

Role of mouse spleen cell HMG proteins and their poly-ADP-ribosylation in betelnut induced carcinogenesis

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The role of high mobility group (HMG) proteins and their poly-ADP-ribosylation (PAR) in betel nut induced initiation of carcinogenesis in mice has been studied. A known carcinogen, diethylnitrosamine (DEN) was used as a positive control. Swiss albino mice were chronically exposed to aqueous extract of betel nut (AEBN) or DEN at low doses for up to 4 weeks. The poly-ADP-ribosylation (PAR) of spleen cell HMG proteins was monitored using [32P]-NAD⁺. Parallel to this, chromatin was subjected to DNase I cleavage and the organizational state of the chromatin was monitored. The PAR of HMG proteins showed a marked progressive reduction at different times following AEBN- or DEN treatment. HMG proteins isolated from the control and carcinogen treated mice were allowed to reassociate with the untreated spleen cells chromatin. The reassociated chromatin showed progressive relaxation in its superstructure. The results suggest that under the influence of potential carcinogens AEBN or DEN, the mouse spleen cell HMG proteins created molecular conditions favourable to initiation of cancer.

A significantly large section of the world population, estimated to be over 600 million, masticate betel or areca nut (*Areca catechu*, L.) in one form¹ or the other. Its mastication is strongly implicated in human carcinogenesis and several of its constituents, particularly an alkaloid, arecoline, are reported to be suspected mutagens and carcinogens²⁻⁷. In an effort to understand the betel nut-induced carcinogenesis at molecular level, this study looks into the high mobility group (HMG) proteins as there is a strong evidence that they are involved in organization of chromatin. These proteins are proposed to be gene regulatory proteins, which modify the structure of chromatin to allow replication or transcription to occur. Of the four major such proteins, HMG 1 & 2 are involved in DNA replication and function as a transcription factor, whereas HMG 14 and 17 are associated with transcriptionally active genes and have regulatory functions^{8,9}. Given their close association with discreet regions of chromatin and their capacity to interact with DNA in the nucleosome core, it is possible that HMG binding to nucleosomes might be regulated by post-translational modifications as observed in case of histone and other chromosomal proteins. Of several modifications, poly-ADP-

ribosylation (PAR) is of interest due to its special characteristics^{10,11}. Many non-histone proteins including the HMG proteins are reported to be poly-ADP-ribosylated. Since HMG proteins are associated with transcriptionally active genes^{12,13}, their PAR may also influence gene activity and, thereby the process of carcinogenesis^{6,7,14}. In addition, as PAR of chromosomal proteins influences the organization of chromatin, it is quite likely to affect mutations, gene rearrangement, and gene amplification, etc. which are integral part of the process of carcinogenesis. Therefore, studying the effects on HMG proteins, their status of PAR and chromatin organization after carcinogen treatment may help to understand the process of carcinogenesis. The present study was undertaken to look into these aspects in an *in vivo* system, using aqueous extract of betel nut (AEBN) as a carcinogen. A known hepatocarcinogen, diethylnitrosamine (DEN), was used as a positive control¹⁵.

Materials and Methods

Chemicals and Animals

All chemicals used were of analytical grade or of highest purity grade available. Deionized glass double distilled water was used to prepare reagents and solutions. Six to eight weeks old, female Swiss albino mice (Balb/c strain) were used. Standard mouse pellet and drinking water were provided *ad libitum*.

Carcinogens and Administration Protocol

AEBN was prepared as described earlier⁵ and

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Abbreviations used: AEBN, aqueous extract of betel nut; DEN, diethylnitrosamine; HMG, high mobility group; PAR, poly-ADP-ribosylation.

administered at a concentration of 0.5 mg ml⁻¹. DEN was administered¹⁵ at a dose of 0.0167 mg ml⁻¹. Both carcinogens were prepared in drinking water and administered chronically on groups of 3 to 4 mice for 1, 2, 3 or 4 weeks. Age-matched control mice received normal drinking water. Mice were sacrificed by cervical dislocation. Experiments were repeated at least three times for each set.

Assay of PAR of total HMG proteins

Spleen cells (8.3×10^6) and [³²P]-NAD⁺ were used for assay of PAR by method described earlier¹¹. After reaction step involving [³²P]-NAD⁺, HMG proteins were isolated from the spleen cells following the method described earlier¹⁶ with some modifications. The isolated HMG fraction was collected on GF/C filter discs and washed extensively with cold 15% trichloroacetic acid. The radioactivity on filter discs was counted in a scintillation counter.

