A STUDY OF METABOLIC FATE OF A NEW CENTRAL ANALGETIC: 1-(M-METHOXY PHENYL)-2-DIMETHYLAMINOMETHYLCYCLOHEXANOL (1) HCl (CG-315)

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Abstract—Subcutaneously or per orally administered CG-315 was rapidly absorbed and widely distributed in rat whole body, especially in the lungs, spleen, liver and kidneys etc. Most of the administered drug and its metabolites was excreted in the urine within 24 hr with a small amount in the feces. Part of absorbed CG-315 may be mainly metabolized in liver to O-, N- or both demethylated forms, some of which are conjugated with glucuronates. No difference was found in metabolism of CG-315 between non-tolerant and tolerant rats.

Pharmacological properties of CG-315 have been already demonstrated in our laboratory (1–4); i.e. the drug revealed a potent antitussive effect, though the analgetic action was weaker than that of morphine. However, the mode of action of CG-315 on EEG was different from that of morphine (5–8), and the response to catecholamine was also rather opposite that of the latter (9–12). Physical dependence liability on CG-315 in rats was not observed, but tolerance to the drug did develop moderately (13).

Present experiments were performed to determine distribution, metabolic fate and excretion in rats using 3H-labeled CG-315.

METHODS AND MATERIALS

Male rats of Donryu strain weighing 240–260 g were used. 3H-labeled CG-315 (1 mc/mg) was provided by Tokyo Institute of Kowa Co. Ltd., Japan and the given dose of the drug was fixed at 60 mg/kg regardless of the route of administration.

For the study of tissue distribution of CG-315 and its metabolites, 3H-CG-315 was previously mixed with cold CG-315 to contain 85 μc of 3H-CG-315. Amounts administered to rats, were dissolved in 0.1 ml of 0.9% NaCl per 100 g of body wt. Under light anesthesia with Nembutal (50 mg/kg, i.p.), blood samples were obtained by cardiac puncture, and organs were removed, weighed and homogenized with 0.5 N HCl. Red cells washed with ice chilled 0.85% NaCl solution and an aliquot of blood samples were de-
colorized with 30% H$_2$O$_2$ and the thereafter added to Bray's scintillator (14). Acidified homogenates above mentioned were neutralized with NaOH and then solubilized with DOC and thereafter added to Bray's scintillator. The radioactivity of each sample was counted by Packard's Liquid Scintillation spectrometer Model 3310, quenching being corrected according to the external standard method (15).

A procedure for extraction of CG-315 and its metabolites from tissue homogenate and subcellular fractions is illustrated in Fig. 1 and details for cell fractionation (16) are shown in Fig. 2.

For the study of urinary excretion of CG-315 and its metabolites, the excreted urine was collected and an aliquot of the urine was used for analytical examination, as shown in Fig. 3.

![Figure 1](image1.png)

**Fig. 1.** A procedure for extraction of CG-315 and its metabolites from tissue homogenate and subcellular fractions.

![Figure 2](image2.png)

**Fig. 2.** A procedure for cell fractionation.
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Paper chromatogram was developed ascendingly with mixed solvent (ethylacetate: pyridine: acetic acid: Aq. dist.=15: 10: 2: 6) on Toyo filter paper No. 51A (2×40 cm). For thin layer chromatography, alumina oxide F 254 neutral (Type E, Merck 5550/0025, 20×20 cm) was used and the developing solvent was a mixture of isopropyl alcohol: methanol: petroleum ether (60: 20: 1).

CG-315 allied compounds, its phenol derivative (M1), desmonomethyl derivative (M2) and phenol derivative of desmonomethyl-CG-315 (M5) were provided by Grünenthal Co. Ltd., West Germany.

Chemical formulas are as follows:

- **Desmonomethyl derivative of CG-315 (M2)**
- **Phenol derivative of desmonomethyl CG-315 (M5)**
- **Phenol derivative of CG-315 (M1)**
- **CG-315**

RESULTS

1) **Distribution of CG-315**

When CG-315 in a dose of 60 mg/kg was administered s.c. to rats, as shown in
Table 1 and in Fig. 4, a certain amount of the drug was found to be already distributed in various organs 1 hr after administration, reaching a maximum level in 3 hr later. Thereafter, there was a gradual decline in the distribution and the amount distributed in organs 12 hr after administration decreased one fifth to one sixth that at 3 hr after but below one tenth in plasma, and only a trace was found 24 hr later. In view of the distributed concentration, the highest distribution of CG-315 was found in the lungs, followed by spleen, kidneys, liver and adrenal glands, the lowest being the in plasma.

