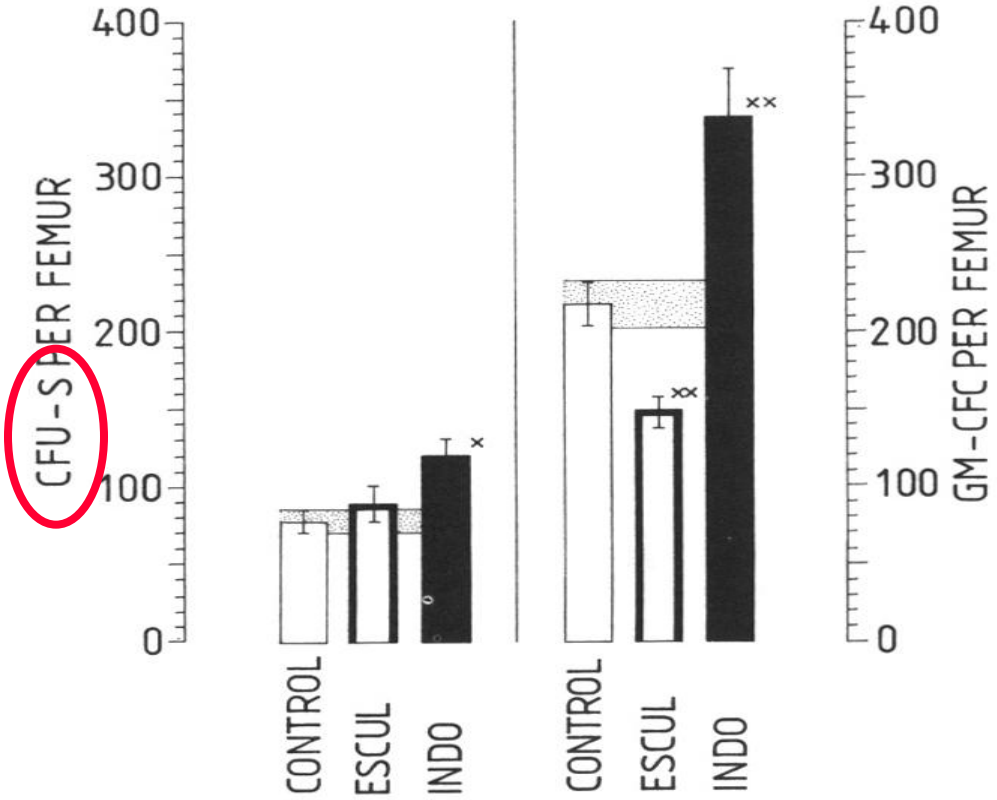


Figure 2. Mean ± SEM numbers of CFU-S and GM-CFC in the femoral bone marrow of the 8·5 Gy-irradiated and bone marrow-transplanted mouse determined on day 6 after transplantation. On days 3, 4 and 5 after transplantation mice were injected with indomethacin (INDO, 0·025 mg/mouse and dose) or esculetin (ESCUL, 0·0125 mg/mouse and dose), in two daily doses (a total of six injections were administered). Control mice (open columns and dotted areas) were treated in the same way with the vehicle. Two independent experiments were performed and the data were pooled. Twelve mice per group were used. Statistical significance: x, $p < 0{\cdot}05$; and xx, $p < 0{\cdot}01$ as compared with controls.

Příklad aplikace inhibitorů metabolismu AA v „terapeutickém režimu“

INT. J. RADIAT. BIOL., 1994, VOL. 65, NO. 3, 369–377

Effects of drugs inhibiting prostaglandin or leukotriene biosynthesis on postirradiation haematopoiesis in mouse

A. KOZUBÍK*, J. HOFMANOVÁ, M. POSPÍŠIL, J. NETÍKOVÁ, J. HOLÁ and A. LOJEK

(Received 21 May 1993; revised 31 August 1993; accepted 15 October 1993)

