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MODELING THE PROTECTIVE EFFECT OF AN ANTIMETABOLITEINTERCALATED IN Zn-Al LAYERED DOUBLE HYDROXIDES ON THE EARLY PHASES OF COLON CARCINOGENESIS

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Abstract

Background: Nanomaterials benefit from numerous fields of application, including drug delivery.
This study investigated the chemopreventive potential of an antimetabolite (ATM, with antioxidant properties) intercalated in a lamellar matrix double hydroxides (ZnAl-HDL), on early stages of colon carcinogenesis.
Methods: ZnAl-HDL were synthesized by co-precipitation technique and characterized by XRD, FTIR and SEM to confirm the intercalation of the antimetabolite into ZnAl-HDL.
Multiorgan organ carcinogenesis was induced in adult mice (10/group) with 1,2-dimethylhydrazine (DMH) and arsenic trioxide (As2O3) for 7 weeks. Mice were then given NaCl (control) or 20 mg/kg ATM once a day, for 4 consecutive weeks. After mice sacrifice, blood and colon was harvested and analysed.
Results: Free ATM had no significant effect on body and organ weight, food and water consumption. DMH combined to As2O3 resulted in aberrant crypt foci (ACF) formation and inflammatory cells infiltrate, and oxidative stress in colon mucosa at week 12. ATM, markedly improved colon damage with decreased inflammatory infiltration. The lipid peroxides (malondialdehyde, MDA) level and myeloperoxidase (MPO) activity were enhanced by 248% ($p < 0.001$) and 67% ($p < 0.05$), respectively, while glutathione level (GSH) was decreased by 40% ($p < 0.01$). ATM improved crypts disruption, leukocyte influx, and oxidative stress, thereby restoring colonic homeostasis. The characterization results showed a good intercalation of antimetabolite in our HDLs, without altering the specific properties of HDLs.
Conclusion: ATM intercalated in ZnAl-HDL can efficiently attenuate early features of chemically-induced multiorgan carcinogenesis in mice. Further research would decipher the molecular mechanisms underlying this chemoprotective effect
Keywords: Antimetabolite, 1,2-dimethylhydrazine, Arsenic trioxide, Oxidative stress, ZnAl-HDL

Introduction

- **Early diagnosis of cancer ensures more effective treatment and prolonged patient survival. Significant research efforts are currently directed toward the identification and elimination of precancerous lesions [(1)].**
- **The short-term multi-organ carcinogenesis bioassay is a suitable model for inducing early preneoplastic lesions on multiple target organs, enabling the rapid and simultaneous**
- **screening of anticancer pharmacological molecules [(2)].**
- **Antimetabolite (ATM), an electrophilic ester. This antioxidant exerts its effects by activate the erythroid nuclear factor 2 (Nrf2) pathway and induce expression of antioxidant proteins [(3)].**
- **Nanomaterials are used as nanovectors for the transport of biomolecules. This improves bioavailability, pharmacokinetics and makes it possible to reduce the necessary dose of the biomolecule thanks to its targeted release [(4)]. Among the available nanovectors, we chose lamellar double hydroxides "HDL", these are anionic clays in the form of sheets separated by an interlamellar space [(5)].**

→This study investigated to confirm the possibility of vectorization of ATM in an HDL type nanovector as well as the therapeutic potential of ATM in the early stage of colon carcinogenesis using 1,2 dimethylhydrazine (DMH, a colon carcinogen) combined with arsenic trioxide (As2O3), in a mouse model of multi-organ carcinogenesis.

