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Cell fusion
heterokaryon
pederine

Cell Fusion Induced by Pederine

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Extract

Pederine, a natural product extracted from beetles, induces cell fusion among human skin fibroblasts grown in tissue culture. Heterokaryons are produced when pederine is added to mixtures of human diploid fibroblasts and HeLa cells. The efficiency of cell fusion exceeds that achieved with other available agents. The technique is simple and the results are reproducible. Cells exposed to pederine under conditions that cause fusion retain their growth potential, which indicates that the treatment does not damage the cells. The technique should prove useful in research into mechanisms of membrane fusion, as well as research in which cell fusion is used as an investigative tool.

Speculation

Lysolecithin is believed to induce cell fusion by perturbing the molecular structure of cellular membranes. Pederine is more effective at concentrations less than one thousandth that of lysolecithin. The mechanism of pederine-induced cell fusion may provide insight into the physiologic processes which maintain membrane integrity.

Introduction

The experimental induction of cell fusion among cells grown in tissue culture has facilitated studies of the mechanism of membrane fusion as well as research into other fields in which cell fusion is used as an investigative tool (4, 10, 19,22). Unfortunately, the available techniques are cumbersome and the results are erratic. A simple, effective and reproducible method for inducing cell fusion should be of great advantage to investigators in many disciplines.

Pederine is a natural product extracted from the beetle, Paederus fuscipes Curt. With its aid, it is possible to consistently induce cell fusion among 40-60% of human diploid skin fibroblast cells growing in monolayer.

Methods

Pederine induced cell fusion

Crystalline pederine was dissolved in sterile, distilled water and the pH adjusted to 7.0 with sodium bicarbonate. The solution was passed through a millipore filter and stored at -70°C until ready for use.

Human diploid skin fibroblasts or equal mixtures of fibroblasts and HeLa cells were grown to a confluent monolayer in Waymouth's medium supplemented with 15% fetal calf serum and antibiotics (penicillin 50 units, streptomycin 50 µg, kanamycin 30 µg/ml) (3). The medium was decanted and fresh medium containing pederine, 1 nanogram/ml, was added. The cells were incubated for 18 to 24 hrs. The medium was decanted, the cells washed with Puck's Saline A solution, and a suspension formed by brief exposure to 0.04% trypsin and 0.02% EDTA (versene) in Puck's saline A solution (3). The cells were diluted 1:10 in fresh medium and transferred to Leighton tubes containing coverslips, where they were permitted to attach to the glass. The coverslips were removed, washed in Hanks' buffered salt solution, fixed in methyl alcohol and stained with Giemsa. The cells were examined for cytotoxic effects and the extent of cell fusion was determined. Two hundred nuclei were counted and fusion was scored as the percent of nuclei within multinucleated cells.

The scoring method indicates the percent of the original cell population that have fused.

Pederine was prepared from beetles as previously described (1). Waymouth's medium, fetal calf serum and trypsin (1:200) were obtained commercially (30).

Sendai virus induced cell fusion

Sendai virus was cultivated in the allantoic cavity of nine day old embryonated chicken eggs. The virus was harvested by removing the allantoic fluid on the fifth day of incubation. It was then centrifuged at 2000 g and the supernatant decanted into tubes for ultracentrifugation at 16,000g. The pellet was resuspended in phosphate buffered saline and 1% balanced salt solution and centrifuged again. The supernatants were pooled and the live virus was inactivated with β-propiolactone (17). Fusion studies followed the method of Velasquez et al (29). Skin fibroblasts were suspended in nutrient medium supplemented with 10% immunoprecipitin tested fetal calf serum (30) and allowed to grow to confluency. Replicate cultures of confluent monolayers were incubated with the inactivated Sendai virus (250-1250 hemagglutinating units/Leighton tubes) for 18-24 hr. Subculturing, fixing and staining was done as with pederine treated cultures.