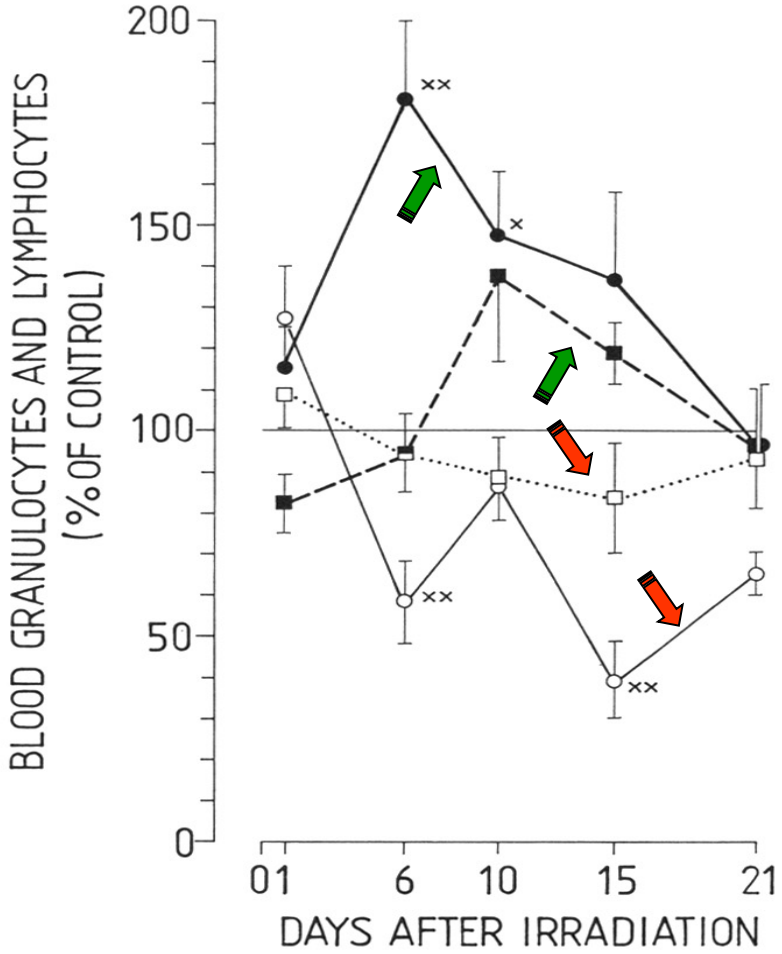


Figure 3. Percentage changes (related to 100% of irradiated control) of peripheral blood granulocytes (●, ○) and lymphocytes (■, □) in selected intervals after 5-Gy irradiation of mice. One hour before irradiation, mice were injected i.p. with indomethacin (0·3 mg/mouse, closed symbols) or esculetin (0·15 mg/mouse, open symbols). Control mice were injected with the vehicle. Ten mice from two independent experiments per point were used (mean ± SEM). Statistical significance: x, $p < 0{\cdot}05$; and xx, $p < 0{\cdot}01$ as compared with control.

Inhibitory
cyklooxygenáz stimulují (+)
a lipoxygenáz inhibují (-)

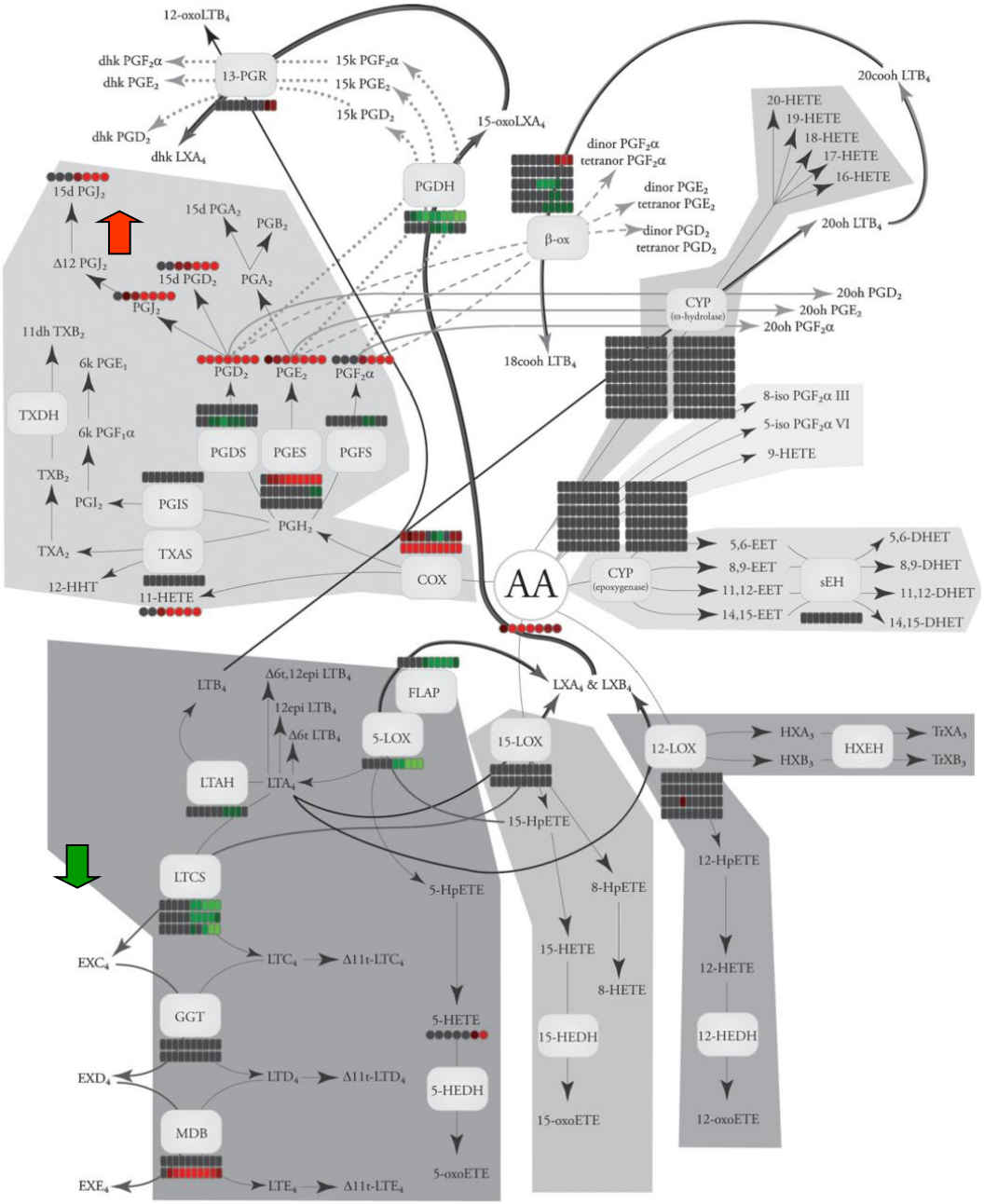
granulopoézu ○●
i
lymfopoézu □■

INT. J. RADIAT. BIOL., 1994, VOL. 65, NO. 3, 369–377

Effects of drugs inhibiting prostaglandin or leukotriene biosynthesis on postirradiation haematopoiesis in mouse

