Sodium salicylate modulation of urokinase plasminogen activation system in breast cancer cells

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Introduction

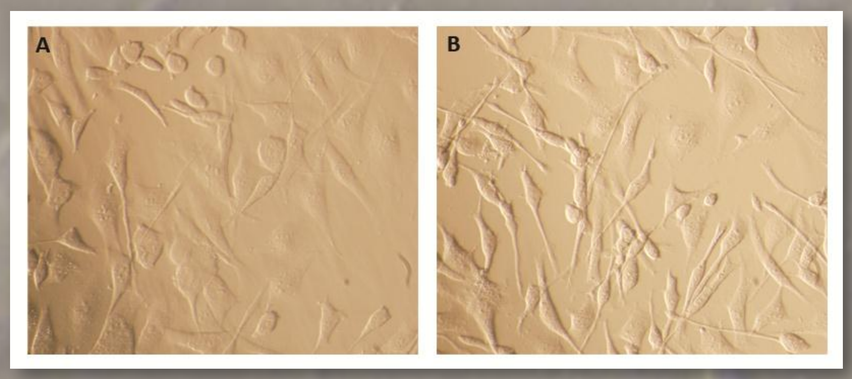
Urokinase plasminogen activation system is a precisely regulated system important for controlled proteolysis of extracellular matrix. It is involved in various physiological and developmental processes, as well as in invasive growth of tumors and metastasis[1]. The system consists of extracellular protease, urokinase plasminogen activator (uPA), its inhibitors PAI-1 and PAI-2 and specific cell receptor, uPAR, involved in localized proteolysis.

Aim

- To investigate the effect of sodium salicylate (NaS) on the plasminogen activation system.

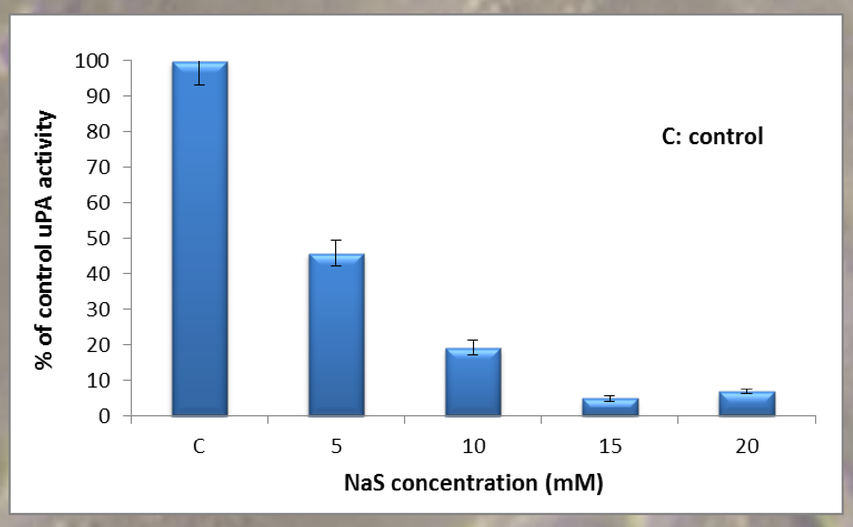
Materials and methods

- MDA MB-231 human breast cancer cells produce high levels of uPA.
- Sodium salicylate (NaS) is a derivative of acetylsalicylic acid which acts as non-steroidal anti-inflammatory drug (NSAID) due to its ability to inhibit cyclooxygenases. Sodium salicylate has been shown to modulate the COX-independent biological responses predominately through alternations on the activity of kinases involved in different signaling pathways, such as MAP kinases and IkB[2].
- Urokinase activity was assesed by radial caseinolysis, plasminogen dependent caseinolytic reaction, and its value estimated by comparison with calibration curve of human urokinase and normalized according to protein concentration



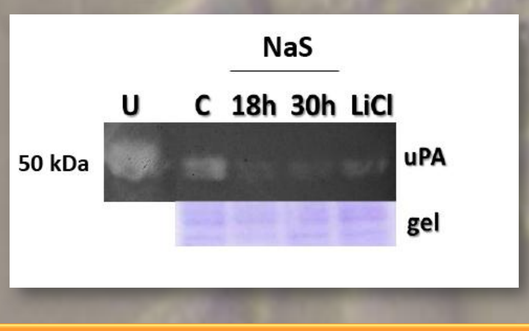
Effect of sodium salicylate on MDA MB-231 cell morphology

A: control cells; **B:** cells treated with 15 mM of NaS for 24 hours.
MDA MB-231 cells exhibit epithelial like morphology. We observed cell morphology changes upon treatment with 15 mM sodium salicylate for 24 h. The degree of cell morphology changes increased with the increase in sodium salicylate concentration and treatment period (data not shown).



