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YEARLY REVIEW

GENETIC EFFECTS OF FLUORESCENT LAMP RADIATION ON EUKARYOTIC CELLS IN CULTURE

Introduction

The biological effects of fluorescent lamp radiation (FLR)* have not been reviewed before in this format. It has only recently been widely recognized that the strong mercury vapor lines present in fluorescent lamp emission spectra might represent a biological hazard. The irradiance in the emission lines of mercury can be as much as 100-fold more intense than that in the neighboring fluorescent continuum. However, a more accurate understanding of the biological significance of FLR must take into account the relative biological effectiveness of the wavelengths involved. A review of relative biological effectiveness, or action spectra, for a variety of effects in mammalian cells exposed to ultraviolet light has recently been published [9] in which it was noted that action spectra for mammalian cellular functions seem to clearly indicate that many important cellular parameters are most influenced by DNA. Damage to DNA, and subsequent repair of damage, determine a cell's response to such parameters as inactivation, mutation, transformation, latent virus induction, cellular viral capacity and ultraviolet enhanced viral reactivation [9].

We have limited the scope of this review to genetic effects of FLR on eukaryotic cells in culture. Specifically *not* covered in this review are *in vivo* studies, photoimmunological aspects of FLR, and effects on virus induction and enhanced reactivation. Finally, we will not attempt to address the current controversy surrounding the question of whether 'balanced' light (simulating the spectral distribution of sunlight) is necessary for human health. Recent reviews are available on *in vivo* photocarcinogenesis [16], phototherapy [35] and non-therapeutic effects of phototherapy [34], photoimmunology [29, 31] and on induction and enhanced reactivation of mammalian viruses by light [3]. For a general review of the actinic effects of light, see [52].

Since this is a Yearly Review, we will restrict the discussion to papers published since 1980. However, much information has accumulated prior to 1980 on the ability of radiation emissions from a variety of fluorescent lamps to cause cell killing [11, 33, 50, 53–56] and mutation [6, 7, 23–26], chromosomal changes [15, 17, 30, 36–39, 43–45], and DNA damage [5, 18] in mammalian cells. This earlier work has formed the basis for the work to be discussed here, and has served to increase awareness of the biological importance of radiation emissions from fluorescent lamps.

Published work since 1980 on the genetic effects of FLR can be roughly grouped into the following categories: (1) lethal and non-lethal effects of FLR, i.e. cell death, mutation, transformation, chromosomal and growth disturbances. Non-lethal effects have serious implications for multicellular organisms such as man; (2) mechanisms of DNA damage and the relationships of the damage to lethal and non-lethal events. This category also includes several studies on the ability of FLR to actually repair damage induced in cells; and (3) studies predicting the effects of fluorescent sunlamp radiation on man, and attempts to relate hazards encountered in using sunlamps and other fluorescent lamps to those associated with sunlight exposure.

Lethal and non-lethal effects

Previous studies have shown that photoproducts form in Dulbecco's modified Eagle's medium (depleted of phenol red) (MEM) after exposure to FLR as well as radiation from a black light fluorescent lamp, and these photoproducts are responsible for human, mouse, and hamster cell killing [33, 50, 53–56]. A recent report describes a similar effect with irradiated Fischer's medium on mouse cells [19]. The irradiation time necessary to cause the effect with Fischer's medium is much shorter than with MEM. Other differences in the effect of FLR on the two media were discussed.

The mutagenic potential of FLR is well established, and was shown to be due to the ultraviolet radiation emitted by the lamps [5, 25]. This is not surprising since ultraviolet light, a potent inducer of pyrimidine dimers, has long been known to be a mutagen. That FLR is able to induce malignant transformation in mouse embryo cells was recently demonstrated by Kennedy *et al.* [27] and Sanford *et al.* [43, 45]. Previous work had shown that FLR induces DNA sites

Abbreviations: BP, benzo(*a*)pyrene; FLR, fluorescent lamp radiation, defined here as radiation emitted from fluorescent lamps used for general illumination. Studies utilizing special purpose fluorescent lamps (e.g. black lights, sunlamps) are identified as such in the text; UVA, (near UV)—UV in the wavelength range 320–400 nm; UVB, UV in the wavelength range 290–320 nm.