A. KOZUBÍK*, J. HOFMANOVÁ, M. POSPÍŠIL, J. NETÍKOVÁ, J. HOLÁ and A. LOJEK

(Received 21 May 1993; revised 31 August 1993; accepted 15 October 1993)



Genomické a lipidomické studie:

změny v biosyntéze eikosanoidů u makrofágů RAW264.7

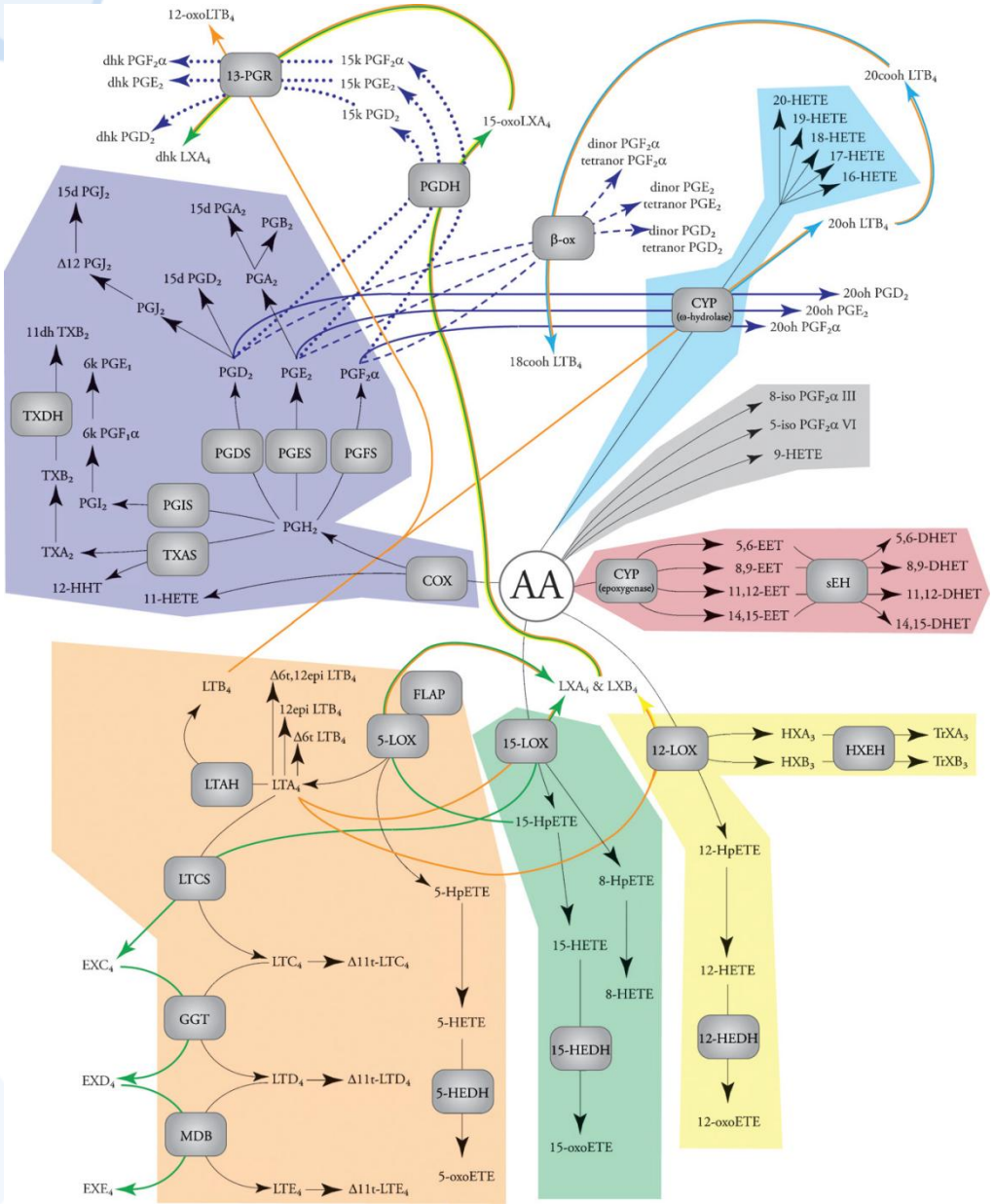
(jako reakce na Kdo_2-Lipid A stimulaci)

Vyšší intenzita červené ukazuje zvýšené hladiny,

Vyšší intenzita zelené ukazuje snížené hladiny,

Šedá – nezměněné hladiny

J Lipid Res. 2009 June; 50(6): 1015–1038.



Hlavní dráhy biosyntézy eikosanoidů (metabolity hlavních os barevně):

COX (purpurová),
5-LOX (oranžová),
15-LOX (zelená),
12-LOX (žlutá),
CYP epoxygenase (červená),
CYP ω-hydroxylase (světle modrá),
and nonenzymatic oxidation (šedá)

J Lipid Res. 2009 June; 50(6): 1015–1038.

