Effect of retinoic acid on asbestos induced plasminogen activator activity of peritoneal macrophages

D LISON, B KNOOPS, R LAUWERYS

From the Unité de Toxicologie Industrielle, Faculté de Médecine, Université Catholique de Louvain, 1200 Brussels, Belgium

On the basis of epidemiological, morphological, and biochemical evidence, it has been proposed that asbestos acts as a tumour promotor. Different phenomena are associated with tumour promotion, including production of reactive oxygen species and induction of various enzymatic activities such as ornithine decarboxylase and plasminogen activator (PA). Asbestos, like the classic tumour promoting agent, phorbol 12-myristate 13-acetate (PMA), stimulates the release of active oxygen species and stimulates plasminogen activation (PA) activity in macrophages both in vitro and in vivo.

Retinoids are potent inhibitors of the tumour-promoting effects of PMA in different cellular systems (for a review see ref 4). Retinoic acid inhibits the production of reactive oxygen metabolites in human leukocytes stimulated by PMA and inhibits phorbol ester induced mouse epidermal ornithine decarboxylase. We have also observed (unpublished results) that retinoic acid prevents the in vitro PMA induction of membrane bound PA activity in macrophages. We have therefore investigated whether retinoic acid also modulates the induction of PA in mouse peritoneal macrophages stimulated by asbestos in vitro.

Materials and methods

The influence of retinoic acid (10⁻⁴M) was tested under three different conditions: (a) before chrysotile stimulation (this incubation was performed during 15 minutes in BSS), (b) during the chrysotile stimulation (2 h in DMEM + 0·1% lactalbumin), and (c) after stimulation (retinoic acid was added to the substrate medium).

Measure of PA activity
After removing the stimulation medium and thorough washing, the cells were incubated for three to six hours in a medium containing S-2251 (0·3 mM) and human plasminogen (0·165 CU/ml) in BSS. Induced PA activity was assayed by measuring the amount of p-nitroaniline released in the medium (405 nm). Each assay was run in triplicate with a plasminogen free blank.

Statistical analysis
Statistical analysis was performed using analysis of variance and Scheffe’s multiple comparisons test.
**Results and discussion**

In vitro treatment of mouse peritoneal macrophages by asbestos stimulates the expression of plasma membrane bound PA activity. In our system exposure of the macrophages to chrysotile for two hours stimulates the PA activity by a factor of 1.5. The influence of retinoic acid on this stimulation is summarised in the table. Preincubation of the macrophages during 15 minutes with retinoic acid (10^{-6} M) before exposure to chrysotile significantly depresses the induction of PA activity. This inhibition cannot be reversed by washing the cells before stimulation. Coincubation of the macrophages with retinoic acid and asbestos also prevents the induction of PA activity. Preincubation is slightly more efficient than coincubation with the inducer; binding of retinoic acid to the fibres may explain this observation. Retinoic acid alone incubated for 15 minutes with macrophages also slightly depresses the PA activity (about 70% of the control value). By contrast, addition of retinoic acid after the cells have been stimulated by chrysotile has no effect on the induced PA activity. Likewise, incubation of the purified enzyme during 30 minutes with retinoic acid (10^{-5} M) before addition of substrate does not affect its activity (data not shown). The inhibitory effect of retinoic acid on the induction of PA is independent of a global cytotoxic action; monitoring LDH release shows no cytotoxic effect of retinoic acid on macrophages up to 10^{-5} M (data not shown).

These results indicate that a low concentration of retinoic acid inhibits the basal and the asbestos induced turnover of PA in macrophages but has no direct effect on the preformed enzyme; this inhibition occurs rapidly and is irreversible. Induction of PA activity in macrophages and other cell types is recognised as an important change accompanying tumour promotion and transformation of cultured cells.

Inhibition of the induction of PA in macrophages may thus partly explain the antipromoting effect of retinoids.

The present results justify further studies on the chemopreventive role of retinoids, not only in asbestos induced neoplasias but also in asbestos induced lung fibrosis, since macrophages seem also to play a prominent part in the initiation of this lung reaction.

**References**

Effect of retinoic acid on asbestos induced plasminogen activator activity of peritoneal macrophages.
D Lison, B Knoops and R Lauwerys

doi: 10.1136/oem.46.7.496

Updated information and services can be found at:
http://oem.bmj.com/content/46/7/496.citation

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/