Sodium salicylate inhibited basal uPA activity in dose-dependent manner

Cells were treated with sodium salicylate for 24 h, followed by 6-hour incubation with sodium salicylate in serum-free medium. Conditioned medium was analyzed by radial caseinolysis to assess uPA activity. uPA activity was inhibited proportionally with sodium salicylate concentration treatment until the maximum inhibition was reached at 15 mM.



uPA zymography from sodium salicylate-treated cells

Conditioned serum free media from control and treated cells were collected, concentrated 14x and analyzed by zymography. Treatment with LiCl was used as a positive control for uPA activity inhibition. After electrophoresis under non-denaturing conditions, the gel was laid on agarose plate containing plasminogen and casein as substrates.
Gel: corresponding protein extracts were stained with Coomassie Brilliant Blue.
U: commercial urokinase (10^{-2} U), C: control, NaS: 15 mM NaS, LiCl: 20 mM LiCl.

Zymography (electrophoretic caseinolysis)[3] demonstrated that casein degradation was uPA-dependent.

References

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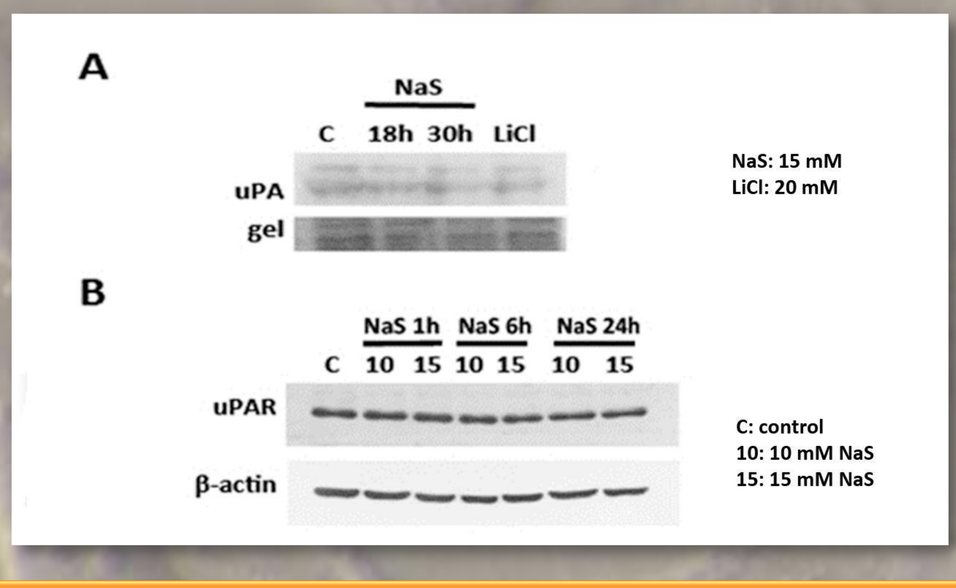
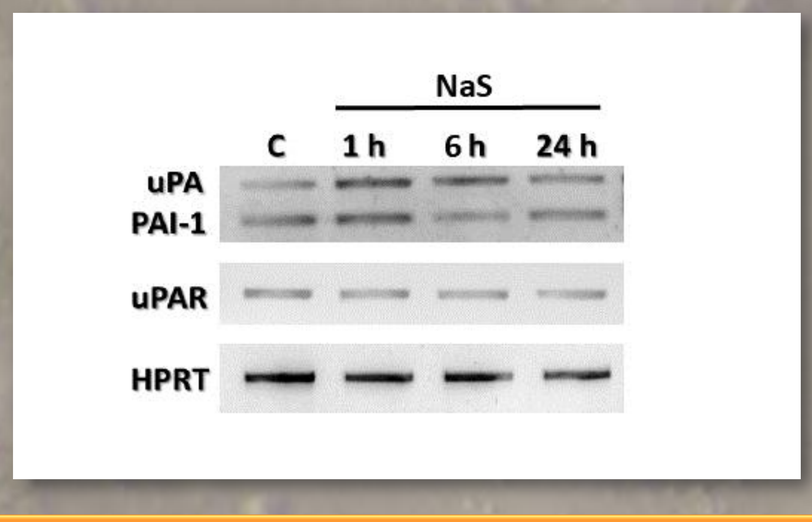


