RESEARCH ARTICLE

Effect of Mirtazapine on MNNG-Induced Gastric Adenocarcinoma in Rats

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Abstract

<u>Objective</u>: In this study, anticancer effects of mirtazapine on rats were investigated in an adenocarcinoma model induced by N-methyl-N-nitro-N-nitrosoguanidine (MNNG) and compared with those of cisplatin. <u>Materials and Methods</u>: For this purpose, 10 mg/kg doses of mirtazapine were administered orally to one group of rats, while 1 mg/kg doses of cisplatin were administered intraperitoneally to another group. At 1 hour after administration, 200 mg/kg doses of MNNG were given orally to both groups. MNNG administration was repeated once every 10 days through 3 months, after which period, gastric tissue was taken and pathologically evaluated. <u>Results</u>: Mirtazapine prevented adenocarcinoma induction by MNNG in rats to a greater extent than cisplatin. Some of the rats receiving cisplatin demonstrated severe dysplasia in gastric samples and others exhibited mild dysplasia. Rats given mirtazapine were not observed to suffer severe dysplasia, only mild dysplasia being observed. <u>Conclusion</u>: For adenocarcinoma induced by MNNG on rats, mirtazapine was determined more effective than cisplatin. In order to make statement about mechanism of anticancer activity of mirtazapine, wider studies are required.

Keywords: Gastric cancer - rat - MNNG - mirtazapine - cisplatin - prevention

Introduction

Gastric cancer is one of the most important causes of cancer-associated mortality all around the world (Chan et al., 2001). Adenocarcinomas account for about 90% of gastric cancers. Development of gastric cancer is a complex condition associated with multifactorial etiology (Crew et al., 2006). H. pylori, high-salt diet, smoking, obesity, exposure to radiation and Epstein-Barr virus are among factors which increase cancer risk (Tatematsu et al., 1975; Thompson et al., 1994; Levine et al., 1995; Chow et al., 1998; Hishida et al., 2010; Liu et al., 2012). In addition, life style and environmental factors have a considerable amount of contribution in etiology of cancer (Crew et al., 2006). Among these, the most frequently indicated factor in cancer patients is major depression. Major depression affects quality of life and treatment compliance of the patient; in time, it also affects severity, prognosis and treatment response of cancer (Berard, 2001). Studies show that risk of mortality is high in cancer patients with major depression (Onitilo et al., 2006). Therefore, treatment of major depression in cancer patients is crucial. Depression is not only seen in cancer disease; it's also seen during other diseases which are not associated with cancer, e.g.

depression associated with psychosomatic and somatic symptoms was seen in majority of patients with GIS diseases (Guldahl, 1977).

Tatsuta et al. (1989) reported that supression of sympathetic nervous system inhibits carcinogenesis while stimulation of sympathetic nervous system activity increases carcinogenesis (Tatsuta et al., 1989;1992). It's well known that main mechanism of action of antidepressant agents is oriented to increase noradrenergic, serotonergic and dopaminergic activity (Baldessarini, 2005). It's reported that nialamide, a monoaminooxygenase inhibitor, increases incidence of cancer by causing an elevation in norepinephrine concentration of gastric wall (Tatsuta et al., 1989). It's known that norepinephrine and other catecholamines induce their effects through α and ß adrenergic receptors (Baldessarini, 2005). Increase in incidence of gastric cancer was observed following long-term administration of α adrenoreceptor agonists (Iishi et al., 1998). Besides, it was determined that phenylephrine, an α receptor agonist, increases severity of gastric cancer induced by MNNG (Tatsuta et al., 1998). Data from literature indicates that administration of antidepressant activity through α adrenergic receptor blockage in combination with antidepressant therapy may

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be beneficial.

In due course, purpose of our trial is to investigate effect (if any) of mirtazapine, an antidepressant with α adrenergic receptor blocking activity, on MNNG-induced gastric adenocarcinoma in rats.

Materials and Methods

Experimental animals

Rats used in our trial were supplied by Ataturk University Experimental Medical Application and Research Center. Thirty male rats of albino wister species with weights between 110-120 grams were used during this experiment. During pre-trial period, rats were kept and fed in groups in the laboratory at room temperature (22° C).

Chemicals

N-methyl-N0-nitro-N-nitrosoguanidine (MNNG), thiopental sodium, cisplatin and mirtazapine for this trial were obtained from ABRC (Germany), IE Ulagay (Turkey), Koçak Farma (Turkey) and Organon (Turkey), respectively.

