Black Tea Polyphenols Target Matrix Metalloproteinases, RECK, Proangiogenic Molecules and Histone Deacetylase in a Rat Hepatocarcinogenesis Model

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Abstract. Background: The aim of this study was to evaluate the chemopreventive effects of black tea polyphenols (Polyphenon-B) on markers of invasion and angiogenesis during dimethylaminoazobenzene (DAB)-induced hepatocarcinogenesis. Materials and Methods: Male Sprague-Dawley rats were divided into four groups. The rats in groups 1 and 2 were given 0.06% DAB in the diet for 3 months followed by the normal diet. The rats in group 2 received in addition 0.05% Polyphenon-B in the basal diet. The group 3 animals were given 0.05% Polyphenon-B alone in the basal diet. The group 4 animals served as the control. Results: The dietary administration of DAB induced well-differentiated hepatocellular carcinomas (HCC) that showed increased expression of the markers of invasion, angiogenesis and epigenetic histone deacetylation compared with the controls. The administration of Polyphenon-B significantly reduced the incidence of DAB-induced hepatomas as evidenced by modulation of the markers of invasion (matrix metalloproteinase, MMP-2, MMP-9, tissue inhibitor of matrix metalloproteinase, TIMP-2, and reversion-inducing cysteine rich protein with Kazal motifs RECK) and angiogenesis (hypoxia inducible factor 1α, HIF1α, vascular endothelial growth factor, VEGF, and VEGF receptor, VEGFR1) as well as the expression of histone deacetylase HDAC-1. Conclusion: The results of the present study provide evidence that Polyphenon-B has potential as a chemopreventive agent.

Key Words: Angiogenesis, black tea polyphenols, chemoprevention, hepatocellular carcinoma, histone deacetylation, invasion.
and DNA in the dimethylaminoazobenzene (DAB)-induced hepatocarcinogenesis model (11). Here, the modulatory effects of Polyphenon-B on markers of invasion (MMP-2, MMP-9, TIMP-2 and RECK) and angiogenesis (HIF-1α, VEGF, and VEGFR1 (vascular endothelial growth factor receptor 1) as well as the expression of histone deacetylase-1 (HDAC-1) in the DAB-induced rat HCC model are reported.

Materials and Methods

Chemicals. DAB was purchased from Sigma Chemical Company, St. Louis, MO, USA. All the other reagents used were of analytical grade.

Animals and diet. All the experiments were carried out with male Sprague-Dawley rats, aged 6-8 weeks, weighing 100-110 g obtained from the Central Animal House, Annamalai University, India. They were housed four to a polypropylene cage and provided food and water ad libitum. The animals were maintained under standard conditions of temperature and humidity with an alternating 12-hour light/dark cycle in accordance with the guidelines of the Indian Council of Medical Research and approved by the Ethical Committee of Annamalai University. The experimental diet was prepared every day by mixing DAB (0.06%) and Polyphenon-B (0.05%) to a preweighed standard pellet diet (Mysore Snack Feed, Mysore, India). The diet was replenished every day and the food consumption was recorded.

Treatment schedule. The animals were randomized into experimental and control groups and divided into four groups of eight animals each. The rats in group 1 were given DAB (0.06%) in the diet for 3 months followed by the normal diet. The rats in group 2, administered DAB as in group 1, received in addition 0.05% Polyphenon-B in the diet (11). The group 3 animals were administered 0.05% Polyphenon-B alone in the diet as in group 2, but without DAB. Group 4 received the basal diet and tap water throughout the experiment and served as the untreated control. The experiment was terminated at the end of 24 weeks and all the animals were sacrificed by cervical dislocation after an overnight fast. For RNA isolation, the tissues were immediately frozen in liquid nitrogen and for Western blot analysis, the tissues were processed using lysis buffer.

Reverse transcriptase (RT) reaction and PCR amplification. The total RNA was isolated and 1 μg reverse-transcribed to cDNA as described previously (11). The nucleotide sequences of the primers (Sigma Genosys, Bangalore, India) were: for amplification of MMP-2: 5’-GGCCCTGTCACCTCTGAGAT-3’ (sense), 5’-GGCATCCAGGTTATCGGGGA-3’ (antisense); MMP-9: 5’-ATCTTTGCTTGTCGCGGAG-TACGAC-3’ (sense), 5’-GTTTTGCAATGCAGACGTAG-3’ (sense), 5’-TACATGAGCGCTTCCGGCAC-3’ (antisense); TIMP-2: 5’-GTTTTGCAATGCAGACGTAG-3’ (sense), 5’-ATCGGGGA-3’ (antisense); MMP-9: 5’-AGTTTGGTGTCGCGGAG-3’ (sense), 5’-GGCATCCAGGTTATGTCGCGGAG-3’ (sense), 5’-GTTTTGCAATGCAGACGTAG-3’ (sense), 5’-ATCGGGGA-3’ (antisense); MMP-9: 5’-ATGAACTTTCTGCTGTCTTGG-3’ (sense), 5’-TCACCGCCTCGGCTTGATGTCAAGAAACTCTGCTT-3’ (antisense); VEGF: 5’-ATGAACTTTCTGCTGTCTTGG-3’ (sense), 5’-TCACCGCCTCGGCTTGATGTCAAGAAACTCTGCTT-3’ (antisense); VEGFR1: 5’-AGGAGAGGACCTGAAA-3’ (sense), 5’-TCACCGCCTCGGCTTGATGTCAAGAAACTCTGCTT-3’ (antisense); VEGFR1: 5’-AGGAGAGGACCTGAAA-3’ (sense), 5’-TCACCGCCTCGGCTTGATGTCAAGAAACTCTGCTT-3’ (antisense); VEGFR1: 5’-AGGAGAGGACCTGAAA-3’ (sense), 5’-TCACCGCCTCGGCTTGATGTCAAGAAACTCTGCTT-3’ (antisense); VEGFR1: 5’-AGGAGAGGACCTGAAA-3’ (sense), 5’-TCACCGCCTCGGCTTGATGTCAAGAAACTCTGCTT-3’ (antisense); VEGFR1: 5’-AGGAGAGGACCTGAAA-3’ (sense), 5’-TCACCGCCTCGGCTTGATGTCAAGAAACTCTGCTT-3’ (antisense). The PCR amplification reaction mixture (final volume 25 μl) contained 1 μl of cDNA, 0.5 μl each of the forward and reverse primers, and 10 μl of Hot Master Mix (2.5X) (Eppendorf, Hamburg, Germany). The PCR was carried out in a thermal cycler (Eppendorf). Negative controls without cDNA were also performed. The amplification products were analysed by electrophoresis in a 2% agarose gel containing ethidium bromide with 100 bp DNA ladder. The PCR products were visualized as bands with a UV-transilluminator and photographs taken using a gel documentation system (GelDocMega™, Devon, UK).

