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THE TUMOR PROMOTING PHORBOL DIESTER, 12-O-TETRADECANOYLPHORBOL-13-ACETATE (TPA) INCREASES GLYOXALASE I AND DECREASES GLYOXALASE II ACTIVITY IN HUMAN POLYMORPHONUCLEAR LEUKOCYTES

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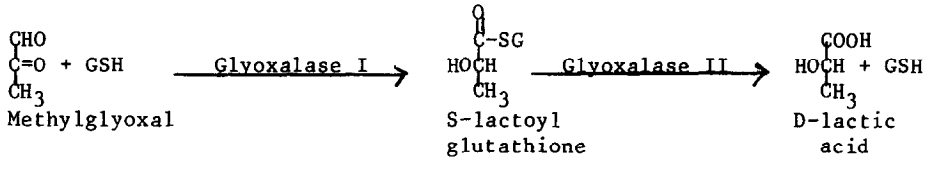
SUMMARY: Glyoxalase I and II catalyze the formation and breakdown of S-lactoylglutathione respectively. Recent studies have implicated this compound as a possible mediator of immune and inflammatory responses. Incubation of human polymorphonuclear leukocytes with the tumor promoter, 12-O-tetradecanoylphorbol-13-acetate has been found to affect the activities of both glyoxalase enzymes in an interrelated manner. The diester either increases the activity of glyoxalase I or decreases the activity of glyoxalase II or has both effects. It is suggested that a subsequent increase in S-lactoylglutathione might mediate some or all of the effects of the phorbol diesters.

The glyoxalase enzyme system catalyzes the conversion of methylglyoxal to D-lactic acid (1,2) with the formation and destruction of a stable intermediate, S-lactoylglutathione (3), as follows:



Recent studies from this laboratory (4-6) have led to the hypothesis that the function of the two glyoxalase enzymes is the regulation of the level of S-lactoylglutathione and have provided evidence that this compound is involved in modulating immune and inflammatory responses. It has been shown that S-lactoylglutathione potentiates histamine release from human

ABBREVIATIONS: Con A, concanavalin A; PDA, phorbol-12, 13-diacetate; PDD, phorbol-12,13-didecanoate; PMNs, polymorphonuclear leukocytes; TPA, 12-O-tetradecanoyl-13-acetate

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leukocytes while inhibitors of glyoxalase I inhibit the process (6). S-lactoylglutathione has also been implicated as a modulator of microtubule assembly *in vitro* (4). In addition, the mitogen, concanavalin A (Con A), has been shown to increase the activities of both glyoxalase I and II in lymphocytes and polymorphonuclear leukocytes (5). It was suggested in this last study that the primary effect of Con A was to increase glyoxalase I and thereby increase S-lactoylglutathione levels with the increase in glyoxalase II activity being a secondary compensatory response.

12-O-Tetradecanoylphorbol-13-acetate (TPA), the most potent of the active components of croton oil, causes the formation of tumors in mouse skin previously treated with subthreshold concentrations of a carcinogen (7-10). It also is an inflammatory compound (7-10). With respect to this second property, Con A and TPA have been shown to elicit similar or identical responses in a variety of leukocyte populations. Both are mitogenic for human lymphocytes (11-13), both induce plasminogen activator in macrophages (14), both release histamine from human basophils (15,16), and both release lysozyme, but not β-glucuronidase from polymorphonuclear leukocytes (17-20) and also increase the occurrence of cytoplasmic microtubules in this cell type (19-20). Because of these numerous similarities, it was of interest to determine the effect of TPA on the two glyoxalase enzymes. The present report describes the enhancement of glyoxalase I and the inhibition of glyoxalase II by TPA in human polymorphonuclear leukocytes. This response is different from the one induced by Con A where the activities of both the enzymes were found to increase. Given constant methylglyoxal formation, it seems probable that TPA increases intracellular S-lactoylglutathione levels.

MATERIALS AND METHODS

Experimental procedures were essentially as previously described (5). Polymorphonuclear leukocytes (PMN) were prepared from the blood of normal individuals by density gradient centrifugation in Hypaque-Ficoll mixtures. Contaminating red blood cells were removed by hypotonic lysis with ice-cold

water. Cells (4 to 5 x 10^6) were incubated at 37° in 12 x 75 mm plastic tubes (Falcon Plastics, Oxnard, CA). The medium consisted of (mM).: NaCl, 110; KCl, 5; $MgCl_2$, 1.0; $CaCl_2$, 1.0 and PIPES, 25 adjusted to pH 7.3 with NaOH. After incubation, cells were centrifuged (1000 x G, 2 min) and resuspended in 1.2 ml 10 mM HEPES buffer, pH 7.0, containing 0.02% Triton X-100. The cells were disrupted by sonication (30 sec; microtip of a Branson Power Company sonicator, intensity of 40 watts).

