[13] observed a competitive type of inhibition of spermidine uptake by L 1210 cells with a series of diamine homologues of putrescine and triamine homologues of spermidine and it was suggested that these polyamine homologues were interfering with polyamine transport. They further observed that one of the polyamine analogues could inhibit the cell proliferation with $IC_{50} < 10$ mM [14]. Though in the present study polyamines do not show a competitive type of inhibition of DIAM 3 uptake by U 251 cells, nevertheless, by considering the results on the similarities between polyamine [9] and DIAM 3 uptake by these cells, we may speculate that DIAM 3 would be utilizing the polyamine transporter protein to enter the cells.

We do not know about the metabolism of DIAM 3 by U 251 cells since the studies on this drug are limited. In another experiment, after 10% trichloroacetic acid precipitation of U 251 cells containing [^{14}C]DIAM 3, we observed no radioactivity in the acid soluble material and this indicates towards the covalent binding of DIAM 3 with cell proteins. As regards the efflux of the drug, we observed that after transfering the [^{14}C]DIAM 3 loaded cells into a drug free RPMI 1640 medium, no radioactivity was lost during the chase of every 15 min till 2 hr.

Further studies are needed on the localization and catabolism of this new anticancer drug by U 251 or other mammalian cells.

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Effect of 5-aminosalicylic acid on ferrous sulfate-mediated damage to deoxyribose

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Ulcerative colitis is a recurrent inflammation of the colon and rectum characterized by diffuse ulcerations, crypt abscesses, decreased mucous production, subepithelial hemorrhage, and infiltration of large numbers of phagocytic leukocytes (monocytes, macrophages, and granulocytes). Oral administration of sulfasalazine (SAZ) has been proven to be effective in attenuating the mucosal injury associated with this inflammatory disease. Sulfasalazine passes unmodified through the upper gastrointestinal tract into the colon where it is metabolized by enteric bacteria to yield 5-aminosalicylic acid (5-ASA) and sulfapyridine (SP). Although it is now well accepted that 5-ASA is the pharmacologically active moiety of SAZ [1], the mechanisms by which 5-ASA exerts its beneficial effect remain speculative.

Several groups of investigators have proposed that 5-ASA acts to inhibit either cyclooxygenase [2] or lipoxygenase [3, 4] activities, thereby attenuating the production of arachidonate-derived, pro-inflammatory mediators. Other studies suggest that 5-ASA may act to scavenge free radicals [5–7] as well as decompose hypochlorous acid ([8]; HOCl). The rationale for these studies is based upon the fact that the inflamed mucosa contains numerous phagocytic leukocytes that are capable of generating substantial quantities of superoxide (O_2^-), hydrogen peroxide (H_2O_2) and HOCl. Neither O_2^- nor H_2O_2 is particularly toxic to cells; however, they will interact with certain transition metals such as iron to yield potentially injurious oxidants such as the hydroxyl radical ($^{\cdot}OH$). In biological systems, iron is bound to a variety of ligands including proteins, carbohydrates, nucleic acids, and inorganic anions. Depending upon the coordination chemistry of this iron–ligand interaction, O_2^- and/or H_2O_2 may interact with the metal to yield $^{\cdot}OH$. Because of the highly reactive nature of $^{\cdot}OH$, it will not move more than 1–5 molecular diameters from the site of its formation [9]. Thus, $^{\cdot}OH$ formation will occur at the site of metal binding, resulting in site-specific damage to the metal binding compound. Because some salicylate derivatives are known to chelate iron and because iron is thought to play a role in inflammatory tissue injury, we assessed the possibility that 5-ASA may behave as an iron chelator and thus may protect deoxyribose from iron-catalyzed $^{\cdot}OH$-mediated damage.

