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Evaluation of the analgesic activity and safety of ketorolac in whole body fractionated gamma irradiated animals

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ABSTRACT

This study was performed to evaluate the analgesic activity and the toxicity of ketorolac in normal and fractionated (1.5 Gy/day/4 days) γ-irradiated animals. Determination of brain serotonin content and serum prostaglandin level were also undertaken. The analgesic activity was tested using formalin test, at three dose levels (15, 30 and 60 mg/kg) after 1 and 7 days post radiation exposure. LD_{50} determinations and assessment of liver and kidney function tests were performed. Our results indicated marked analgesic effects on the early and late phases of nociception. Double treatment with ketorolac and irradiation increased brain serotonin content. The acute LD_{50} of ketorolac was decreased in irradiated animals as compared to the LD_{50} of normal animals. Double treatment with ketorolac and irradiation induced an elevation of gastric mucin content, urea and BUN levels on the 1st day post irradiation, whereas, albumin level was lowered and globulin level was elevated after 7 days post irradiation. Depending on this study the dose of ketorolac used for treating cancer patients addressed to radiotherapy should be reduced, however, this requires further clinical confirmation.

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1. Introduction

Radiotherapy is the most common modality for treating human cancers. About 80% of the patients need such treatment either for curative or palliative purpose [1]. Radiation exposure may produce many harmful effects on living organisms [2]. Gamma irradiation initiates chemical changes in the cellular components as a result of immediate consequence of the penetration of ionizing radiation through the living systems. The biological effect differs according to irradiation dose [3]. It was observed that renal damage may be produced as evidenced by the reduction of renal function at all doses above 4 Gy [4]. Furthermore, the whole body exposure of rats to γ-radiation at a dose of 3.5 Gy causes an increase in the levels of AST and ALT [5].

Pain is one of the most common associated symptoms of cancer which may be a result of tumors expansion, nerve compression, infiltration by the tumor, or infections in malignant ulcers [6]. Radiation exposure may also lead to pain in which chemical stimuli are released from the damaged cells [7]. These stimuli act on the nociceptors resulting in central or peripheral pain. However more than 80% of those patients treated with radiation report an analgesic effect, usually during the first days after treatment, that is sustained for months thereafter [8,9]. Theories, mostly originating from clinical studies, attempting to explain the antinociceptive effect of radiation include a decreased number of carcinomatous cells, an inhibition of osteoclasts, decreases in local algogenic substances, immune response, and neural transmission [10–12].

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most useful medications for peripheral nerve injury as it is accompanied by inflammatory response. This response involves increased production of prostaglandins (PGs) which enhances sodium current and calcium influx in peripheral nociceptive neurons [13] and increased central neurotransmitter release and depolarization of second order nociceptive neurons. NSAIDs inhibit cyclooxygenase COX-1 or COX-2 or both and block the production of prostaglandins [14,15]. Ketorolac is a non-selective COX − inhibitor [16], may be strong enough to be used in treating cancer pain.

Therefore the aim of the present study was to evaluate the analgesic activity of ketorolac in relation to pain mediators (brain serotonin and serum prostaglandin) which associated with pain perception. Drug safety in terms of toxicological examination will be also performed in normal and irradiated animals.

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2. Material and Methods

2.1. Chemicals

Ketorolac is a pyrrolizine carboxylic acid derivative used as ketorolac tromethamine and was obtained from Amrya Pharmaceuticals Company, Alexandria, Egypt. It was used as an aqueous solution. Other chemicals were obtained from Sigma Aldrich Chemical (Egypt). Kits used in these experiments were purchased from Bio-Diagnostics (UK).

2.2. Animals

Male albino mice each weighing 20–25 g were used for toxicological studies (acute LD_{50} determination), while male Wistar rats, each weighing 120–150 gm, were used for pharmacological, biochemical and histopathological studies. Animals were obtained from the animal breeding unit of the National Research Centre, Giza, Egypt. They were housed under appropriate conditions of controlled humidity, temperature and light. Animals were allowed free access to water and fed a standard pellet rat diet. They were acclimatized in the animal facility of the National Centre for Radiation Research and Technology (NCRRT)-Atomic Energy Authority, Cairo, Egypt for at least one week before subjecting them to experimentation. This study was conducted in accordance with the guidelines set by the European Economic Community (EEC) regulations (Revised Directive 86/609/EEC) and approved by the Ethical Committee at NCRRT.

2.3. Radiation process

Animals were exposed to fractionated whole body irradiation at dose of 6 Gy divided into 4 doses (1.5 Gy/day) daily, using gamma rays by Cesium 137 irradiation unit, National Center for Radiation Research and Technology (NCRRT), with the dose rate 0.758 rad/sec. The radiation process of animals has been carried out at the central position of the sample chamber using a special designed polyethylene plates with a polyethylene cover. This place is actually calibrated using alanine dosimeter relative to a primary standard.