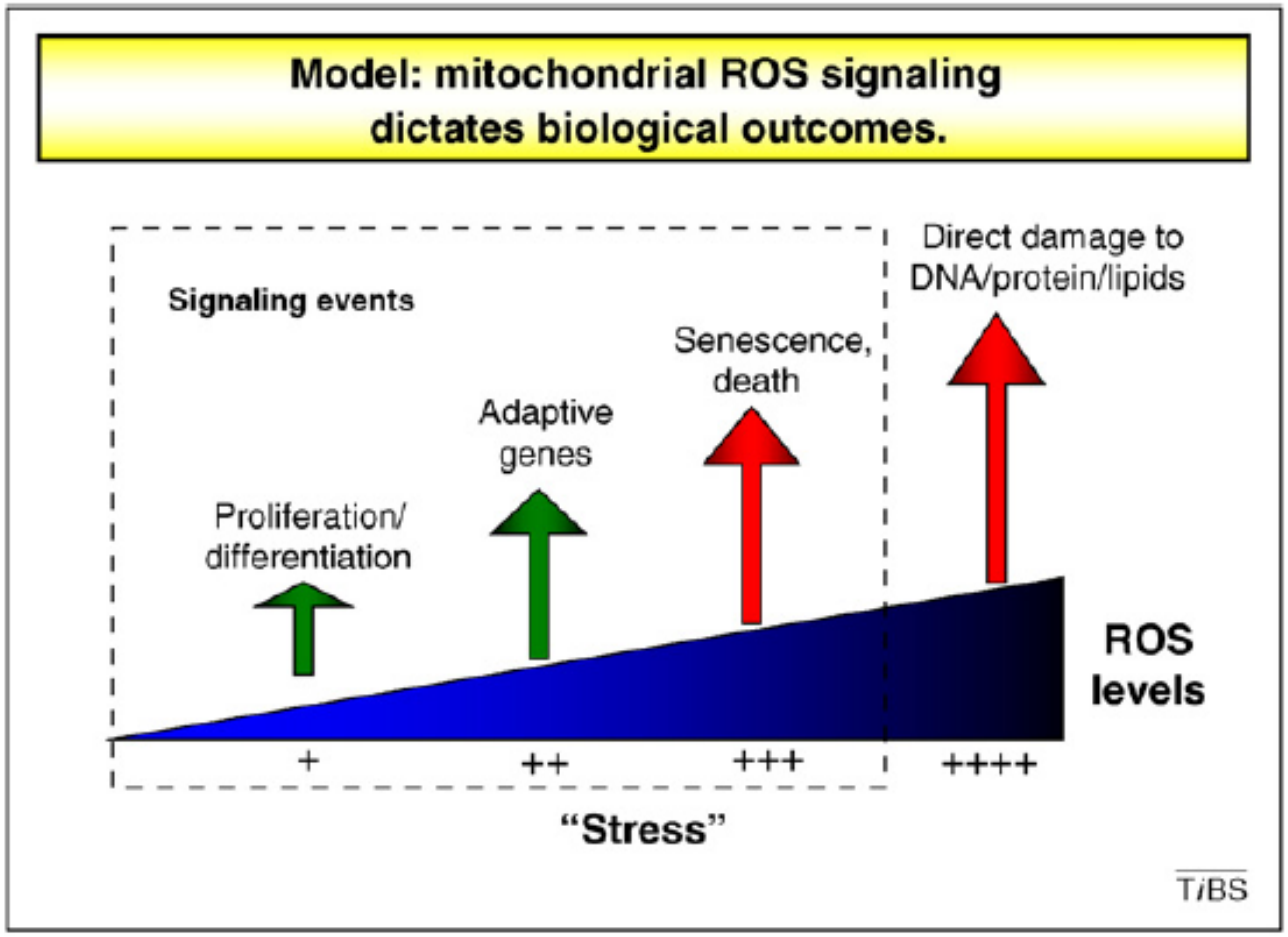


Figure 4. Mitochondrial ROS levels are crucial for biological outcomes. Low levels of mitochondrial ROS production are required for cellular processes such as proliferation and differentiation. An induction in ROS production will lead to adaptive programs including the transcriptional upregulation of antioxidant genes. Even higher levels of ROS will signal the initiation of senescence and apoptosis. Non-signaling, irreversible damage to cellular components is only observed under the highest levels of cellular ROS.

Hamanaka RB Chandel NS

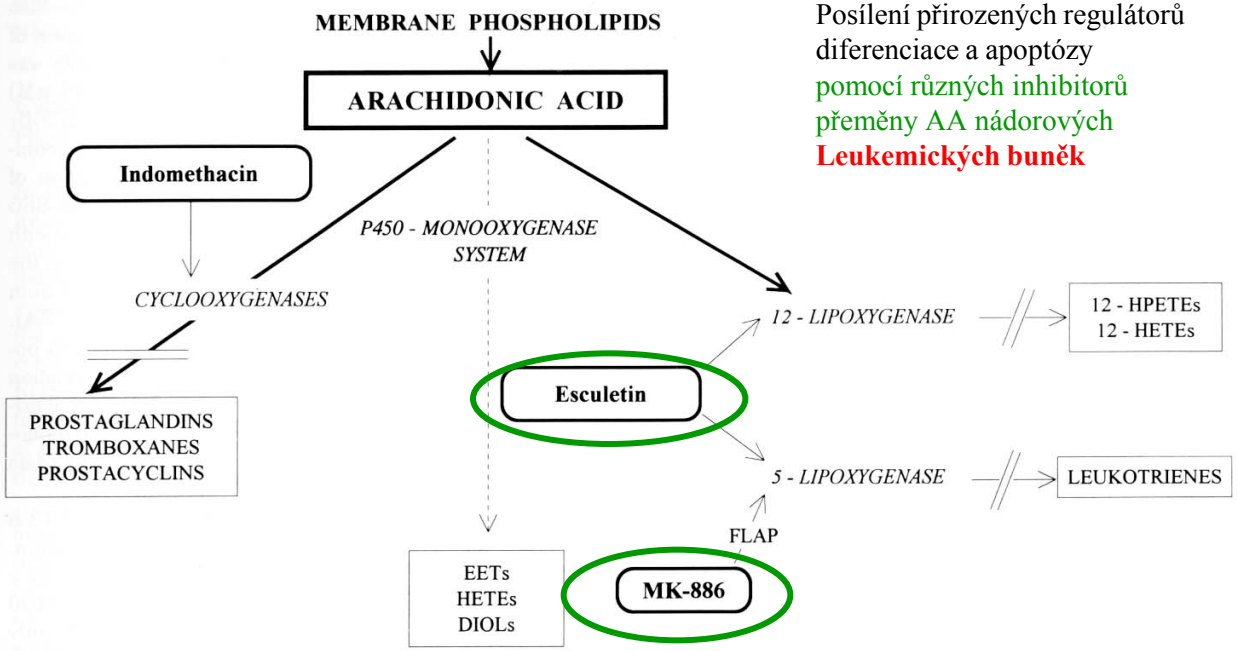
240 Journal of Leukocyte Biology Volume 62, August 1997

