The Furocoumarin Photosensitizing Effect on the Virus-producing Graffi Leukaemia Cells

F. Bordin and F. Baccichetti

Istituto di Chimica Farmaceutica dell'Università, Centro di Studio sulla Chimica del Farmaco e dei Prodotti Biologicamente Attivi del Consiglio Nazionale delle Ricerche, Padova

(Z. Naturforsch. **29 c**, 630–632 [1974]; received March 29/May 28, 1974)

Graffi Virus Leukaemia, Photosensitizing Furocoumarins

The irradiation at 365 nm in the presence of the skin-photosensitizing furocoumarins of Graffi leukaemia cells, producers of an oncogenic RNA virus, determines the arrest of cell duplication and of the synthesis of the nucleic acids; the virus, on the other hand, remains equally oncogenic for newborn mice and still capable of reproduction.

We have previously reported [1] that cells of the Graffi leukaemia, an experimental tumor of the mouse, irradiated with long wave UV light in the presence of skin-photosensitizing furocoumarins (psoralens), lose their tumor-transmitting capacity when transplanted into adult mice. This result can be explained by the C_4-photocycloaddition that these drugs yield to the pyrimidine bases of DNA and RNA [2–5], with formation of covalent linkages; in addition, in the double-stranded DNA inter-strand cross-linkages occur [6]. Therefore, it may be foreseen that this photochemical interaction might interfere strongly with the macromolecular synthesis as previous observed in other cell lines [7–9].

These cells contain and produce the Graffi virus, an oncogenic RNA tumor virus [10]; actually, the leukaemia is also transferable by injecting cell-free filtrates into newborn mice [11]. Moreover, while some aspects only of the furocoumarin photosensibilization on a living cell are still not yet clear, the effects on the Graffi virus properties as well on the virus-cell interactions, *i. e.* the infecting and transforming ability or the capacity of the cell to produce new viruses, are entirely unknown.

Thus, in this paper we have studied the furocoumarin photosensitizing effect on the Graffi virus-cell system, using psoralen, the parent compound of natural origin, and 8-methylpsoralen and 4,4′,8-trimethylpsoralen, two synthetic products, all three drugs with strong photosensitizing activity.

Results and Discussion

At first, the Graffi leukaemia cells, in balanced saline solution ($2 \cdot 10^6$ cells/100 μl) were irradiated on crushed ice with a Philips HPW 125 lamp (365 nm; intensity of irradiation $2.02 \cdot 10^{16}$ quanta/sec) as already described [1], and then injected subcutaneously into adult and newborn C_{57}BL/6 mice ($2 \cdot 10^7$ cells for adult, $2 \cdot 10^6$ for newborn animals).

As shown in Table I, in none of the adult mice is mortality noted; it is clear that the capacity to transmit leukaemia by transplantation has been lost and that cell replication is arrested. Newborn mice injected in the same way develop leukaemia but with latent periods of considerable lenght; this result is significant, because leukaemia induction by cell transplantation requires only a few days, while by virus several weeks are necessary [11]. Therefore, these animals develop leukaemia as if they have been injected with the virus and not with whole cells *.

This picture can be explained assuming that cell replication is stopped in consequence of photobinding of furocoumarin to cellular nucleic acids, while the Graffi virus is, on the contrary, unaffected.

To prove this assumption we have performed the following experiments.

The Graffi leukaemia cells were irradiated in the presence of furocoumarins, as above, washed with Hank's solution and then incubated at 37 °C in the same medium ($3.5 \cdot 10^6$ cells/100 μl) containing 3 μCi/ml of [^{3}H]thymidine or [^{3}H]uridine (specific activity 5 Ci/mmol and 4.6 Ci/mmol respective-

Requests for reprints should be sent to Dr. F. Bordin, Istituto di Chimica Farmaceutica dell'Università — Centro di Studio Via Marzolo 5, *35100 Padova*-Italy.

* That no leukaemias were obtained in adult mice is not surprising in view of the fact that the Graffi virus is known to be incapable of inducing the disease in adult mice [11].