The glyoxalase enzymes were assayed by a modification of the spectrophotometric procedure described by Racker (3) which is based on the fact that S-lactoylglutathione absorbs light at 240 nm. Portions (300 µl) of the cell supernatants were combined in a total volume of 0.5 ml with either methylglyoxal and glutathione (final concentrations 2.0 and 2.5 mM) or with S-lactoylglutathione (final concentration 1mM) for the assays of glyoxalase I and II, respectively. The incubation temperature was 37° C and the times were 15 min for glyoxalase I and 60 min for glyoxalase II. The substrates were prepared in a 100 mM HEPES buffer, pH 7.0. At the end of the incubation the reaction was stopped by the addition of 1.5 ml of 0.133 M HCl, and optical density at 240 nm was measured.

Methylglyoxal (Sigma Chemical Co., St. Louis, MO) was purified by steam distillation. The phorbol esters were obtained from Consolidated Midland Corp., Brewster, NY. S-lactoylglutathione was freshly prepared for each experiment by reacting methylglyoxal (20 mM) and glutathione (25 mM) with glyoxalase I (4 units/ml, Sigma) for 30 min or longer at 20° C in 100 mM HEPES buffer, pH 7.0. Under these conditions (i.e., 25% excess glutathione) all the methylglyoxal was converted to S-lactoylglutathione. The excess glutathione did not interfere with the assay for glyoxalase II.

RESULTS

The effects of different concentrations of TPA on the activities of the two glyoxalase enzymes are shown in Figure 1. A partial response to TPA is seen at a concentration of 10^{-8} M. The observed dose-response relationship is virtually identical to that noted by others (17-19) for the release of lysozyme. The effects of other phorbol esters have been determined. Phorbol (10^{-5} M), had no effect on the activity of either enzyme. Phorbol-12,13-didecanoate (PDD) and phorbol-12,13-diacetate (PDA) were compared with TPA and with each other in experiments utilizing the cells of six different individuals. Both compounds caused the same maximum change in enzyme activities as did TPA although both were less potent than was TPA. Half the maximum effect was seen at a PDD concentration of 1-2 x 10^{-7} M and at a PDA concentration of 4-5 x 10^{-7} M.

The time course of the response to TPA is shown in Figure 2. Changes in both enzyme activities occur within the first 2-5 minutes of incubation

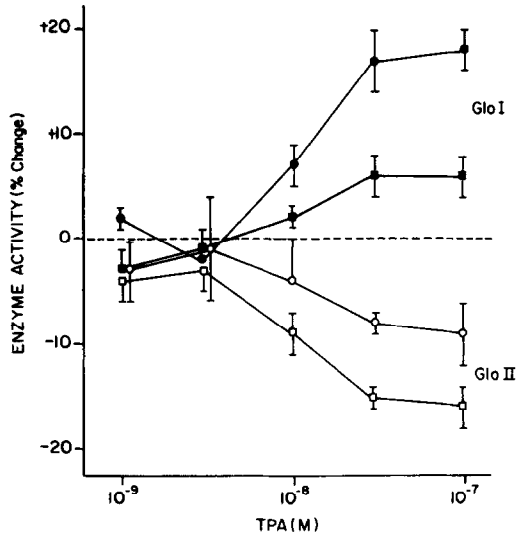

Fig. 1: Effects of different concentrations of TPA on glyoxalase (Glo) I and II. Polymorphonuclear leukocytes were incubated in quadruplicate with TPA at the concentrations indicated for 30 min. Cells were processed and glyoxalase enzyme activities assayed as described under METHODS. Points and vertical bars represent the mean ± S.E.M. for two experiments using the cells of different donors. o ●, expt. 1; □ ■ , expt. 2.

and continue unchanged for up to 60 minutes. The effects of TPA require that TPA be incubated with whole cells. In three experiments with cell sonicates TPA (10^{-8} or 10^{-7} M) failed to cause the changes illustrated.

The effects of TPA on the two glyoxalase enzymes in 14 consecutive experiments carried out with the cells of ten Caucasian donors are depicted in Fig. 3. In the majority of the experiments, TPA caused an increase in the activity of glyoxalase I and a decrease in the activity of glyoxalase II as illustrated in Figs. 1 and 2. With the cells of some individuals, however, a larger increase in glyoxalase I was observed which was accompanied by a smaller decrease in glyoxalase II whereas with the cells of other individuals the reverse pattern was the case, i.e., a large change (decrease) in glyoxalase II was accompanied by a small or negligable increase in glyoxalase I. This relationship was analyzed statistically and found to be significant ($r = 0.77$, p below .001). This inter-relationship