Materials and Methods

Sulfasalazine and sulfapyridine were purchased from the Sigma Chemical Co. (St. Louis, MO). 5-ASA and *N*-acetyl 5-ASA (NASA) were provided by Dr. Thomas Berglindh, Pharmacia AB (Uppsala, Sweden). All other reagents were of the highest quality available. The iron-catalyzed, site-specific generation of $^{\cdot}OH$ was measured using ferrous sulfate-mediated degradation of deoxyribose to yield thiobarbituric reactive substances (TBARS) as described by Gutteridge [10]. It has been shown that Fe^{2+} associates with deoxyribose where it auto-oxidizes to generate $^{\cdot}OH$ on the surface of the carbohydrate (i.e. site specific). Only those scavengers capable of removing Fe^{2+} from deoxyribose and reducing its reactivity toward H_2O_2 and O_2^- will be effective inhibitors of this system [10, 11]. Each reaction volume (0.5 mL) contained the following compounds which were added in this order: 2 mM deoxyribose, 0.1 mM ferrous sulfate, various concentrations of drug and 10 mM potassium phosphate buffer (pH 7.4). Following a 30-min incubation period at 37°, reactions were terminated by the addition of catalase (20 μg/mL). TBARS were then quantified by the sequential addition of 0.5 mL trichloroacetic acid (2.8%) and 0.5 mL thiobarbituric acid (TBA) (1% in 0.05 N NaOH), and the tubes were heated at 100° for 15 min in a boiling water bath. The tubes were then cooled and the absorbance was determined at 532 nm [10]. Each absorbance value was corrected for nonspecific development that occurred in the absence of iron.

In a second set of studies, $^{\cdot}OH$ was generated in free solution by exposing deoxyribose to a Fe^{2+} chelate in the presence of H_2O_2. In these experiments Fe^{2+} was prevented from associating with deoxyribose by first chelating it to diethylenetriaminepentacetic acid (DTPA). Thus, any $^{\cdot}OH$ generated from the interaction between Fe^{2+}-DTPA and H_2O_2 that escaped interaction with DTPA would have equal access to all components of the reaction volume. The Fe^{2+}-DTPA was prepared by the method of Cohen [12] in which 1 mM ferrous sulfate was added to a solution containing 2 mM DTPA and 50 mM potassium phosphate buffer (pH 7.4). Unlike free Fe^{2+}, this iron chelate is relatively stable in the presence of phosphate buffer [12]. Each reaction volume (0.5 mL) contained the following compounds which were added in this order: 2 mM deoxyribose, 0.1 mM Fe^{2+}-DTPA (0.1 mM Fe^{2+}-0.2 mM DTPA), various concentrations of drug, and 10 mM potassium phosphate buffer (pH 7.4). Addition of 0.2 mM H_2O_2 was used to initiate the reaction. Following a 10-min incubation period at 37° reactions were terminated by the addition of catalase, and TBARS were determined as described above.

To directly assess the ability of 5-ASA to chelate Fe^{2+}, ferrous sulfate (0.2 mM) was added to a solution of 5-ASA (1 mM) in 0.1 M NaCl (pH 7.4), and the absorbance spectrum was determined. The ability of 5-ASA to decompose H_2O_2 was measured using GSH and GSH peroxidase to measure H_2O_2 [13]. GSH peroxidase was chosen as the catalyst because of its specificity for GSH as the electron donor which eliminates the interference due to 5-ASA.

Results

Figure 1 illustrates the effects of SAZ or its metabolites on ferrous sulfate-induced degradation of deoxyribose to yield TBARS. 5-ASA was the most effective compound in inhibiting the $^{\cdot}OH$-mediated production of TBARS. The concentration of 5-ASA required to inhibit the formation of TBARS by 50% (IC_{50}) was approximately 300 μM, whereas all other compounds possessed IC_{50} values in excess of 1000 μM. Figure 2 demonstrates the effects of these same compounds on Fe^{2+}-DTPA catalyzed degradation of deoxyribose. These data demonstrate that all drugs became equally effective in inhibiting the formation of TBARS when Fe^{2+} was prevented from binding to deoxyribose by chelating it to DTPA. The IC_{50} values for all drugs ranged from 300 to 500 μM. Because catalase inhibits ferrous sulfate-mediated formation of TBARS from deoxyribose [14], it could be argued that 5-ASA exerts its protective effect by decomposing H_2O_2. Therefore, we assessed the ability of 5-ASA to decompose H_2O_2 under our experimental conditions. We found that incubation of 5-ASA (2.0 mM) with H_2O_2 (0.2 mM) for 30 min at 37° resulted in less than 5% decomposition of H_2O_2 (data not shown). The ability of 5-ASA to chelate Fe^{2+} was monitored by measuring the absorbance spectrum between 400 and 700 nm. Figure 3 shows that the interaction between Fe^{2+} and 5-ASA in the absence or presence of deoxyribose yielded a purple chromophore with a wavelength maximum (λ_{max}) of 535 nm. These data suggest that 5-ASA chelates Fe^{2+} directly.