Effect of Mirtazapine on MNNG-induced gastric adenocarcinoma in rats

In rats, N-methyl-n-nitrosoguanidine is utilized to induce gastric adenocarcinoma (Sugimura et al., 1998). In this trial, oral mirtazapine was given in doses of 10 mg/kg in one group of rats while intraperitoneal (IP) cisplatin in doses of 1 mg/kg was administered to another group. Distilled water was given to rats in control group as vehicle. One hour after administration of drugs, oral MNNG of 200 mg/kg was administered in all rat groups through a catheter. MNNG administration was repeated every 10 days for three months (Suleyman et al., 2010). Mirtazapine and cisplatine were administered daily, as indicated above, for three months. At the end of third month, rats were exterminated by high dose anesthetics (thiopental sodium 50 mg/kg) and stomachs were dissected. After initial macroscopic examination, histopathological (microscobic) examinations were performed on gastric samples. Gastric samples of drug-administered rat groups were compared with the control group to evaluate anticancer activity.

Histopathological examination

Gastric tissue samples were fixated in 10% formaline solution for one day. On fixated gastrectomy materials, samples were obtained from areas where mucosa folds were flattened or irregular. These samples were dehydrated by alcohol solutions at various degrees and embedded in paraffine. Afterwards, cross-sections of 5 mm were obtained. These cross-sections were stained by H&E and examined under light microscobe olympus Bx-51.

Results

In macroscobic examination, protuberant and firm thickening was seen in certain areas of gastrum (corpus, fundus) in control group who were fed MNNG only. Gastric tissue lost its elasticity; it was firm and thickened. No ulcerative defect was observed in gastric mucosa. Mild thickening in corpus and fundus were seen in mirtazapine-administered rats. Gastric tissue was more elastic as compared to control group. Among some rats in cisplatin group, thickening and protuberation in gastric tissue were more prominent, while it was less severe in others. Mildly protuberant and firm gastric tissues were more elastic as compared to other samples.

Microscopic examination

As seen in Figure 1A, atypical epithelial cells with large hyperchromatic nuclei, prominent nucleoli and enlarged eosinophilic cytoplasm with no polarity constituted gland-like structures in stomach of MNNG-administered control group rats. These findings (only in gastric tissue samples of MNNG-administered control group rats) were considered as gastric carcinoma in situ. Mild dysplasia was seen in gastric tissue samples of mirtazapine-administered rats (Figure 1B). In cisplatin group, severe dysplasia was determined in some rats while mild dysplasia was seen in others (Figure 1C).

Discussion

In our trial, effect of an antidepressant, mirtazapine, on MNNG-induced gastric adenocarcinoma in rats
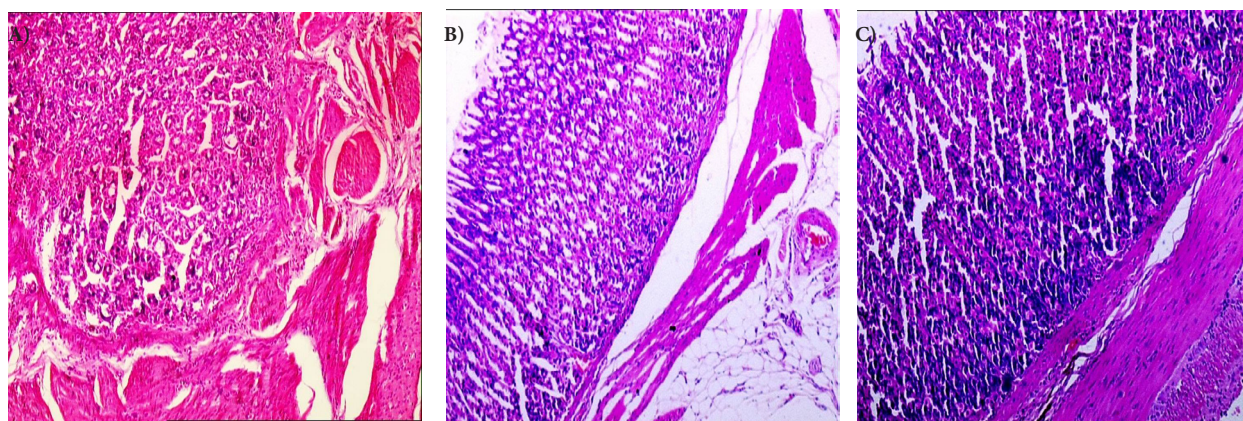

Figure 1. Light Microscopic Appearance of Stomach Sample from A) Control Group that Received MNNG Only, B) Rat Group that Received Mirtazapine and MNNG, C) Rat Group that Received Cisplatin and MNNG.

was investigated. Anticancer activity of mirtazapine was evaluated by comparison with cisplatin. Results showed that mirtazapine prevented MNNG-induced adenocarcinoma in rats. It was determined that supression of development of adenocarcinoma was higher with mirtazapine as compared to cisplatin. In gastric samples of some of the cisplatin-administered rats, severe dysplasia was observed whereas in others, mild dysplasia was seen. No severe dysplasia was seen in gastric samples of mirtazapine-administered rats. In gastric samples of all mirtazapine-administered rats, only mild dysplasia was observed.

