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RESEARCH ARTICLE

Hematological and toxicological parameters after metformin treatment during pancreatic carcinogenesis

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ABSTRACT

Pancreatic cancer is the fourth-highest cancer killer among both men and women worldwide. In Egypt, pancreatic cancer incidence and mortality rates has been increasing in the last few years due to unknown reasons. The biguanides metformin is the most widely used first-line therapy for type 2 diabetes (T2D). Numerous observational studies have supported a protective role for metformin against a variety of cancer types as breast, liver and prostate cancer. This study aimed to investigate the anti-tumor effects of metformin treatment against pancreatic cancer induced in Syrian golden hamsters.by subcutaneous injection of A/-nitrosobis (2-oxopropyl) amine (BOP). Gemcitabine was used as a conventional drug used for treatment of pancreatic cancer. The results show that metformin has no toxic effect against chemically induced pancreatic carcinogenesis of hamsters caused by BOP induction.

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INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer death, where the five-year survival rate remains at only about 5%. Typical presenting symptoms of pancreatic cancer include abdominal or mid-back pain, obstructive jaundice, and weight loss. About 25% of patients with pancreatic cancer have diabetes mellitus at diagnosis and roughly another 40% have impaired glucose tolerance (Chari et al., 2008). There are five stages of pancreatic cancer, Stage 0 (Tis, N0, M0), Stage IA (T1, N0, M0), Stage IB (T2, N0, M0), Stage IIA (T3, N0, M0), Stage IIB (T1, N1, M0; T2, N1, M0; T3, N1, M0), Stage III (T4, any N, M0), Stage IV (any T, any N, M1). Patients with Stage I/II disease should undergo surgical resection followed by adjuvant therapy while patients with Stage III borderline resectable cancers should undergo neoadjuvant therapy prior to resection (Evans et al., 2008). Patients with stage III locally advanced disease should be treated with chemotherapy and/or chemo radiotherapy. Patients with Stage IV and good performance status may receive systemic therapy and those with poor overall health should be given supportive therapy.

The biguanide metformin is the most widely used first-line therapy for type 2 diabetes (T2D), a diuretic used as an adjuvant for the treatment of diabetes taken by an estimated 150 million individuals worldwide. The first link between metformin treatment and reduced cancer risk in diabetic patients was reported in 2005 (Evans et al., 2005). This finding stimulated a great deal of another research and numerous observational studies which supported the protective role of metformin

against a variety of cancer types as colorectal cancer, hepatocellular carcinomas, prostate cancer and breast cancer.

Gemcitabine is one of the most widely used anticancer drugs in the treatment of several types of solid tumors, including pancreatic cancer, non-small cell lung cancer, breast cancer, head and neck squamous cell carcinoma, and cervical, bladder, ovarian, and thyroid cancers (Mini et al., 2006).

A/-Nitroso (2-hydroxypropyl) (2-oxopropyl) amine (HPOP) and A/-nitrosobis (2-oxopropyl) amine (BOP) proved to be a potent carcinogen in Syrian golden hamsters. The ability of both Syrian golden hamster and Fischer rat pancreatic acinar cells to convert BOP and HPOP to mutagens for V79 cells was investigated in order to examine the basis for species specificity. Acinar cells of both species were capable of activating BOP and HPOP to mutagens for V79 cells in a dose-dependent manner. BOP is a considerably more potent mutagen than HPOP after activation by either cell type. The Syrian golden hamster has proven to be a valuable animal model for investigating the induction of ductal adenocarcinoma of the pancreas (Pour et al., 1974).

The Syrian hamster (Mesocricetus auratus) is a member of the subfamily Cricetinae (Carleton 2005) used in experimental studies of infectious diseases and cancer including, metabolic diseases, non-cancer respiratory diseases, cardiovascular diseases, infectious diseases, and general health concerns. The size of adult animals ranges from 5 to 7 in (13 to 18 cm) long, with a lifespan of two to three years (3–4 years in domestic homes, 2–3 years in the wild). Body mass is in the range of 120-125 g. In 2006-07, Syrian hamsters accounted for 19% of the total animal research participants in the United States (United States Department of Agriculture 2008).

This study aimed to investigate the anti-tumor effects of metformin treatment against pancreatic cancer induced in Syrian golden hamsters.by subcutaneous injection of A/-nitrosobis (2-oxopropyl) amine (BOP).

