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ORIGINAL ARTICLE

Lighting during the day and night: possible impact on risk of breast cancer

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Abstract

Risk of breast cancer varies by about 5-fold among societies, and incidence and mortality have been increasing worldwide for many decades. Migrants from low-risk Asian societies to the U.S. suffer elevated risk of breast cancer in their own lifetimes, and the second or third generation Asian-Americans attain the high risk of the multi-generational European immigrants [1,2]. Something about a modern Western lifestyle apparently increases risk dramatically.

Madigan et al. [3] estimate that 41% of the new U.S. cases of breast cancer are explained by 'known risk factors'; these include the reproductive factors of age at first birth, menarche, menopause. They ascribe about 30% to reproductive factors when they are analyzed alone. 'High income' is estimated to account for about 19% when analyzed by itself. The 41% is an analysis taking all the factors together, and since they are related, the total is less than the sum of estimates for the individual items.

By itself, 'high income' has no biological interpretation and must reflect attributes of lifestyle and/or environment that increase risk. So, the proportion of breast cancer cases in the U.S. that can be accounted for by known biological risk factors is about one third. Therefore, at least half of breast cancer risk in the U.S., and other Westernized/industrialized societies, is in excess of that found in non-industrialized societies and is without any agreed-upon explanation. Many candidate factors exist, each with a cadre of proponents. The sum of these may turn out to explain the bulk of the excess risk in modern societies. On the other hand, they may not, and worse, may fall woefully short.

Circadian Disruption

Disruption of the circadian rhythm may alter hormones relevant to well-being, particularly to breast cancer risk. It is becoming increasingly clear that circulating estrogen is a key factor in elevated breast cancer risk [4]. Its effects are probably mediated by influencing the growth, differentiation, and turnover of the normal breast epithelial cells at risk of malignant transformation, although there may also be a free radical component to its actions [5]. The circadian hormone melatonin may also play a role both directly and perhaps through effects on estrogen production or function. The altered lighting from use of electricity in modern societies may disrupt circadian hormone rhythms [6]. Experimental work can address various exposure scenarios and impacts on mammary tumorigenesis.

Circadian Rhythms

Circadian rhythms are found in virtually all organisms on the planet, from cyanobacteria to human beings [7]. These rhythms depend on a bright, broad spectrum day (from Sun), and dark nights. In order to examine whether disruptions of human circadian rhythms may be implicated in disease, particularly in breast cancer risk, it is worthwhile to contemplate the elements of this system.

Takahashi [8] describes three essential elements of a circadian system for all organisms: 1) ability to detect environmental input, 2) the molecular mechanism of the clock itself, and 3) physiological output of the clock. For mammals (e.g., humans) each of these includes, 1) phototransduction to entrain the clock, 2) clock proteins and feedback loops in the suprachiasmatic nucleus as well as in

the clock mechanism in each cell in the body, and 3) rhythms of gene expression throughout the organism and timing of hormone production and release.

Figure 1 shows how these three elements of the circadian rhythm might intersect with breast cancer risk. It begins with circadian phototransduction.

Phototransduction

An as yet unresolved consideration is the nature of the phototransduction mechanism for the circadian system. It seems not to be vision [9]. Recent evidence has implicated a cell type distinct from rods or cones in the retina; rat retinal ganglion cells are depolarized by light [10], and it has been noted that these cells contain melanopsin, a candidate for the photopigment for the circadian system [11]. However, these cells also contain cryptochrome [12], another candidate circadian photopigment which contains vitamin B2 as chromophore as opposed to vitamin A, as in the opsins.

There is conflicting evidence from knockout mice on whether an opsin or cryptochrome is the primary circadian photopigment: a cry1/cry2 double-knockout still responds to light during subjective night [13], and a retinol binding protein knockout mouse maintained on vitamin A deficient diet also responds to light [14].

Discovering the underlying biology of circadian phototransduction is fascinating in its own right, but it also has implications for designing experiments and epidemiological studies of light and breast cancer risk. For example, if the circadian photopigment is an opsin such as melanopsin, then vitamin A ingestion and metabolism may be relevant to light sensitivity; whereas if cryptochrome is the photopigment, then vitamin B2 is relevant. Also, understanding the biophysics of circadian phototransduction should better define what aspects of nocturnal light exposures can disrupt circadian rhythms such as spectrum, timing, duration, and intensity; as well, what aspects of day lighting best maintain a healthy rhythm?

Constant light and mammary tumorigenesis on rats

In figure 1, downstream of the master circadian CLOCK, is communication of circadian time to the rest of the organism and the effect of this on hormone production and release. These hormones may then affect mammary carcinogenesis.

Anderson et al. [15] attempted a replication of an experiment by Shah et al. [16] in which female rats exposed to constant light were compared to similar female rats exposed to a 12:12 light:dark cycle in the effectiveness of a chemical carcinogen, DMBA, to induce mammary tumors. The Shah et al. [16] experiment was based on the idea that constant light would suppress melatonin and thereby increase mammary tissue cell turnover by effects on estrogen and prolactin [17]. Shah began light exposure in utero, that is to say, female rats were bred in constant light, gestation took place in constant light, and the female pups were reared in constant light. As predicted, the females rats under this constant light paradigm had greater terminal end bud concentration in the mammary tissue at age 55 days than did the 12:12 light:dark controls, and yielded more mammary tumors after a dose of DMBA than controls.

