STUDY OF THE MUTAGENIC POTENTIAL OF LOW POWER MICROWAVES BY DIRECT DNA ANALYSIS

Soma Sarkar, Sher Ali, B.K.Thelma and J.Behari

Defence Institute of Physiology and Allied Sciences, Lucknow Road, Delhi 110 054, India.

INTRODUCTION

Man is constantly exposed to electromagnetic radiation which is a constituent part of the biosphere. Due to the technological progress and numerous man-made sources such as those used in industry, traffic, medicine, defence, communication network as well as some home appliances, the character of the natural electromagnetic field has altered significantly. These sources of nonionizing radiation cause atmospheric pollution similar to the pollution from various industrial sources. Unambiguous detection of the mutagenic potential of these radiations is necessary for genetic well being of future generations.

DIRECT DNA ANALYSIS

DNA was isolated from brain and testis tissues of male Swiss albino mice exposed to low power microwave ($1 mW/cm^2$, 2.45 GHz continuous wave) for 2 hrs daily for a period of about 200 days. A variant of the restriction fragment length polymorphism (RFLP) approach was developed to study the effect of at the DNA sequence level using a single restriction enzyme digest and hybridization with a short synthetic oligodeoxynucleotide probe specific for a simple repetitive sequence $(GACA)_9$.

RATIONALE: Eukaryotic genome harbors two principally different classes of DNA: a) single copy DNA present once per haploid chromosome set; b) repetitive DNA existing from a few to a few hundred thousand (or million) copies. The whole array of repetitive sequences may comprise from less than 10% to more than 90% of the genome in different animal and plant species (in man it is about 30%). Short sequence motifs (less than 10 bases long) are ubiquitous repetitive components of eukaryotic genome (1). The number of repeat units of such simple sequences vary considerably causing allelic variation. When sequences complimentary to these repeat units are used as hybridization probes, polymorphism at several loci is simultaneously detected and individual specific hybridization patterns are generated (2). Of the various types of hybridization patterns obtained, multilocus monomorphic band profile is particularly useful because any loss or gain of band due to sequence rearrangement or sporadic mutations can be easily detected by the technique.

CYTOGENETIC ANALYSIS

The sensitive analysis of sister chromatid exchange (SCE) induction , which represents a sensitive and qualitative index of mutagen-carcinogen-induced DNA damage in eukaryotic chromosomes, was used as a cytogenetic end point to assess the mutagenic potential of microwaves. Bromodeoxuridine (BrdU) tablets (25 mg) were implanted subcutaneously. 19 hrs posttablet implantation, mitotic arrests were established by administration of colchicine (25 µg/animal). Metaphase plates prepared from bone marrow, were stained by modified fluorescent plus Giemsa (FPG) technique (3).

RESULTS

A multilocus monomorphic band profile was demonstrated with Southern blots in both the tissues of brain and testis studied. Hybridization profile of the brain DNA showed a sharp band (mol. wt 8.2 kb) in both the control and the exposed group of animals (Fig.1, arrow). In the exposed animals, an additional band at 7.7.kb appeared below this particular band. Gel track analysis showed a sharp peak marked 1 (Fig. 3 a,b) in both the control and the exposed animals and the appearance of a second peak marked 2 (Fig. 3 d-g) in all the exposed animals.

The hybridization profile in the testis DNA is not a reflection of sharp band difference in this region between exposed and control animals but rather a broadening of the band width. Both control and exposed animals revealed a sharp band in the testis DNA in the region of 8.1. kb (Fig. 2, arrow). The track histogram of both control and exposed animals showed a sharp peak marked 1, corresponding to 8.1 kb (Fig. 4, a-c). In the exposed animals, there was a change in the peak profile with the appearance of a second peak marked 2 corresponding to 7.7 kb (Fig. 4, d-g).

It is interesting to note that amidst a large number of bands at identical positions in DNA of both controls and exposed group of animals, rearrangement of DNA is consistently observed in all the exposed animals in the same region between 7-8 kb, irrespective of the duration of the exposure.

No statistically significant differences in the SCE frequency were recorded between the control and the irradiated animals exposed for various intervals of time. The mean value of SCEs in both control and exposed group of animals fell between 2.47+1.80 to 3.53+1.76; range 0-7 . The distributions were reasonably continuous and unimodal so that the difference in mean value could be evaluated in terms of standard statistical parameters of normal distribution. One level of ANOVA test was applied for the evaluation of the SCE frequencies using epistat software programme.

