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Low dose aspirin like analgesic and anti-inflammatory activities of mono-hydroxybenzoic acids in stressed rodents

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ABSTRACT

Aims: To compare analgesic and anti-inflammatory activities of aspirin and mono-hydroxybenzoic acids after their daily oral doses.
Main methods: Efficacies of repeated daily stress response suppressing low oral doses (20 mg/kg) of aspirin and 2-, 3-, and 4-hydroxybenzoic acids in mice hot plate test for centrally acting analgesics, and in acetic acid induced writing test were compared. Effects of their same daily doses and treatment regimen in cotton pellet granuloma and carrageenan edema test for anti-inflammatory drugs in stressed rats were compared in a second experiment. Effects of treatments on body weights, basal rectal temperatures, organ weights and plasma glucose, insulin and cortisol levels in stressed animals were compared also.
Key findings: Although stress response suppressing effects of aspirin and all the three hydroxybenzoic acids in both mice and rats were almost equal, effectiveness of 3- and 4-hydroxybenzoic acids as analgesic and anti-inflammatory agents were lower than those of aspirin or salicylic acid.
Significance: Observations made after single oral doses of aspirin or of mono-hydroxybenzoic acids are not very reliable predictors of their pharmacologically interesting bioactivity profiles and efficacies. Prostaglandin synthesis inhibition is not involved in low dose anti-inflammatory activities of 3- and 4-hydroxybenzoic acids. After their repeated daily low oral doses they are almost as potent stress response desensitizers as aspirin or salicylic acid.

1. Introduction

Long before analgesic and anti-inflammatory activities of aspirin became known, numerous plants enriched in 2-, 3- or 4-hydroxybenzoic acids and their metabolic precursors have been used for prevention and cure of diverse chronic diseases. Amongst them the 2-hydroxybenzoic acid (salicylic acid) is pharmacologically the most well studied one, and it is now well recognized that aspirin and other salicylic acid derived products can be used for prevention and cure of diverse metabolic disorders, including cancer, and associated mental health problems [1]. Salicylic acid is a plant hormone also involved in ecological survival processes of numerous plants [2], and together with 4-hydroxybenzoic acid it is often considered to be a bioactive constituent of numerous traditionally known medicinal plants often pharmacologically classified as adaptogenic or stress resistance improving herbs [3, 4]. Although the 3-hydroxybenzoic acid is also encountered in several plants often used in traditionally known systems of medicine and dietary therapies, it still remains to be one the least pharmacologically explored one [5, 6].

In all dietary and traditionally known herbal therapies, plant phenolics are regularly used for medicinal or health care purposes, and it is now well recognized that repeated daily oral doses of plant extracts enriched in them is necessary for observing their therapeutically interesting bioactivities in animal models [7, 8]. Observations in our laboratories have revealed that repeated daily oral doses of aspirin or of mono-hydroxybenzoic acids lower than 30 mg/kg can suppress diverse physiological stress responses triggered by daily handling and repeated testing, or on repeated exposures of experimental animals to stressful experimental conditions [3, 9]. Their daily dose dependant activity profiles in suppressing diverse adaptive stress responses quantified in those experiments, or as potential antidepressants or anxiolytics, were not always identical, and depended also on the numbers of treatment days.

Biological processes and mechanisms regulating adaptive stress responses, or allostatic load, are also involved in etiology, pathogenesis and progression of numerous chronic diseases commonly associated with inflammation and pain [10–12]. Preventive potential of regular intake of low dose aspirin against cardiovascular and other diseases are well recognized, and its diverse brain function modulating effects are now also well known [1]. However, as yet very little concentrated efforts have been made to assess anti-inflammatory and analgesic

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activities of low dose aspirin or to compare its efficacies with salicylic and other two naturally occurring mono-hydroxybenzoic acids. It has recently been reported though, that unlike several other phenolic acid with anti-inflammatory activities in animal models, the 3- or 4-hydroxybenzoic acids do not inhibit prostaglandin synthesis [13].

Results of experiments conducted to verify the possibility that stress response desensitizing low daily oral doses of the three mono-hydroxybenzoic acids and aspirin are also effective in conventionally known rodent bioassays for aspirin like anti-inflammatory drugs are described and discussed in this communication. Choices of the doses and treatment regimen used in the experiments were based on the observations made in our earlier exploratory experiments [3, 10]. Stress biomarkers and other parameter quantified were also same as those used in earlier studies with mono-hydroxybenzoic acids and other known bioactive plant metabolites and extracts [3, 10, 14, 15].

2. Materials and methods

2.1. Animals

Adult male Swiss mice (20 ± 5 g) and wistar rats (150 ± 50 g) were obtained from Central Animal House of Institute of Medical Sciences, Banaras Hindu University, Varanasi (Registration number Dean/2014/CAEC/607). At least one week before starting the experiment all animals were acclimatized to constant laboratory conditions. They were randomly selected and group-housed (six animals per cage) in polypropylene cages (28 × 19 × 12.5 cm) maintained at an ambient temperature (25 ± 1 °C) and relative humidity (50 ± 10%) with a 12:12 h light/dark cycle (light on at 06:00 and off at 18:00). Animal cages were provided with husk and they were routinely cleaned. Except when mentioned, all animals were always provided with standard rodent diet and water *ad libitum*. Prior approval from the Central Animal Ethical Committee of the University was obtained (Dean/2014/CAEC/344, dated May 30, 2014) for experimental protocols. In both the experiments, six randomly selected groups of six animals each were used, and all experimental groups in a given experiment were tested in parallel (*i.e.* on the same days of the experiments). A trained observer made the observations blind to the treatments given to the animals, and body weights and basal rectal temperatures of all animals were recorded (before drug administration) on all observational days. Except when mentioned, all tests were conducted 1 after the day's oral treatments.

