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Differential binding of conA and WGA on the cell surface, the role of sialic acid in their expression and the increased activity of sialidase after cis-Platin treatment

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Summary. It is reported that concanavalin A (conA) and wheat germ agglutinin (WGA) have a differential binding pattern on normal mouse spleen lymphocytes and the surface of Dalton's lymphoma cells. It is suggested that sialic acid on the cell surface controls the expression of lectin binding sites. Further, it has been observed that the increased release of sialic acid from cell surfaces after *cis*-dichlorodiammine platinum (II) (cis-Platin) treatment is due to the increased activity of sialidase.
Key words. Mouse spleen cell; mouse lymphoma; concanavalin A binding; wheat germ agglutinin binding; sialic acid.

cis-Dichlorodiammine platinum (II) (cis-Platin) a potent antitumor compound[2-5] has a definite effect on the surface of tumor and normal cells, and particularly affects lectin binding sites and cell agglutination. Normal lymphocytes (NL), which agglutinate very little with conA and WGA show weak fluorescence with lectin-FITC (fluorescein isothiocyanate). NL when incubated with cis-Platin show a gradual increase in the degree of cell agglutination with concanavalin A (conA) and wheat germ agglutinin (WGA), and a significant increase in the fluorescence intensity with lectin-FITC is observed after 10–30 min of treatment. Maximum cell agglutination and fluorescence intensity are observed after 30 min of treatment, and 60 min treatment results in sharp decrease in both cell agglutination and fluorescence intensity, to the level of untreated NL. On the other hand, Dalton's lymphoma (DL) cells, which show a high degree of cell agglutination with lectins and bright fluorescence with lectin-FITC, when treated with cis-Platin gradually show a significant decrease in the cell agglutination and fluorescence intensity from 10 to 60 min of treatment. The least cell agglutination and weakest fluorescence are observed after 60 min of treatment[6-8]. Various ultrastructural and biochemical studies have shown that in vivo and in vitro cis-Platin treatment of the cells causes a gradual removal of cell surface sialic acid and mucopolysaccharides[6,7,9]. This removal of sialic acid from the cell surface was observed to have a role in the differential increase or decrease in the degree of agglutination of the cells with lectins, and neuraminidase treatment of normal and tumor cells resembled closely the results seen with cis-Platin[6,7]. The present fluorescence and biochemical studies were undertaken to provide direct evidence for a) the different binding patterns of conA and WGA to the cell surfaces, b) the role of sialic acid in the expression of lectin binding sites on normal and tumor cells, and c) the possible mechanism of release of sialic acid from the cell surfaces after cis-Platin treatment.

It is reported that there is a differential binding behavior of conA and WGA on cell surfaces; the presence of sialic acid moieties is apparently correlated with the expression of lectin binding sites and removal of sialic acid from the cell surface seems to be due to an increase in sialidase activity after cis-Platin treatment.

Materials and methods. Single-cell suspensions of normal spleen lymphocytes and Daltons's lymphoma ascites cells from Dba mice were prepared in PBS (without Ca^{++} and Mg^{++}) as described earlier[7]. Ganglioside (type IV), conA-FITC, WGA-FITC, neuraminidase (type V), sialic acid (type VI) were purchased from Sigma Chemical Co., USA. All other chemicals used were of analytical grade; cis-Platin was thoroughly mixed in 0.89% NaCl in the dark 10–15 min before the experiments. For fluorescence microscopy the cell count was adjusted to 2×10^6 cells/ml. 3.0 ml of cell suspensions were incubated with cis-Platin (25 µg/ml) or with neuraminidase (2.5 units/ml) and without cis-Platin or neuraminidase (controls) for 20 and 30 min at 37 °C. After incubation, the cell suspensions were centrifuged and the pellets were suspended in one fourth of the original volume of PBS, fixed in 2% glutaraldehyde and labelled with conA-FITC (100 µg/ml) or WGA-FITC (50 µg/ml) as previously described[7]. In another set of experiments NL and DL cells were treated with cis-Platin (25 µg/ml) or neuraminidase (2.5 units/ml) for 30 and 60 min, respectively. After incubation, the cells were centrifuged and the pellets were suspended in the same volume of PBS containing 2 mg/ml of sialic acid for 20 min at room temperature. The cells were washed twice with PBS and then processed for fluorescence microscopy.