MATERIALS AND METHODS

Experimental Animals

Sixty healthy, 6-week-old male Syrian Golden Hamsters were obtained from The Holding Company for Biological products & Vaccines (Vaccera), Helwan-Egypt. Animals were housed in plastic cages covered with metal grids with wood chips for bedding and allowed to acclimate for 1 week in the animal house conditions at the Faculty of Science, Tanta University, Egypt before being divided into the experimental groups. The experimental design was approved by the institutional animal care committee at Zoology Department, Faculty of science, Tanta University- Egypt. Target values for temperature and relative humidity were about 22±1ºC and 55±5% respectively, and light- dark (day/night) cycle was achieved. The hamsters were given normal experimental pelleted animal food ad libitium. Animals were carefully observed every day and their body weights.

Chemicals and drugs

N-Nitrosobis (2-oxopropyl) amine (BOP) was a purchased from SIGMA ALDRICH (USA). It was dissolved in physiological saline and injected at two consecutives subcutaneous (s.c.) injections at doses of 60 and 30 mg/kg b.wt. once a week for consecutive 2 weeks, respectively.

The biguanides metformin was purchased from Sigma Aldrich. It was administered into hamster by intraperitoneal (i.p.) injection at 200 mg/ kg d in saline until the animals were killed (Del Prete et al., 1999(. Metformin was administered to hamsters after 1 week of the carcinogen administration and continued to the end of the experiment (26 weeks of start).

Gemcitabine (2'-2'-difluorodeoxycytidine (dFd)) was purchased from Eli Lilly and company Indianapolis (USA). Hamsters were given gemcitabine by i.p. injection twice /week at dose of 25mg/kg b.wt. It was administered to hamsters at the end of first week after the carcinogen administration and will continue to the end of the experiment after 26 weeks of start.

Experimental groups

After 1 week of acclimation period at the animal facility conditions, hamsters were divided according to body weights to minimize the standard errors between groups as follows:
Group I (BOP-treated): hamsters were treated by BOP only by two consecutives s.c. injections at doses of 60 and 30 mg/kg b.wt. at 0 and 1st week respectively. These hamsters were served as positive controls.
Group II (BOP/metformin): hamsters were i.p. administered with metformin at the end of week 1 after the carcinogen administration for 3 times in weeks (200 mg/kg b.wt./day).
Group III (BOP/gemcitabine): hamsters were i.p. administered with gemcitabine for 2 times in weeks (25mg/kg b. wt) at the end of week 1 after the carcinogen administration.
Group IV (PBS treated): non treated hamsters injected with the vehicle (0.09% saline) and served as a negative control group.
Group V (metformin treated): hamsters were administered 0.09% saline then metformin.
Group VI (gemcitabine-treated): hamsters were administered 0.09% saline then gemcitabine.
Fifteen hamsters were used in the first 3 groups and five hamsters for the other 3 groups.

All hamsters t was sacrificed after 26 weeks under excess of ethyl ether anesthesia. Gross examinations were performed macroscopically of all hamsters during sacrifice. Percentages of absolute and relative organ weights (organ wt /b. wt × 100) of all hamsters were taken for the pancreas, livers, kidneys and spleens after organs being necropsied.

Sample collection

Blood samples were collected at necropsy from the abdominal aorta of each hamster from all groups, in either heparinized or non-heparinized glass tubes. Whole blood was used for complete blood count (CBC) test.

Hematological investigations

Complete blood counts (CBC)

Complete blood count was analyzed by the automated method using Dirui BCC-3600, MA, USA automated hematology analyzer.

Statistical analyses

Data were expressed as means ± S.D. and were analyzed by t-test. The data were analyzed by X2 (Chi-squared) test using Graph Pad Prism software, version 6.0, USA. $P<0.05$ was considered as significant value for all the statistical analyses in this study.

RESULTS

Toxicological effects indicated by average body, absolute and relative organs weights

The weekly body weight curves are shown in Figure 1. The initial body weights, final body weights, absolute liver, kidneys, spleen, testis and pancreases weights, as well as calculations for their relative organ weights (%) for hamsters in experiment are shown in Table 1. The final body weights of all groups were significantly decreased when compared to the negative control in group 4. The absolute weights of pancreas were significantly decreased in group 2 compared to positive controls in group 1. The absolute testis weights were significantly decreased in group 2 and 3 as compared to positive controls in group 1. The absolute liver weights were significantly decreased in group 5 as compared to negative controls in group 4.