In case of oral administration of CG-315, the distribution was similar to that in the s.c. administered groups (Table 2).

**Table 1.** Time course of distribution of radioactivity in organ tissues following s.c. administration of $^3$H-CG-315.

| Organ Tissues       | 1     | 3     | 6     | 12    | 24 hr |
|---------------------|-------|-------|-------|-------|-------|
| **Brain**           | 433± 47 (5) | 435± 47 (5) | 239± 40 (5) | 71± 6 (3) | 7± 2 (3) |
| **Heart**           | 249± 13 (5) | 310± 37 (5) | 212± 39 (5) | 62± 8 (3) | 5± 0 (3) |
| **Lungs**           | 1150±139 (5) | 1892±184 (5) | 997±186 (5) | 333±58 (3) | 74± 5 (2) |
| **Liver**           | 813± 92 (5) | 1418±118 (5) | 921±172 (5) | 331±65 (3) | 45±13 (3) |
| **Kidneys**         | 1294±203 (5) | 1434±306 (5) | 990±215 (5) | 265±15 (3) | 37± 9 (3) |
| **Spleen**          | 1018±120 (5) | 1594± 96 (5) | 948± 31 (5) | 244±58 (3) | 27± 2 (3) |
| **Adrenal glands**  | 1187±220 (4) | 1244± 78 (4) | 817±31 (4) | 317±60 (3) | 103±19 (3) |
| **Hypophysis**      | 682± 47 (4) | 721± 30 (4) | 396± 75 (4) | 157±24 (2) | 60±13 (3) |
| **Skeletal muscles**| 177± 10 (5) | 289± 10 (5) | 167± 17 (5) | 50± 3 (3) | 4± 0 (3) |
| **Plasma**          | 108± 12 (5) | 272± 64 (5) | 100± 17 (5) | 25± 3 (2) | 7± 3 (3) |

All values are expressed as $\times 10^3$ dpm/g or ml and average± s.e.
The number in parenthesis is the experimental number.

**Fig. 4.** Time course of distribution of total radioactivity in organ tissues following s.c. administration of $^3$H-CG-315.
2) Observations for distributions of CG-315 and its metabolites

The results above noted were obtained by measuring the total radioactivity of tritium. Consequently, it is difficult to define with certainty whether or not all the radioactivity

![Graph showing distribution of total radioactivity in organ tissues between CHCl₃ extractable and water soluble fractions after s.c. administration of ³H-CG-315.]

Fig. 5. Distribution of total radioactivity of organ tissues between CHCl₃ extractable and water soluble fractions after s.c. administration of ³H-CG-315.

TABLE 2. Time course of distribution of radioactivity in organ tissues following oral administration of ³H-CG-315.

| Organ Tissue     | 1        | 3        | 6        | 12       | 24 hr    |
|------------------|----------|----------|----------|----------|----------|
| Brain            | 227±47   | 357±46   | 163±43   | 143±49   | 8±1 (2)  |
| Heart            | 208±80   | 261±70   | 196±38   | 114±14   | 8±2 (2)  |
| Lungs            | 1039±285 | 1489±537 | 853±301  | 235±13   | 37±1 (2) |
| Liver            | 733±327  | 1272±164 | 735±199  | 257±38   | 100±9 (2) |
| Kidneys          | 875±322  | 1224±382 | 710±197  | 132±4    | 44±4 (2) |
| Spleen           | 801±330  | 1263±94  | 545±81   | 369±56   | 26±3 (2) |
| Adrenal glands   | 666±63   | 996±217  | 541±15   | 491±22   | 57±0 (2) |
| Hypophysis       | 615±285  | 868±235  | 515±15   | 285±47   | 141±27 (2) |
| Skeletal muscles | 125±42   | 249±63   | 178±36   | 73±3     | 45±6 (2) |
| Plasma           | 95±13    | 179±27   | 154±24   | 67±2     | 36±7 (2) |

All values are expressed as ×10⁶ dpm/g or ml and average±s.e.

The number in parenthesis is the experimental number.
in organ tissues really represents total distributed amounts of \(^3\)H-CG-315 itself, as the administered drug may be partly metabolized and thereafter redistributed. Details will be described in the next chapter. Thus, the distribution of both CHCl\(_3\) extracted radioactive portions and water soluble ones in several main organs were assayed again, as CG-315 itself was very easily extracted with CHCl\(_3\).