5-Lipoxygenase inhibitors potentiate effects of TGF-β_1 on the differentiation of human leukemia HL-60 cells

Alois Kozubík,* Jiřina Hofmanová,* Ladislav Dušek,† and Eva Musilová*

*Institute of Biophysics, Academy of Sciences of the Czech Republic; and †Department of Environmental Studies, Masaryk University, Brno, Czech Republic

Příklad využití inhibitorů biosyntézy eikosanoidů *in vitro*



Posílení přirozených regulátorů diferenciace a apoptózy pomocí různých inhibitorů přeměny AA nádorových **Leukemických buněk**

Fig. 1. Formation of arachidonic acid metabolites by lipoxygenases, cyclooxygenases, and P450-monooxygenase system and mechanism of action of specific inhibitors of selected metabolic pathways. HPETEs, hydroperoxy acids; HETEs, monohydroxy acids; EETs, epoxy-eicosatrienoic acids; FLAP, 5-lipoxygenase-activating protein.

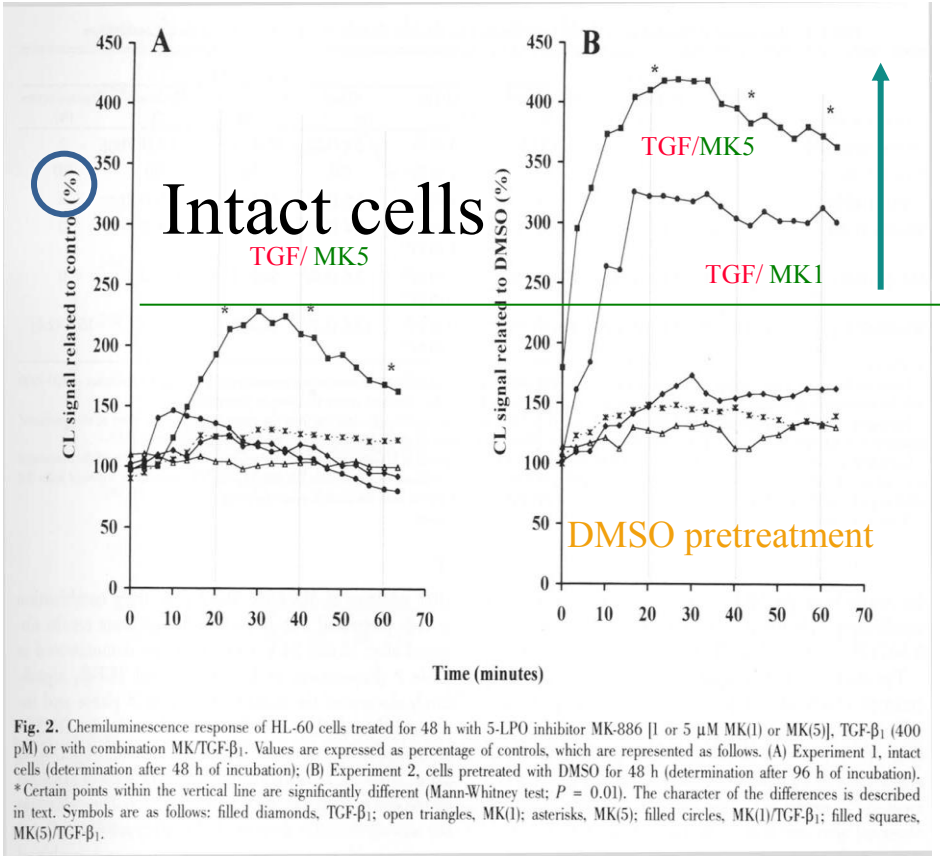


Fig. 2. Chemiluminescence response of HL-60 cells treated for 48 h with 5-LPO inhibitor MK-886 [1 or 5 μM MK(1) or MK(5)], TGF-β_1 (400 pM) or with combination MK/TGF-β_1. Values are expressed as percentage of controls, which are represented as follows. (A) Experiment 1, intact cells (determination after 48 h of incubation); (B) Experiment 2, cells pretreated with DMSO for 48 h (determination after 96 h of incubation). *Certain points within the vertical line are significantly different (Mann-Whitney test; $P = 0.01$). The character of the differences is described in text. Symbols are as follows: filled diamonds, TGF-β_1; open triangles, MK(1); asterisks, MK(5); filled circles, MK(1)/TGF-β_1; filled squares, MK(5)/TGF-β_1.

