Studies on OPSPA

III. Distribution and Excretion of Radioactivity Following Administration of OPSPA-C\(^{14}\) and OPSPA-P\(^{32}\) to Humans*

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Following the recognition that N-ethylene-substituted phosphoramides possess tumor-inhibitory properties against transplanted tumors in animals (cf. 8), there have been a number of clinical studies reported in the literature (1-6, 11-13). These have usually dealt with the action of TEPA and thio-TEPA primarily against leukemias. OPSPA itself has undergone extensive clinical evaluation against solid tumors in the University Hospitals (4, 5), and a detailed clinical report will be given elsewhere. In none of these studies has any information on the excretion, distribution, and metabolism of the drugs in man been obtained. In the case of OPSPA, a compound that causes complete regression of established Flexner-Jobling carcinomas with a single, nontoxic dose (8), such studies in human cancer patients have been carried out concurrently with these in tumor-bearing rats (9). The present paper reports the results of studies on the distribution, excretion, and blood levels of radioactivity following administration of OPSPA-C\(^{14}\) and -P\(^{32}\) to human patients by several routes.

MATERIALS AND METHODS

The synthesis of the labeled compounds has been described (9).

Six patients were chosen for these studies, all white females of roughly comparable ages and weights. The first group of three who received the carbon-labeled drug had metastatic skin tumors following radical mastectomy for breast carcinoma. The others receiving the P\(^{32}\)-labeled compound had various other malignancies: the patient receiving OPSPA intramuscularly in oil for 5 successive days had an inoperable ovarian adenocarcinoma with metastases to the peritoneum; the intraperitoneally treated case had an adenocarcinoma of the breast with pulmonary metastases; and the woman given OPSPA intramuscularly in saline suffered from an inoperable bronchogenic carcinoma. All diagnoses were confirmed histologically. Insofar as the dose was concerned, the amount of OPSPA-C\(^{14}\) that could be given was limited by the maximum radioactivity authorized by the Atomic Energy Commission (250 μc.), while with the P\(^{32}\)-labeled OPSPA it was limited by the maximum tolerated dose of 0.4 mg/kg/day. Thus, OPSPA-C\(^{14}\) was given in a single dose of 5.0 mg. in the intravenous- and intramuscularly-treated cases and 3.5 mg. in the intratumoral-treated patient. The OPSPA-P\(^{32}\) was given in single injections, intramuscularly and intrapleurally, the dose in each case depending upon the body weight. Intravenous, intratumoral, and intrapleural injections were given in saline; intramuscular injections were given either in saline or corn oil.

Following administration of the labeled drug, blood samples were withdrawn at 10 and 20 minutes, and 1, 2, 4, 8, 12, 24, and 48 hours afterwards. Urines were collected as voided or by catheter. Tissue samples were obtained by biopsy. Radioactivity assays were carried out as described (9).

To determine the rates of disappearance of OPSPA-P\(^{32}\) from the site of intramuscular injection, a Geiger tube was held against the carefully marked site on the skin, to assure reproducible geometry.

RESULTS

Chart 1 shows a semilogarithmic plot of the plasma levels of radioactivity against time in three patients given OPSPA-C\(^{14}\) intravenously (I.V.), intramuscularly in oil (I.M.), and intratumorally (I.T.). It appears from the graph that, except for the intravenous case, the initial rate component marks the passage of the drug from the site of injection into the blood stream; this takes approximately 20 minutes in the intravenous case, 2 hours in the intratumoral, and 4 hours in the intramuscularly injected patient. During the next 2-8-hour period the drug and its metabolites decrease in the blood stream and probably become equilibrated in the tissues. This process has an approximate half-time of 4 hours in each case. By 8 hours a component in the curve appears, representing a much slower rate, which is the same within ex-
Experimental error in all three patients. The halftime of this process is 29 hours. It seems reasonable to suppose that this slow component represents the gradual release of the drug or its metabolites from the tissues. From the point of view of maintaining a blood level, there would appear to be no advantage in any one of these modes of administration.

