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6,7-dimethoxy-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid attenuates colon carcinogenesis via blockade of IL-6 mediated signals

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ABSTRACT

In this study, we investigated the *in vivo* antiproliferative activity of 6,7-dimethoxy-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid (M1) in dimethylhydrazine (DMH) induced colorectal carcinoma (CRC) using albino Wistar rats. M1 was administered to DMH induced CRC rats at 10 and 25 mg/kg doses for 15 days. Various physiological, oxidative parameters, histopathology, ELISA, gene and protein expression studies were conducted to evaluate the anti-CRC potential of M1. The histopathology and biochemical tests indicated the protective action of M1 in DMH-induced colon cancer. ELISA confirms that M1 reduced the increased concentration of IL-6 more prominently than those of IL-2 and COX-2. Gene expression analysis revealed that M1 attenuated the increased mRNA over-expression of IL-6, JAK2 and STAT3. The result obtained from quantitative western blot analysis demonstrated that the CRC condition was produced by the IL-6 induced activation/phosphorylation of JAK2 and STAT3 and further down-regulated with M1 treatment. This evidence was supported well with the application of data-based mathematical modeling. Applying the fitted model, we predicted the quantitative behavior of STAT3 populations not accessible to experimental measurement. Later, ^{1}H NMR based serum metabolic profiling was carried out using rat sera to investigate the impact of M1 on CRC-induced metabolic alterations. M1 showed its ability to restore the perturbed metabolites in CRC condition. Altogether, our study provided the first time evidence that M1 exhibits anti-CRC potential through the blockade of IL-6/JAK2/STAT3 oncogenic signaling.

1. Introduction

The annual incidence rate of colorectal carcinoma (CRC) is ~4% per ten million populations [1] and it is the third most common cancer worldwide [2]. Most of synthetic drugs used for CRC treatment, produces chemo-resistance or the resistance due to gene mutation and lesser drug deposition at the site of action [3,4]. The drugs from natural origin are very few in number and synthetic drugs are costly as well as highly toxic to human body. Natural products have been used for the treatment of CRC since ancient times; however, very few compounds were isolated from natural origin for the treatment of CRC [5].

Previous literature revealed that the isoquinoline alkaloids are present in higher amount in *Mucuna pruriens* seeds [6] and have good antiproliferative properties [7,8]. Later, our research group isolated one isoquinoline alkaloid from *Mucuna pruriens* seeds (namely M1, 6,7-dimethoxy-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid (Fig. 1), and the obtained results demonstrated that M1 exhibited dramatic role in anti-proliferation against human hepatoma (Huh-7) cells *in vitro* [9]. In another experiment, we found good anti-neoplastic properties of M1 *in vivo* using diethylnitrosamine induced hepatic carcinoma in rats [10]. In the present study, we investigated its anticancer potential using human colorectal carcinoma cells (HT-29) *in vitro* where we found that

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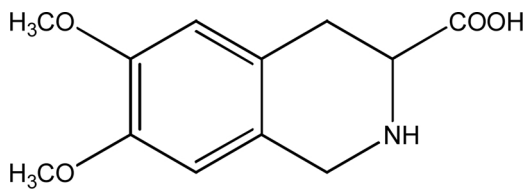

Fig. 1. Structure of M1 (6,7-dimethoxy-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid).

M1 exhibited well antiproliferative properties against HT-29 cells with IC_{50} of 7.76 μM (Supplementary materials, Fig. S1). So, it is questionable whether M1 has any effect against CRC *in vivo*.

Therefore, the present study was undertaken to find out the *in vivo* antiproliferative effect of M1 in colorectal-carcinogenic albino Wistar rats. Various oxidative stress parameters, scanning electron microscopic (SEM) and histopathological studies of the colon tissues were performed to evaluate the protective effect of M1. The tissue concentration of the important inflammatory mediators (IL-2, IL-6) and enzyme (COX-2) were measured using ELISA, where M1 was found to be more effective to attenuate the concentration of IL-6. Thus, to elucidate the mechanism of M1 action at molecular level, gene expression study was carried out on IL-6, JAK2 and STAT3 to confirm the m-RNA expression levels, whereas protein expression study was performed on IL-6, JAK2, p-JAK2, STAT3 and p-STAT3 to confirm the level of phosphorylation of JAK2 and STAT3. Further, inspired by previous literatures [11,12], we applied mathematical modeling concept and put the data of quantitative western blot into a system of differential equations formulated for individual steps of the IL-6 induced JAK2-STAT3 signaling pathway. Applying this model, the quantitative behavior of different STAT3 populations could be determined that was not accessible to experimental measurement. In addition, proton nuclear magnetic resonance (^{1}H NMR) based serum metabolic profiling was performed to discover the metabolic modulations in CRC rats treated with M1.