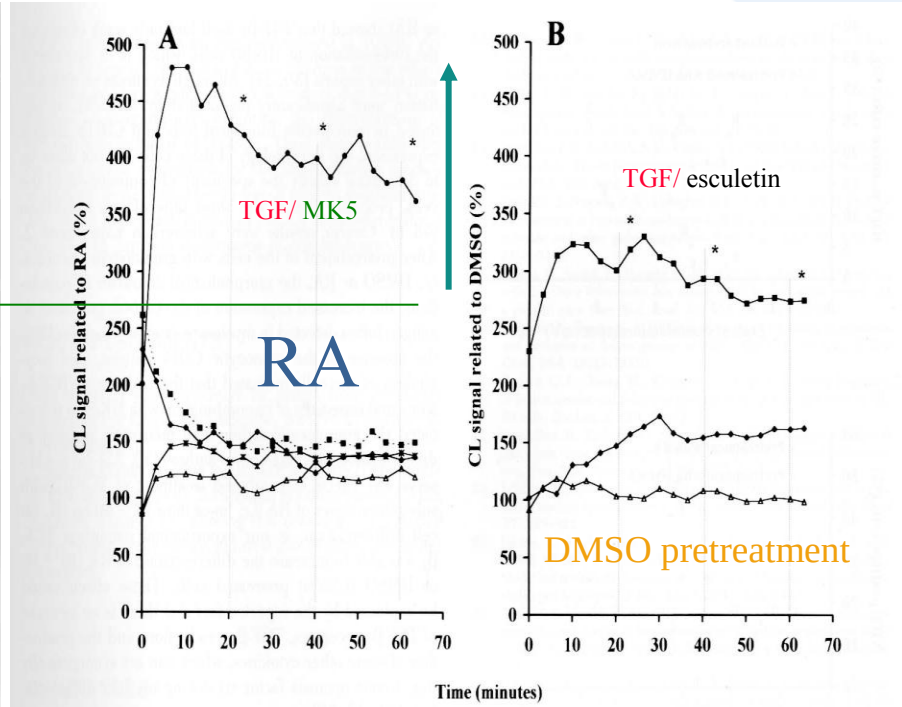


Fig. 3. Chemiluminescence response of HL-60 cells treated for 48 h with 5-LPO inhibitors, i.e. esculetin (12.5 μM), MK-886 [5 μM; MK(5)], TGF-β_1 (400 pM), or with their combinations. Values are expressed as percentage of controls, which are represented by cells pretreated for 48 h with RA (A) or with DMSO (B). All data were obtained after 96 h of incubation. *Certain points within the vertical line are significantly different (Mann-Whitney test; $P = 0.01$). The character of the differences is described in the text. Symbols are as follows: filled diamonds, TGF-β_1; open triangles, esculetin; asterisks, MK(5); filled circles MK(5)/TGF-β_1; filled squares, esculetin/TGF-β_1.

ELSEVIER

European Journal of Pharmacology 350 (1998) 273–284

ejp

Inhibitors of lipoxygenase metabolism exert synergistic effects with retinoic acid on differentiation of human leukemia HL-60 cells

Jiřina Hofmanová [a,*], Alois Kozubík [a], Ladislav Dušek [b], Jiří Pacherník [a]

[a] *Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, CZ-612 65 Brno, Czech Republic*
[b] *Department of Environmental Chemistry and Ecotoxicology, Faculty of Sciences, Masaryk University, Kotlářská 2, CZ-11 37 Brno, Czech Republic*