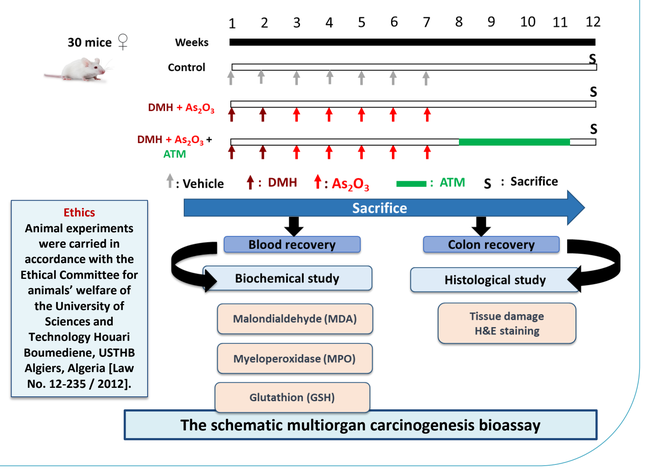
Materiels and Methods

- For the nanoparticle part, we synthesized our nanoparticles then we characterized them as follows
- **HDL synthesis:** Preparation of a ZnAl-HDL hydrotalcite type compound.

Intercalation of the active ingredient in the HDL matrix: The sample is denoted ZnAl-HDL-ATM.
HDL characterization methods:

- **DRX:** makes it possible to observe the obtaining of the HDL host matrix and to verify the incorporation of the bioactive molecule in the interlamellar space of the matrix.
- **SEM:** allows the specific composition of phases, impurities, inclusions or crystallographic defects to be determined
- **FTIR:** used in the context of the identification of substances and compounds of a solid

- To test our molecule at the pharmacological level, we have established an experimental protocol for multiorgan carcinogenesis



The schematic multiorgan carcinogenesis bioassay

Results

1. Nanopharmacology

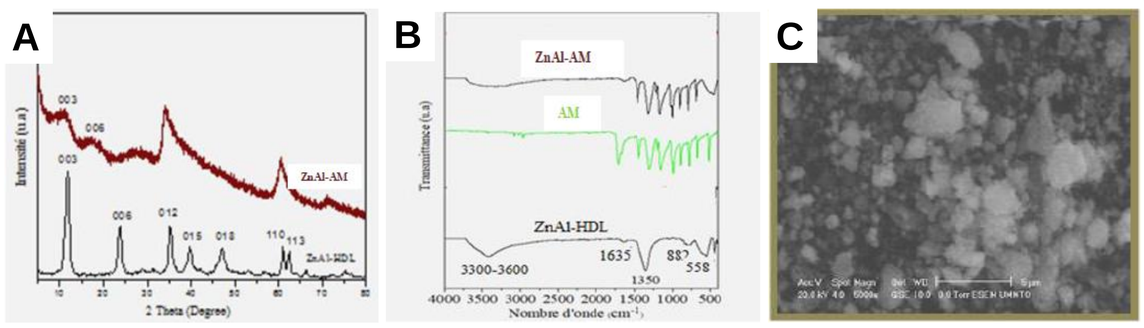


Fig.1. (A) Difractogram of ZnAl-HDL samples and ZnAl-ATM hybrid compound by X-ray diffraction (XRD). (B) Infra-red spectra of ZnAl-HDL, ATM and ZnAl-ATM samples. (C) sample Scanning electron microscopy (SEM) images of the ZnAl-HDL (x5000).

→ **ZnAl-ATM**, maintains the pre-deformed hydrotalcite structure as well as an increase in the value of the intersheet distance parameter (c) from 2.53 nm to 3.13 nm compared to ZnAl-HDL (fig 3 A).

→ Presence of vibration bands characteristic of **ATM** as well as a clear reduction in vibration bands respectively at 3420 cm-1 relating to the OH- of the HDL framework and at 1650 cm-1 corresponding to C=O carbonyl groups of ATM for **ZnAl -ATM** compared to ZnAl-HDL (fig 3 B).

→ Presence of fine particles of fairly regular shape, with obvious interplatelet porosity (fig 3 C).