Radioautographic studies

HeLa cells were grown in Leighton tubes for 72 hr. in Waymouth's medium containing 0.2 µCi/ml of 3H-thymidine (specific activity 6.7 Ci/mmole). The radioactive medium was decanted and the cells were washed five times with Hanks' balanced salt solution. Human skin fibroblasts were inoculated onto the HeLa cell cultures. The fibroblasts were permitted to attach and form a confluent monolayer with the HeLa cells. The cultures were treated with pederine for 18 hrs. and subcultured as described above. Radioautographs were prepared as previously described (3) except the emulsion was Kodak NTB.

Results

In table 1 are presented the results of 5 experiments demonstrating the consistently high incidence of fusion of human skin fibroblasts induced by low concentrations of pederine (1 ng/ml). In two experiments replicate cultures were treated with Sendai virus with a fusion rate of 8 and 13%. Following pederine treatment, as with Sendai virus, the most frequent class of multinucleated cells are those that contain two nuclei.

A homokaryon of human skin fibroblasts and a heterokaryon formed from fibroblasts and HeLa cells are presented in figures 1 and 2, respectively. The heterokaryon is easily identified by the distinctive morphological features of the human diploid and the HeLa heteroploid nuclei. The HeLa cell nucleus is also identified by the radioactive label present as the result of previous incorporation of 3H-thymidine.

Pederine is a known inhibitor of protein synthesis in eukaryotic organisms (1) and therefore it was important to determine if in the dose used to produce fusion, it irreversibly damaged the cells. To investigate this possibility, cells were treated with pederine according to the method used to produce fusion and then subcultured and permitted to grow to confluency. The growth rate of unfused cells was not detectably different from that of replicate untreated cultures.

Discussion

Pederine has been studied intensively in the laboratories of Pavan (21) and Brega et al (1). The material has been purified and crystallized, and its structure determined (2). Metabolically, it inhibits protein (1, 27) and DNA synthesis (1). Its remarkable capability for inducing fusion among cells grown in tissue culture has not been previously reported. Pederine induced cell fusion is easily documented through the production of heterokaryons recognized by the distinctive cytological characteristics of nuclei derived from different cell types and the presence of 3H-thymidine in one parental nuclei.

Cell fusion has attracted considerable attention from investigators in recent years. The phenomenon of membrane fusion is involved in a multitude of physiological processes including fertilization, pinocytosis and the formation of syncytia. It is also a common event in pathological conditions such as viral infections and the response to foreign bodies. It is not surprising that the mechanism of membrane fusion is now being subjected to close scrutiny (13, 23, 24).

Cell fusion is also proving to be a useful tool for investigators interested in the control of cell metabolism. Harris and his colleagues have exploited the formation of heterokaryons in a series of studies on nuclear-cytoplasmic interactions (6, 9, 11). Geneticists have resorted to cell fusion for chromosome mapping (15, 25, 28) and gene complementation (12, 14, 26), and oncologists use cell fusion to explore controlling factors of cell growth (5, 8).

The most commonly employed agent to induce cell fusion under controlled laboratory conditions has been inactivated Sendai virus (17, 18, 19). Although potentially very effective, the techniques that are required are cumbersome and the results are erratic. Each step of the long process, begining with the source of the virus and the condition of the eggs used for culture and continuing to the final exposure of the cells to the inactivated virus, involves a large number of variables which affect the results. Moreover, fusion of human diploid skin fibroblasts is not as efficient as with heteroploid lines (20). In our experience the fusion rate has varied from 7 to 30%, depending on unknown factors that influence the efficiency of the virus. Fusion rates as high as 50% have been reported, but they are distinctly unusual (16, 29).

Lysolecithin has also been used for cell fusion (4, 22). It has the advantage of being a chemical that is simply obtained. It appears to act directly on the cell membrane without metabolic intervention. However, it damages the cell and, in our hands, has not been very effective.