Received 24 November 1997; revised 25 March 1998; accepted 31 March 1998

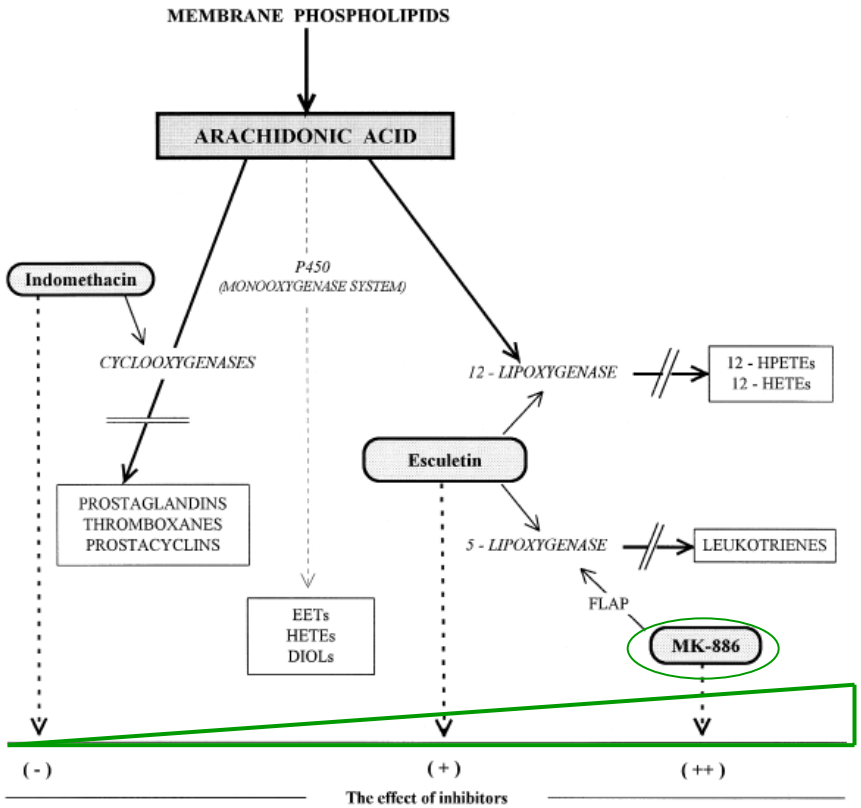
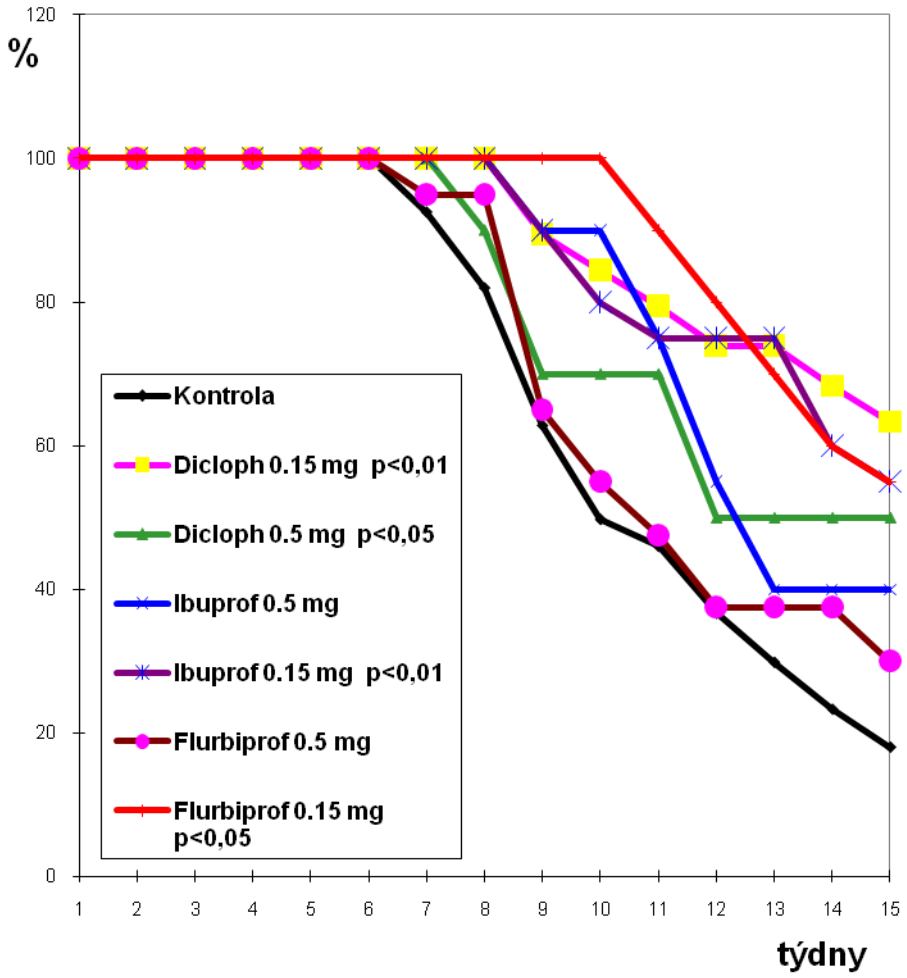
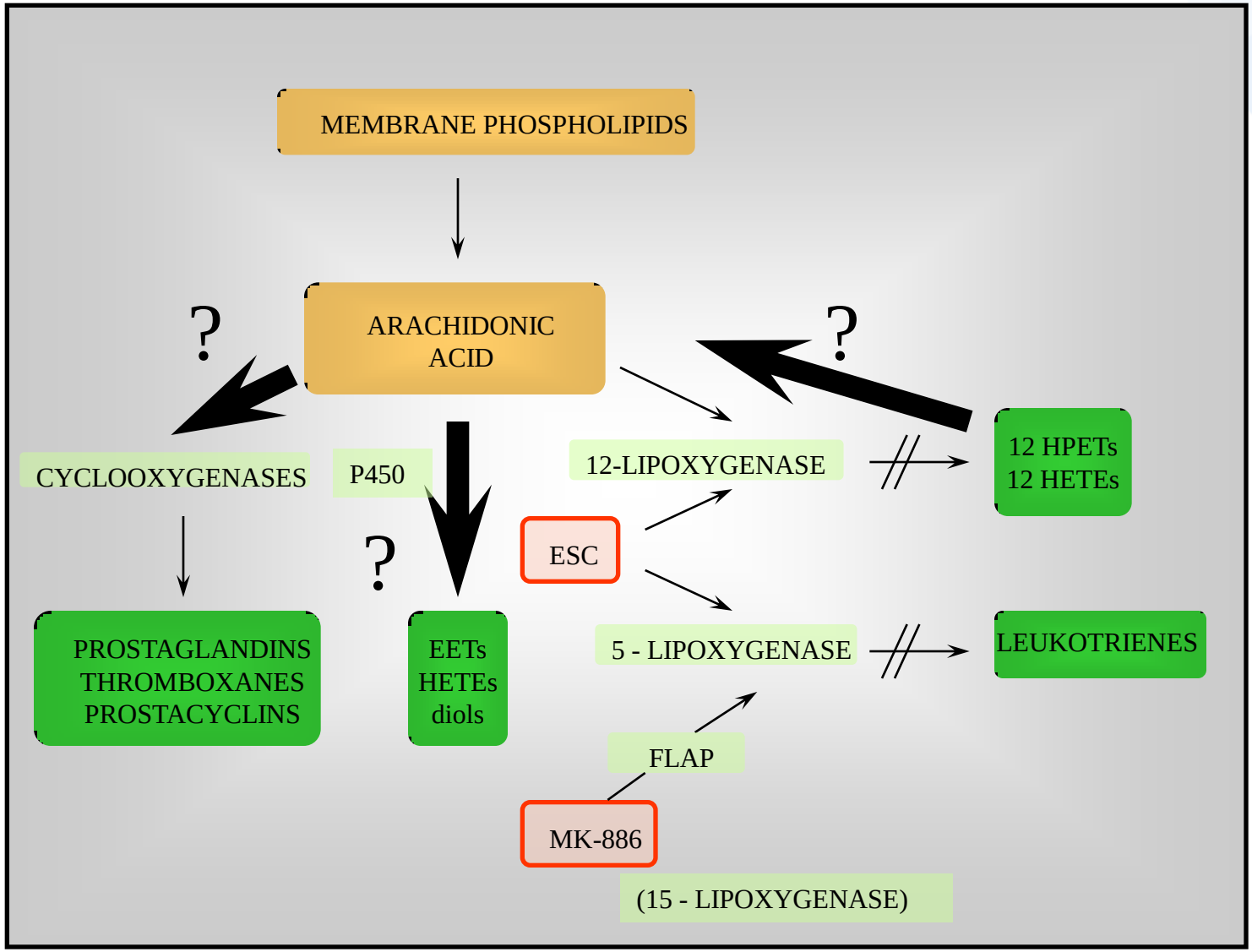