Hematological investigations of complete blood count

Figure 2 shows data for complete blood count (CBC) in experimental groups. All blood parameters showed no significant changes except for the WBCs numbers of groups 1 and 5 were significantly increased as compared to group 4 while in group 3 was decreased as compared to group 1.

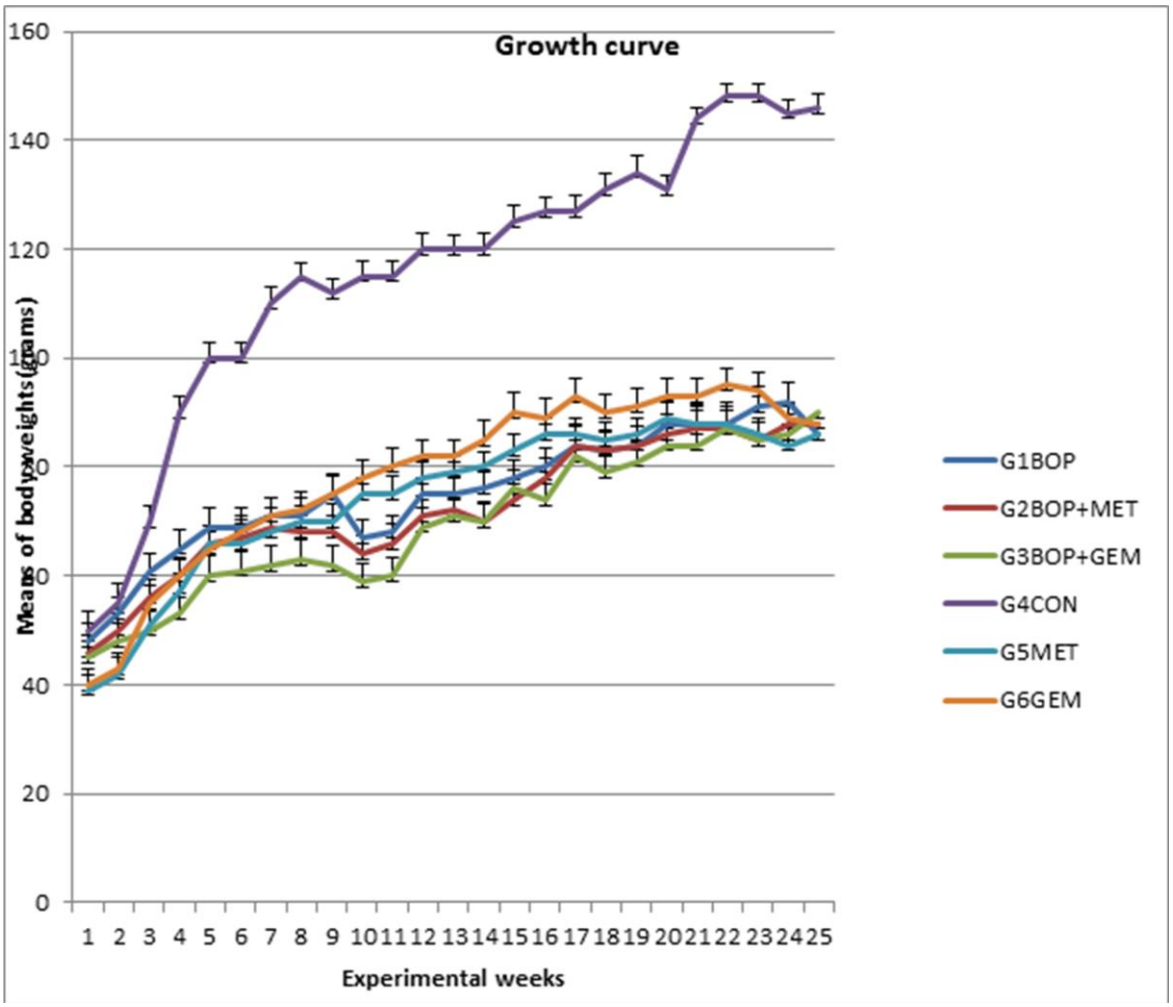

Figure 1: Growth curves of hamsters in the experimental groups

Table 1. Initial and Final Average Body Weights, Absolute and Relative Organ Weights.