For sialidase assay in NL and DL cells (4×10^6 cells/ml) treated with cis-Platin (25 µg/ml) for 30 min at 37 °C or without cis-Platin (controls) the method of Schengrund and Rosenberg[10] was used with some modifications: duplicate samples of the cell suspensions (1.0 ml) were centrifuged and the pellets were suspended in 1.0 ml of 0.02 M acetate buffer, pH 4.0, containing different concentrations (100, 200, and 500 µg/ml) of substrate (brain ganglioside type IV). A substrate blank and an enzyme blank were also prepared omitting the substrate or the cells from the reaction mixture. The reacting mixture was incubated for 90 min at 37 °C. The reaction was stopped by adding 0.15 ml of 0.1 N NaOH. Then the cell suspensions were

centrifuged and the sialic acid content was estimated in the supernatant by the method of Warren[11]. The mean optical density obtained at 549 and 532 nm was directly plotted on the graph. The values represent the comparative release of sialic acid in the samples.

In another set of experiments the ganglioside type IV (200 µg/ml) was directly treated with cis-Platin (25 and 100 µg/ml) or with neuraminidase (2.5 units/ml) for 30 min and then the free sialic acid released was estimated. The complete set of experiments was repeated thrice, and there was not much variation in the results.

Results. Normal lymphocytes (NL) when incubated for 10–60 min without cis-Platin show weak, evenly distributed fluorescence with conA-FITC and WGA-FITC (fig. 1, A, B). After cis-Platin treatment of NL from 20 to 30 min there is increased intensity of fluorescence (fig. 1, C, D, E, F). The pattern of lectin binding is different for conA-FITC and WGA-FITC. ConA-FITC labeling shows a bright fluorescent rim around the cell (Fig. 1, C, E), whereas in WGA-FITC labelling there is the appearance of small granular fluorescent patches (fig. 1, D, F). After 20 min of cis-Platin treatment, NL cells show an uneven distribution of fluorescent patches concentrated in the two halves of the cell (fig. 1, D). These small fluorescent patches are more evenly distributed at 30 min of treatment (fig. 1, F). When NL cells treated for 30 min with cis-Platin or neuraminidase are incubated with sialic acid, washed and then labeled with conA-FITC or WGA-FITC, NL again shows weak fluorescence (fig. 1, G, H), similar to that of controls (fig. 1, A, B). Dalton's lymphoma cells (DL) when incubated without cis-Platin for 10–60 min show bright fluorescence intensity with conA-FITC and WGA-FITC (fig. 1, I, J). ConA-FITC labeled DL cells have a bright fluorescent rim around the cells (fig. 1, I), while in WGA-FITC labeled cells this rim is absent (fig. 1, J). DL cells treated with cis-Platin for 20 and 30 min show a gradual decrease in the fluorescence intensity (fig. 1, K, L, M, N) with some fluorescent patches in WGA-FITC. labeled cells (fig. 1, L, N). When cis-Platin or neuraminidase-treated DL cells are incubated with sialic acid before conA-FITC and WGA-FITC labeling, there is significant increase in the fluorescence intensity with the appearance of a fluorescent rim in conA-FITC labeled cells (fig. 1, O, P). When NL and DL cells treated with cis-Platin or neuraminidase for 30 and 60 min are incubated in PBS without sialic acid for the same period as these incubated with sialic acid and then labeled with FITC conjugated lectins, the pattern of fluorescence remains the same as that of the cells treated with cis-Platin or neuraminidase and immediately labeled with lectin-FITC (figures not included).

Neuraminidase treatment of NL and DL cells results in the same differential pattern of fluorescence with conA-FITC and WGA-FITC as observed for cis-Platin treated cells (figures not included).

Sialidase activity is found to be higher in DL cells than that of NL (fig. 2). The enzymatic activity is significantly enhanced in both NL and DL cells after cis-Platin acid treatment (fig. 2). When type IV ganglioside used as sialidase substrate is directly incubated with cis-Platin, only a very small amount of free sialic acid is released (fig. 3). On the other hand neuraminidase incubation of ganglioside results in a high release of free sialic acid (fig. 3).

Discussion. It has been reported by us that the low agglutinability of NL with conA and WGA, and the weak fluorescence intensity with conA-FITC and WGA-FITC, increase appreciably after cis-Platin treatment from 10 to 30 min. On the other hand DL cells under similar conditions, which have a high degree of agglutinability and bright fluorescence intensity, show a gradual decrease in the fluorescence intensity as well as in the degree of agglutination from 10 to 60 min of cis-Platin treatment[6–9]. Prasad and Sodhi[6] reported the increased release of sialic acid from cells after cis-Platin treatment, and that neuraminidase treatment of NL and DL cells has similar results as observed for cis-Platin[6,7]. It was proposed that sialic acid plays a role in the lectin binding to the cell surface. To confirm the role of sialic acid moieties in the expression of lectin binding sites, the present studies were undertaken. Greenway and Levine[12] reported that the treatment of transformed cells with neuraminidase abolished their ability to be agglutinated by WGA. Other workers have also suggested a role of sialic acid in cell agglutination with lectins[13–19]. Masking of lectins RCA-I and II by sialic acid has been reported[14,16,17]. Maylie-Pfenninger and Jamieson[18] showed that after neuraminidase treatment and labeling with lectin-ferritin the number of receptor sites for SBA and RCA-II on endocrine cells increased dramatically, and suggested that the sialic acid masks the receptor sites for SBA and RCA-II. Burger and Goldberg[15] noted that neuraminidase treatment of L-1210 cells abolished their agglutinability by WGA, thus implicating membrane sialic acid residues in the agglutination process. Ganguly and Fossett[19] reported that ^{125}I-WGA binds to the surface of human platelets and leads to their agglutination, but platelets treated with neuraminidase showed a markedly reduced agglutination with WGA as compared to that of controls. They proposed that the decreased binding of WGA to neuraminidase-treated platelets may be due to reduction in the number of binding sites or an altered affinity. Bhavanandan