Fig. 1. Formation of arachidonic acid metabolites by lipoxygenases, cyclooxygenases and the P450-monooxygenase system and the mechanism of action of specific inhibitors of selected metabolic pathways. The effects of inhibitors on HL-60 cell differentiation induced by retinoic acid or DMSO in the experiments presented are shown schematically under the figure: (−) no effect; (+) the level of potentiation. HPETEs = hydroperoxy acids; HETEs = monohydroxy acids; EETs = epoxy-eicosatrienoic acids; FLAP = 5-lipoxygenase activating protein.

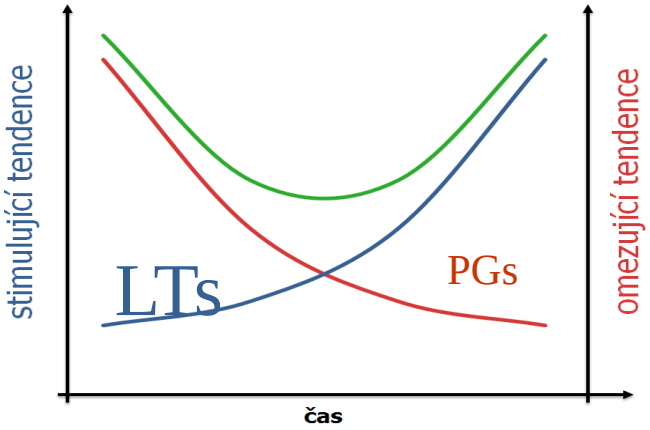
Příklad působení NSAIDs na nádorové buňky *in vivo*



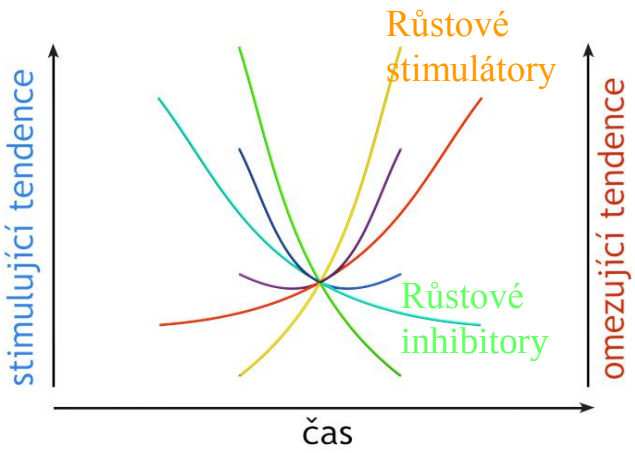
PŘEŽÍVÁNÍ ZVÍŘAT s nádorem (G:5:113) PO TERAPII S INHIBITORY CYKLOOXYGENÁZ

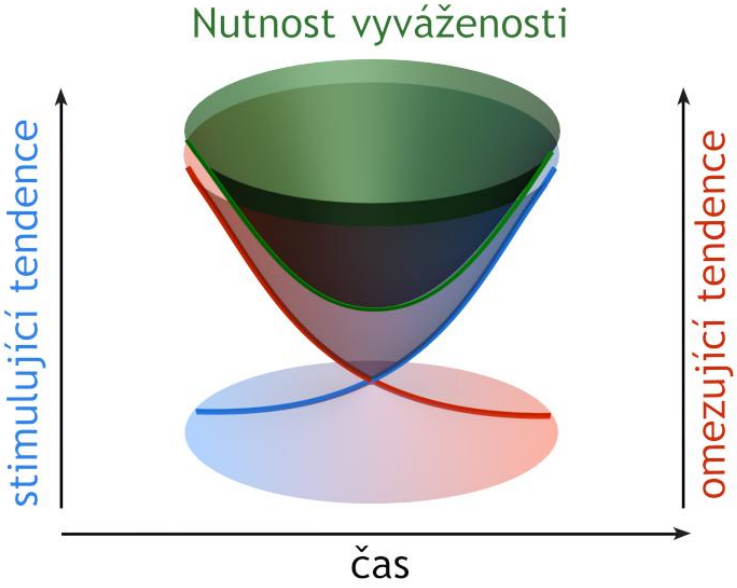


Homeostáza jako výsledek mnohačetných zpětných vazeb a protichůdně působících regulátorů a tendencí.



Významnou úlohu zde hrají lipidové složky výživy - VNMK a jejich metabolity





„Ideální stav“