NOTE BREVII

CYTOTOXIC EFFECTS OF FURAZOLIDONE ON HEp–2 CELL LINE

A. STAMMATI, F. ZAMPAGLIONI and A. MACRI

Laboratorio di Tossicologia Comparata ed Ecotossicologia, Istituto Superiore di Sanità, Roma

Summary. — The effects of Furazolidone (N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone) on cell growth and colony-forming ability of a continuous cell line (HEp–2) have been studied. Almost complete death of cell culture was observed at the concentration of 50 μg/ml Furazolidone, while 0.5 μg/ml treated cells did not show any effect on viability and cell growth. In the presence of 5 μg/ml Furazolidone, cells exhibited a dose- and time-related growth inhibition. This effect was partially reduced by removing the drug from the cultures. The colony-forming ability of treated cells was almost completely inhibited.

Riassunto (Effetti citotossici del Furazolidone sulla linea cellulare HEp–2). — Sono stati studiati gli effetti del Furazolidone (N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone) sulla crescita e la capacità di formare colonie di una linea cellulare (HEp–2). Alla concentrazione di 50 μg/ml il Furazolidone ha causato la morte quasi totale delle cellule, mentre le colture trattate con 0,5 μg/ml non hanno mostrato alcun effetto avverso sulla vitalità e sulla crescita. Nelle cellule trattate con 5 μg/ml di Furazolidone è stata osservata un’induzione della crescita dose- e tempo-dipendente. Questo effetto veniva parzialmente ridotto se si allontanava il farmaco dalle colture. La capacità di formare colonie delle cellule trattate è stata inibita quasi completamente.

Materials and methods

Cell Culture. — The HEp–2, an epithelial cell line derived from human carcinoma of the larynx, was used. Cells were routinely cultured in monolayer at 37 °C in 5% CO2 in air, in disposable plastic flasks (Falcon) containing Minimum Essential Medium supplemented with Earle salts, 5% fetal bovine serum (Flow Laboratories), 4mM glutamine, 0.2% sodium bicarbonate, 100 U.I./ml penicillin and 100 μg/ml streptomycin.

Furazolidone. — The nitrofuran was furnished by F.L.S. in Vicenza (Italy) with a purity grade of 99.52%. For the cytotoxicity tests Furazolidone was dissolved in DMSO (0.5% v/v) and serial dilutions from this solution were made.

Cell Growth. — Cells were seeded in Falcon dishes (35 mm) at a density of 5x10⁴ cells/dish, in a medium containing 50, 5, 0.5 and 0 μg/ml Furazolidone; the cultures were incubated at 37 °C in 5% CO2 in air and daily observed at light microscopy. On the 3rd and 6th...
Fig. 1. – Effect of 50 μg/ml Furazolidone on HEP-2 cell line after 24 hrs treatment x 100. a) Control culture. b) Treated cultures.

Fig. 2. – Effect of 5 μg/ml Furazolidone on the colony-forming ability of HEP-2 cell line after 8 days treatment x 100. a) Control cultures. b) Treated cultures.
day of culture, two dishes of each concentration were treated for protein determination by Lowry method [8].

Reversibility. – In order to observe a possible reversibility of the adverse effects of Furazolidone, the medium of some dishes containing the nitrofuran was removed on the 3rd day of treatment and substituted with control medium while to other dishes fresh medium still containing Furazolidone was added; the same procedure was carried out for the control cells. On the 6th day of culture, the cells of all dishes were treated for the protein determination by Lowry method.

Colony-forming ability. – The colony-forming ability of the cells treated with 5 μg/ml Furazolidone was almost completely inhibited (94.7%) as compared to the control cultures and the size of the observed colonies was also reduced (Fig. 2).

Conclusions

The deleterious action of Furazolidone, already known in vivo on laboratory animals and man, has been confirmed in vitro on HEp-2 cell line by the observation of a reduced cell growth. This adverse effect, which is clearly dose- and time-related and has shown to be partially reduced when the drug is removed from the cells, is probably due to death of the cells exposed to the nitrofuran, rather than to growth inhibition. This hypothesis is supported by the results of the colony-forming ability of the treated cells. In fact, after 8 days continuous exposure, there are very few surviving cells able to produce colonies, thus demonstrating that Furazolidone probably does not interfere with some mechanisms of cell division, but causes cell death during the contact with the cells, according to its time-dependent action; obviously the ratio cell number/ Furazolidone concentration will be critical. The results of the reversibility test could be interpreted in the same way and indicate that the effect is not reversible, being due to cell death. In fact, the apparent and partial reversibility of the cell damage observed when Furazolidone is removed from the cultures can be explained as the cells not affected by the drug can normally replicate.

The in vitro system used in this work has shown to be sensitive to the presence of low concentrations of Furazolidone and consequently seems to be promising for the study of the mechanism of action of this drug, which is not yet known in vivo and for the screening of other related compounds employed as feed additives. Moreover, it is known that 5-nitrofurans undergo metabolic modification in vivo [1], thus missing some of their biological activities. In particular, Furazolidone looses its mutagenicity when given to rats by gavage [11]. Therefore in vitro systems could be advantageous if the parameters used to reveal cytotoxicity are able to show the toxic effects of residues present in tissues or products of treated animals.

Results

Cell growth. – In the presence of 50 μg/ml Furazolidone all the cells were severely damaged after 24 hrs treatment and disintegrated with the formation of cellular debris and granular material (Fig. 1), while the 0.5 μg/ml treated cells did not show any alteration in viability and cell growth (evaluated as total protein content), compared to the controls, either in the 3rd or in the 6th day of culture (Tab. 1). In the cells treated with 5 μg/ml Furazolidone a growth inhibition was observed, compared to the controls (Tab. 1).

Table 1. – Effect of Furazolidone on cell growth. Growth inhibition (%) 

<table>
<thead>
<tr>
<th>Furazolidone</th>
<th>3rd day of culture</th>
<th>6th day of culture</th>
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</thead>
<tbody>
<tr>
<td>50 μg/ml</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td>5 μg/ml</td>
<td>32.5 ± 4.2</td>
<td>70.6 ± 2.9</td>
</tr>
<tr>
<td>0.5 μg/ml</td>
<td>0</td>
<td>0</td>
</tr>
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a = each value is the mean of 6 experiments. 
b = each value is the mean of 3 experiments. 
c = medium changed on the 3rd day of culture with normal medium. 
d = medium changed on the 3rd day of culture with fresh medium containing 5 μg/ml Furazolidone.

Reversibility. – The test was carried out with the concentration of 5 μg/ml Furazolidone and the treated cells, whose medium was changed with control medium after three days treatment, showed a reduced growth inhibition in respect to the cells exposed for 6 days (Tab. 1).

Colony-forming ability. – The colony-forming ability of the cells treated with 5 μg/ml Furazolidone was almost completely inhibited (94.7%) as compared to the control cultures and the size of the observed colonies was also reduced (Fig. 2).

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REFERENCES