Statistical analysis. The data are expressed as mean±standard deviation (SD). The data for densitometric analysis was analysed using analysis of variance (ANOVA) and the group means were compared by the least significant difference test (LSD). The results were considered statistically significant if the p<0.05.

Results

Tumour incidence. The tumour incidence was the same as described previously (11). At the end of the experimental period, the tumour incidence in group 1 was 100% with a multiplicity of 4.90 per rat and tumour burden of 542.02 mm3. Multiple metastatic lung deposits in addition to HCC were observed in one of the 8 animals in group 1. The administration of Polyphenon-B decreased the tumour incidence to 12.5 per cent with a multiplicity of 1.56 per rat. Furthermore, the tumours were significantly smaller (mean tumour burden 15.85 mm3) compared to group 1. No tumours were observed in groups 3 and 4.

RT-PCR analysis. Figure 1 shows the RT-PCR data for the markers of invasion and angiogenesis in the liver tissue of the control and experimental animals. A significant increase in the expression of MMP-2, MMP-9, VEGF and VEGFR1 with decreased expression of TIMP-2 was seen in the DAB treated rats (group 1) compared to the untreated controls. The dietary administration of Polyphenon-B decreased the expression of MMP-2, MMP-9, VEGF and VEGFR1 and increased TIMP-2 expression as compared to group 1. In the animals administered Polyphenon-B alone, the mRNA expression of the markers analysed was not significantly different from that in the untreated controls. β-Actin was used as a loading control.
Western blot analysis. Figure 2A shows the Western blot analysis of the markers of invasion and angiogenesis and HDAC-1 in the liver of the control and experimental animals. A significant increase in the expression of MMP-2, MMP-9, VEGF, HIF-1α and HDAC-1 with decreased expression of TIMP-2 and RECK was seen in the DAB-treated group 1 rats compared to the untreated control. The dietary administration of Polyphenon-B decreased the expression of MMPs, HDAC-1 and the proangiogenic factors, and increased TIMP-2 and RECK as compared to group 1. No significant changes in protein expression were observed in the group 3 animals compared to the control. Analysis of these markers in the rat that harboured a secondary neoplasm in the lung showed changes in protein expression that were similar to those observed in the primary liver tumour (2B). The protein expression was not significantly different in the lung tissues of the group 2 and group 3 animals compared to the control.

Discussion

A positive correlation was observed between MMP overexpression and increased risk of intrahepatic and pulmonary metastases attributable to the release of proangiogenic molecules (12-14). Higher expression of HDAC-1 was observed in HCC compared to adjacent non-malignant cirrhotic nodules in human hepatitis B virus-associated HCC specimens (15). The increased expression of MMP-2 and -9, HIF-1α, VEGF and VEGFR1 and HDAC-1 with the down-regulation of TIMP-2 and RECK seen in the DAB-induced hepatomas in the present study indicated ECM degradation and neovascularization and may have been responsible for the lung metastasis.

The down-regulation of proinvasive and angiogenic proteins with the up-regulation of their inhibitors by Polyphenon-B observed in the present study is in line with reports by us and other workers. In a recent study, black tea extract and theaflavins have been reported to inhibit prostate cancer in athymic nude mice by down-regulating VEGF expression (16). Theaflavin and theaflavin digallate have been reported to block tumour cell invasion in vitro by inhibiting MMP-2 and -9 expression of the highly metastatic mouse Lewis lung carcinoma LL2-Lu3 cells (17). Epigallocatechin gallate has been reported to diminish the synthesis of MMPs, HIF-1α and VEGF and enhance TIMP expression (18-20). Recent reports from this laboratory have demonstrated the antiinvasive and antiangiogenic potential of Polyphenon-B (8-10).

The present study demonstrated that black tea polyphenols modulate the expression of several key molecules that regulate invasion and angiogenesis. Although agents that target MMPs and VEGF signaling have entered clinical trials, the therapeutic potential of RECK has remained largely unexplored despite the strong correlation between the extent of RECK expression and improved prognosis in multiple carcinomas (4, 21, 22). The inhibition of HDAC-1 by Polyphenon-B observed in the present study is of significance in the context of the emerging interest in cancer epigenetics in general and the potential anticancer effects of HDAC inhibitors in particular. HDAC inhibitors are known
to exert antimetastatic and antiangiogenic activity both in vitro and in vivo (23). Since malignant tumors have the propensity to switch to alternate pathways to circumvent chemointervention, agents such as Polyphenon-B that target multiple molecules involved in invasion and angiogenesis are an attractive option for preventing tumour progression.

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References

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