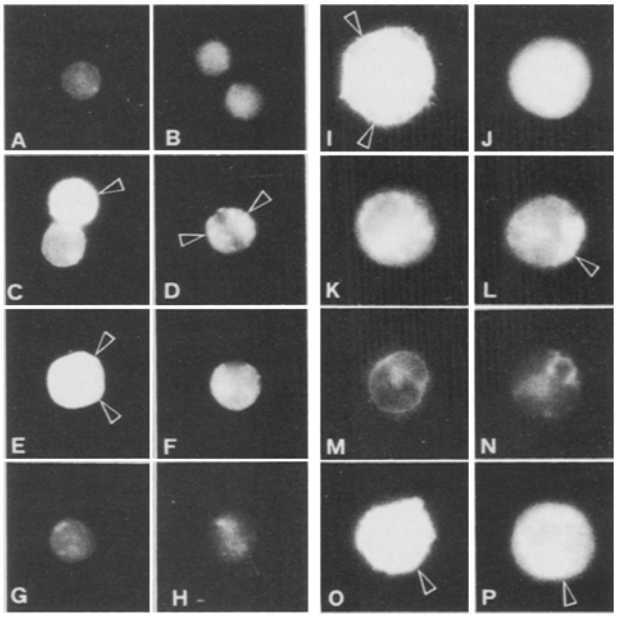

Figure 1 *A, B* Control NL showing weak, evenly distributed fluorescence with conA-FITC (A) and WGA-FITC (B). *C, D* 20 min cis-Platin treated NL showing increased fluorescence intensity. Note the fluorescent rim around the cell with conA-FITC (C, arrow), and appearance of small granular fluorescent patches on the two halves of the cell with WGA-FITC (D, arrows). *E, F* 30 min cis-Platin treated NL. Note the further increase in the fluorescence intensity, with fluorescent rim in conA-FITC (E, arrows), and even distribution of fluorescent granular patches with WGA-FITC (F). *G, H* Sialic acid incubated NL after 30 min of cis-Platin treatment. Note the significant decrease in the fluorescent intensity. conA-FITC (G), WGA-FITC (H). *I, J* Control DL cells showing a bright fluorescence with conA-FITC (I) and WGA-FITC (J). Note the presence of a fluorescent rim in conA-FITC (arrows) and fine granular distribution of fluorescence in WGA-FITC. *K, L* 20 min cis-Platin treated DL cells. Note the decrease in the fluorescence intensity and appearance of fluorescent patches in WGA-FITC (L, arrow). *M, N* 30 min cis-Platin treated DL cells showing further decrease in fluorescence intensity with conA-FITC (M) and WGA-FITC (N). *O, P* Sialic acid incubated DL cells after 60 min of cis-Platin treatment. Note the increase in the fluorescence intensity with conA-FITC (O) and WGA-FITC (P). The fluorescent rim is evident in conA-FITC (O, arrow) and fluorescent granular patches in WGA-FITC (P, arrow). × 860.

and Katlic[20] also suggested that the topography of the N-acetyl neuraminic acid on the cell surface is important for the formation of a stable association between WGA and the cell surface. In the present fluorescence microscopical studies it was shown that when cis-Platin or neuraminidase treated NL and DL cells are washed and incubated with sialic acid before the conA-FITC and WGA-FITC labeling, the fluorescence intensity observed is similar to that of controls (fig. 1, A, B, G, H, I, J, O, P). This suggests that sialic acid has a regulatory role in the expression of lectin binding sites on the cell surface, and that this regulatory role of sialic acid in the expression of lectin binding sites could be due to the steric or topographical interactions of sialic acid with lectin binding sites. The removal of sialic acid after cis-Platin or neuraminidase treatment differentially alters the affinity of lectin receptors on the cell surface, which results in the initial increase and then decrease in the lectin binding to cell surface of NL, and a significant decrease in the binding of lectins to DL cells. One observation significant for this differential binding of lectins to NL and DL is that the untreated DL cells show a bright fluorescent rim with conA-FITC (fig. 1, I). When NL are treated with cis-Platin for 20 and 30 min, the increased fluorescence with conA-FITC is accompanied by the appearance of a fluorescent rim (fig. 1, C, E), similar to the one observed in untreated DL cells. This suggests that the molecular organization (at least with reference to conA binding sites) is similar in untreated DL cells and cis-Platin treated NL. And it is at this stage, if cis-Platin treatment of NL is continued, that a marked decrease in the lectin binding to their cell surfaches is observed, as is also observed for DL cells treated for 10–60 min with cis-Platin.