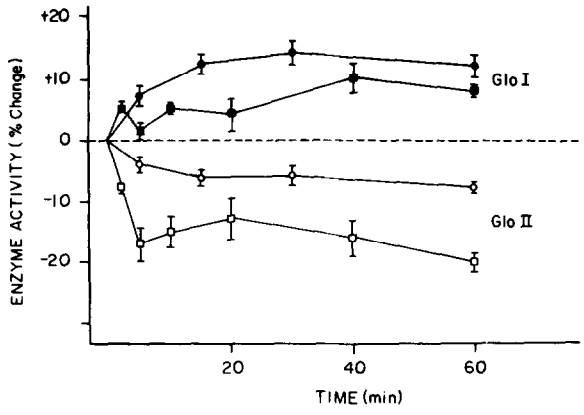

Fig. 2: Time course of the TPA effect on glyoxalase (Glo) I and II. Polymorphonuclear leukocytes were incubated in quadruplicate for 60 min. TPA was added at the times needed to give a TPA exposure of 2-60 min. The final TPA concentration was 10^{-7} M. Cells were processed and enzymes assayed as described under METHODS. Points and vertical bars represent the mean ± S.E.M. for two experiments using the cells of different donors. o ●, expt. 1; □ ■, expt. 2.

between the effects of TPA on the two enzymes was unexpected and places constraints on the system in that it suggests that the regulation of these two enzymes by TPA is interdependent. It is also compatible with the idea that changes in the activity of either enzyme can alter the level of S-lactoylglutathione.

DISCUSSION

Glyoxalase enzyme activity was first described in 1913 (1,2) and is now considered to be ubiquitous (21). In the 1920s this enzyme system was extensively investigated as it was thought that methylglyoxal and D-lactic acid might be intermediates in glycolysis. In 1932 Lohmann demonstrated that glutathione was required for glyoxalase enzyme activity but did not affect glycolysis (22) making it clear that this hypothesis was incorrect. In the mid-1960s a second hypothesis concerning the enzyme system was proposed by Szent-Gyorgyi and his colleagues who suggested that these enzymes were involved in the regulation of growth (23-25). More specifically Szent-Gyorgyi suggested that a six carbon ketoaldehyde, 2-keto-3-deoxyglucose, was a growth retarding substance and postulated that the

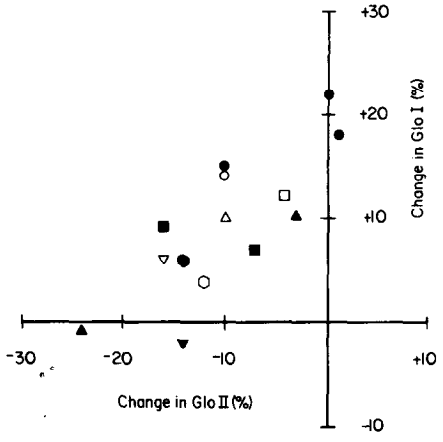

Fig. 3: Effects of TPA on glyoxalase (Glo) I and II in the cells of different individuals. Polymorphonuclear leukocytes were incubated with or without TPA (10^{-7} M) in quadruplicate for 30 min, processed, and the glyoxalase enzymes assayed as described under METHODS. The average standard error of the mean throughout these experiments was 3.1% for glyoxalase I and 2.7% for glyoxalase II. The different symbols indicate different donors. The correlation coefficient, r, is 0.77 with p below 0.001.

glyoxalase enzymes would therefore be growth promoting. It was subsequently found that this ketoaldehyde was not a naturally occurring compound and also was not a substrate for glyoxalase (26,27). Despite these negative data, the hypothesis that the glyoxalase enzymes might be involved in the control of growth has stimulated research in this area. Jerzykowski and his colleagues have studied glyoxalase II activity in a series of tumors (28,29). No enzyme activity was detected in approximately two-thirds of the tumors studied, while in the remaining one-third it was low compared to the activity of normal tissues. Assuming constant methylglyoxal formation, S-lactoylglutathione levels in these tumor tissues might well be elevated. Other investigators have synthesized inhibitors of glyoxalase I (30-34). Some of these compounds have been shown to be cytotoxic. While this result could be due to the buildup of methylglyoxal or other unidentified analogues, it could also be due to inhibition of the formation of S-lactoylglutathione.

The finding that TPA both increases glyoxalase I and decreases glyoxalase II activity in polymorphonuclear leukocytes is compatible with

the hypothesis that increased levels of S-lactoylglutathione are a concomitant of inflammation and possibly represents the mechanism whereby TPA exerts its inflammatory actions. It is also possible that an increased S-lactoylglutathione level is related to the tumor promoting properties of this compound. Studies of the role of the glyoxalase enzymes in the control of growth within the context of the hypothesis that their function is regulation of S-lactoylglutathione would seem warranted.

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