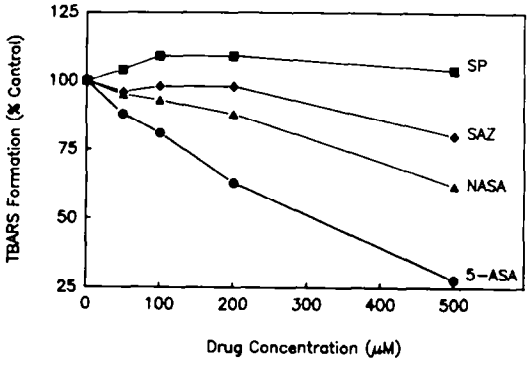

Fig. 1. Effects of 5-aminosalicylic acid (5-ASA), *N*-acetyl 5-ASA (NASA), sulfasalazine (SAZ) and sulfapyridine (SP) on ferrous sulfate-mediated degradation of deoxyribose to yield thiobarbituric reactive substances (TBARS). Reaction conditions are described in Materials and methods. Each data point is the mean from at least four determinations and varied by less than ±5%. A mean absorbance value of 0.218 was achieved in the absence of drug and was designated as 100%.

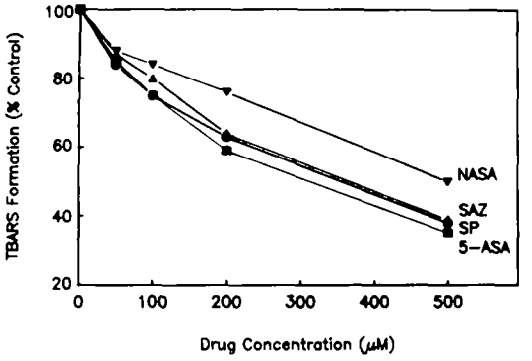

Fig. 2. Effects of 5-aminosalicylic acid (5-ASA), *N*-acetyl 5-ASA (NASA), sulfasalazine (SAZ) and sulfapyridine (SP) on the Fe^{2+}-DTPA-mediated degradation of deoxyribose to yield thiobarbituric reactive substances (TBARS). Reaction conditions are described in Materials and methods. Each data point is the mean from four determinations and varied by by less than ±5%. A mean absorbance value of 0.426 was achieved in the absence of drug and was designed as 100%.

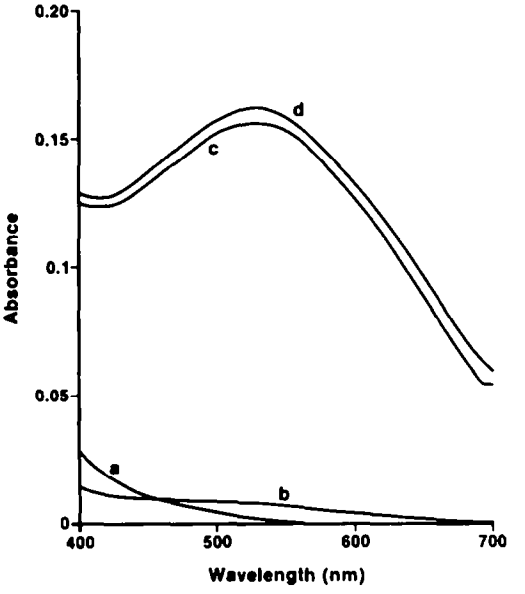

Fig. 3. Absorbance spectrum of the Fe^{2+}-5-ASA chelate in the absence or presence of deoxyribose. Ferrous sulfate (0.2 mM) was added to a solution containing 1 mM 5-ASA and 0.1 M NaCl, pH 7.4, in the absence or presence of 4 mM deoxyribose and the absorbance spectrum determined. Key: a, b, c and d represent Fe^{2+}, 5-ASA, Fe^{2+} + 5-ASA, and Fe^{2+} + 5-ASA in the presence of deoxyribose respectively.

Discussion

Several groups of investigators have demonstrated that the concentration of low molecular weight, redox active iron increases in the exudate or extracellular fluid in certain inflammatory diseases states such as rheumatoid arthritis [15–17]. It is felt that this type of iron may play a role in pathogenesis of inflammatory tissue injury since some iron chelators have been shown to attenuate inflammatory tissue injury in certain models of inflammation [16–18]. Because of the highly reactive nature of 'OH coupled to the fact that iron is bound to certain biological compounds, several investigators have proposed that O_2^- and/or H_2O_2-dependent cytotoxicity is mediated by the site-specific formation of 'OH [11, 19–21]. The auto-oxidation of deoxyribose (DOR)-associated Fe^{2+} provides a useful model to investigate the site-specific damage of an important biomolecule. The following reactions describe the proposed mechanism by which 'OH is generated on the surface of the carbohydrate.

