Prevention of colon carcinogenesis with a combination of Sulindac, 3,3'-diindolylmethane and Curcumin or Polyethylene Glycol in *Apc*-mutated Pirc rats

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The administration of drugs or natural compounds to prevent or at least to slow the process of colon carcinogenesis (chemoprevention) in patients at risk (familial adenomatous polyposis (FAP) and sporadic colorectal cancer (CRC) patients), may be a suitable strategy to reduce the number of late-stage cancers for which chemotherapy is not completely successful. The rat strain Polyposis in Rat Colon (Pirc) carries a germline mutation in *Apc,* the key genetic event in both FAP and CRC (1). Notably, at variance with genetic models like *ApcMin* mice developing tumours mostly in the small intestine, Pirc rats develop tumours also in the colon, thus resembling CRC and FAP and potentially standing as a robust model of colon cancer. Significant side effects of drugs like sulindac (SU) or celecoxib, two of the most effective chemopreventive drugs, hampered their chronic use in FAP and sporadic CRC patients suggesting the need for alternative strategies. Experimental studies have shown that a combination of low doses of drugs is often more effective that using individual agents. Chemopreventive effects of NSAID like SU or celecoxib as well as natural compounds such as curcumin or 3,3'-diindolylmethane, are mediated by the induction of apoptosis. Studies in rodents have also shown that Polyethylene glycol (PEG), an osmotic laxative, is one of the most powerful agents in reducing chemically-induced carcinogenesis in rat colon (2). However, contrasting results obtained in *ApcMin* mice, dampened the enthusiasm on this potentially strong, and virtually safe, tumour chemopreventing agent. Based on these premises, the aims of our work were 1) to validate Pirc rats as a reliable model to identify chemopreventive agents and 2) to find out new chemopreventive strategies. To these purposes we studied 1) the effect of sulindac at 320 ppm in the diet, corresponding to a clinically used dose, in a short-term carcinogenesis experiment. 2a) the effect of a combination of different doses of sulindac (SU) (80 and 160 ppm) in combination with 3,3'-diindolylmethane (DIM) (250 ppm) and curcumin (CUR) (2000 ppm) in a long-term carcinogenesis experiments. 2b) the effect of PEG-8000 (PEG, 5% in drinking water) in short and long-term carcinogenesis experiment. Results:1) Sulindac, 320 ppm in the diet, significantly reduced the number of the precancerous lesions MDF (Mucin Depleted Foci) after 1 and 4 months of treatment (MDF/colon after 4 months were 156±8 and 38±6 in controls and sulindac-treated animals, respectively, mean±SE, $P<0.001$). 2a) Colon tumours assessed after 6 months of treatment were significantly reduced by SU 320 ppm, by the combination of DIM and CUR without or with SU 80 and 160 ppm but not by SU 80 ppm. Apoptosis in the normal mucosa was significantly increased by SU 320 ppm, and slightly, by DIM and CUR with or without SU. A slight reduction in *Survivin-Birc5* expression was observed with all the treatments compared to Controls. 2b) Precancerous lesions were dramatically reduced by PEG treatment (MDF/colon after 2 months of treatment were 99 ±17 and 12±8 in Controls and PEG-treated rats, respectively; $p < 0.001$; mean ± SD). Similarly, colon tumors were significantly reduced after 6 months. Colon proliferation, a parameter correlated to cancer risk, was also significantly lower in PEG-treated rats than in Controls. Conclusions. The results obtained with a clinically relevant dose of Sulindac validate Pirc rats as a reliable model to identify chemopreventive agents. The efficacy of PEG or the DIM-CUR combination in lowering colon tumours, suggests alternative strategies to be exploited in patients at risk.

References: 1) Amos-Landgraf JM et al., PNAS 2007 Mar 6;104(10):4036. 2) Corpet DE et al., Cancer Res 2000; 60:3160.

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