2. Materials and methods

2.1. Materials

Dimethylhydrazine (DMH), glutathione (GSH) and 2,4-dinitrophenylhydrazine (DNPH) were purchased from Sigma-Aldrich, USA. ALT and AST kits were acquired from the TransasiaBiomedicals Pvt. Ltd., Baddi, India. Interleukins (IL-2, IL-6, ELISA) were commercially availed from Sigma-aldrich, USA. COX-2 ELISA kit was obtained from Bioseps Technology Co., LTD, China. All other chemicals were procured from Himedia, Mumbai, India. All the solvents and chemicals were of analytical grades with 99% purity and in house distilled water was used throughout the experiment. All the antibodies were procured from Thermo Fisher Scientific, Waltham, MA, USA. M1 was isolated form seeds of *Mucuna pruriens* previously by our research group [9].

2.2. Procurement of M1

M1 was isolated previously from *Mucuna pruriens* seeds [9] and synthesized by our research group previously [10]. The details of synthetic procedures and characterization were also published previously [10]. On comparing both of these, the characterization data of our synthesized M1 were found to be in good agreement with the previously published data of naturally isolated M1. The percentage purity of synthesized M1 was found to be 99.8% and this M1 was used for further experiments.

2.3. Experimental animals

Male albino Wistar rats (80–100 g) were used for this experiment and Institutional Animal Ethical Committee approved the protocol previously (Ref no. AEC/PHARM/1601/07/2016/R2). Standard laboratory conditions (temperature 25 ± 5 °C and light/dark cycle of 12 h) were maintained with free access to commercial pellet diet and water *ad libitium*. Animals were kept for one week before experiment.

2.4. Experimental design

All animals were randomly divided into 5 groups of 8 animals each (n = 8). The groups were then divided as follows: Group I was the normal control rats, for which 0.25% CMC (2 mL/kg) in pure drinking water was supplemented daily through oral gavage. Group II was the carcinogen control group, for which DMH (40 mg/kg body weight) was injected subcutaneously, once in a week for 4 weeks [13,14]. Group III was the positive control group, for which DMH (40 mg/kg body weight) was injected once in a week for 4 weeks, subsequently treated with 5-FU (10 mg/kg body weight, water for injection, WFI) intraperitoneally, daily for 15 days. Group IV was test group, for which DMH (40 mg/kg body weight) was injected once in a week for 4 weeks, subsequently M1 (10 mg/kg body weight, suspended in 0.25% CMC solution) was supplemented through oral gavage, daily for 15 days. Group V was another test group similar to Group IV, but with different dose of M1 (25 mg/kg body weight).

Next, all the groups were supplemented with similar vector i.e. 0.25% CMC solution. The Group III, where 5-FU was administered with WFI, was also supplemented with 0.25% CMC as vector separately in the same routine. At the end of the experimental period, animals were euthanized by cervical decapitation and colons were dissected out immediately, rinsed in ice cold saline and stored at −80 °C for further studies. The serum was collected, processed and stored for further analysis.

2.5. Estimation of various physiological parameters

Body weight changes were measured at the initial and final days of experiment. Tumour incidence no. was calculated via percentage of animals having tumours. The colons cut along with the longitudinal axis, the inner surface was examined macroscopically and tumours of each colon were counted. The length (l), width (w) and height (h) of tumour were measured using a vernier caliper with 0.1 mm graduations, and tumor volume was calculated ($l \times w \times h \times \pi/6$). The pH of colonic content was measured using a pH meter. Total acidity was determined through 0.1N sodium hydroxide using phenolphthalein as indicator in the similar experiment.

2.6. Estimation of serum enzyme levels and biochemical estimations in colon

The enzyme levels in serum like aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) were also measured in serum using commercially available kit. The biochemical parameters like catalase (CAT) [15], protein carbonyl (PC) [16], superoxide dismutase (SOD) [17], glutathione (GSH) [18] and thiobarbituric acid reactive substances (TBARS) [15] were estimated in CRC tissue in the similar experiment.

2.7. Estimation of cytokine and inflammatory mediators in colon tissue

ELISA was performed for interleukins (IL-2 and IL-6) and COX-2 as per the protocols provided by manufacturer.

2.8. Histopathological studies and scanning electron microscopy (SEM) of colon

The procedures adopted for histopathology and SEM were described previously [19].