$$2\text{DOR-Fe}^{2+} + 2\text{O}_2 \rightleftharpoons 2\text{DOR-Fe}^{3+} + 2\text{O}_2^-$$

$$2\text{O}_2^- + 2\text{H}^+ \longrightarrow \text{H}_2\text{O}_2 + \text{O}_2$$

$$\text{DOR-Fe}^{2+} + \text{H}_2\text{O}_2 \longrightarrow \text{DOR-Fe}^{3+} + \text{OH}^- + \text{OH}^{\cdot}.$$

Ferrous iron induced degradation of deoxyribose is inhibited by catalase, desferrioxamine (an iron chelator), mannitol and dimethylthiourea (DMTU) but not by SOD or dimethyl sulfoxide (DMSO) [14]. The differences in efficacy observed among the different 'OH scavengers (mannitol, DMSO, DMTU) have been suggested to result from differences in the abilities of these scavengers to remove iron from the surface of the detector (deoxyribose) and render the metal poorly redox active [11, 20–22]. Indeed, it is known that deoxyribose, mannitol and possibly DMTU bind iron whereas DMSO does not [11, 20]; Grisham MB and Kvietys PR, unpublished data). Therefore, only those 'OH scavengers capable of binding iron and preventing its ability to redox cycle would be effective in inhibiting the oxidative damage to deoxyribose even though all scavengers are equally effective in scavenging 'OH generated in free solution either chemically or by pulse radiolysis [8, 11]. Not all iron chelators (e.g. EDTA or DPTA) are effective in inhibiting iron-mediated deoxyribose degradation as they allow iron to effectively generate 'OH in free solution. Based upon reports using drugs with a similar structure, we proposed that 5-ASA may be an effective iron chelator and thus could inhibit the site-specific damage to important biomolecules such as deoxyribose. We found that 5-ASA was the most effective inhibitor of ferrous sulfate induced degradation of deoxyribose possessing an IC_{50} of 300 μM (Fig. 1). It is interesting to note that N-acetylation of 5-ASA to yield NASA reduced its effectiveness in this assay, even though the carboxylic and hydroxyl groups remained unmodified. The reasons for this phenomenon are unclear. Aruoma *et al.* [8] have demonstrated recently that 5-ASA, SAZ and SP are all equally effective in scavenging 'OH which is generated in free solution using either pulse radiolysis or chemically by the interaction between EDTA-chelated Fe^{3+} and ascorbate. We have confirmed these observations using Fe^{2+}-DTPA and H_2O_2 (Fig. 2). Taken together, our data demonstrate that the same 'OH scavenger may have very different efficacies in scavenging 'OH depending upon whether 'OH is generated site specifically or in free solution. Furthermore, our data suggest that 5-ASA inhibits Fe^{2+}-mediated decomposition of deoxyribose by chelating Fe^{2+} and rendering it poorly redox active since the drug does not decompose H_2O_2 under the experimental conditions of the assay. Indeed, we found that 5-ASA bound Fe^{2+} (in the absence or presence of deoxyribose) to generate a purple chromophore with a λ_{max} of 535 nm (Fig. 3).

In summary, we have demonstrated that 5-ASA is much more effective at inhibiting Fe^{2+}-catalyzed, site-specific damage of deoxyribose than NASA, SP or SAZ. If, however, Fe^{2+} is first bound to the chelator DTPA, then all four drugs become equally effective in inhibiting deoxyribose damage. Because 5-ASA does not decompose H_2O_2 under the conditions of our assay, we reasoned that it may exert its protective effect by chelating iron and scavenging 'OH. Thus, we have demonstrated in this study that 5-ASA chelates Fe^{2+} to yield a purple chromophore with a λ_{max} of 535 nm.

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Differences in plasma carboxylesterase activity: relevance to anticholinesterase sensitivity

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Russell and Overstreet [1] have discussed recently the mechanisms underlying sensitivity to the organophosphorus anticholinesterase agents. In the section on noncritical binding proteins, they pointed out that serum butyrylcholinesterase may be involved in the sex but not the strain differences in anticholinesterase sensitivity [2–4]. Carboxylesterase has also been implicated as a protein to which anticholinesterases may bind, thereby reducing the amount available to bind acetylcholinesterase and produce physiological effects [5, 6]. The present study was a pre-