ANITITUMOR ACTIVITY OF PLATINUM(II) COMPLEXES OF 1,2-DIAMINO-
CYCLOHEXANE ISOMERS*1

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Dichloro, dibromo, oxalato, malonato, dinitrato, sulfato and mono and bis-
(D-glucuronato) platinum(II) complexes of 1,2-diaminocyclohexane (dach)
isomers were prepared and tested on L1210 mouse leukemia employing the NCI
protocol for evaluation of Pt analogs.

A large number of long-term survivors were observed with certain analogs,
though the therapeutic indices (optimal dose/minimum effective dose) were not
large. Among the analogs tested, the oxalato, malonato, dinitrato and mono-
(D-glucuronato) Pt(II) complexes of trans-l,2-diaminocyclohexane were found
to be particularly effective. The glucuronato Pt complexes appear to be prom-
ising candidates for clinical trial since they have the highest solubility in water.

Key words: 1,2-Diaminocyclohexane platinum(II) complexes — Leukemia
L1210 — Antitumor activity — Water-soluble Pt(II) complexes

Since the discovery of antitumor plati-
num(II) complexes, cis-dichlorodiammineplat-
inum(II) (cis-Platinum) by Rosenberg et al.,8) various Pt(II) complexes have been prepared
and tested on various tumor systems. Among
them, the dichloro Pt(II) complex of 1,2-
diaminocyclohexane (dach) was reported to
be active by Cleare,1) Connors,2) Gale3) and
Speer.10) These early Pt dach complexes were
isomeric mixtures, and we succeeded for
the first time in separating them into two
geometrical isomers, cis and trans, followed
by resolution of the trans isomer into two
optical isomers, trans-d and trans-l. Their
absolute configurations are: cis=1S, 2R-dach;
trans-d=1S, 2S-dach; trans-l=1R, 2R-dach.

The Pt(II) complexes prepared were the
dichloro, dibromo, oxalato, malonato, sulfato,
dinitrato, and mono and bis(D-glucuronato)
Pt(II) complexes of the 3 dach isomers. The
first 4 analogs are water-insoluble and the
rest are water-soluble.

In order to select the most potent analog,
determinations of T/C% and therapeutic
index (TI) were carried out with L1210 mouse leukemia according to the protocol
for the study of Pt analogs recommended by
the National Cancer Institute, U.S.A. Some
of them exhibited high antitumor activity

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and TI values greater than that of cis-Platinum. Our evaluation was based on T/C values, as well as the numbers of long-term survivors of the treated mice bearing L1210, with reference also to similar experimental data with P388 mouse leukemia.\(^5,6\)

**Materials and Methods**

Separation of the geometrical isomers of dach and the resolution of trans-dach were carried out according to the method described by Saito and Kidani\(^9\) and Galsbøl et al.\(^4\).

Dichloro platinum(II) complexes of dach isomers were synthesized according to the usual method by mixing K\(_2[PtCl_4]\) and dach isomers in a 1:1 ratio in water. Dibromoplatinum(II) complexes were prepared by treating dinitrato Pt(II) complexes of dach isomers with KBr. Carboxylato Pt(II) complexes of dach were obtained by treating dinitrato Pt(II) complexes of dach with the corresponding carboxylates. Dinitrato and sulfato Pt(II) complexes were obtained by treating dichloro Pt(II) with AgNO\(_3\) and Ag\(_2\)SO\(_4\), respectively.

Mono and bis(D-glucuronato) Pt(II) complexes of dach isomers were obtained by mixing sodium glucuronato and dinitrato Pt(II) complexes of dach in 1:1 and 1:2 ratios in water. The mixtures were allowed to stand at room temperature for about 3 weeks and the resulting solutions were evaporated to dryness at 40\(^\circ\)C under reduced pressure. The solids thus obtained were washed with small amounts of EtOH.

Preparation of suspensions of the insoluble Pt complexes: Suspensions of the water-insoluble analogs were prepared in physiological saline with the addition of a few drops of Tween 80.

**Evaluation of Antitumor Activity** DBA/2 and CD2F\(_1\) (BALB/c × DBA/2)\(_1\) mice were supplied by Simonsen Laboratories, California, and Laboratory Supply Co., Inc., Indianapolis. L1210 tumor was maintained by serial passage in male DBA/2 mice. L1210 cells (10\(^5\)) were transplanted intraperitoneally into CD2F\(_1\) mice on day 0, and the samples were given intraperitoneally on days 1 and 5, or 1, 5 and 9. From the mean survival time (days) of both treated (T) and control (C) mice, T/C\(\%\) values were calculated. The lowest dose at which T/C\(\%\) exceeded 125 was designated as the minimum effective dose (MED), and the dose which produced the maximum T/C\(\%\) as the optimal dose. TI was expressed as the ratio of the optimal dose to MED. A dose at which the T/C value was less than 85\% was designated as a toxic dose.