In the early 1980s, guidelines for the diagnosis and grading of GED were developed and a three-tiered classification of mild, moderate and severe dysplasia was proposed. Dysplasia was defined as "unequivocally neoplastic epithelium that may be associated with or give rise to invasive adenocarcinoma" (Morson et al., 1980; Ming et al., 1984; Ren et al., 2011). Gastric dysplasia is usually observed on the basis of long-term chronic irritation of mucosa. Extent of dysplasia is parallel to severity of chronic irritation (Owen, 1997). Gastric dysplasia is the gastric lesion most frequently associated with development of gastric carcinoma. In practice, it may be regarded as an atypical differentiation of gastric mucosa with a potential for malignancy (Fenoglio-Preiser, 2000; Carter, 2004). In classification of low, middle and high grade cases, it was determined that a great majority of low-grade dysplasia cases regress in time. This indicates that these lesions rather have a reactive nature. On the contrary, it was shown that only 5% of high-grade dysplasia cases regress while 80% progress to carcinoma (Bearzi et al., 1994).

In literature, it was suggested that increase in sympathetic nervous system activation may be associated with an increase in gastric carcinogenesis (Tatsuta et al., 1991). Diet low in calcium causes a considerable increase in incidence of gastric cancer by elevating norepinephrine concentration in the gastric wall (Tatsuta et al., 1993). Thyrosine methyl ester also produced a carcinogenic effect by increasing norepinephrine concentration in gastric wall, without affecting gastric pH or serum gastrin levels (Tatsuta et al., 1991). Severity of MNNG-induced cancer was found to be higher than animals in whom DOCA+NaCI was administered (Tatsuta et al., 1991). In DOCA+NaCI-administered animals, blood pressure was elevated and this elevation was associated with increase in cathecolamine synthesis (Champlain et al., 1969). On the other hand, in some trials, it was shown that supression of sympathetic system has no role in anticancer activity; e.g. it was found that clonidine, a sympatholytic agent, has no anticancer activity (Tatsuta et al., 1998). This, in turn indicates that sympatholytic effect is not important in terms of anticancer activity. Besides, it's well known that there is no association between antidepressant effect and sympathetic activity. This can be further explained by existence of antidepressant agents which increase noradrenergic and dopaminergic activity (amoxapine, reboxetine) and, on the other hand, by existence of agents which have no effect (trimipramin) or even agents which supress this activity (tianeptin) (Baldessarini, 2005).

Anticancer activity of mirtazapine may not be due to its antidepressant effect because relevance of importance related to antidepressant activity seen in humans for animals is not known. It was suggested that use of antioxidants decreases risk of cancer. On the other hand, cancer can not be prevented by using natural or synthetic nutritive substances or drugs with antioxidant properties. Even though it was reported that antioxidants like vitamin C, E and carotene may decrease risk of gastric cancer (Crew et al., 2006), degree of efficacy against cancer is controversial.

In experimental animals, it was shown that mirtazapine supresses oxidant parameters and increase antioxidant parameters in gastric tissues (Bilici et al., 2009). It was found that mirtazapine and a number of antidepressant agents have a preventive effect in gastrum (Bilici et al., 2009; Dursun et al., 2009; Suleyman et al., 2009). Nevertheless, this does not mean that gastroprotective properties are associated with antidepressant activity. This is further supported by the fact that certain antidepressant drugs from the same group do not have gastroprotective effects or even cause gastric damage (Suleyman et al., 2009). In this trial, we argue that mirtazapine possess anticancer activity in addition to antidepressant and α antagonistic properties. It was shown that mirtazapine is not a toxic agent and it even prevents (antagonizes) toxic effect of indomethacin on gastric tissue. On the other hand, drugs used in cancer therapy are known as cytotoxic agents (Chabner et al., 2006). In conclusion, it was found that prevention against MNNG-induced gastric adenocarcinoma model in rats is more prominent by mirtazapine, as compared to cisplatin. Further extensive studies are required in order to explain mechanism of action of mirtazapine related to anticancer activity.

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