(pyrimidine dimers) that are sensitive to *Micrococcus luteus* endonuclease [35]; Kennedy *et al.* [27] suggest that the pyrimidine dimer is the causal agent in transformation by both germicidal ultraviolet light and FLR from comparing equivalent dose response curves obtained for FLR- and germicidal ultraviolet light-induced transformation plotted against numbers of endonuclease-sensitive sites. This suggestion is supported by recent work in which it was shown that the action spectrum for transformation in Syrian hamster cells is the same as the action spectrum for pyrimidine dimer formation in the same cells [12]. Kennedy *et al.* [27] also suggest that exposure to FLR could contribute on a small scale to human skin carcinogenesis (see below).

Previous studies on the production of abnormal chromosomes resulting from chromatid breaks and exchanges that were induced by FLR showed that the effective wavelengths were in the visible range, coinciding with the mercury vapor emission peak at 405 nm [39]. In contrast, filtration of wavelengths shorter than 390 nm eliminated the induction of sister chromatid exchanges (SCEs) observed after fluorescent lamp irradiation of human fetal lung fibroblasts [30]. Media photoproducts were shown to be a contributing factor to SCE induction [30].

More recent work concerned with possible genetic effects of phototherapy lamp radiation reported a two-fold increase in the frequency of SCEs in cells exposed to blue light compared to that of cells exposed to red light [46, 47]. Corning colored glass filters were used to isolate selected spectral bands from daylight fluorescent lamps.

Transmittance through the filter was so low below 400 nm that it was not measurable. However, inspection of the data shows that even the highest (blue light) value for SCE induction did not reach a doubling over background, so the interpretation of these results is not easy.

Potential hazards from fluorescent lamps used in phototherapy were addressed in a recent review [48], in which it was indicated that phototherapy has DNA-modifying properties and has the potential for inducing genetic and carcinogenic effects. Speck *et al.* [49] subsequently studied the effect of radiation from fluorescent phototherapy lamps on the development of sea urchins. Exposure of unfertilized oocytes and spermatazoa to radiation from the lamps resulted in dose-dependent abnormalities in fertilization and development. Both white and blue light were used in conjunction with glass and plastic filters to prevent ultraviolet light from reaching the specimens. It was suggested that a photochemical reaction involving an intracellular chromophore with an absorption maximum in the region of 450 nm (possibly riboflavin) may be responsible.

Mechanisms for DNA damage

Some of the most exciting work undertaken during the time period covered by this review examines the mechanisms for DNA damage induced by FLR. Hydrogen peroxide (H_2O_2) had formerly been identified as a photoproduct toxic to cells, which was produced in tissue culture medium that had been exposed to FLR [55]. It was shown to have been formed through riboflavin-sensitized photooxidation of tryptophan and tyrosine [33, 55]. Recent work provides evidence that H_2O_2, produced by irradiation of growth medium with a fluorescent black-light lamp (i.e. near UV radiation) induces single strand (SS) breaks in cellular DNA of intact human cells [56]. Cells incubated in H_2O_2 solutions previously inactivated with catalase showed no toxic effect or SS breaks.

Catalase treatment of black-light-induced photoproducts also eliminated SS break induction, but did not completely eliminate lethality. Thus two classes of photoproducts have been identified: H_2O_2 and non-H_2O_2. H_2O_2 is responsible for all SS breaks but only some loss of viability, while non-H_2O_2 photoproduct(s) give no SS breaks, and are responsible for the remaining loss of viability [56].

H_2O_2 was also shown to be largely responsible for the induction of chromatid breaks and exchanges produced by irradiation with FLR in normal (i.e. non-neoplastic) human fibroblasts [41]: addition of catalase during exposure prevented chromatid damage, and exogenous H_2O_2 induced chromatid breaks. H_2O_2 does not appear to be responsible for the increased susceptibility of malignant mouse cells to FLR-induced chromatid damage however, since catalase activity levels could not be correlated to neoplastic state with any consistency. Rather, the effect of caffeine, an inhibitor of postreplication repair, indicated that increased susceptibility of malignant mouse cells to FLR-induced chromatid damage appears to be due to impaired DNA repair capacity [40].