**Results**

The antitumor activities of the dichloro and dibromo platinum(II) complexes of dach isomers on L1210 are listed in Table I, in comparison with that of cis-Platinum. Therapeutic indices for dach isomers seemed to be generally superior to those of cis-Platinum; the dichloro Pt(II) complex of trans-\(l\)-dach isomer had a greater TI of 32 and the dichloro Pt(II) complexes of cis and trans-\(d\)-dach isomers had TI values of 16, while TI of cis-Platinum was 8. However, TIs of these compounds on P388-bearing mice were in the range of 4 to 8, all being rather similar.\(^\text{12}\)

As shown in Table I, the effective dose ranges of the dihalogeno Pt(II) complexes were higher without a significant decrease of T/C values at the optimal dose if the chlorine atoms of dichloro complexes were replaced by bromine atoms. In the case of the dibromo Pt(II) complex of trans-\(l\)-dach, 5 out of 6 treated mice survived the 30-day observation period with T/C of 343\% at a dose of 225 mg/kg (2 injections on days 1 and 5) (Fig. 1).

This dibromo Pt(II) complex of trans-\(l\)-dach showed higher activity against P388 than the dichloro complex, and seemed to be one of the most effective Pt(II) complexes, though its solubility in water was poor. However, as shown in Fig. 2, the T/C value of the dibromo compound did not exceed 229\%, with only one long-term survivor out of 6 mice, if the mice were given 3 injections on days 1, 5 and 9.

A similar result was also observed with dichloro Pt(II) complex of trans-\(l\)-dach, as shown in Fig. 2, as well as with cis-Platinum, and may be due to cumulative toxicity of the water-insoluble analogs with 3 successive injections. Since we hoped to find an active Pt(II) complex which has low cumulative toxicity and reduced side-effects on the kidneys, the screening protocol of 3 injections was employed for further investigation.
Table I. Comparison of the Antitumor Activities of Pt(Halogeno)$_2$ (dach) Complexes against Leukemia L1210

<table>
<thead>
<tr>
<th>NSC No.</th>
<th>Dach</th>
<th>Leaving groups</th>
<th>Treatment schedule (days)</th>
<th>Toxic dose$^a$ (mg/kg)</th>
<th>Optimal dose$^b$ (mg/kg)</th>
<th>T/C$^c$%</th>
<th>MED$^c$ (mg/kg)</th>
<th>T/C$^c$%</th>
<th>TI$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>119875</td>
<td>cis-Pt</td>
<td>Cl$_2$</td>
<td>1, 5</td>
<td>25</td>
<td>12.5</td>
<td>278</td>
<td>1.56</td>
<td>146</td>
<td>8</td>
</tr>
<tr>
<td>259917</td>
<td>cis</td>
<td>Cl$_2$</td>
<td>1, 5</td>
<td>50</td>
<td>12.5</td>
<td>211</td>
<td>0.78</td>
<td>139</td>
<td>4</td>
</tr>
<tr>
<td>263459</td>
<td>trans-d</td>
<td>Cl$_2$</td>
<td>1, 5</td>
<td>50</td>
<td>6.25</td>
<td>336 (3)</td>
<td>0.39</td>
<td>127</td>
<td>4</td>
</tr>
<tr>
<td>263460</td>
<td>trans-l</td>
<td>Cl$_2$</td>
<td>1, 5</td>
<td>50</td>
<td>12.5</td>
<td>379 (3/3)</td>
<td>≤0.39</td>
<td>133</td>
<td>4</td>
</tr>
<tr>
<td>289558</td>
<td>cis</td>
<td>Br$_2$</td>
<td>1, 5</td>
<td>100</td>
<td>25</td>
<td>227 (2)</td>
<td>1.56</td>
<td>127</td>
<td>16</td>
</tr>
<tr>
<td>289759</td>
<td>trans-d</td>
<td>Br$_2$</td>
<td>1, 5</td>
<td>100</td>
<td>12.5</td>
<td>312 (3)</td>
<td>≤1.56</td>
<td>149</td>
<td>8</td>
</tr>
<tr>
<td>289559</td>
<td>trans-l</td>
<td>Br$_2$</td>
<td>1, 5, 9</td>
<td>100</td>
<td>25</td>
<td>343 (3)</td>
<td>1.56</td>
<td>129</td>
<td>4</td>
</tr>
<tr>
<td>289759</td>
<td>cis</td>
<td>Br$_2$</td>
<td>1, 5, 9</td>
<td>100</td>
<td>25</td>
<td>188</td>
<td>6.25</td>
<td>151</td>
<td>4</td>
</tr>
<tr>
<td>289559</td>
<td>trans-d</td>
<td>Br$_2$</td>
<td>1, 5, 9</td>
<td>100</td>
<td>6.25</td>
<td>252</td>
<td>1.56</td>
<td>140</td>
<td>4</td>
</tr>
<tr>
<td>289759</td>
<td>trans-l</td>
<td>Br$_2$</td>
<td>1, 5, 9</td>
<td>100</td>
<td>25</td>
<td>229 (1)</td>
<td>0.78</td>
<td>133</td>
<td>22</td>
</tr>
</tbody>
</table>