Test/group	G1 (BOP)	G 2 (BOP+MET)	G 3 (BOP+GEM)	G4 (Control)	G5 (MET)	G6 (GEM)
No. of animals	15	15	15	5	5	5
Initial b. weighs (g)	48±5.1	46±5.0	45±5.2	50±5.6	39±4.5	40±4.9
Final b. weights (g)	86±8.7	88±8.5	90±8.6	145±12.6	86±8.2	88±8.3
Liver weight	4.08±0.91[a] (4.7)[b]	3.98±1.07 (4.5)	4.15±1.07 (4.6)	5.79±1.16 (3.9)	2.77±0.53* (3.2)	4.59±0.95 (5.2)
Right kidney	0.47±0.18 (0.54)	0.44±0.11 (0.50)	0.39±0.10 (0.43)	0.56±0.23 (0.38)	0.51±0.16 (0.59)	0.45±0.10 (0.51)
Left kidney	0.42±0.11 (0.48)	0.44±0.10 (0.50)	0.38±0.10 (0.42)	0.55±0.13 (0.37)	0.49±0.07 (0.56)	0.43±0.13 (0.48)
Spleen	0.14±0.02 (0.16)	0.21±0.04* (0.23)	0.16±0.02 (0.17)	0.17±0.04 (0.11)	0.16±0.02 (0.18)	0.28±0.12 (0.31)
Pancreas	0.57±0.17 (0.66)	0.16±0.03* (0.18)[b]	0.17±0.07 (0.18)	0.82±0.17 (0.56)	0.14±0.04 (0.16)	0.15±0.058 (0.17)
Testis	1.41±1.08 (1.6)	0.19±0.03* (0.21)	0.34±0.32* (0.37)	2.86±0.60 (1.97)	0.21±0.012 (0.24)	0.45±0.22 (0.51)

Each reading represents Means±S.D. of Hamsters. * Significant vs. group1 at $P<0.05$, * Significant vs. group4 at $P<0.05$, a) the absolute organs wts, b) relative organs wts. (Ratio of organ wt/body wt. X100)