As shown in Fig. 5, 6 hr after s.c. administration (60 mg/kg), total radioactivity distributed into brain, heart, lungs, kidneys and spleen was almost extractable with CHCl\(_3\), whereas CHCl\(_3\)-extractable radioactivity in liver and blood (same in plasma) was approx. 50\%, the remaining being water-soluble. These findings indicate the possibility of distribution of metabolites from CG-315.

3) **Subcellular distribution of CG-315 and its metabolites in several organ tissues**

3 hr after s.c. administration of \(^3\)H-CG-315, irrespective of solubility in CHCl\(_3\) or water, the distribution of the radioactivity per mg protein was found predominantly in the supernatant fraction in brain, heart, liver and kidneys, while those in other fractions such as microsomes, mitochondria and nuclei were very low (Fig. 6).

4) **Excretion of CG-315**

i) **Excretion into urine**

After s.c. or oral administration of CG-315, as shown in Fig. 7, radioactivity corresponding to approx. 50\% of the administered amounts of the drug was detected in excreted urine for 12 hr, with the total excretion rate reaching approx. 75\% in 24 hr, thereafter which only a trace was seen. 70-80\% of the total excreted amounts was CHCl\(_3\)-extractable (Fig. 8).
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ii) Excretions into bile and feces

Approx. 16% of the administered amounts of the drug was excreted into bile within 24 hr, of which a greater part was water-soluble. On the other hand, approx. 5% of the administered dose of CG-315 was detected in feces within 24 hr. The results above noted are summarized in Table 3. From these experiments, it appears that most of the administered CG-315 which would be metabolized in the body was excreted into the urine through the kidney with some in the feces, though a part of the absorbed CG-315 and metabolites was excreted into bile with some being reabsorbed through the intestinal walls.

FIG. 7. Urinary excretion of radioactivity (percent of injected amounts) following s.c. administration of ³H-CG-315 in normal rats.

FIG. 8. Changes in the ratio of water soluble and CHCl₃ extractable radioactivity in the urine during the course of excretion following s.c. or oral administration of ³H-CG-315 in normal rats.

TABLE 3. Excretion of CG-315 and metabolites into feces, bile and urine following s.c. administration of ³H-CG-315.

|        | 0--12 hr | 0--24 hr |
|--------|----------|----------|
|        | Recovery (%) | CHCl₃ extr. | Water soluble | Recovery (%) | CHCl₃ extr. | Water soluble |
| Feces  | 0.9±0.5  | 71.4±4.0  | 28.6±4.0  | 5.4±0.4   | 78.9±4.8   | 21.1±4.8    |
| Bile   | 13.4±0.5 | 3.4±0.6   | 96.6±0.6  | 17.2±0.5  | 2.5±0.4    | 97.5±0.4    |
| Urine  | 52.8±2.7 | 76.8±4.2  | 24.3±4.2  | 75.3±5.5  | 76.5±2.0   | 23.4±2.0    |

* is % of total radioactivity administered
** is % of recovery in CHCl₃ extractable and water soluble radioactivity
Each value represents an average ±s.e.
5) **Analysis for metabolic products of CG-315**

**i) Paper chromatography**

Excreted urine was collected for 24 hr after s.c. administration of $^3$H-CG-315. An aliquot of collected urine was used for paper chromatography. As shown in Fig. 9, the two spots on the paper having 0.84 and 0.24 of Rf value were obtained. However, using the remained urine previously treated with CHCl$_3$, only a spot with 0.24 Rf value was present on the paper. This is presumed to be a product yielded from the administered CG-315, as the untreated urine added $^3$H-CG-315 had only a spot with 0.84 Rf value on the paper.

**ii) Thin layer chromatography**

When the urine collected for 24 hr from rats administered $^3$H-CG-315 was shaken with CHCl$_3$, approx. 65% of total radioactivity in the urine was extracted into CHCl$_3$, as illustrated in Table 4. Thereafter, CHCl$_3$ extracted fraction was developed by thin layer chromatography using alumina oxide; i.e., as shown in Fig. 10 and Table 5, the four spots showing 0.75, 0.67, 0.52 and 0.17 of Rf value were obtained, indicating that metabolic products other than CG-315 would be extracted into CHCl$_3$ fraction. In addition, the two spots with 0.67 and 0.17 of Rf value revealed positive phenol reaction (17). On the other hand, CG-315, under the same procedure, produced a spot showing 0.75 Rf-value. Moreover, of CG-315 allied derivatives, M$_1$, M$_2$ and M$_5$ produced the spots with Rf-values 0.67, 0.52 and 0.17, respectively.