2.2. Drugs, chemicals, and test kits

Aspirin, salicylic acid, 4-hydroxybenzoic acid and 3-hydroxybenzoic acid were purchased from HiMedia laboratories Pvt. Ltd. Mumbai, India; carboxymethyl cellulose (CMC) from Central Drug House Pvt. Ltd., New Delhi, India; acetic acid and carrageenan from Sigma Aldrich, Bengaluru, India. Plasma glucose level was estimated by biochemical enzyme test kit (ERBA diagnostics Mannheim GmbH, Germany) and plasma insulin level was estimated using Enzyme-Linked Immunosorbent Assay (ELISA) test kit (Chemux BioSciences, Inc, USA). Plasma cortisol was estimated using ELISA kit (DSI S.r.l., Italy). All other chemicals and reagents used were from other laboratory suppliers and of highest analytical quality available in India.

For oral administrations, aspirin and 2-, 3- and 4-hydroxybenzoic acid were suspended in 0.3% CMC, and the application volume was always 10 ml/kg. Except when mentioned, the control groups of the experiments were always similarly treated with 0.3% CMC only.

2.3. Design of the experiments

Male mice used for comparing analgesic activities of aspirin and the three mono-hydroxybenzoic acids were preselected (one day before the start of the experiment) for their reaction times on a hot plate maintained at 55 ± 1 °C. Only those mice reacting within 15 s after placing them on the hot plate on the pre-selection day, and which did not show large variation when tested on four separate occasions (each 15 min apart) on that day, were randomly assigned to the six test groups used in this experiment. Although this pre-selection procedure is necessary for improving reproducibility of observation, such animals are also mildly stressed and repeatedly handled ones. One of the experimental groups (the reference group) was further handled and daily treated orally with 0.3% CMC, but was not subjected to hot plate test on the days 1, 5, 7 and 10 of the experiment. The others were treated daily either with 0.3% CMC (control group), or with 20 mg/kg/day of aspirin, or 2-, or 3-, or 4-hydoxybenzoic acids for 12 consecutive days and subjected to hot plate test on those days of the experiment. Except for the reference group, all others were subjected to acetic acid writhing test on the 11th experimental day. On the 12th day of the experiment, all animals of all groups were subjected to the tail suspension test for evaluating antidepressant activity, and the day thereafter they were sacrificed (without treatments) for estimating their blood glucose, insulin, and cortisol levels and weights of their spleen and adrenal glands.

In another experiment conducted to compare anti-inflammatory activities of aspirin with mono-hydroxybenzoic acids in stressed rats, a similar experimental procedure was used (see Fig. 1b). Except for the animals of the CMC treated reference group used in this experiment, pre-weighed (50 ± 1 mg) cotton pellets (for cotton pallet granuloma test described later) were implanted (one day before the 1st day of the experiment) in all other animals of other groups, and the reference group was not subjected to foot shock stress triggered hyperthermia test on days 1, 5, 7 and 10 of the experiment. The other five groups were daily treated (orally) either with CMC (control group), or with 20 mg/kg/day aspirin, or 2-, or 3-, or 4- hydroxybenzoic acids for 12 consecutive days and subjected to foot shock stress induced hyperthermia test on those days of the experiment. On the 11th observational day, all animals of all groups were subjected to carrageenan induced paw edema test. On the next day (1 h after the days treatments) animals of the five cotton pellet implanted groups were weakly ether anesthetized for removing the their cotton pellets covered by granuloma tissue. On this last day of the experiment, all animals of all groups were sacrificed by decapitation for estimating their blood glucose, insulin and cortisol levels, and weights of their spleen and adrenal glands.

2.4. Experimental procedures

2.4.1. Hot plate test

This test was conducted 1 h after recording their basal core temperatures (using a rectal probe and calibrated electronic thermometer) and oral treatments on the 1st, 5th, 7th and 10th day of the experiments. In this test, an individual mouse of a group was placed on a hot plate maintained at 55 ± 1 °C and its reaction time in seconds for forepaw licking or jumping was recorded [16]. For preventing any thermal injury, maximum time the mouse was allowed to stay on the hot plate was 30 s. Immediately after the test, the mouse was returned to its home cage, and 10 min thereafter its core temperatures were recorded again. The animals of the CMC treated reference group were not subjected to hot plate test, but were also placed on a similar plate maintained at room temperature for 15 s and then returned to their home cage and 10 min thereafter their temperatures were also recorded again.

2.4.2. Acetic acid writhing test

On the 11th day of the experiments, and 1 h after oral treatments and basal core temperature measurements, all mice of all experimental groups (except those of the REF group) of the experiment were intraperitoneally injected with 0.7% (v/v) aqueous acetic acid (10 ml/kg). Number of writhes for the following 5 min of observational period was counted [17].