Pederine shares the advantages offered by lysolecithin in being a chemical with known structure. It is simple to use, and is considerably more effective than lysolecithin at concentrations less than one-thousandth that customarily used for lysolecithin. Under the conditions used to induce fusion, the cells appear to be undamaged by pederine, as measured by their growth potential. If cells which have been treated with pederine are subcultured, the growth rate of unfused cells is comparable to untreated cells.

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Table 1. Cell fusion induced by pederine

	Controls Experiment					Pederine-treated Experiment				
	A	B	C	D	E	A	B	C	D	E
No. of nuclei/ cell										
1	98	97	98	96	97	61.5	60.8	46.5	38	47.7
2	2	3	2	4	3	22	32	36.3	38	40.7
3						11.25	7.5	13	12	10.5
4						3		0.7	11	0.7
5						--		2.5	--	0.8
over 5						2.75		1.0	1.5	--
%fused cells	2	3	2	4	3	39	40	53	62	53

Fused cells, presented as per cent of original cell population. Human diploid skin fibroblasts growing in monolayer were exposed overnight to pederine, 1 ng/ml in nutrient medium. The cells were washed, removed with trypsin, diluted 1:10 in medium and transferred to coverslips for examination. At least 200 nuclei on each coverslip were counted and the extent of fusion was scored. Pederine experiments were performed in duplicate or triplicate. Replicate cultures unexposed to pederine served as controls.

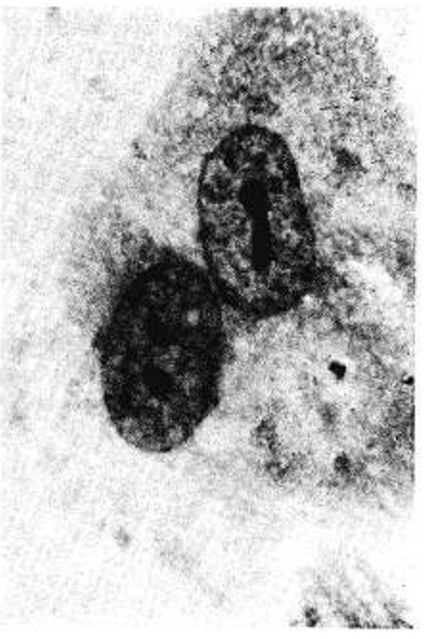

Figure 1. Pederine-induced fusion of two human skin fibroblasts to form a homokaryon with two nuclei sharing a common cytoplasm. The fibroblast nuclei are oval shaped and contain one or several nucleoli.

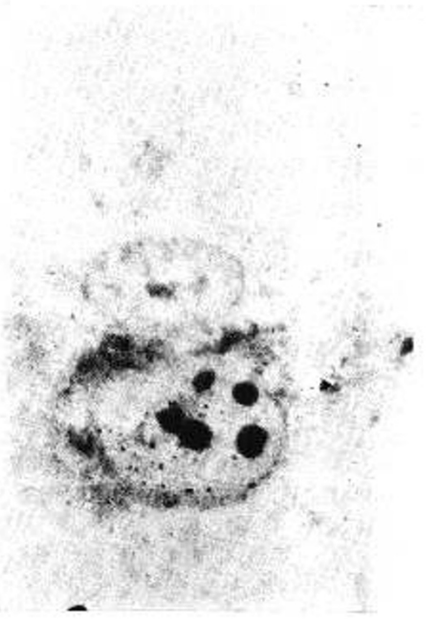

Figure 2. Pederine-induced fusion of a skin fibroblast and a HeLa cell forming a heterokaryon containing a HeLa and a fibroblast nucleus. The HeLa cell nucleus is large, round and contains four or five large nucleoli. The HeLa cell nucleus is further identified by the presence of radioactive grains produced by the previous incorporation of 3H-thymidine. The human skin fibroblast nucleus is smaller, oval-shaped and contains one or two nucleoli. The photograph is slightly out of focus since the nuclei and the radioactive grains in the overlying emulsion are in different focal planes.

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