$^a$ Toxic dose: Dose at which T/C is less than 85%
$^b$ Optimal dose: Dose which produces the maximum T/C%.
$^c$ MED: The lowest dose at which T/C% exceeds 125
$^d$ TI: Therapeutic index (optimal dose/MED)

$\text{cis}-\text{Pt} = \text{cis}-\text{dichlorodiimineplatinum(II)}$

**Fig. 1.** Antitumor activities of PtBr$_2$ (trans-l-dach) against L1210: Drugs were administered on days 1 and 5. The numbers in parentheses indicate 30-day survivors out of 6 mice.

**Fig. 2.** Comparison of the antitumor activities of PtCl$_2$ (trans-l-dach) and PtBr$_2$ (trans-l-dach) against L1210: Drugs were administered on days 1 and 5, or days 1, 5, and 9 (open symbols). The numbers in parentheses indicate 30-day survivors out of 6 mice.
Contrary to expectation, the oxalato and malonato (=PHM) Pt(II) complexes were not readily soluble in water. However, the oxalato Pt(II) complex of trans-l-dach exhibited a maximum T/C value of 308% with 4 long-term survivors among 6 L1210-bearing mice, treated at a dose of 12.5 mg/kg. In a similar experiment with the malonato Pt(II) complex of trans-l-dach, the maximum T/C value was 355% with 4 long-term survivors among 6 mice at a dose of 50 mg/kg, though the TIs of these carboxylato complexes were not large. As shown in Table II, the toxic doses of malonato complexes were about 8 to 16 times greater, and their MED approximately 4 times greater than those of the oxalato complexes (Fig. 3).

In order to obtain more water-soluble complexes by modifying the leaving groups, dinitrato and sulfato (=SHP) Pt(II) complexes of the dach isomers were prepared. Sulfato Pt(II) complexes of the 3 dach isomers

### Table II. Comparison of the Antitumor Activities of Pt(dicarboxylato)(dach) Complexes against Leukemia L1210

<table>
<thead>
<tr>
<th>NSC No.</th>
<th>Dach</th>
<th>Leaving groups</th>
<th>Toxic dose&lt;sup&gt;a)&lt;/sup&gt; (mg/kg)</th>
<th>Optimal dose&lt;sup&gt;b)&lt;/sup&gt; T/C%</th>
<th>MED&lt;sup&gt;c)&lt;/sup&gt; T/C%</th>
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<tbody>
<tr>
<td>266038</td>
<td>cis</td>
<td>ox</td>
<td>50</td>
<td>25</td>
<td>217</td>
</tr>
<tr>
<td>266042</td>
<td>trans-d</td>
<td>ox</td>
<td>25</td>
<td>12.5</td>
<td>179</td>
</tr>
<tr>
<td>266046</td>
<td>trans-l</td>
<td>ox</td>
<td>25</td>
<td>12.5</td>
<td>308 (4)</td>
</tr>
<tr>
<td>266039</td>
<td>cis</td>
<td>mal</td>
<td>400</td>
<td>50</td>
<td>308 (3)</td>
</tr>
<tr>
<td>266043</td>
<td>trans-d</td>
<td>mal</td>
<td>400</td>
<td>50</td>
<td>195 (1)</td>
</tr>
<tr>
<td>266047</td>
<td>trans-l</td>
<td>mal</td>
<td>400</td>
<td>50</td>
<td>355 (4)</td>
</tr>
</tbody>
</table>

