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Short communication

Hyperbaric oxygen treatment reduces carrageenan-induced acute inflammation in rats

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Abstract

The present study was designed to assess the anti-inflammatory activity of hyperbaric oxygen treatment by comparing it with that of diclofenac, a nonsteroidal anti-inflammatory drug, and also to investigate whether hyperbaric oxygen treatment enhances the anti-inflammatory effect of diclofenac in carrageenan-induced paw edema which is commonly employed as an acute inflammation model in rats. Hyperbaric oxygen treatment and diclofenac (20 mg/kg) markedly reduced the carrageenan-induced paw edema in rats. In other words, they displayed anti-inflammatory activity. On the other hand, hyperbaric oxygen treatment did not consistently modify the anti-inflammatory effect of diclofenac in this model.

Keywords: Hyperbaric oxygen; Diclofenac; Carrageenan; Inflammation, acute; (Rat)

1. Introduction

Hyperbaric oxygen treatment is currently used in various conditions such as carbon monoxide intoxication, air embolism, radiation osteonecrosis, osteomyelitis, necrotizing fasciitis and traumatic peripheral ischemia (Caplan, 2000). Through a significant elevation of tissue oxygen concentration, hyperbaric oxygen aids in treating these pathological conditions (Leach et al., 1998).

Despite extensive studies conducted on the involvement of hyperbaric oxygen treatment in various disorders, there is only limited information about its effect on inflammatory response. Interestingly, Lukich et al. (1991) reported that hyperbaric oxygen treatment considerably diminishes clinical symptoms in patients with rheumatoid arthritis responding poorly to nonsteroidal anti-inflammatory drugs (NSAIDs). Warren et al. (1979) also showed that hyperbaric oxygen treatment suppresses adjuvant arthritis in rats. However, to the best of our knowledge, hyperbaric oxygen treatment has not yet been studied extensively in acute inflammation models.

Thus, the present study was undertaken to assess the anti-inflammatory activity of hyperbaric oxygen treatment by comparing it with that of diclofenac, a NSAID, and also to investigate whether hyperbaric oxygen treatment alters the anti-inflammatory effect of diclofenac in carrageenan-induced paw edema which is commonly employed as an acute inflammation model in rats.

2. Materials and methods

2.1. Animals

Adult male Sprague Dawley rats (200–250 g), obtained from the Experimental and Medical Research Centre of Istanbul University, were housed in a temperature-controlled (21 ± 1 °C) colony room, with 12 h light/dark cycle 1 week before the experiments. The animals were handled according to guidelines for research on experimental pain with conscious animals (Zimmermann, 1983) and in compliance with EEC Council Directive 86/609.

2.2. Drugs

Carrageenan (Sigma) and diclofenac sodium (Ciba-Geigy) were used. Diclofenac, freshly dissolved in physio-

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logical saline, was injected intraperitoneally (i.p.,) in doses of 10 and 20 mg/kg in a volume of 10 ml/kg body weight. The doses quoted refer to saline. Control rats were treated with saline (10 ml/kg).

2.3. Hyperbaric oxygen treatment

Hyperbaric oxygen treatment was carried out in hyperbaric chamber. The pressure and duration of hyperbaric oxygen treatment were selected according to the previous protocol (Jain, 1990), which does not cause toxic effects. Each hyperbaric oxygen exposure lasted 90 min at 2.4 absolute atmosphere pressure with 10 min of air and 20 min of 100% oxygen breathing periods.

2.4. Carrageenan-induced acute inflammation

The rats were fasted for 18 h but had free access to water. Inflammation was induced by intraplantar injection of 0.05 ml homogenous suspension of 1% carrageenan in pyrogen-free saline into the right hind paw, as described by Vinegar et al. (1987).

Paw volume up to the ankle joint was measured by means of a volume displacement method using a plethysmometer (Ugo Basile, Italy). Basal volume of each rat paw was taken as 100% and variations from this volume were given as percentage differences.

2.5. Experimental procedure

On the test day, the basal volume of each rat paw volume was measured. Then, either saline or diclofenac was administered 30 min before the carrageenan injection. Hyperbaric oxygen treatment was initiated immediately after carrageenan injection and lasted 90 min for corresponding groups. Following carrageenan challenge, paw volume was measured at second, third, fourth, fifth and sixth hours in order to determine the inflammatory activity. Each group consisted of five to eight rats.

2.6. Data analysis

The results expressed as a means ± S.E. One-way analysis of variance (ANOVA) was conducted to analyse the influence of various treatments on the development of paw edema after carrageenan injection. Statistical significance was determined by Student's *t*-test and the differences of $P < 0.05$ were considered statistically significant.