2.4. Experimental design

2.4.1. Anti-nociceptive activity

2.4.1.1. Formalin test in rats. It was performed according to the method described by Dubuisson & Dennis [17] and modified by Tjolsen et al. [18] Injection of 50 μl of 2.5% formalin solution subcutaneously in the rat paw induced a licking response. Animals (n = 6)were divided into three groups, the first group is non-irradiated (control), and the second and third groups were exposed to fractionated gamma irradiation (1.5 Gy × 4 days) to attain total dose of 6 Gy. The test was carried out after 1 day for the second group and 7 days for the third group. Each of these groups were sub-divided into four subgroups each consists of six rats and received the same treatment as follows: 1st subgroup represent control group and received saline, the 2nd, 3rd and 4th subgroups received i.p injection of ketorolac at dose levels of 15 mg/kg, 30 mg/kg and 60 mg/kg respectively. One hour after treatments, rats were injected with 50 μl of 2.5% formalin solution subcutaneously under the plantar surface of the left hind paw. They were then placed under observation for half an hour. The severity of the pain response was evaluated by recording and counting the licking response. Antinociceptive effect was determined in two phases, the early phase from 0 to 5 min whereas; the late phase from 15 to 30 min with lag period of 10 min between the two phases [19].

2.4.2. Acute LD_{50} determinations

Lethal dose 50 (LD_{50}) was detected according to Spearman-Karber method [20] using male Swiss albino mice (20–25 gm). The test was carried out in normal and irradiated (1.5 Gy × 4 days) animals.

2.4.3. Biochemical evaluation

2.4.3.1. Experimental procedure. In this study animals were divided into seven groups each of 6 rats. Animal groups were classified as follows **control group:** received saline. **Control irradiated (1 day) group**: exposed to fractionated whole body gamma irradiation with total dose of 6 Gy (1.5 Gy daily × 4 days). **Ketorolac (1 day) treated group**: given Ketorolac as a single dose of 30 mg/kg. **Ketorolac treated irradiated (1 day) group:** exposed to a fractionated dose of 6 Gy (4 × 1.5 Gy) and then animals were treated with ketorolac (30 mg/kg) as a single dose. **Control irradiated (7 day) group**: exposed to fractionated whole body gamma irradiation with the dose of 6 Gy (4 × 1.5 Gy) and sacrificed after 7 days. **Ketorolac treated (7 day) group**: given Ketorolac dose of 30 mg/kg daily for 7 days. **Ketorolac treated irradiated (7 day) group:** exposed to a fractionated dose of 6 Gy (4 × 1.5 Gy) and then animals were treated with ketorolac (30 mg/kg) daily for 7 days. Ketorolac dose level of 30 mg/kg was selected based on the study of J.-P Vit et al. [21] such dose level was the best effective dose tested in the formalin test which used for the evaluation of the analgesic activity of ketorolac in this study.

2.4.4. Collection of blood and serum preparation

At the end of each study, all rats involved in the biochemical studies were anesthetized with urethane at a dose of 1.2 g/kg [22], blood samples were collected by heart puncture in dry centrifugation tubes and centrifuged at 3000 rpm for 20 min at room temperature using universal 16R/Germany centrifuge. Serum was separated and used for determination of biochemical parameters involved in this study.

2.4.5. Tissue sampling and collection of gastric juice

Brain was excised from the rats and both brain cortex and brain stem areas were homogenized using a homogenizer (universal laboratory AID type MPW- 309, Poland). Brain homogenate was used for evaluation of serotonin content in brain cortex and stem areas. Whereas, gastric juice was collected and centrifuged, the supernatant was used to measure mucin conte.

2.4.5.1. Biochemical determinations. Brain homogenate was used for determination of serotonin content in both brain cortex and stem areas using HPLC technique according to the method of Pegal and Wolf [23].

Serum prostaglandin E_2 ($PG E_2$) level was measured according to the method described by Green et al. [24].

Gastric juice was collected and centrifuged; the supernatant was used to measure mucin content according to the method of Winzler [25].

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes as well as serum concentrations of total protein, albumin, globulin, urea and creatinine in serum were evaluated. Total protein content was assayed by Biuret method [26], albumin content was determined by the method of Doumas et al. [27] ALT and AST activities were examined according to the method of Reitman and Frankel [28]. Serum urea was also performed according to the method of Kaplan [29]. Creatinine level was evaluated according to the method of Tietz [30]. BUN was calculated from serum urea level using the following equation:

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