Anderson et al. [15] undertook to replicate the Shah finding. However, these authors found, unexpectedly, significantly fewer mammary tumors in the constant light group. They also found, also unexpectedly, that 29 of the 50 female rats on constant light had gross evidence of milk production in their mammary glands at the termination of the experiment (age about 140 days). In contrast to Shah et al., Anderson et al. began constant light at age 26 days, not in utero. This difference probably explains the difference in the tumor yield between the two experiments, and underscores the importance of mammary tissue development on risk of malignant transformation [18].

In Utero exposure

Hilakivi-Clarke et al. [19] tested the hypothesis that elevated in utero estrogen exposure increases mammary gland mass in rats, and increases susceptibility to chemically-induced mammary tumorigenesis. Their findings confirmed their prediction both for direct estrogen administration to pregnant rat dams, and by feeding a high PUFA diet; the high PUFA diet also increased circulating estrogen in pregnant rats. Stevens and Hilakivi-Clarke [20] have suggested that perhaps ethanol ingestion could have a similar effect by raising estrogen in pregnant rats. This experiment is currently underway.

These findings with estrogen may explain the difference in results between Shah et al. and Anderson et al. In the Shah experiment, there may have been higher mammary gland mass at birth, and also accelerated development such that by the 55 day age at DMBA dosing, a greater tumor yield was obtained. In Anderson, however, constant light began at age 26 days, thereby not affecting mammary gland mass, but only, perhaps, having pushed the mammary tissue to terminal differentiation at age 55 days and thereby reducing its susceptibility to transformation. Future experiments are planned to address these possibilities.

Master Clock Interaction with Cells

In addition to effects on hormones, there may also be more direct communication from the master clock in the SCN to cellular clocks and cell cycle regulatory mechanisms (figure 1).

Cell cycle regulation received the Nobel prize in 2001 (Paul Nurse, Lee Hartwell, and Tim Hunt). The role of the cellular clock mechanism in regulation of cell cycle kinetics has not been investigated, and the influence of the master clock of the SCN on both is unclear. Cyclin D1 appears to be a "G1 cyclin" which functions to push a cell through this cell-cycle checkpoint [21].

Cyclin D1 overexpression has been implicated in a variety of human tumors [21]. In particular, overexpression occurs in 30 to 50% of breast cancer cases [22]. In 12–13% of breast cancers, the overexpression is due to amplification of the cyclin D1 gene. Mice lacking cyclin D1 (Cyl-1(–/–)) are small and show mammary tissue developmental retardation [23,24] suggesting reduced susceptibility to mammary carcinogenesis. Thus, overex-

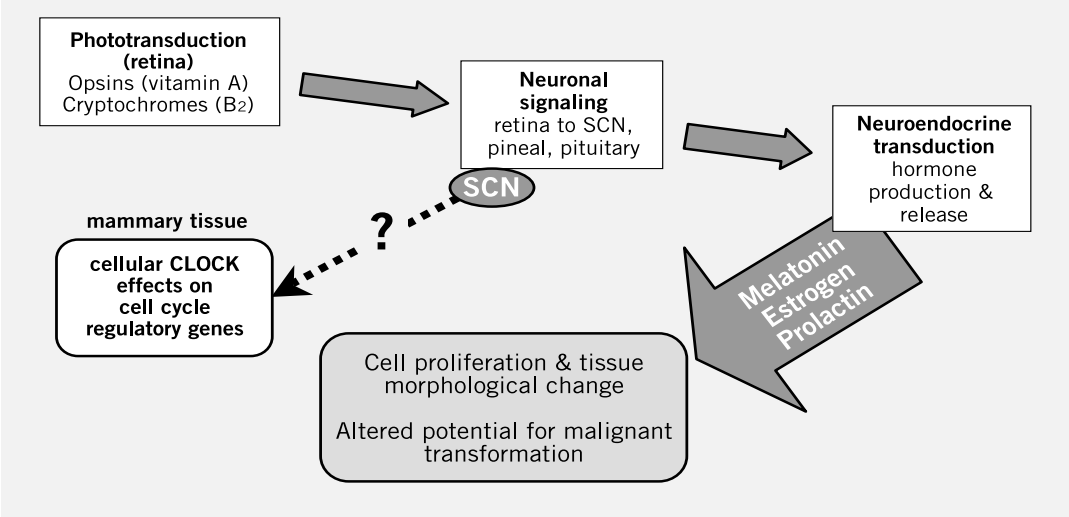

Figure 1. Graphic representation of the 3 elements of the circadian system and howthese might be related to risk of breast cancer. Aspects of light which matter areintensity, spectrum, timing, and duration.

pression of a normal cyclin D1 product, through various mechanisms, contributes to neoplasia and/or progression to the malignant phenotype [25].