3. Results

As shown in Fig. 1, hyperbaric oxygen and 20 mg/kg diclofenac markedly reduced the carrageenan-induced edema in rat paw. The inhibition started 2 h after carrageenan injection and was maintained throughout the

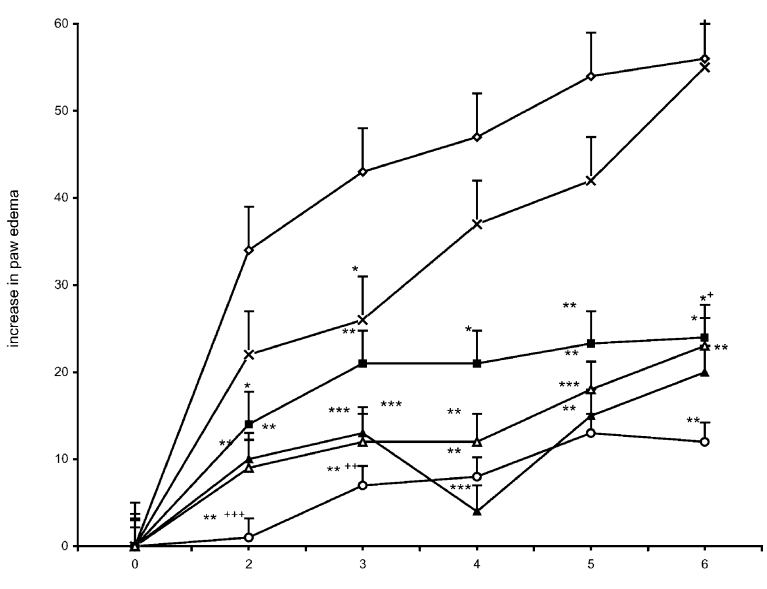

Fig. 1. The effects of saline (◇), hyperbaric oxygen (△), diclofenac at doses of 10 mg/kg (×) and 20 mg/kg (▲), and the combination of hyperbaric oxygen with 10 mg/kg diclofenac (■) and 20 mg/kg diclofenac (○) on the development of paw edema after intraplantar injection of carrageenan. Diclofenac and saline were given i.p., 30 min before carrageenan injection. Hyperbaric oxygen treatment initiated immediately after carrageenan challenge and lasted 90 min for corresponding groups. The basal volume of each rat paw was taken at 100% and variations from this value were given as percent difference. Vertical bars denote S.E. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. saline (control). $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ vs. either diclofenac 10 mg/kg or 20 mg/kg.

observation time. Diclofenac, at a dose of 10 mg/kg also caused a significant reduction but only in third hour of the study. When compared with their separate applications, the co-administration of diclofenac at doses of 10 mg/kg or 20 mg/kg with hyperbaric oxygen produced greater inhibition in paw edema in sixth, second and third hour of the experiment, respectively.

4. Discussion

Both HBO and 20 mg/kg diclofenac treatments significantly reduced carrageenan-induced paw edema in rats. In other words, hyperbaric oxygen treatment has an anti-inflammatory activity comparable to that of diclofenac. Nevertheless, the question is by which mechanism hyperbaric oxygen exerts this effect.

Carrageenan-induced paw edema in rats has been widely used as a simple and reliable model to assess the anti-inflammatory activity of various agents (Winter et al., 1962; Vinegar et al., 1987; Gürsoy et al., 1989; Raza et al., 1996). The mechanisms underlying this model are not fully understood yet, though several inflammatory processes such as activation of complement, release of histamine and serotonin due to degranulation of mast cells, release of reactive oxygen or nitrogen species by activated phagocytes, kinins, proinflammatory cytokines and prostaglandins have been suggested to be involved in the development of acute inflammation in this model (Hirschelmann and Bekemeier, 1981; Vinegar et al., 1987). Even if a neurogenic component has also been reported for carrageenan-induced inflammation (Nagahisa et al., 1992).

Diclofenac, which is commonly used in inflammatory models as a reference drug, inhibits cyclooxygenase, the rate-limiting enzyme for the synthesis of prostaglandins. However, recently, it was discovered that there are two isoforms of cyclooxygenase (Xie et al., 1991); cyclooxygenase-1 has been described as a "house-keeping enzyme" and is observed in almost all tissues under basal conditions. Cyclooxygenase-2 is usually absent or present in only small amounts in cells under basal levels. However, it can be induced in cells involved in inflammation by inflammatory stimuli or cytokines (Dubois et al., 1998). Diclofenac, like other conventional NSAIDs, inhibits both isoforms (Cryer and Feldman, 1998). Thus, the anti-inflammatory effect of diclofenac in this model seems to be related to its inhibitory action on cyclooxygenase-2. However, it cannot be proposed in any mechanism for anti-inflammatory action of hyperbaric oxygen since inflammatory or proinflammatory mediators were not analysed in the present study. Nevertheless, Inamoto et al. (1991) reported that the release and production of interleukin-6, which also enhances prostaglandins by acting on phospholipase A_2 enzyme, and prostaglandin-E_2, a prominent prostaglandin in inflammation, decreased after a 5-day hyperbaric oxygen exposure at 2.5 atmospheres of pressure in mice.