![Fig. 9. Paper chromatogram of urine excreted for 24 hr after s.c. administration of $^3$H-CG-315 in normal rats.](image-url)

| Route | Water sol. $\times 10^5$ cpm (M±S.E.) | CHCl$_3$ sol. $\times 10^5$ cpm (M±S.E.) | Total $\times 10^5$ cpm | Ratio % (W : C) |
|-------|-----------------------------------|------------------------------------------|------------------------|----------------|
| s.c.  | 2738±128                          | 4575±123                                 | 7313                   | 37 : 63        |
| p.o.  | 2787                              | 4653                                     | 7440                   | 37 : 63        |
iii) Treatment with β-glucuronidase

After extracting treatment with CHCl₃, the remaining urine was dried up under reduced pressure and then extracted with methanol. Thereafter, the condensed methanol extract was incubated with β-glucuronidase. Thus, as shown in Table 5, approx. 70% of the water soluble radioactivity became CHCl₃ extractable again by treatment with the enzyme. Furthermore, the CHCl₃ re-extract had two spots with 0.67 and 0.17 of Rf-value on the thin layer chromatogram, which would be M₁ and M₅, respectively. Consequently, it appears that M₁- and M₅-glucuronides remained in the water layer after the first extracting treatment with CHCl₃.

6) Metabolism of CG-315 in tolerant rats

Rats were made tolerant by injection of CG-315 for 2 wk. No difference was observed in the metabolism of CG-315 between tolerant and non-tolerant rats.

### Table 5. Percentage of CG-315 and metabolites on thin layer chromatogram of CHCl₃ extracted fraction of urine excreted from nontolerant rats.

| Route | Excretion % (Mean ± S.E.) |
|-------|---------------------------|
|       | CG 315  | M₁    | M₅    | M₅     |
| s.c.  | 31 ± 2.2 | 11 ± 0.7 | 38 ± 2.0 | 13 ± 0.9 |
| p.o.  | 19 ± 0.5 | 15 ± 2.5 | 46 ± 0.5 | 15 ± 2.0 |

### Table 6. Percentage of CHCl₃ re-extractable radioactivity from the water layer of urine by β-glucuronidase treatment.

| Route | Water sol. cpm | CHCl₃ sol. cpm | Total cpm | Ratio % (W : C) |
|-------|-----------------|----------------|------------|-----------------|
| s.c.  | 66008 ± 4025    | 169583 ± 7305  | 235591     | 28 : 72         |
| p.o.  | 78425           | 214058         | 292484     | 27 : 73         |
DISCUSSION

When CG-315 (³H-labeled) in a dose of 60 mg/kg was administered s.c. or orally to rats, the drug was widely distributed in the whole body one hr after administration, reaching a maximum level 3 hr later. Thereafter it gradually disappeared with only a trace remaining in the body 24 hr later. In view of the distributed amounts, the highest concentration of the radioactivity was found in lungs, followed by spleen, kidneys, liver and adrenal glands, the lowest being in the plasma. Subcellular distribution of ³H-CG-315 was highest in the supernatant fraction, being common to all organs examined.

On the other hand, approx. 50% of the administered dose of CG-315 was excreted into the urine within 12 hr after administration and about 70% after 24 hr. 5% was found in the feces, although over 15% was excreted into the bile during 24 hr.

From the chromatographical examinations together with CHCl₃ extracting treatment for urine, feces or bile, the metabolites of CG-315 that is, M₁, M₂, M₃, M₁-gluc., and M₂-gluc. were found. The urinary excretion rates of CG-315 and its metabolites are summarized in Table 7. It particularly seems reasonable that M₁-and M₂-glucuronides would be present in liver and plasma to a greater extent, as the radioactivity of these two remained abundantly in the water layer even after CHCl₃ treatments and the radioactivity excreted into bile was hardly CHCl₃ extractable.

Finally, it is presumed that the administered CG-315 was rapidly absorbed and widely distributed in the whole body and mostly metabolized in liver to O-, N- or both demethylated forms and further in part conjugated with glucuronates. CG-315 and its metabolites were then excreted into the urine within 24 hr as well as some into the feces, though some of the metabolites in the liver were excreted with bile into the intestinal canal, most being reabsorbed into the blood.

| Route | CG-315 | Free base | Conjugated metabolites | Unknown |
|-------|--------|-----------|------------------------|---------|
|       |        | M₁ | M₂ | M₃ | M₁-Gluc. | M₂-Gluc. |         |         |
| s.c.  | 22     | 8  | 27 | 9  | 10       | 12       | 4       | 8       |
| p.o.  | 12     | 10 | 32 | 10 | 9        | 13       | 5       | 8       |

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