Extraction of chromatin from spleen cells and chromatin activity assay

The method published elsewhere was employed to isolate chromatin from mouse spleen cells and perform chromatin activity assay⁷. Diphenylamine and Bradford's methods were used to estimate DNA and protein, respectively.

Results and Discussion

Though the *in vitro* studies have made significant insight into the process of carcinogenesis, there are obvious problems of extrapolation of these results into *in vivo* situation. The present study was carried out to elucidate molecular insight into the process of carcinogenesis in *in vivo* system. To achieve this goal, Swiss albino mice were chronically exposed to betel nut constituents at low dose levels through drinking

water. This protocol mimicked human betel nut masticator who is also constantly exposed to betel nut components at low doses. Therefore, this report has special significance as the results have direct bearing on human carcinogenesis related to betel nut chewing.

On the whole, there was no distinguishable sign of abnormality in the AEBN- or DMN-exposed mice throughout the period of experimentation. No change in the body weights of treated mice was observed either. These observations are consistent with the fact that during initiation stage of carcinogenesis no apparent abnormality or body weight change is observed. These changes are expected to occur only after prolonged period of promotion^{12,14}.

Fig. 1 shows a marked progressive reduction in total PAR of HMG proteins at different times following AEBN or DEN treatment. The slope of the curve indicates that the lowering of PAR of HMG proteins was persistent but variable during the period of investigation. ADP-ribose polymer being negatively charged, its reduction from the HMG proteins following exposure to AEBN or DMN (Fig. 1) suggests that the net positive charge of the HMG protein molecules should rise. This would alter the interaction between the HMG proteins and the nucleosomes, thereby causing change of chromatin organization⁹. Thus, these results (Fig. 1) show that the lowering of PAR of HMG proteins could potentially cause change in the nucleosome superstructure during the process of initiation of carcinogenesis. It is known that DMN causes cancer¹⁷ and AEBN is a suspected potent carcinogen implicated in 2/3rd of mouth cancer in humans^{1,2}. Prolonged and persistent exposure to any of the two agents eventually causes cancer^{1,2,17}. Besides, PAR of chromosomal proteins affects chromosome organization^{7,10,12,13}. Therefore, the results suggest that AEBN- or DMN-induced change in the superstructure of chromatin is mediated by PAR of HMG proteins, in favour of initiation of carcinogenesis. In other words, the progressive suppression of PAR of HMG proteins during first 8 weeks of exposure may suggest that AEBN or DEN creates a molecular condition in such a manner that it favours the commitment of cells for eventual transformation.

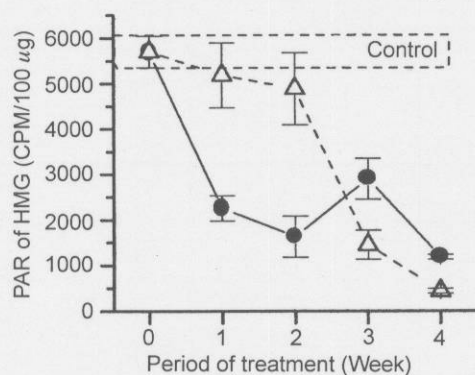


Fig. 1—The level of total poly-ADP-ribosylation [mean \pm SD] of HMG proteins of mouse spleen cells [in untreated control (0 week); and exposed to AEBN (●—●) or DEN (▲—▲)]

Fig. 2 shows the DNase I cleavage of chromatin in 2 min at 37°C following reassociation of normal spleen cell chromatin with the HMG proteins isolated from AEBN or DEN treated or from untreated mice. It is assumed that the exogenously added HMG proteins interact with the chromatin and, therefore, the

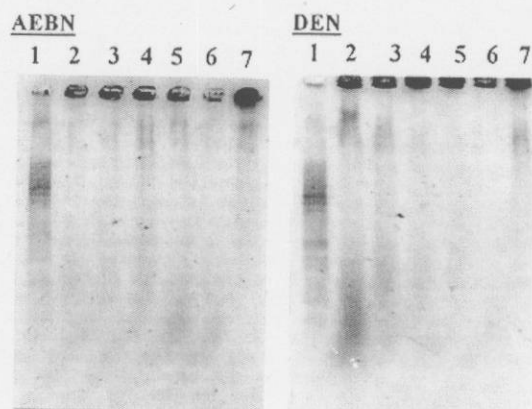


Fig. 2—Agarose gel electropherogramme of untreated (control) mouse spleen cell chromatin without or with DNase I cleavage for 2 min at 37°C following reassociation with HMG proteins isolated from liver of (a), AEBN and (b), DEN treated mice [lanes 1-5 with chromatin fragmented by DNase I: lane 1, 4 week; lane 2, 3 week; lane 3, 2 week; lane 4, 1 week; lane 5, control; lane 6, undigested chromatin; lane 7, standard marker]