Table I. Incidence of leukaemia in $C_{57}BL/6$ mice after transplantation of Graffi leukaemia cells ($2 \cdot 10^6$ for newborn, $2 \cdot 10^7$ for adult mice) irradiated in the presence of furocoumarins. The latent period correponds to the age of the mice at death. The adult mice were observed up to 60 days.

Furocoumarin	Concentration [μg/ml]	Irradiation time [min]	Mice age [days]	Leukaemia incidence [leukaemic/injected mice]	Laten period [weeks]	
					Median	Range
None (controls)	0	0	60–90	10/10	1.4	1–1.6
		30	60–90	10/10	1.4	1.1–1.6
Psoralen	15	0	60–90	10/10	1.35	1–1.5
		30	60–90	0/20	—	—
		30	1	13/13	17	11–26
8-Methylpsoralen	10	30	60–90	0/20	—	—
		30	1	4/4	18	15–24
4,4′,8-Trimethyl-psoralen	1	32	60–90	0/30	—	—
		16	60–90	0/10	—	—
		8	60–90	0/10	—	—
		30	1	16/16	16	12–23

ly, from the Radiochemical Centre, Amersham, England).

After 30 min, DNA and RNA were extracted with 10% NaCl solution and counted with a Beckman LS-150 liquid scintillation spectrometer as already described [8]. The nucleic acids content was determined by the diphenilamine [12] or orcinol reactions [13].

Table II. DNA and RNA synthesis in Graffi leukaemia cells after long wave UV irradiation (30 min) in the presence of furocoumarins. The irradiated cells were incubated in the presence of [^{3}H]thymidine or [^{3}H]uridine, and then the nucleic acids were extracted and their specific activity determined.

Furocoumarin	Concentration [μg/ml]	[^{3}H]DNA [cpm/μg DNA]	[^{3}H]DNA [percent of controls]	[^{3}H]RNA [cpm/μg RNA]	[^{3}H]RNA [percent of controls]
None, controls	—	710	—	500	—
Psoralen	15	85	12	85	17
8-Methyl-psoralen	10	38	5	18	4
4,4′-8-Trimethyl-psoralen	1	115	16	135	27

The results of these experiments (Table II) show clearly that DNA and RNA synthesis is almost completely abolished; the [^{3}H]nucleoside incorporation is so much depressed that the radioactivity values are at times so low as to be distinguishable with difficulty from the background.

To ascertain if the Graffi virus is really unaffected by furocoumarin activity we prepared cell-free filtrates according to Fiore-Donati and Chieco-Bianchi [11]; these fluids, with the added furocoumarin, were irradiated at 365 nm in the usual way and then injected into newborn $C_{57}BL/6$ mice. In these experiments care has been taken so that every mouse was always injected with the extract of $2 \cdot 10^6$ starting cells. As shown in Table III, the incidence of leukaemia as well as the latent periods are the same as observed in the untreated controls, for all the drugs tested.

These results show clearly that even after furocoumarin photosensitization the Graffi virus infects and transforms cells.

We remember that the irradiation of certain other RNA tumor viruses such as various strains of Rous sarcoma virus or avian sarcoma virus, by short wave UV light [14] or by gamma rays [15], yields two types of mutants: Oncogenic viruses, which transform cells but are incapable of reproduction and viruses which have lost their transforming and oncogenic capacity but are still able to replicate. This fact derives from the loss of certain viral genetic functions, very probably as a consequence of damage to the viral RNA. Therefore, it is possible that the Graffi virus after furocoumarin photosensibilization can transform cells but is incapable of reproduction, *i. e.*, the transformed cells are unable to produce new oncogenic viruses.