As known, NSAIDs are associated with several adverse reactions, primarily gastrointestinal toxicity such as hemorrhagia and ulcerations (Feldman and McMahon, 2000). Among others, the administration of higher doses of NSAIDs was also established as a risk factor in NSAID-related gastrointestinal complications (Wolfe et al., 1999). Accordingly, one might expect to eliminate this risk factor by a strategy which provides an effective treatment with a dose of NSAIDs as low as possible. Thus, our second aim was to assess whether hyperbaric oxygen exposure enhances the anti-inflammatory activity of diclofenac. Nevertheless, hyperbaric oxygen treatment did not consistently enhance anti-inflammatory activity of either 10 or 20 mg/kg doses of diclofenac (Fig. 1). However, these data are not sufficient to propose either effectiveness or ineffectiveness of hyperbaric oxygen as an agent to reduce anti-inflammatory doses of NSAIDs since hyperbaric oxygen treatment itself already seems to be very effective.

References

Caplan, E.S., 2000. Hyperbaric oxygen. J. Pediatr. Infect. Dis. 19, 151–152.

Cryer, B., Feldman, M., 1998. Cyclooxygenase-1 and cyclooxygenase-2 selectivity of widely used nonsteroidal antiinflammatory drugs. Am. J. Med. 104, 413–421.

Dubois, R.N., Abramson, S.B., Crofford, L., Gupta, R.A., Simon, L.S., Van de Putte, L.B.A., Lipsky, P.E., 1998. Cyclooxygenase in biology and disease. FASEB 12, 1063–1073.

Feldman, M., McMahon, A.T., 2000. Do cyclooxygenase-2 inhibitors provide benefits similar to those of traditional nonsteroidal antiinflammatory drugs, with less gastrointestinal toxicity? Ann. Intern. Med. 132, 134–143.

Gürsoy, A., Eroğlu, L., Ulutin, Ş., Taşyürek, M., Fessi, H., Puisieux, F., Devissaguet, J.P., 1989. Evaluation of indomethacin nanocapsules for their physical stability and inhibitory activity on inflammation and platelet aggregation. Int. J. Pharm. 52, 101–108.

Hirschelmann, R., Bekemeier, H., 1981. Effects of catalase, peroxidase, superoxide dismutase, and 10 scavengers of oxygen radicals in carrageenan edema and adjuvant arthritis of rats. Experientia 37, 1313–1314.

Inamoto, Y., Okuno, F., Saito, K., Tanaka, Y., Watanabe, K., Morimoto, I., Yamashita, U., Eto, S., 1991. Effect of hyperbaric oxygenation on macrophage function in mice. Biochem. Biophys. Res. Commun. 179, 886–891.

Jain, K.K., 1990. Hyperbaric oxygen in traumatology and orthopedics. In: Jain, K.K., Neubauer, R., Correa, J.G. (Eds.), Textbook of Hyperbaric Medicine. Hogrefe & Huber, Toronto, pp. 355–365.

Leach, R.M., Rees, P.J., Wilmshurst, P., 1998. ABC of oxygen. Hyperbaric oxygen therapy. BMJ 317, 1140–1143.

Lukich, V.L., Poliakova, L.V., Sotnikova, T.I., Belokrinitskii, D.V., 1991. Hyperbaric oxygenation in the comprehensive therapy of patients with rheumatoid arthritis. Fiziol. Zh. 37, 55–60.

Nagahisa, A., Kanai, Y., Suga, O., Taniguchi, K., Tsuchiya, M., Lowe, J., Hess, H.J., 1992. Antiinflammatory and analgesic activity of a nonpeptide substance P receptor antagonist. Eur. J. Pharmacol. 217, 191–195.

Raza, M., Dhariwal, M.A.H., Ageel, A.M., Qureshi, S., 1996. Evaluation of the antiinflammatory activity of sodium valproate in rats and mice. Gen. Pharmacol. 27, 1395–1400.

Vinegar, R., Turuax, J.F., Selph, J.L., Johnston, P.R., Venable, A.L.,

Mckenzie, K.K., 1987. Pathway to carrageenan-induced inflammation in the hind limb of the rat. Fed. Proc. 46, 118–126.

Warren, J., Sacksteder, M.R., Thuning, C.A., 1979. Therapeutic effect of prolonged hyperbaric oxygen in adjuvant arthritis of the rat. Arthritis Rheum. 22, 334–339.

Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Carrageenan-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Proc. Soc. Exp. Biol. Med. 111, 544–547.

Wolfe, M.M., Lichtenstein, D.R., Singh, G., 1999. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N. Engl. J. Med. 340, 1888–1899.

Zimmermann, M., 1983. Ethical guidelines for investigation of experimental pain in conscious animals. Pain 16, 109–110.

Xie, W.L., Chipman, J.G., Robertson, D.L., Erikson, R.L., 1991. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc. Natl. Acad. Sci. U. S. A. 88, 2692–2696.