Administered to CDF<sub>1</sub> mice on days 1, 5 and 9

<sup>a)</sup> Toxic dose: Dose at which T/C is less than 85%
<sup>b)</sup> Optimal dose: Dose which produces the maximum T/C%
<sup>c)</sup> MED: The lowest dose at which T/C% exceeds 125

Numbers in parentheses indicate 30-day survivors out of 6 mice.

ox = oxalate ion; mal = malonate ion

![Fig. 3. Antitumor activities of Pt(oxalato)(dach) (solid lines) and Pt-(malonato)(dach) (broken lines) against L1210](image)

Drugs were administered on days 1, 5, and 9. cis-dach (x), trans-d-dach (○), trans-l-dach (●). The numbers in parentheses indicate 30-day survivors out of 6 mice.
ANTITUMOR ACTIVITY OF PLATINUM(II)

Table III. Comparison of the Antitumor Activities of Water-soluble Pt(II) Complexes against Leukemia L1210

<table>
<thead>
<tr>
<th>NSC No.</th>
<th>Dach</th>
<th>Leaving groups</th>
<th>Toxic dose(^a) (mg/kg)</th>
<th>Optimal dose(^b) T/C% (mg/kg)</th>
<th>MED T/C%</th>
<th>TI</th>
</tr>
</thead>
<tbody>
<tr>
<td>289542</td>
<td>cis</td>
<td>SO(_4)</td>
<td>25</td>
<td>6.25</td>
<td>180</td>
<td>0.78</td>
</tr>
<tr>
<td>289543</td>
<td>trans-d</td>
<td>SO(_4)</td>
<td>12.5</td>
<td>3.12</td>
<td>219</td>
<td>0.78</td>
</tr>
<tr>
<td>289544</td>
<td>trans-l</td>
<td>SO(_4)</td>
<td>12.5</td>
<td>3.12</td>
<td>189</td>
<td>0.78</td>
</tr>
<tr>
<td>290133</td>
<td>cis</td>
<td>(NO(_3))(_2)</td>
<td>25</td>
<td>6.25</td>
<td>223</td>
<td>0.39</td>
</tr>
<tr>
<td>290131</td>
<td>trans-d</td>
<td>(NO(_3))(_2)</td>
<td>25</td>
<td>6.25</td>
<td>287 (2)</td>
<td>0.78</td>
</tr>
<tr>
<td>290132</td>
<td>trans-l</td>
<td>(NO(_3))(_2)</td>
<td>25</td>
<td>6.25</td>
<td>335 (3)</td>
<td>0.39</td>
</tr>
<tr>
<td>289548</td>
<td>cis</td>
<td>(glucu)</td>
<td>≥50</td>
<td>50</td>
<td>212 (2)</td>
<td>6.25</td>
</tr>
<tr>
<td>289550</td>
<td>trans-d</td>
<td>(glucu)</td>
<td>50</td>
<td>25</td>
<td>313 (2)</td>
<td>1.56</td>
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<tr>
<td>289561</td>
<td>trans-l</td>
<td>(glucu)</td>
<td>50</td>
<td>25</td>
<td>313 (4)</td>
<td>1.56</td>
</tr>
<tr>
<td>289549</td>
<td>cis</td>
<td>(glucu)(_2)</td>
<td>≥50</td>
<td>25</td>
<td>154</td>
<td>6.25</td>
</tr>
<tr>
<td>289551</td>
<td>trans-d</td>
<td>(glucu)(_2)</td>
<td>50</td>
<td>25</td>
<td>274 (1)</td>
<td>1.56</td>
</tr>
<tr>
<td>289562</td>
<td>trans-l</td>
<td>(glucu)(_2)</td>
<td>100</td>
<td>50</td>
<td>330 (2)</td>
<td>6.25</td>
</tr>
</tbody>
</table>

\(^a\) Toxic dose: Dose at which T/C is less than 85%  
\(^b\) Optimal dose: Dose which produces the maximum T/C%  
The numbers in parentheses indicate 30-day survivors out of 6 mice.

were not especially effective, with no long-term survivors, though the animal test against L1210 leukemia carried out at the Wadley Institutes of Molecular Medicine, Dallas, Texas, showed exceedingly potent activity.\(^7\) Dinitrato Pt(II) complexes, which were soluble in water, showed high TI with excellent T/C values, as shown in Table III. In particular, the trans-l-isomer showed a T/C value of 335% with 3 long-term survivors out of 6 mice treated at a dose of 6.25 mg/kg (Fig. 4).