DISCUSSION

In the present study, the DNA analysis from exposed animals with nine repeat 5'-GACA-3' probe show similar profiles as those produced from the control animals with the notable exception of a prominent 7.7 kb Hinf I fragment specific to all exposed animals. Since this particular fragment is not present in the control animals but appears after microwave exposure, it is suggested that probably in the unexposed animals, the copy number of these repeat sequences is not sufficient to form a distinct band. Microwave exposure may have led to the amplification of these tandem sequences generating more copies of GACA sequences in this particular region. Although it is not known at present whether exposure to a mutagenic agent or a specific class of mutagens increases the mutation rate in the region of these tandem repeat units, it is known that stress induces amplification by extra replication of DNA segments in the non coding repeat sequences (4). The observed change of DNA rearrangement could be attributed to some sort of non-specific stress created by low intensity microwave field.

However low power microwave did not induce increased incidences of SCE formation in the exposed animals compared to the controls. This may be explained on the basis that though both SCEs and amplification phenomenon form a part of recombinational events, mechanisms of their formations are different. Interestingly, a parallel can be drawn at this juncture.

between microwaves and ionizing radiation (IR), which although a powerful mutagen (and a carcinogen), is a poor inducer of SCEs [5,6].

The technique of direct DNA analysis, which seems to monitor even minute sequence variation in the genome, therefore, appears to be a powerful technique for the assessment of the nonionizing radiation effects. Furthermore, based on the present study and other recent documents (7), what seems imperative is the (re)evaluation of the recommended safe limits of microwave exposure from the personnel and people who are exposed.

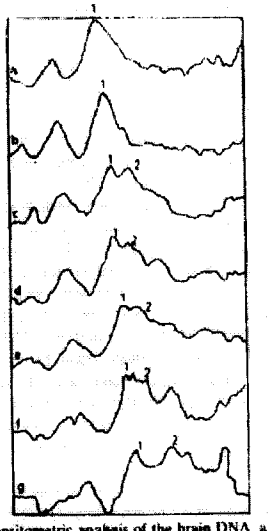

Fig. 3. Densitometric analysis of the brain DNA. a and b are control DNA; c–g are DNA from exposed animals. Peak 1 is present in both control and exposed animals while peak 2 appears in all the exposed animals.

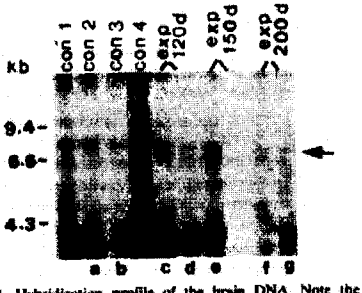

Fig. 1. Hybridization profile of the brain DNA. Note the appearance of a band below the position marked with an arrow in the exposed groups of animals. Lanes marked a–g are the ones which have been traced densitometrically as shown in Fig. 3.

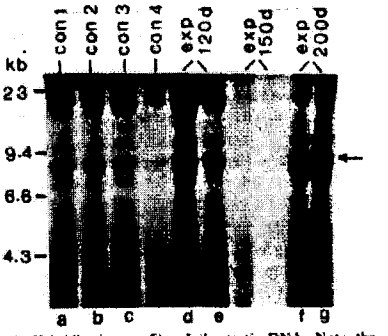

Fig. 2. Hybridization profile of the testis DNA. Note the broadening of the band width in the position marked with an arrow in the exposed animals. Lanes a–g are the ones which have been traced densitometrically as shown in Fig. 4.

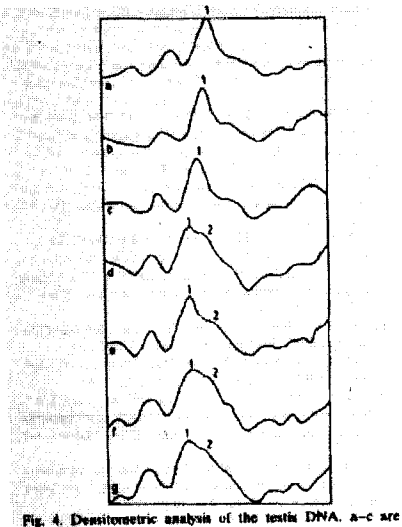

Fig. 4. Densitometric analysis of the testis DNA. a–c are control DNA; d–g are DNA from exposed animals. Peak 1 is present in both control and exposed animals while peak 2 appears in all the exposed animals.

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