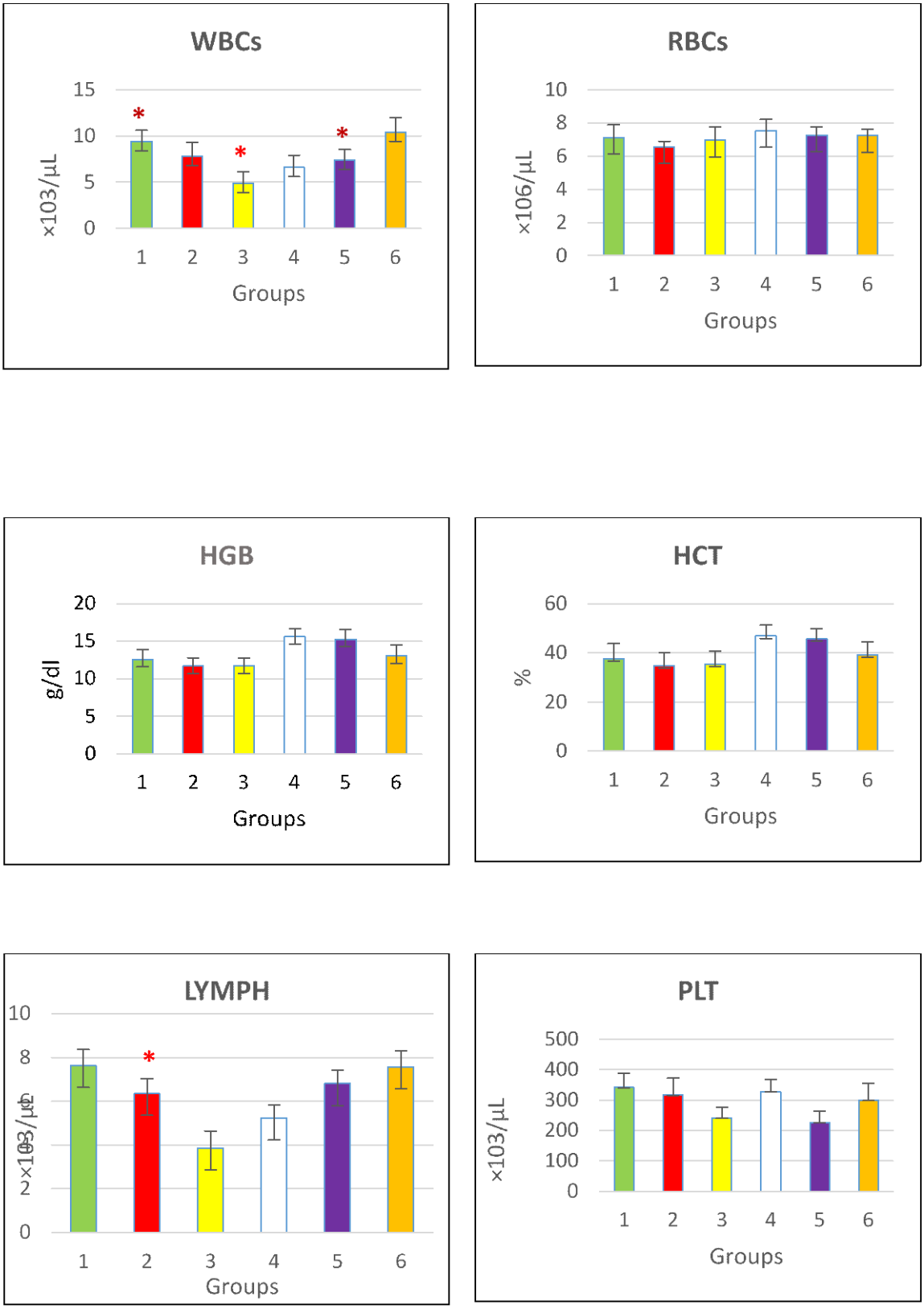

Figure 2. Changes in CBC count parameters in different groups under the study. * Significant vs. group1 at *P*<0.05. * Significant vs. group4 at *P*<0.05

DISCUSSION

The current study showed a robust protective effect of metformin on hematological and toxicological parameters induced in male Syrian golden hamster model by s.c. injection of the carcinogen N-nitrosobis (2-oxopropyl amine) BOP. We compared the results with another drug used for treatment of pancreatic cancer, gemcitabine that is considered as the standard drug for the treatment of pancreatic cancer over the world (Mini et al., 2006).

When hamsters treated with metformin and gemcitabine were compared, we did not observe any significant difference in the final body weights of the animals. However, significant difference was observed for the absolute and relative pancreas weight which were decreased in the metformin treated group (0.16 ± 0.037 (0.18) as compared to the positive group (0.57 ±0.17 (0.66).

In a previous study (Nozawa et al., 2012) the potential effect of the porcine pancreatic extract (PPE) was observed in controlling pancreatic carcinogenesis in the hamster models treated with a single s.c. injection of N-nitrosobis-(2-oxopropyl) amine (BOP) at a dose of 40 mg/kg BW. The body weights in the PPE group were lower, but without significant value (104 ± 21 g; P=0.217), when compared to the control group (109 ± 20 g). In the subsequent weeks neither the body weights differed significantly between the groups, nor was the relative weight of the pancreas (PPE group: 0.27 ± 0.05 g/100g; controls: 0.28 ± 0.21 g/100g; P = 0.735). While in another study (Schneider et al., 2001) a reduced final body weight gain was reported with no coincided effect on the animal physical activity and behavior.

Additional study examined the possible chemopreventive effects of Fermented brown rice by Aspergillus oryzae (FBRA) on N-nitrosobis (2-oxopropyl) amine (BOP)-induced pancreatic tumorigenesis in male Syrian golden hamsters (Kuno et al., 2015). That study found no significant differences in the kidney weight among the groups while the mean body and liver weights of the hamsters in FBRA groups were significantly higher as compared to those of the positive control group (BOP alone).

In the present study, the hematology investigations (CBC analyses) showed no significant difference between the control and treatment groups except for the WBCs in gemcitabine-treated group which showed decrease values as compared to the positive control.

In view of the results presented here, it can be speculated that no toxic effects of metformin treatment against pancreatic cancer induced in Syrian golden hamsters. However, more studies are required to study the anti-tumor effects of metformin on pancreatic cancer.

CONCLUSION

These controversial results might be interpreted with the variation in degree of diabetes, sample size, age, environmental factors or population ethnicity. Also, the type of treatment can affect the level of cytokines. Therefore, it can be recommended that more studies with larger sample size can give results that are more precious.

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