Mono and bis(D-glucuronato) complexes of the dach isomers were synthesized in the hope of obtaining reduced toxicity in the kidneys, owing to their high solubility in water. In fact, they were freely soluble in water (solubility: >150 mg/ml at room temperature), though the exact chemical structures of these complexes have not yet been determined. Among mono(D-glucuronato) Pt(II) complexes of the dach isomers, trans dach was superior to cis dach, as shown in Table III, but there was little difference in T/C values between trans-d and trans-l (Table III and Fig. 5). The trans-l complex gave a T/C of 313% with 4 long-term survivors among 6 mice treated at a dose of 12.5 mg/kg, while the trans-d complex showed a T/C of 313% with 2 long-term survivors among 6 mice at a dose of 25 mg/kg. The TI of the former was 8

Fig. 4. Antitumor activities of Pt(NO\(_3\))\(_2\)(dach) complexes against L1210

Drugs were administered on days 1, 5, and 9. cis-dach (x -- x), trans-d-dach (O -- O), trans-l-dach (● -- ●). The numbers in parentheses indicate 30-day survivors out of 6 mice.
Fig. 5. Antitumor activities of Pt(d-glucuronato) (trans-dach) (solid lines) and Pt(d-glucuronato)₂(trans-dach) (broken lines) against L1210. Drugs were administered on days 1, 5, and 9. The numbers in parentheses indicate 30-day survivors out of 6 mice.

Fig. 6. Model structures of dichloro-cis-, and trans-l-dach plantinum(II) complexes
(a) Dichloro cis-1,2-diaminocyclohexaneplatinum(II)
(b) Dichloro trans-1,2-diaminocyclohexaneplatinum(II)

and that of the latter was 16. Bis(d-glucuronato) complexes also showed excellent activity against L1210; the trans-l complex seemed more than the trans-d complex. As far as dach isomers are concerned, trans-l-dach Pt(II) complexes seem to be the most potent so far tested.

**DISCUSSION**

It has already been reported by others that several Pt(II) complexes of isomeric mixtures of dach showed antitumor activity superior to that of cis-Platinum. Upon separation of dach into 3 isomers, it became apparent that the antitumor activities of the Pt(II) complexes of the dach isomers all differed. Trans-dach Pt(II), and especially trans-l-dach Pt(II), were found to be generally more potent than cis-dach Pt(II).

These phenomena can be explained in terms of the conformational differences among the Pt(II) complexes of dach isomers, as visualized in Fig. 6. Trans-l-dach Pt(II) complex takes a lambda-gauche form, while trans-d-dach takes a delta-gauche form.

Since the final targets of antitumor Pt(II) complexes are considered to be the bases of DNA molecules, the conformational change is expected to affect the interaction between Pt(II) complexes and DNA.

From a clinical standpoint, the cumulative toxicity of certain Pt complexes to the kidney was very serious. As demonstrated in Table I and Fig. 2, the antitumor activities of the dichloro and dibromo Pt(II) complexes of dach isomers were high when administered twice (on days 1 and 5), while the activities were markedly reduced on administration by 3 successive injections on days 1, 5 and 9, possibly because of cumulative toxicity. Since the cumulative toxicity might be related to the water solubility, several Pt(II) complexes of each dach isomer having water-soluble leaving groups were prepared and their antitumor activities were compared.

Though the solubility of carboxylato Pt complexes was poor, contrary to our expectation, oxalato and malonato Pt complexes
of trans-l-dach showed strong antitumor activity against L1210. Sulfato Pt complexes were soluble in water, but did not show any marked activity in our animal test.

Malonato and sulfato Pt(II) complexes of trans-l-dach are already in Phase I clinical study at the Wadley Institutes of Molecular Medicine, Dallas, Texas as “neo-PHM” and “neo-SHP”, respectively. The former was reported to be very active against acute myelogenous leukemia.\textsuperscript{11)

Other water-soluble Pt complexes of interest were dinitrato and mono D-glucuronato trans-l-dach Pt(II); in particular, the latter was freely soluble in water. Their antitumor activities were high, with many long-term survivors.

Judging from experiments using mouse leukemias L1210 and P388, oxalato, malonato, dinitrato and mono D-glucuronato Pt(II) complexes of trans-l-dach seemed to show reduced toxicity and high potency, and in particular mono D-glucuronato Pt(II) of trans-l-dach appeared to be a promising candidate for clinical trial in view of its high solubility in water.

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