The H_2O_2 story is complicated by data which show that H_2O_2 is not mutagenic in Chinese hamster V-79 cells, and thus cannot account for the mutagenicity of FLR [4]. H_2O_2, which is cytotoxic but not mutagenic, was compared to X-rays which are both. At equitoxic doses, H_2O_2 gave more SS breaks than X-rays. Hydroxyl radical scavengers reduced the number of SS breaks induced by both agents, thus implicating hydroxyl radicals in the formation of SS breaks. Since both H_2O_2 and X-rays produce hydroxyl radicals, but only X-rays are mutagenic, the authors contend that hydroxyl radicals are not necessarily mutagenic in V-79 cells. It is interesting that hydroxyl radicals were shown to be at least partially responsible for chromatid damage in human fibroblast cells. Mannitol, a hydroxyl radical scavenger, significantly decreased the number of chromatid breaks induced by exposure of cells to FLR [41]. The results of these two studies suggest that hydroxyl radical-induced chromatid breaks may not be responsible for mutagenesis.

Two very interesting papers attempted to relate mutagenesis to physical events in the DNA. The first examined the effects of FLR at wavelengths greater

than 295 nm on human and hamster cells [14]. Light at wavelengths greater than 295 nm from cool white fluorescent lamps, a fluorescent sunlamp, and the sun produced SS breaks, some of which could be eliminated by filtering the radiation through para-aminobenzoic acid (PABA) solution to eliminate wavelengths less than 345 nm. PABA filtration eliminated all mutagenesis. Radical scavengers also protected from some breaks, but not from mutagenesis. H_2O_2, and riboflavin and tryptophan photoproducts produced DNA breaks, but no mutagenesis. These results led the authors to conclude that several types of lesions can be produced in DNA by different mechanisms. Some of these lesions are responsible for toxicity and mutagenicity, and others are not. In a related paper, the ability of a fluorescent sunlamp to induce pyrimidine dimers in Chinese hamster ovary cells was compared to that of ultraviolet radiation at 254 nm (germicidal lamp) [57]. Radiation from the sunlamp was filtered so that only wavelengths greater than either 290 or 310 nm were transmitted. On the basis of equal numbers of pyrimidine dimers induced, more cells were killed by light of wavelengths greater than 310 nm than by light of wavelengths greater than 290 nm. Both were more cytotoxic than 254 nm light. Light at wavelengths greater than 310 nm induced 5–6 times more mutants per dimer than did light at wavelengths than 290 nm, but light at wavelengths greater than 290 nm induced approximately the same number of mutants per dimer as that at 254 nm. Differences in repair were excluded: removal of sites susceptible to UV dimer-specific endonuclease was the same for cells with equal numbers of dimers induced by radiation at 254 nm and in the near ultraviolet. In addition, identical amounts of repair replication were present in each case. It was concluded that near ultraviolet radiation emitted by the sunlamp induces both pyrimidine dimers and other lesions of biological significance.

FLR is also capable of reversing damage done to DNA by germicidal ultraviolet light or chemical carcinogens. Rat kangaroo cornea cells and Herpes simplex virus-1 inactivated by radiation at 254 nm (germicidal lamp) could be reactivated by either 'white fluorescent light' or sunlight (with wavelengths less than 375 nm eliminated by filtration) [21]. However, concomitant inactivation by light at these wavelengths was also noticed. Radiation at wavelengths greater than 475 nm and greater than 560 nm did not damage cells and was able to photoreactivate UV-induced damage, although at lower efficiency than at the shorter wavelengths. Evidence was also given that, in rat kangaroo cells, filtered sunlight at wavelengths greater than 375 nm produces photorepairable damage in DNA, which is fully repaired by photoreactivation with the same light. Thus the lethal effects of sunlight at wavelengths greater than 375 nm in rat kangaroo cornea cells may result from non-photorepairable damage. Cells from placental mammals, including humans, showed up to 90% repair of (photoenzymatically repairable) lesions in an *in vitro* photorepair system, when illuminated with 'white fluorescent light' [22]. The relevance of these and other studies on photorepair to human UV exposure was demonstrated in a recent paper which showed photoreactivation of UV dimers, induced in human skin *in vivo*, by light at wavelengths greater than 455 nm [10]. However, man may differ from mouse in the qualitative aspects of the ability to photoreactivate. Exposure of neonatal BALB/c mice to photoreactivating cool white FLR caused a reduction in the number of pyrimidine dimers induced with FS40 sunlamps; however, the effect could only be seen in neonates, and only in the dermis [2]. The human study was done on adult volunteers; however, it is not possible to determine which skin layer was involved because the specimens consisted of epidermis plus dermal tissue from the highest tips of the papillary dermis. For a more general discussion of photoreactivation, see [51].