The cumulative urinary excretion of radioactivity in the same three patients is shown in Chart 2. Only minute quantities of isotope were present in the feces. By 72 hours, 42 per cent of the total injected radioactivity appeared in the urine following intravenous injection, whereas a somewhat higher amount (53 per cent) was excreted in the intratumorally injected patient and 56 per cent in the intramuscularly treated case. It is interesting to note that there was a delayed excretion during the first 24-hour period in the intramuscular case, but that all the values leveled off by the 3d day. During the first 12-hour period, the intravenously and intratumorally injected drugs were excreted at the same rates. These results on urinary excretion are very similar to those found in rats (9).

Chart 3 represents the semilogarithmic plot of the disappearance of radioactivity from the site of the injection of OPSPA in corn oil and in saline into the deltoid muscle. The high energy of the $P^{32}$-particle made direct counting of the site possible. When the drug was injected in oil, the halftime of disappearance of radioactivity from the site of injection was about 1 hour, but in the saline-injected patient the half-time was estimated at 25 seconds, a remarkably rapid absorption.

In Chart 4 is shown the semilogarithmic plot of...
the plasma levels of radioactivity of these two patients, together with that of a woman given the drug by intrapleural injection. It is clear that the blood picture correlates with the disappearance from the site of intramuscular injection in that the maximum specific activity was reached within 40 minutes when the injection was given in saline, whereas it took 4–6 hours to reach the maximum when oil was the vehicle. However, the plasma level dropped more rapidly when the drug was given in saline. In the intrapleurally injected patient, the rise in the blood level was as rapid as in the intramuscular saline case, which demonstrated a very rapid absorption from the pleural cavity; the decay was somewhat slower than in the intramuscular case.

As indicated in Chart 5, there was a much more rapid excretion of radioactivity in the urine of the intrapleurally treated patient than in the one given OPSPA intramuscularly in oil, in agreement with the blood-level data.

Since the most usual therapeutic course of treatments with OPSPA involved five consecutive daily intramuscular injections in oil, a patient was thus treated with OPSPA-P₃², and the plasma level of radioactivity was measured at various time intervals for 7 days. The results given in Chart 6 show that it required three daily injections for the maximum plasma level of 150 counts/min/ml of blood to be reached, which subsequently fell to a value of 50 counts/min/ml. These maximum and minimum values represent radioactivities equivalent to 0.25–0.40 µg of OPSPA or metabolites circulating/ml of blood. Once the maximum value of 150 counts/min/ml was reached, the fluctuation between the high and low values was remarkably constant for a 3-day period. It can definitely be concluded that repeated doses of OPSPA will cause a blood level considerably higher than that obtained with a single dose, indicating that there is some sort of cumulative effect.

The results of the radioactivity assay of biopsy samples obtained from patients treated with OPSPA-C¹⁴ are given in Table 1. The specific activity of the P₃²-labeled drug was too low to obtain statistically valid counts in tissues obtained by biopsy or surgery. It is clear from the data that the uptake by the tumor was small in comparison with that of liver when the drug was not injected locally, a result comparable to that found in rats (9). The directly injected tumor, however, had a comparatively high specific activity, although this represented only a minute amount of total retention. The adjacent fat had a higher specific activity, probably as a result of the lipophilic nature of the drug and the lack of vascularity of the fatty tissue, because when OPSPA was given intramuscularly the fat had only a small uptake of isotope. Since both tumors were similar and the fatty tissues were obtained from the same location relative to the tumors, these comparisons appear justifiable.

DISCUSSION

It is inevitable from the nature of clinical experimentation that blood-level and excretion data are collected from individual patients. While there is undoubtedly some quantitative variation from patient to patient, nevertheless the similarity of the blood-level curves following administration of both P₃² and C¹⁴-labeled OPSPA intramuscularly in oil shows that the shapes of the curves are probably valid. Some blood-level and excretion data based on patients given labeled 6-mercaptopurine have been reported by Hamilton and Elion (7).