To deal with this problem, the leukaemic cells obtained from experiment number 2 of Table III, *i. e.*, the leukaemias induced by cell-free filtrates irradiated with psoralen, were transplanted into adult

Experiment No.	Furocoumarin	Concentration [μg/ml]	Irradiation time [min]	Leukaemia incidence [leukaemic/injected mice]	Latent period [weeks] Median	Range
1	None (controls)	0	0	18/19	17	10–27
			30	4/4	14	13–18
2	Psoralen	15	0	4/4	15	12–20
			30	8/8	16	14–20
3	8-Methylpsoralen	10	30	8/8	15	13–19
4	4,4′,8-Trimethylpsoralen	1	30	12/14	21	14–30
5	* None	0	0	11/12	16	15–23

Table III. Incidence of leukaemia in $C_{57}BL/6$ mice after injection at birth of Graffi leukaemia cell-free filtrates irradiated in the presence of furocoumarins. The latent period corresponds to age of the mice at death.

* Extracts prepared using cells of leukaemias induced in newborn mice by cell-free filtrates irradiated with psoralen (experiment No. 2).

$C_{57}BL/6$ mice; after the 10th transplant the resulting cells, were used for the preparation of cell-free filtrates that were injected without other treatments into newborn animals (experiment number 5 of Table III).

These extracts were equally oncogenic and therefore the starting cells contain and produce an oncogenic virus.

All these results show that while cell replication is stopped by furocoumarin action, the properties of the Graffi virus remain unchanged and it can infect cells and transform them into leukaemic and virus-producing cells.

Experiments of furocoumarin photosensibilization performed previously with certain non-oncogenic DNA and RNA viruses showed that the former were inactivated while the latter were very resistant [16].

The explanation of these results is still not clear because the photosensitizing furocoumarins are capable of photoreacting with both DNA and RNA *in vitro* [17].

We are now investigating the reason of this failed inactivation and in particular whether the furocoumarins are able to photoreact with Graffi virus RNA, whether such damage is insufficient to interfere with the functions of viral genome, or wether on the other hand the furocoumarins are unable to run through the virion envelope and therefore reach the viral RNA.

We believe that another very interesting question is whether the furocoumarins, by their selective action, are useful in the study of the molecular activity of an RNA tumor virus in a transformed cell.

We thank Prof. Luigi Musajo for his helpful discussions and suggestions.

[1] L. Musajo and F. Baccichetti, Europ. J. Cancer **8**, 397 [1972].
[2] C. H. Krauch, D. M. Krämer, and A. Wacker, Photochem. Photobiol. **6**, 341 [1967].
[3] L. Musajo, F. Bordin, G. Caporale, S. Marciani, and G. Rigatti, Photochem. Photobiol. **6**, 711 [1967].
[4] L. Musajo, F. Bordin, and R. Bevilacqua, Photochem. Photobiol. **6**, 927 [1967].
[5] L. Musajo, G. Rodighiero, F. Dall'Acqua, F. Bordin, S. Marciani, and R. Bevilacqua, Rend. Accad. Naz. Lincei **42**, 457 [1967].
[6] F. Dall'Acqua, S. Marciani, L. Ciavatta, and G. Rodighiero, Z. Naturforsch. **26 b**, 561 [1971].
[7] J. E. Trosko and M. Isoun, Int. J. Radiat. Biol. **19**, 87 [1971].
[8] F. Bordin, F. Baccichetti, and L. Musajo, Experientia **28**, 148 [1972].
[9] F. Bordin, F. Baccichetti, R. Bevilacqua, and L. Musajo, Experientia **29**, 272 [1973].
[10] M. A. Rich and R. Siegler, Ann. Rev. Microbiology **21**, 529 [1967].
[11] L. Fiore-Donati and L. Chieco-Bianchi, J. Nat. Cancer Inst. **32**, 1083 [1964].
[12] K. Burton, Biochem. J. **62**, 315 [1956].
[13] H. N. Munro and A. Fleck, Analyst **91**, 78 [1966].
[14] K. Toyoshima, R. R. Friis, and P. K. Vogt, Virology **42**, 163 [1970].
[15] A. Golde, Virology **40**, 1022 [1970].
[16] L. Musajo, G. Rodighiero, G. Colombo, V. Torlone, and F. Dall'Acqua, Experientia **21**, 22 [1965].
[17] G. Rodighiero, L. Musajo, F. Dall'Acqua, S. Marciani, G. Caporale, and L. Ciavatta, Biochim. Biophys. Acta **217**, 40 [1970].