Another type of FLR-induced 'repair' was discovered in a study designed to detect possible deleterious photoproducts, caused by visible light, of the ubiquitous photosensitive carcinogen benzo(a)pyrene (BP) [8]. However, it was found that low intensity, intermittent 'white fluorescent light' *reduced* the formation of covalent BP adducts in cells and completely eliminated cytotoxicity in cells treated with 1 μg BP per mℓ. At low BP doses (0.1 μg/mℓ) covalent adducts were produced, but their formation was not influenced by light. These adducts persisted for at least 7 days, and the authors suggested that chronic low level exposure to BP may lead to an accumulation of DNA damage.

Sunlamps, fluorescent lamps and human health

The appearance and rapid proliferation of suntan salons and other suntan products in the last several years has increased the risk of cutaneous and ocular injury from the UV radiation emitted by the fluorescent sunlamps used. The most common type of suntan booth installation in 1980 utilized a UVB source which emits intense radiation between 290 and 340 nm. Increasingly, UVA sources, emitting radiation primarily between 320 and 400 nm, are coming into use. Problems associated with the use of suntan booths have been enumerated [13]: loss or lack of protection due to genetic factors, presence of endogenous or exogenous photosensitizers, specific photosensitive diseases not dependent on the former, and increase in total UV exposure lifetime burden (cumulative effects). It has been estimated [32] that the irradiance from two unfiltered UVB-type fluorescent sunlamps can be from 7.5 to 14 times more effective in causing DNA damage and from 3.9 to 6.5 times more effective in producing erythema than noontime sunlight when skin transmittance and latitude are taken into account. It was also noted that fluorescent sunlamps produce more DNA damage per unit of erythema than sunlight.

Carcinogenesis is among the cutaneous effects associated with UV exposure. Mutation induction has been linked to carcinogenesis, and in a recent report, sunlight-induced mutagenesis was measured and related to that caused by fluorescent lamp and reflector type sunlamp radiation, all in the same line of cultured mouse lymphoma cells [28]. When relative intensities of radiation were taken into account, the mutagenic effect of light from cool white- and daylight-type lamps was about 1/25 that of sunlight, that of 'incandescent' fluorescent and Vita-Lite* about 1/200 that of sunlight, and that of a reflector type sunlamp 8-fold greater than that of sunlight. The basis for the comparison was the exposure time required to raise the induced mutation frequency to twice that of the spontaneous mutation frequency. These data allow an ordering of the lamps in terms of their relative biological hazard, and lend support to the suggestion (see above) that exposure to FLR could contribute to human skin carcinogenesis [27]. This suggestion has also been made by others [1, 6].

In a related study, the effects of exposure to two different types of radiation were measured in Chinese hamster cells [20]. Exposure to both UVB-type fluorescent sunlamp radiation and 50 kVp X-rays led to greater killing than would be expected from the effects of each alone. Such combined effects could occur, for instance, in dental practice.

CONCLUSIONS

Exposure to FLR has been shown to be responsible for a variety of genetic effects, including pyrimidine dimer formation and photo-reactivation, SS breaks in DNA, sister chromatid exchanges, chromosomal aberrations, mutations, cytotoxicity, malignant transformation and interference with embryogenesis. Commonly used unfiltered fluorescent lamps emit a biologically significant amount of UV radiation, ranging in wavelength from 290 nm to the visible region. This UV component has been shown to be responsible for the effects observed in a majority of the studies reviewed here. The use of unfiltered fluorescent lamps, both for illumination and for skin tanning, should be carefully considered in view of our increasing awareness of the effects their UV and visible emissions have on cells and cell systems.

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