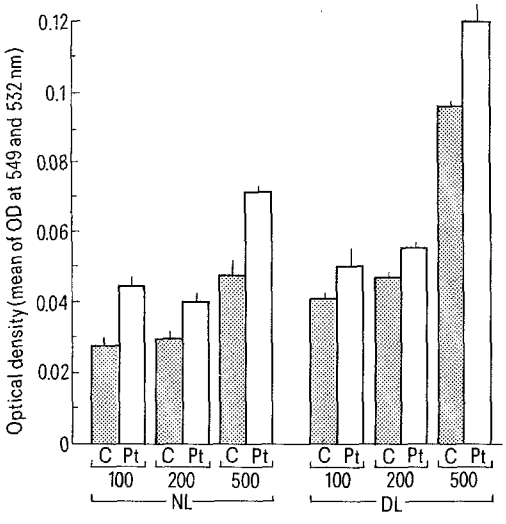

Figure 2. Histogram showing the sialidase activity in normal lymphocytes (NL) and Dalton's lymphoma cells (DL) treated with cis-Platin (Pt) or without (control, C). Note the increased activity of sialidase in the cells after cis-Platin treatment. Substrate (ganglioside type IV) concentrations shown are 100, 200 and 500 µg/ml.

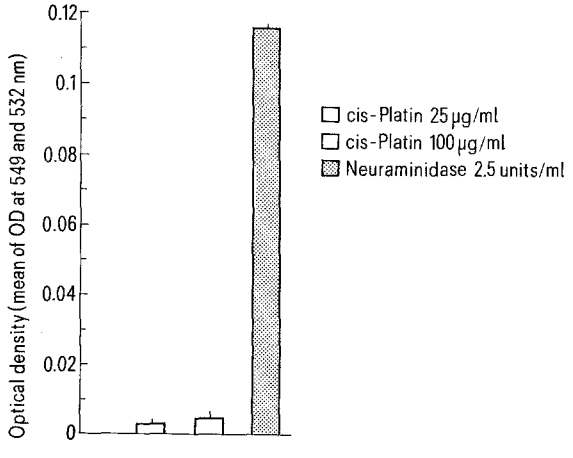

Figure 3. Histogram showing the direct effect of cis-Platin or neuraminidase on the substrate (ganglioside type IV). Note the significant release of free sialic acid from neuraminidase treated substrate, and only a very small amount in the cis-Platin treated substrate.

Another important finding which emerged from the present studies is the differential binding of conA and WGA over the surface of the cells. ConA-FITC labeling always shows a rim around the cells (fig. 1, C, E, I, O), whereas WGA-FITC binding is followed by the appearance of small granular fluorescent patches (fig. 1, D, F, L, N). Although it was observed by us previously by various ultrastructural and biochemical techniques that cis-Platin gradually removes the sialic acid moieties from the cell surface[6,7] the actual mechanism of removal of sialic acid was not investigated. Therefore, it was natural to look at the sialidase activity in NL and DL cells treated with or without cis-Platin, and also at the direct effect of cis-Platin on a substrate of sialidase. Interestingly, in cis-Platin treated cells a significant increase in the sialidase activity was observed (fig. 2). This increase in sialidase activity could be responsible for the release of sialic acid from the cell surface. Further evidence for this suggestion comes from the other set of experiments in which sialidase substrate (ganglioside type IV) was directly incubated with cis-Platin or neuraminidase. The substrate which was incubated with cis-Platin released only a very small amount of free sialic acid (from the substrate). On the other hand, neuraminidase-incubated substrate showed a high release of sialic acid from the substrate. On this basis it is suggested that cis-Platin removes the sialic acid moieties from the cell surface by increasing the sialidase activity in the cell membrane and not by just splitting the terminal sialic acid component from the cell surface. Therefore, the process of increased removal of sialic acid from the cell surface after cis-Platin treatment may well be due to the increased activity of sialidase in the cell membrane.

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