Expression of uPA and uPAR proteins following sodium salicylate treatment

A: Western blot analysis of uPA proteins present in conditioned media from control cells and cells treated with sodium salicylate and LiCl. Media were concentrated 14x. Coomasie stained gel presents equal amounts of cell lysates for quantification.
B: Western blot analysis of uPA receptor (uPAR) in cell extracts obtained from control and treated cells. β-actin was used as a loading control.

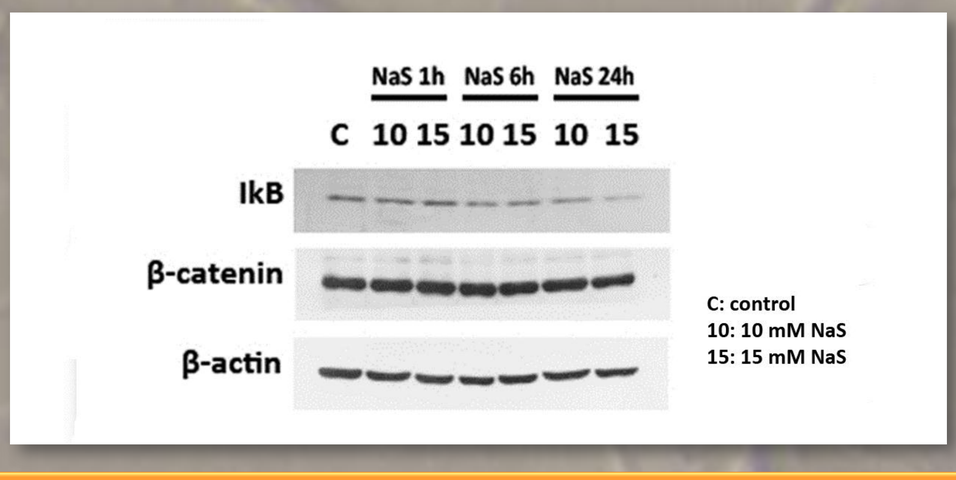
There were no significant differences in uPA and uPAR protein expression in control and treated cells.



Expression of uPA, PAI-1 and uPAR mRNA in cells after sodium salicylate treatment

mRNA expression of uPA system elements was analyzed in cells treated with sodium salicylate by RT-PCR. Housekeeping HPRT gene was used for quantification.
C: control, NaS: 15 mM NaS.

The relative ratio of uPA and PAI-1 did not show significant changes in the samples treated with sodium salicylate in comparison with control. Also, uPAR expression appeared unaffected by the treatment.



Effect of sodium salicylate on signaling pathways

As sodium salicylate was shown to influence several signaling pathways, Western blot analysis of IkB and β-catenin expression in cell extracts obtained from control and treated cells was done.

While the expression of β-catenin, involved in PI3K pathways, remained unchanged, a decrease in IkB activity was observed with maximum IkB inhibition after 15 mM sodium salicylate treatment for 24h. This IkB decrease indicates an increase in basal NF-kB activity.

Conclusions

- Sodium salicylate treatment caused MDA MB-231 cell morphology changes
- MDA MB-231 cell treatment with sodium salicylate was shown to decrease urokinase activity in a concentration and time dependent manner.
- As the level of uPA and PAI mRNA and uPA and uPAR protein level did not show significant changes during sodium salicylate treatment, we suppose the cause of uPA activity inhibition could be the presence of some kind of extracellular inhibition.

Acknowledgement
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