It is evident that, just as in the case of the rat experiments (9), in humans OPSPA is rapidly ab-
sorbed from the site of injection, maintained in the blood stream for a considerable period of time, and eliminated in the urine. The chemical nature of the radioactivity will be dealt with in a separate paper (10). There is no evidence of selective localization of the drug or its metabolites in tumors; the specific activities of liver biopsy samples were higher than tumor biopsies when the compound was not locally injected. Thus, the over-all patterns of distribution, excretion, and metabolism in humans are comparable to those in rats, in which the tumor-inhibitory effect must be due to the presence of a minute amount of OPSPA or metabolite, probably bound with some critical component of the cell (9, 10). Unfortunately, none of the patients in the present metabolic study responded very favorably to OPSPA therapy, so that it is impossible to correlate the metabolic results with clinical effectiveness.

The present work represents an approach to the study of the clinical metabolism and excretion of a potentially tumor-inhibitory drug. From this study we have learned a considerable amount about blood levels, excretion, and tissue distribution of OPSPA in the human under several conditions of dosage, and this information has some general interest. At the outset, it was hoped that the studies would lead to a more satisfactory and logical mode of treatment of the patients. However, at the present state of our lack of knowledge, it must be admitted that such a hope was premature. It is not even known whether or not it is desirable to maintain a high blood level or to slow down absorption and excretion in order to obtain optimal effects against the tumor.

Although OPSPA is a very potent drug against transplanted tumors in rats and mice (8), the lack of consistently favorable clinical responses in patients with solid tumors (4, 5) has been a disappointment, but one that is shared with all those who have tested the other cancer drugs known at present. In the animals the pronounced tumor-inhibitory effect of OPSPA is exerted at a dose at which there is no effect on the bone marrow. However, in human patients there is severe bone-marrow depression at a dose level well below that which might be expected to cause tumor regression. Thus, the primary reason for the lack of conspicuous clinical success with this drug is probably its relatively greater toxicity to humans than to rats. If some means could be devised to protect the hematopoietic system from OPSPA without counteracting its tumor-inhibitory properties, a more effective therapy could be confidently expected.

**TABLE 1**

<table>
<thead>
<tr>
<th>Mode of administration</th>
<th>Material biopsied</th>
<th>Time following administration (hours)</th>
<th>Specific activity (counts/min/mg wet weight)</th>
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</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td>Supra-clavicular metastatic tumor*</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>2.0</td>
</tr>
<tr>
<td>Intratumoral</td>
<td>Subcutaneous metastatic tumor</td>
<td>12</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>Neighboring fat muscle</td>
<td>4</td>
<td>615</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>4</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>15.0</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>Metastatic tumor of skin</td>
<td>4</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>Fat</td>
<td>12</td>
<td>18.0</td>
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<tr>
<td></td>
<td>Liver</td>
<td>8</td>
<td>3.0</td>
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<tr>
<td></td>
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<td>39.2</td>
</tr>
</tbody>
</table>

* For further information about the type of tumor, see "Methods."

favorably to OPSPA therapy, so that it is impossible to correlate the metabolic results with clinical effectiveness.

Work aimed along these lines is under way.

This clinical study adds little to our knowledge of the biochemical mechanism of action of the drug, and few differences in excretion, distribution, and blood level are found between humans, in whom OPSPA has relatively little effect, and rats, in which it is potent. It will remain for more fundamental studies on the mechanism of action of this class of compounds to bring the present data to its greatest usefulness.

**SUMMARY**

1. The blood levels, excretion, and tissue distribution of C¹⁴ and P³¹ labeled OPSPA have been studied in six female cancer patients.
2. The drug was rapidly excreted in the urine, and the rates of urinary excretion correlated well with the blood levels following various modes of administration.
3. OPSPA was more rapidly absorbed from the sites of intramuscular, intratumoral, intrapleural,
and intravenous injection in saline than when given intramuscularly in oil.

4. Repeated daily dosage produced consistently higher blood levels than those observed with individual injections.

5. There was no selective localization in tumor tissue by any mode of administration studied.

REFERENCES


10. ———. Studies on OPSPA. IV. Metabolism of OPSPA in the Rat and Human. Ibid., pp. 296-301.


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