Epidemiological Studies of Light and Breast Cancer

There are a variety of predictions based on the 'light at night' (LAN) component of the circadian disruption hypothesis. For example, breast cancer risk may be increased in: women on shift work schedules for many years; women who read late into the night; women flight attendants; women who's bedrooms are lighted during the night; women sleeping fewer hours than average. Epidemiological studies have been conducted on several of these groups, and generally support a role for LAN. In particular, 3 large studies designed to test the hypothesis that women working on evening or night shifts are at increased risk have recently been reported. An enormous case-control analysis from Denmark found a 50% elevation in risk for women ever working the night shift [26]. This association was not due to confounding by age at birth of first child, number of children, or socioeconomic status. Two more reports appeared later in 2001, one from a case-control study in the Seattle area [27], and the other from the Nurses' Health Study [28], a very large prospective study in the United States. These two studies gave very similar results to the study from Denmark.

Another prediction of the hypothesis that LAN increases risk is that blind women would be at lower risk [29], which has been found in a number of studies [e.g., 30]. In addition, Hansen [31] recently estimated breast cancer risk among photolab workers, who would be predicted to have lower risk also due to a dark work environment; the odds ratio estimate was 0.4 (CI = 0.2 to 0.9) for such workers compared to women in other occupations.

Attributable Fraction

An estimate of attributable risk for rotating shift work was given to ABC News, October 16, 2001 [32]: "...maybe one new breast cancer case per year..." Given that the shift work studies were undertaken as studies of light-at-night, this may be taken as an estimate for LAN. However, this could be far off the mark.

A problem in all studies of LAN and breast cancer is the fact that estimating the degree of risk elevation requires a comparison group. But in fact, these comparison groups undoubtedly contain no women who are not exposed to LAN to some degree. Complete lack of exposure would require life without electricity; there are probably no women in modern societies who retire to bed at dusk and remain in total darkness until sunrise, although this was the norm for the major part of our history on the planet. However, there is one group for whom there is no exposure to LAN: blind women. [Evidence from Czeisler et al. [33] and Lockley et al. [34] shows that about 1/3 of profoundly blind persons can respond to very bright light, such as from the Sun or very high illuminance electric light.]

Breast cancer risk in blind women has been examined in four countries: Finland, Norway, Sweden, and the United States. All found a reduced risk among the blind which ranged from 20 to 50%. If the lower risk of breast cancer among blind women is due to light exposure among sighted women, then estimates of the number of breast cancer cases attributable to LAN, in all its manifestations, are formidably high.

Wacholder et al. [35] argue that in order to properly estimate the proportion of cases of disease attributable to an exposure, there should be a broad definition of exposure. In other words, the referent, or comparison group should be of subjects who are truly not exposed at all. They show, by example, that to include exposed persons in the 'unexposed' comparison group (as is the case in the shift worker studies) underestimates attributable fraction, perhaps greatly. However, to include unexposed in the 'exposed' category does not over- nor underestimate attributable fraction. Greenland [36] argues that it would be rare for the assumptions to be met in practice for the Wacholder et al. method to be unbiased.

Using blind women as the unexposed comparison group yields relative risk estimates ranging from 1.2 to 1.7 (the inverse of the estimates for blind women com-

Table 1. Population attributable fraction where Pe is proportion of population exposed, T is total number of breast cancer cases in 2001, and Te is the number 'attributable' to the exposure. 'Rotating shift work' is a narrow definition of exposure, whereas 'sighted' is a broad definition of exposure. Formula from Rothman & Greenland, *Modern Epidemiology* [37]
Assumptions: risk estimates are accurate, LAN causes differences in risk. If either assumption is false, then the calculations are not valid

	RR	Pe	T	Te
Rotating shift work	**1.4**	**0.01**	**192,000**	**780**
'sighted'	**1.2**	**0.98**	**192,000**	**31,400**

pared to sighted women). Using the formula from Rothman and Greenland [37] which requires an estimate of relative risk, proportion of population exposed, and total number of cases of disease, the table presents estimates of attributable fraction using rotating shift work as the 'exposure' (a very narrow definition), and using 'being sighted' as the exposure (a broad definition). [When the proportion of exposed is very high, as with 'being sighted', then the formula is close to the total number of cases times (RR-1)/RR].

There are two stringent assumptions underling the validity of these calculations: the estimates of relative risk for blind women are accurate, and the ability to perceive LAN causes the higher risk in sighted women. The estimate of new breast cancer cases in 2001 is from the American Cancer Society. As can be seen in the table, even using the modest estimate of 1.2 for relative risk for the broad exposure category of 'sighted', the number of breast cancer cases in the U.S. attributable to LAN would be in excess of 31,000. The estimate based on the very restricted exposure category of 'rotating shift worker' yields 780 cases attributed to LAN; this works out to about "one new case per year" or one new case per 100,000 working women in America (table 1).

Again, these estimates are only valid if both assumptions are true. If either is false (relative risk estimates are not accurate, or LAN is not the cause), then the attributable risk estimates are not valid.

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