reassociated chromatin would behave differently than its form before the reassociation. In other words, the reassociation of chromatin from normal mice with HMG proteins from carcinogen exposed mice is likely to mimic the situation of chromatin in the DMN- or AEBN- exposed mice under the influence of HMG proteins. The presence of HMG proteins from AEBN or DEN treated groups caused progressively more fragmentation of chromatin by DNase I (Fig. 2), except that from the 4 weeks DEN-treated mice which caused resistance to DNase I cleavage. The results suggest that change in the superstructure of chromatin following carcinogen exposure is due to relaxation of chromatin, which is evident by its easy cleavage by DNase I^{7,18}. Also, it is apparent that HMG proteins from DEN exposed mice affected chromatin organization faster than AEBN (Fig. 2). However, DEN caused relaxation of chromatin only upto 3 weeks. The relaxed state of chromatin would facilitate enhanced transcriptional activity – an observation that has been made during initial stages of carcinogenesis induced by DEN¹⁵.

Earlier, conformational changes in HMG proteins have been reported following exposure to DMN or AEBN¹⁵. This report shows progressively lower level of poly-ADP-ribosylation of HMG proteins under similar condition (Fig. 1). Both events alter the properties of HMG proteins such that their characteristic association with chromatin is affected¹⁸. This caused relaxation of chromatin as evident from DNase I digestion patterns (Fig. 2). A relaxed

chromatin is easily accessible to metabolically activated or ultimate carcinogens for adduct formation and mutation. It is interesting to note from the results that two chemically different carcinogens, AEBN and DEN, seem to cause very similar effects on HMG proteins suggesting that early events of carcinogenesis may have commonality. It may not be unexpected since the end point is a full-blown cancer for all carcinogens notwithstanding their chemical differences. In conclusion, the present study shows progressive lowering of PAR of HMG proteins during the early stages of exposure to AEBN or DEN. One possible biological consequence of the lowering was more relaxed chromatin organization. There are indications that these changes favoured initiation of cancer induced by AEBN or DEN.

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References

- 1 Sharan R N (1996) *Cancer J* 9, 13-19
- 2 International Agency for Research on Cancer (1985) in *Monograph on the Evaluation of Carcinogenic Risk of Chemicals to Humans*, pp. 5-269, IARC, Lyon
- 3 Wary K K & Sharan R N (1988) *J Cancer Res Clin Oncol* 114, 579-582
- 4 Wary K K & Sharan R N (1990) *Int J Cancer* 47, 396-400
- 5 Sharan R N & Wary K K (1992) *Mutat Res* 278, 271-276
- 6 Saikia J R, Schneeweiss F H A & Sharan R N (1998) *Cancer J* 11, 94-98
- 7 Saikia J R, Schneeweiss, F H A & Sharan R N (1999) *Cancer Letts* 139, 59-65
- 8 Owen-Hughes T & Workman J L (1994) *Crit Rev Eukaryot Gene Exp* 4, 403-441
- 9 Seyedin S M & Kistler W S (1979) *J Biol Chem* 254, 11264-11271
- 10 Althaus F R (1992) *J Cell Sci* 102, 663-670
- 11 Schneeweiss F H A, Sharan R N & Feinendegen L E (1995) *Indian J Biochem Biophys* 32, 119-124
- 12 Boulikas T (1987) *Int Rev Cytol (Suppl)* 17, 599-684
- 13 Boulikas T (1992) *Anticancer Res* 12, 885-898
- 14 Sugimura T & Miwa M (1990) in *ADP-Ribosylating Toxins and Proteins: Insights into Signal Transduction* (Moss J & Vaughan M, eds), pp. 543-560, American Society for Microbiology, Washington D C
- 15 Pariat T & Sharan R N (1998) *Hepato Res* 12, 177-185
- 16 Sharan R N, Schaffrath R, Laltanpuia & Schneeweiss F H A (1996) *Proc Indian Natl Sci Acad B* 62, 5-10
- 17 Pariat T & Sharan R N (1995) *Life Sci* 57, 2431-2437
- 18 Schneeweiss F H A, Mukherjee A S, Sharan R N, Feinendegen L E & Muller-Gartner H W (1999) *Life Sci* 66, 193-200