2- Pharmaco-oncology

A- Histological analysis

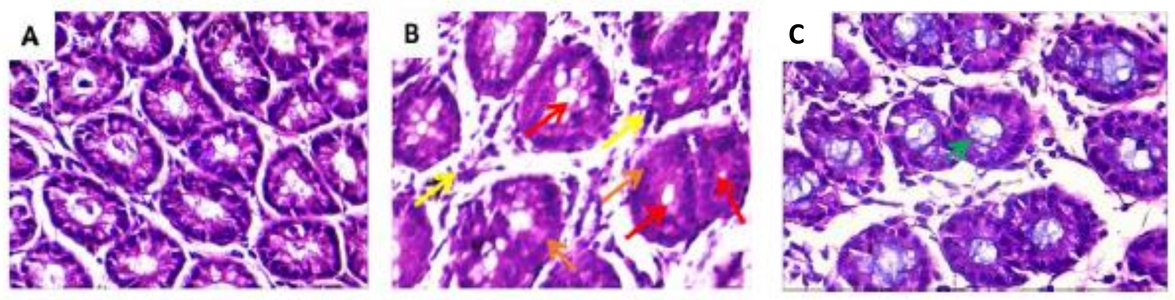


Fig.2. Histological alterations of colon mucosa induced by association of DMH + As_2O_3. Hematoxylin-Eosin-stained, Gx 400. A: Control, B: DMH + As_2O_3, C: DMH + As_2O_3 +ATM. → Light size reduction , → membrane thickening, → inflammatory cell infiltrate, → restore light size.

→ **DMH+ As_2O_3 enhanced** mucosal alteration and inflammatory cells infiltrate in colon.
→**ATM reduced** the signs of inflammation.

B- Biochimical analysis

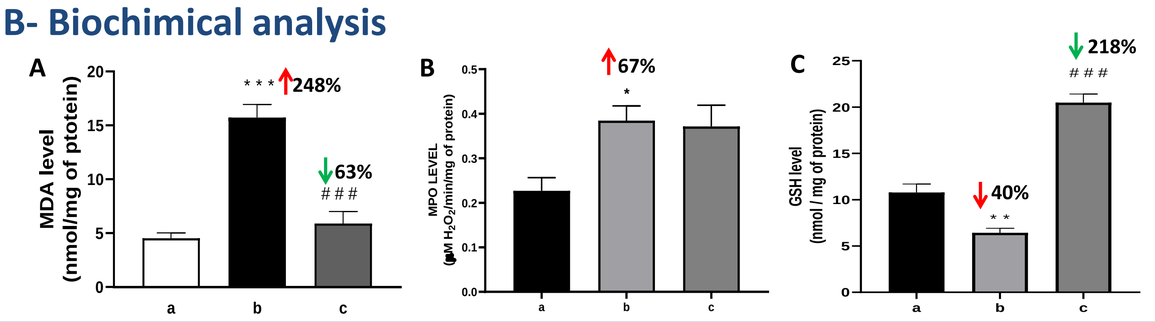


Fig.3. Effect of ATM on the level of MDA (A) and the activity of CAT (B) measured in the colon. The results are given as mean ± standard deviation (P<0.01 vs. Control; ###P<0.001 vs. DMH + As_2O_3). (a) = Ctr, (b)= DMH+ As_2O_3 , (c) = DMH+ As_2O_3 + ATM,**

→ **DMH+ As_2O_3 enhanced** MPO activity and MDA level but **decreased** GSH level.

→**ATM** treatement **reduced** MDA level and increased GSH level while MPO activity was not affected.

Conclusion

→According to these results, ATM warrants further attention as a promising chemo preventive drug for colon cancer.
→The ZnAl-HDL hydrotalcite type compound and the nanohybrid compounds with ATM intercalated showed good intercalation of ATM without having altered its structural integrity.
→ We are continuing our studies to test nanovectors in vivo as well as to determine the molecular pathways underlying the anticancer properties of ATM pathways underlying the anticancer properties of.

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